Chapter 19

Microbial Diversity and Interactions in Natural Ecosystems

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19.1 MICROBIAL COMMUNITIES

Adapting a commonly used phrase, "no microorganism is an island, entire of itself," most environmental microorganisms exist as part of a community. This community may be as simple as two populations coexisting on a fomite (Chapter 30); slightly more complex such as cyanobacteria, sulfate-reducing bacteria and other microorganisms in a stratified, microbial mat (see Information Box 4.4 and Chapter 6); or very complex such as soil, which commonly contains thousands of species per gram. In addition to soils, complex microbial communities are found in essentially all natural ecosystems including plants and surface- and groundwaters. Specialized communities such as rhizospheres or biofilms can have particularly large and diverse community membership (megacommunities). These communities exhibit great diversity as well as great redundancy in terms of potential activity. Both abiotic and biotic pressures drive the evolution of these complex microbial communities so that their composition is highly dynamic, and reflects the rate of change being imposed on the ecosystem—whether it is through natural successional events, climate change or anthropogenic impacts. Abiotic pressures include pH, redox potential, water availability, temperature, salinity and organic matter, all of which can vary from the micro- to the macrosite level. Biotic pressures are imposed by different populations competing for similar nutrient resources, such as organic carbon or nitrogen, which are normally limiting. Although there is great debate over exactly how many different populations there are in a given ecosystem, there is agreement that it is a large number. Soil typically contains 10^8 to 10^{10} bacteria per gram based on direct counts (see Section 4.4.1). If every population was present at 10^6 per gram, this would mean that there are 100 to 10,000 different populations in every gram of soil! These populations exist in close proximity to each other, and either compete or work synergistically for resources.

How do these different populations respond to these pressures? They tend to acquire genes that allow for previously unavailable activities, or enhanced rates of activities that already exist (Pál *et al.*, 2005). Gene acquisition can be through point mutation events that alter regulation or

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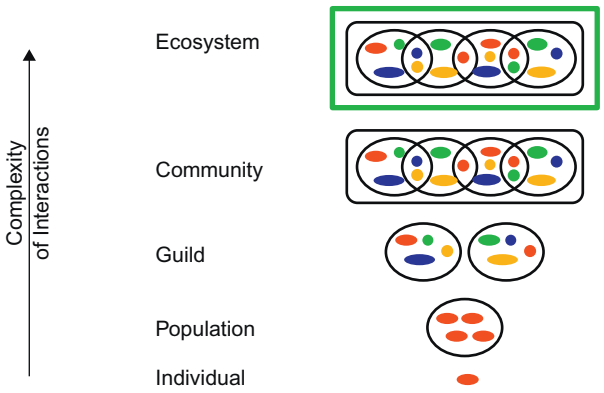


FIGURE 19.1 Ecological organization within a microbial community. Adapted from Atlas and Bartha (1998).

kinetics of enzymes, or alternatively, genes can be acquired through lateral or horizontal gene transfer (Section 2.2.7). The large number of populations present, and their rapid ability to respond to environmental pressures, results in an ever-changing and evolving microbial community within any given ecosystem. In fact, even closely related microorganisms can be quite different. For example, an analysis of four completely sequenced *E. coli* strains showed up to a 29.25% difference in the gene content of the four genomes (Coenye *et al.*, 2005). Thus, diversity in normal healthy soils is necessarily very large to take advantage of numerous niches that can develop. In contrast, diversity in stressed or extreme environments that have very specific, strong selective pressures (e.g., high temperature, low pH) tends to be much lower (Chapter 7).

The importance of these microbial communities is unquestionable. In fact, natural ecosystems provide a myriad of resources that benefit society by helping to maintain Earth's biosphere. These include climate regulation, biogeochemical (nutrient) cycling, waste treatment, water supply and regulation, as well as healthy soils for growing crops. It has been estimated that ecosystems, underpinned by microbial activity, provide at least $33 trillion per year in global services (Costanza *et al.*, 1997) (Information Box 7.1). In addition to this, microbial communities are responsible for a wide array of natural products including antibiotics and anticancer agents that are being explored for the benefit of society. Clearly, then, natural microbial communities are important, and fundamental to the success of these communities in natural ecosystems is their vast diversity, which enables them to adapt to changing conditions. This adaptation response can result in transient community changes in response to new environmental conditions that may be imposed naturally or by anthropogenic activity, or can result in the evolution of new communities in response to long-term ecosystem changes. In this chapter, we will discuss the diversity of environmental microorganisms, how they interact with other organisms and the environment, and how their diversity and interactions impact microbial adaptation, ecosystem function and the discovery of natural products such as antibiotics.

19.2 MICROBIAL DIVERSITY IN NATURAL SYSTEMS

19.2.1 What is a Microbial Community?

Ecologists use a hierarchical classification system to describe organisms and their communities (Figure 19.1). Individual microorganisms that are genetically related and perform the same function in a proximate location (i.e., that occupy the same niche) are referred to collectively as a population. Populations that compete for the same resources are grouped into a guild. All of the guilds (and thus populations) present in a specific environment constitute the microbial community. The community in a defined sample, such as a plant root, is a species assemblage (this is what is commonly referred to as the "microbial community" in research papers since only a portion of the microbial community has typically been characterized). The microbial community along with the other biotic and abiotic components of its environment constitutes an ecosystem.

19.2.2 What is Microbial Diversity?

From a community perspective, microbial diversity is defined as the amount of variation (i.e., genetic, morphological and functional differences) in microbial populations occupying a given environment. A variety of different diversity measures are commonly used for characterizing microbial communities (Information Box 19.1). The basis for these determinations is the number (richness), equitability of distribution (evenness) and identity (community composition) of different organisms (i.e., species) in a sample(s). The most commonly reported microbial diversity data include measures of species richness and/or evenness (often using diversity indices such as Shannon–Wiener; α-diversity) and community composition (β-diversity). It is important to point out that any diversity estimate, including those based upon rRNA genes, are subject to many limitations (Chapter 21). Furthermore, unless the entire microbial community in a sample has been sequenced, the sequence data represents only a portion (sometimes very small) of the entire community. The diversity data from this sample is then extrapolated to estimate the diversity of the community. This is subject to many biases, such as the size of the DNA sequence library used; therefore, diversity estimates should be used carefully and interpreted with caution (Gihring *et al.*, 2012).

Information Box 19.1 Measures of Microbial Diversity

Measure	Description
Alpha (α) diversity	Species diversity in a sample unit. This is often determined using species richness or a diversity index.
Beta (β) diversity	Amount of compositional variation in a sample or set of sample units.
Gamma (γ) diversity	Overall diversity in a collection of sample units.
Species richness	Number of species in a sample unit.
Species evenness	Equitability of species abundance in a sample unit.
Diversity index	Quantitative measure of diversity based upon factors such as species richness and evenness.

Two of the most commonly used indices are Shannon–Wiener and Simpson's.

Information Box 19.2 Criteria for Distinguishing Soil Bacterial Species

Technique	Cutoff for Species Distinction
DNA–DNA hybridization (i.e., the degree of association between the total genomic DNA of two species)	<70% DNA–DNA re-association
16S rRNA gene sequence identity	<97 to 99%

19.2.3 What is a Species?

The discussion of microbial communities and diversity is critically dependent on the concept of what constitutes a bacterial species and how two different species can be distinguished. Thus, before one can address diversity in a given system, the fundamental unit that determines diversity must be defined. This has been the subject of debate for many years. This is especially problematic for bacteria because we cannot easily observe them in their environment, and they can change rapidly through mutation, genome alteration or reduction, and by the acquisition of genes from other, often distantly related, organisms. Therefore, scientists have not been able to develop a classification system for bacteria that is based on evolutionary and ecological processes as they have for higher forms of life. Rather, microbiologists began by classifying microorganisms on the basis of morphology and selected physiological traits, especially those important for human health. The advent of molecular techniques and the increasing availability of rapid and inexpensive DNA sequencing technologies have allowed reexamination of microbial classification. Although there is still not complete consensus, we seem to be slowly moving closer toward a definition of bacterial species.

In the 1970s, before DNA sequencing was available, DNA–DNA hybridization was used to examine whether two organisms were the same or different. In this technique, both differences in gene content and differences in nucleotide sequence in shared genes contribute to the amount of hybridization that occurs. The standard used to differentiate species has been 70% DNA–DNA hybridization. Above this level, the two organisms are considered to be the same species and below this level, they are considered to be different. Although this technique was one of the first developed, results are consistent with newer techniques. The disadvantage of DNA–DNA hybridization is that it is time-consuming because only two organisms are compared at a time.

Currently, because of the ease of the technique, sequence-based techniques are being employed to examine diversity. Here, the criterion for similarity is based on sequence divergence of homologous genes (Cohan and Perry, 2007). The most commonly used target has been the 16S rRNA gene, which is universally found in all bacteria; millions of 16S rRNA sequences are now publicly available from a number of different databases (Table 13.1). The criterion for similarity based upon comparison of 16S rRNA sequences is at least 97% sequence similarly, although more recently it is been suggested that even a 1% difference may indicate different species. Each unique group of sequences (i.e., those with ≥97% matching sequence identity) is classified into an operational taxonomic unit (OTU; also known as a phylotype) which serves as a "species" classification for the purpose of diversity determinations. However, this approach is not without its shortcomings. For example, it has been suggested that 16S rRNA sequences do not discriminate beyond the genus level. When comparing DNA–DNA hybridization and 16S rRNA sequence similarity, it has been shown that less than 97% sequence homology always yields <70% DNA–DNA hybridization. But in some cases, greater than 97% sequence homology may also yield <70% DNA–DNA hybridization suggesting that the hybridization technique is more discriminating (Information Box 19.2). Also, the <97–99% sequence identity for species determination was largely based upon the full-length 16S rRNA genes. However, most current microbial diversity studies using newer sequencing technologies are based upon partial (<500 bp) sequences of 16S rRNA genes. This can greatly impact the taxonomic resolution of the sequence data depending upon the length and region of the 16S rRNA gene that is sequenced (Mizrahi-Man *et al.*, 2013). Furthermore, even the comparison of complete 16S rRNA gene sequences may fail to reflect subtle but

functionally important differences, such as the presence of pathogenicity genes, prophages, etc.

The lack of discrimination provided by 16S rRNA gene analysis has engendered the development of the multilocus sequence typing (MLST) approach to examine diversity among closely related species. MLST involves the sequencing of three to eight genes (each gene is a locus), and comparison of these sequences (Cohan and Perry, 2007). Thus, while the 16S rRNA gene may allow definition to the genus level, MLST can provide further definition to the species level. Furthermore, as DNA sequencing continues to become more affordable, whole-genome sequencing will likely play a greater role in microbial typing in the future. The final step to take is to define a systematic approach for bacteria that is based on ecology and evolution rather than simply on genetic similarity. Additional information on currently used molecular methods for determining microbial diversity and community composition is provided in Chapter 21.

TABLE 19.1 Estimates of Bacterial Diversity (Based Upon 16S rRNA Sequences) in Different Environments

Environment	Diversity Estimate (Species Richness)	Source
Polar desert soil	2935	Fierer *et al.*, 2012
Agricultural soil	3409	Hollister *et al.*, 2013
Diesel-contaminated soil	3259	Sutton *et al.*, 2013
Hypersaline soil	5285	Hollister *et al.*, 2010
Arctic tundra soil	6965	Fierer *et al.*, 2012
North Atlantic Ocean	6997	Sogin *et al.*, 2006
Tropical forest soil	8772	Fierer *et al.*, 2012
Temperate grassland soil	10,253	Fierer *et al.*, 2012
Temperate forest soil	12,150	Fierer *et al.*, 2012

19.2.4 Microbial Diversity in Soil and Ocean Environments

In Section 19.1 we estimated, simply using a numbers game, that the number of different bacterial populations or species in soil could range from 100 to 10,000. However, microbial diversity has actually been measured in soils, and other environments, using a variety of methods. One of the first approaches examined diversity by using DNA-reassociation kinetics of pooled genomic DNA from an environmental sample. This was first applied to a bacterial community from a forest soil sample collected in Norway. This approach yielded an estimate of 4000 different genomes per gram of dry soil (Torsvik *et al.*, 1990). In this case, total counts were 1.5×10^{10} bacteria per gram. A second study used the same approach to compare diversity in an uncontaminated soil from an agricultural field site in Germany, with adjacent soils that were amended with low or high amounts of metal-contaminated sludge. This study estimated 16,000, 6400 and 2000 genomes per gram wet soil for the uncontaminated, low metal and high metal soils, respectively (Sandaa *et al.*, 1999). More recently, the results from the latter study were reexamined using a modified computational analysis of the DNA-reassociation data. This study estimated that there were actually 8,300,000, 64,000 and 7900 genomes in the uncontaminated, low-metal and high-metal soils, respectively (Gans *et al.*, 2005).

At the moment, most diversity estimates are made using rRNA genes (e.g., 16S rRNA) from community DNA samples. Using this approach, diversity estimates are generally somewhat lower than when DNA-reassociation kinetics are used. A statistical analysis is then performed on the number of unique OTUs recovered in comparison to the total number of OTUs sequenced. This approach also involves comparing the total number of OTUs in the community relative to the abundance of the most prevalent OTUs in the community (Curtis *et al.*, 2002). These statistical approaches have provided estimates of bacterial diversity in several different natural soil environments ranging from a few thousand to $>$10,000 OTUs or phylotypes (Table 19.1). Although soil fungi are also diverse, they are usually less diverse than bacterial populations within the same environment. For example, Hollister *et al.* (2013) estimated that an agricultural soil contained $\approx$300 species (phylotypes) of fungi in comparison to $>$3000 species of bacteria.

Natural bacterial communities in marine waters are generally less abundant and diverse than in soils. For example, a survey of paired samples taken from the Juan de Fuca Ridge in the northeast Pacific Ocean showed that microbial counts ranged from 3.9×10^4 to 1.8×10^5 cells per ml water (Sogin *et al.*, 2006). (This can be compared to 10^8 to 10^{10} cells per gram of soil.) This study examined three paired samples taken at the same site but at different depths. In addition, two samples were taken from deep-sea thermal vents. Each sample was 1–2 liters in size and microbes were collected on a filter. Analysis of diversity was based on the 16S rRNA gene and resulted in an estimate that

TABLE 19.2 Impact of Major Environmental Factors on Microbial Diversity

Factor	Impact on Microbial Diversity
pH	Maximum diversity at neutral pH (6 to 8). Extreme pHs result in reduced diversity.
Vegetation	Different plants may stimulate and/or inhibit different microbial populations.
Water content	Greater diversity with moderate water content. Water-saturated conditions decrease diversity due to less spatial isolation of organisms and also the generation of anaerobic conditions.
O_2 concentration	Greater diversity under aerobic conditions.
Temperature	Extremely high or low temperatures reduce diversity.
Organic matter content	Higher organic matter content results in higher diversity.
Soil depth	Decreasing diversity with increasing depth from surface.
Addition of organic substrates	Addition of a single, organic substrate often results in a reduction in diversity due to the stimulation of a subset of the microbial community.
Soil tillage	Decreased diversity due to soil homogenization and reduction in microsite variation.
Addition of organic pollutants	Similar to organic substrates in general, often a reduction in diversity due to stimulation of specific populations but also potentially toxicity of the xenobiotic to other populations.
Addition of metal pollutants	Reduction in diversity due to toxicity to some populations.

between 1184 and 3290 OTUs were present in the samples. A study of the Sargasso Sea, which is located in the North Atlantic Ocean, estimated the presence of 1800 species using a multilocus sequencing approach. Based on rRNA genes alone, this was reduced to 1164 unique sequences (Venter *et al*., 2004). In this case, several hundred liters of water were collected via filtration.

These two studies give very similar estimates of diversity in the marine environment. However, the samples studied were much larger in volume than 1 gram of soil. One theoretical effort to directly compare bacterial diversity in soil and marine samples is provided by Curtis *et al*. (2002). This analysis was based on relating the total number of bacteria in the sample to the number of bacteria in the least abundant species. This approach estimated that oceans have 160 species per mL while soils have 6400−38,000 species per gram. These scientists further extrapolated these numbers to estimate that the entire bacterial diversity of the ocean is 2×10^6 species. This can be contrasted to the diversity in one ton of soil which was estimated to be 4×10^6 species.

19.2.5 Environmental Factors that Impact Microbial Diversity

Many factors can impact soil microbial diversity including pH (Lauber *et al*., 2009), presence and type of vegetation (Lauber *et al*., 2009), contamination (Hemme *et al*., 2010), amendment with organic substrates (Hollister *et al*., 2013), temperature (Castro *et al*., 2010), depth (Hansel *et al*., 2008), water content (Zhou *et al*., 2002), oxygen content (Somenahally *et al*., 2011) and level of CO_2 (Dunbar *et al*., 2012) (Table 19.2). In general, properties that increase the heterogeneous nature of soils (Chapter 4) tend to result in increased microbial diversity. For example, greater microbial diversity is typically found in soils that have well-established structure (i.e., soil aggregates), and that are not saturated with water, since these conditions result in more spatial isolation within the soil communities thus encouraging higher microbial diversity (Torsvik and Øvreås, 2002). Even seemingly benign events, such as sheep urinating in a pasture, may impact microbial communities (Nunan *et al*., 2006). As discussed in Case Study 4.1, Lauber *et al*. (2009) used 16S rRNA sequencing to investigate the bacterial communities in 88 soils from North and South America, and found that that soil pH was one of the main drivers of bacterial diversity, and was more important than other factors such as vegetation type and soil carbon at a continental scale. However, the authors suggested that other factors may be more important at local or regional scales. Fierer *et al*. (2012) expanded upon this by assaying 16 soils from different biomes using both 16S rRNA-based and shotgun metagenomics-based sequencing (Chapter 21). They found a strong correlation between richness of the 16S rRNA-based phylotypes and the metagenomics-based functional genes. Moreover, the grassland and forest environments

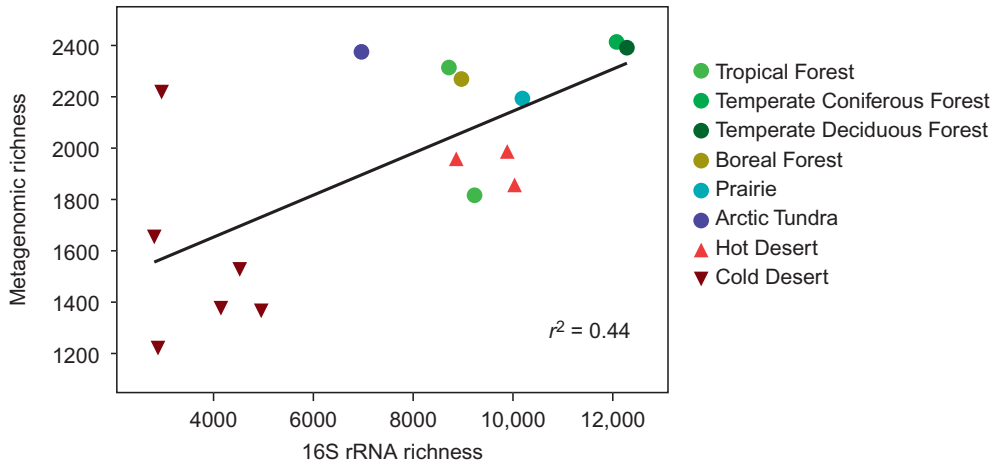


FIGURE 19.2 Diversity of microbial communities in 16 soils from a variety of ecosystems. The y-axis shows the taxonomic richness (number of phylotypes) of the bacterial community. The x-axis shows the functional gene richness. From Fierer *et al.* (2012).

generally contained more diverse microbial communities than either of the more extreme (hot or cold desert) environments (Figure 19.2).

Although dramatic changes such as imposing anaerobic conditions on a previously aerobic soil by flooding it (as commonly used in rice cultivation) can greatly impact microbial communities, the effects of subtle and/or gradual changes, such as may result from climate change, may be more difficult to determine. This is especially true for situations where multiple factors may change simultaneously and even interact. For example, Castro *et al.* (2010) investigated the impact of multiple drivers of climate change (increased atmospheric CO_2, increased temperature and varying precipitation). They found that the level of precipitation had a greater effect than increased CO_2 or temperature on the diversity of the soil bacterial and fungal communities. However, precipitation also had the greatest effect on the abundance and diversity of the plant community, so the observed changes in the microbial community may have been an indirect response to the plant changes, and not directly as a result of the environmental changes. There have been some reported impacts of elevated CO_2 on soil microbial communities for individual ecosystems. For example, one study looked at the effects of 10 years of elevated CO_2 in six ecosystems and found no systematic effect on bacterial biomass, richness or community composition, although ecosystem-specific responses and some trends across sites (e.g., decreased populations of *Acidobacteria* Group 1) were found in response to elevated CO_2 (Dunbar *et al.*, 2012). Additionally, it should be noted that most studies still only characterize the most abundant organisms in a sample. Therefore, even though a study does not detect a response of the microbial community to an environmental disturbance, the impact on less abundant members of the community may be missed unless these populations are specifically targeted (Case Study 4.2).

19.2.6 Functional Diversity and the Resilience of Microbial Communities

Whereas great progress has been made in terms of exploring soil microbial diversity, most of this information has been discovered based on phylogenetic genes (e.g., rRNA genes), which do not provide direct indication of the functional role that these organisms play in the environment. Scientists usually infer potential functional roles for these organisms based upon their relatedness (i.e., similarity of their rRNA genes) to other organisms whose function is known. However, this is complicated by the diversity and widespread distribution of many functional genes. Sequence diversity among functional genes (e.g., nitrite reductase and nitrous oxide reductase) is normally greater than that of ribosomal genes. Further, microbes from all three domains of life (Archaea, Bacteria and Eukarya) have been shown to be able to participate in some of the same processes (e.g., denitrification; Ward, 2002). Therefore, linking phylogeny and function remains a challenging task. However, emerging approaches such as environmental functional gene arrays and metagenomic sequencing are making this more possible (He *et al.*, 2010; Hemme *et al.*, 2010). Additionally, the integration of activity- and community composition-based measurements, such as microradioautography with specific radiolabeled substrates in combination with fluorescent *in situ* hybridization (FISH) of microbial cells, is helping to provide direct linkage between environmental organisms and the processes they facilitate *in situ* (Torsvik and Øvreås, 2002).

Although the relationship between soil microbial diversity and functional diversity remains largely unknown, diverse soil communities are believed to enhance ecosystem stability, productivity and resilience towards stress and disturbance (Torsvik and Øvreås, 2002). Redundancy with respect to functional diversity

may enable soil microbial communities to be active even with environmental parameters that constantly change, including temperature, soil moisture content and nutrient availability. Once a certain level of diversity is reached in a community, all of the functions necessary for ecosystem function exist within members of the microbial community. Beyond this point, additional diversity does not provide additional functions but does instead provide functional diversity and ostensibly ecosystem stability (Figure 19.3).

Following a change or disturbance, a microbial community can have at least four responses: (1) the composition stays the same—known as resistance; (2) the composition is altered but later returns to its original composition—known as resilience; (3) the composition is altered but still performs like the original community due to functional redundancy of the community; or (4) the composition is altered and performs differently than the original community (Figure 19.4).

Allison and Martiny (2008) reviewed over 70 studies that experimentally exposed microbial communities to different disturbances. They found that in the vast majority of cases, the microbial communities were sensitive to the disturbance: 60% to increased CO_2 levels; 84% to nitrogen/phosphorus/potassium fertilization; 82% to temperature; and 83% to carbon amendments. In another example, a study evaluating seasonal and environmental changes on soil microbial community composition found that, although bacterial biomass did not change significantly during the seasons, culturable and molecular techniques did demonstrate significant variations in community composition (Smit *et al.*, 2001). Interestingly, in this study and others, culture-dependent and molecular techniques identify very different microbial populations in soil.

This indicates that microbial communities are very fluid, at least taxonomically, and are not resistant to perturbations. If a change occurs, it is possible for the community to be resilient and later return to the original community composition. However, due to the complexities of microbial communities and interactions, this does not seem likely and data are lacking to support the idea that this occurs regularly. The more likely responses appears to be an impacted microbial community with an altered composition that either does or does not perform like the original community (Case Study 19.1; Table 19.3; Figure 19.5).

Interestingly, anthropogenic activities targeting ecosystem restoration of severely impacted environments can create microbial communities that function similarly to natural ecosystems. For example, mining of ores for copper is a significant industry throughout many regions of the world. Following the extraction of the copper-containing minerals, there is a need to deposit the processed ore. These so-called mine tailings are often piped into desert

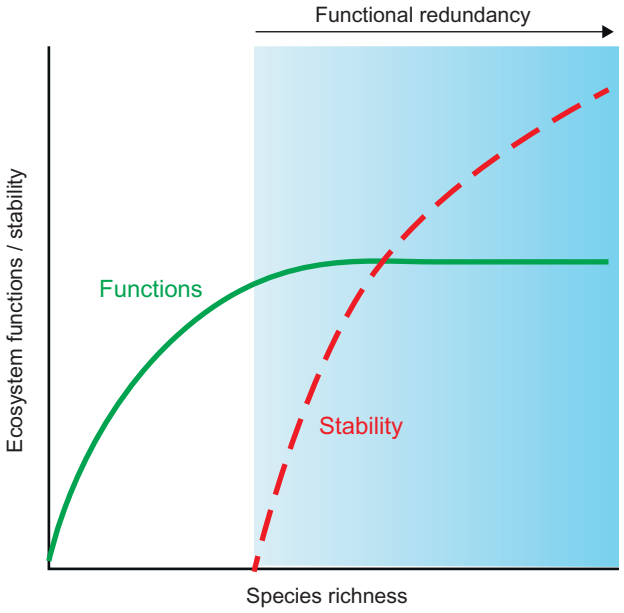


FIGURE 19.3 Relationship between functional redundancy and ecosystem stability. As microbial species are added to an ecosystem, this increases the functional capability of the microbial community. Beyond a point, additional species do not add new functions, but they do serve to increase the functional redundancy and thus stability of the ecosystem. Adapted from Konopka (2009).

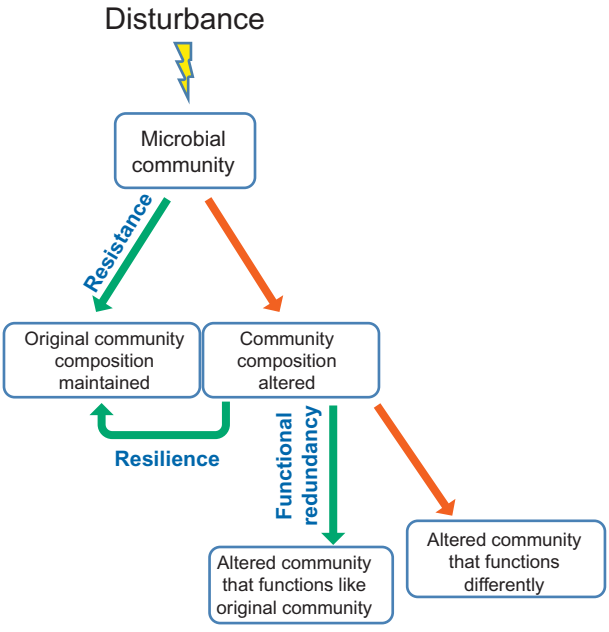


FIGURE 19.4 Illustration of potential microbial community responses to disturbance and the resulting impacts on ecosystem processes. Note that multiple mechanisms (e.g., resistance, resilience and functional redundancy) can help maintain microbial function(s) following a perturbation. Adapted from Allison and Martiny (2008).

Case Study 19.1 Recovery of Soil Microbial Processes, Populations and Communities Following Reclamation of a Lignite Surface Mine

Surface mining for lignite (coal) results in the destruction of the original soil profile characteristics, and therefore alters the physical, chemical and biological conditions. After mining, these sites are reclaimed by backfilling with the previously removed soil (i.e., overburden) and reconstructing the site to its original slopes and contours. In addition, the sites are revegetated with either native or improved (e.g., commercial timber) plant species.

Numerous studies have investigated the recovery of soil chemical and physical properties following reclamation. However, there is very limited information on the recovery of soil microbial communities following reclamation, and even less that correlates this with ecosystem function. To address this knowledge-gap, Ng (2012) conducted a study to determine the amount of time required for recovery of soil physical, chemical and microbial characteristics in a 40-year chronosequence of reclaimed mine soils at the Big Brown lignite mine in East Texas. A similarly vegetated site nearby was used as the unmined reference site.

Following reclamation, many of the soil physical and chemical properties were immediately returned to conditions that met or exceeded those of the soil of the unmined reference site. Nutrient distribution throughout the soil profile required at least 5 years before any stratification was observed. Carbon and nitrogen sustained premined levels through the profile after 15 years. Soil microbial biomass levels and carbon and nitrogen mineralization required 15 to 20 years before returning to unmined levels (Table 19.3). Additionally, numbers of bacteria and fungi (as determined with qPCR) recovered within 20 years. However, the microbial communities (as determined using 16S rRNA gene sequencing and functional gene microarray analysis) did not return to the same composition even after 40 years (Figure 19.5). Interestingly, the 10- and 15-year reclamation soils were more similar to the unmined reference site than the 30- and 40-year post-reclamation sites were. This suggests that the bacterial communities initially became more similar to the reference soil, up to around 15 years, and then deviated into a different bacterial community. Since this corresponded with the recovery of major soil processes (e.g., carbon and nitrogen mineralization), it appears that the functional redundancy of the microbial community contributed to the recovery of soil ecosystem functions, even though the microbial community did not return to its original composition.

Source: Ng (2012).

TABLE 19.3 Recovery of Soil Microbial Processes, Populations and Communities following Reclamation of a Lignite Surface Mine

Years Since Reclamation	Microbial Biomass		Mineralization		Microbial Numbers		Microbial Community Composition
	C	N	C	N	Bacteria	Fungi	
0	X	X	X	X	X	X	?
5	X	X	X	X	X	X	?
10	X	X	X	X	X	X	?
15	√	√	X	X	X	X	?
20	√	√	√	√	√	√	?
30	√	√	√	√	√	√	?
40	√	√	√	√	√	√	?

Adapted from Ng (2012).
X *= Soil quality parameter lower than for unmined conditions.*
√ *= Soil quality parameter equals (or exceeds) unmined conditions.*
? *= Soil quality parameter is different from unmined conditions, but it is unclear if this is better or worse.*

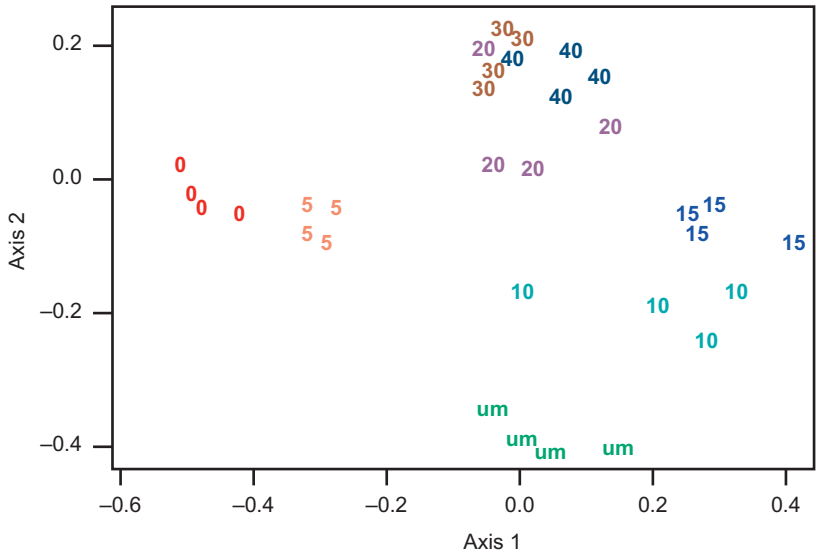


FIGURE 19.5 Changes in soil bacterial communities following reclamation of a lignite surface mine. The graph represents a non-metric multidimensional scaling analysis of 16S rRNA gene pyrosequencing data. Data include four replicate samples of soil from each site along a reclamation chronosequence of 0 to 40 years (0, 5, 10, etc.) along with soil of an unmined (um) reference site. Note that the bacterial community becomes more similar to the original (reference) community up to around 10 years post-reclamation, but then begins to diverge into a relatively stable community that is distinct from the original community. Adapted from Ng (2012).

Case Study 19.2 Community Diversity Dynamics of Mine Tailings Amended with Class A Biosolids

The bacterial community characteristics of mine tailings amended with Class A biosolids were monitored over a 10-year period. Specifically, samples were taken: 3 weeks; 3.5 years; 8 years; and 10 years after biosolid amendment, and subjected to community DNA extraction followed by cloning and sequencing. The 16S rRNA gene sequence analysis showed that the most persistent bacterial populations were members of major soil bacterial phyla including *Proteobacteria*, *Actinobacteria*, *Firmicutes*, *Bacteroidetes* and *Acidobacteria* (Table 19.4). This is consistent with most studies of soil bacterial communities (Kuske *et al.*, 1997; Sun *et al.*, 2004; Janssen 2006). In this study, both the reclaimed mine tailings and the desert soil were comprised of bacteria similar to those others have found in desert soils from the southwestern United States (Kuske *et al.*, 1997) and in most typical soils. It is also of interest that the percentage of unclassified bacteria from the desert soil was in fact slightly higher than the percentage from the other sites. This illustrates the fact that our knowledge of desert soil bacteria is limited and warrants further research.

Initially, sequences affiliated with the phyla *Bacteroidetes* and *Firmicutes* seemed to dominate at the early stages of the study (3 weeks to 3.5 years), but eventually their prevalence diminished with time. This might be due to the fact that *Bacteroidetes* and *Firmicutes*, along with *Actinobacteria*, are known to dominate 80% of identified bacteria in the human gut (Mariat *et al.*, 2009). Thus it could be expected that after the initial application of biosolids, biosolid-associated bacteria (not soil bacteria) would dominate microbial populations. This confirms the hypothesis of this study in which amendment of Class A biosolids into nutrient-poor mine tailings would ultimately lead to the establishment of a functionally redundant soil bacterial population followed by subsequent revegetation. Overall, the results indicated that biosolid-treated mine tailings had eventually acquired diversity levels approaching that of the desert soil.

Source: Pepper et al. (2012).

areas to a depth of 35 m. Mine tailings are essentially crushed rock and resemble soil. However, tailings have very low cation exchange capacities, minimal microbial populations and almost zero organic matter content. They support scant revegetative growth and are subject to wind erosion and dust storms. But, large additions of organic matter supplied as "Class A biosolids" can result in a functional soil with respect to microbial characteristics, which is sustainable over a 10-year period resulting in extensive revegetation of the tailings (Case Study 19.2, see also Case Study 26.1).

Functional diversity can also be an important mechanism that allows for soil microbial communities to successfully respond to anthropogenic-induced changes to the soil environment, as in the case of metal and/or herbicide additions to soil, or other soil amendments. In the case of co-contaminant additions to soil (metal + organic), a metal-resistant bacterium with the appropriate catabolic genes is necessary for effective biodegradation of the herbicide (Roane and Pepper, 2000). Populations of organisms with these twin properties can arise via one of two mechanisms.

TABLE 19.4 Bacterial Community Composition in a Chronosequence of Copper Mine Tailings Amended with Class A Biosolids

Phylum	Mine Tailings				Desert Soil
	Time Since Biosolids Applied				
	3 Weeks	3.5 Years	8 Years	10 Years	
	% of bacterial community				
Proteobacteria	23.9	25.2	30.7	31.4	29.0
Alpha*	21.1	49.3	63.9	57.6	48.1
Beta	59.2	13.4	10.8	12.9	26.6
Delta	1.4	1.5	7.2	1.2	8.9
Gamma	16.9	31.3	12.0	28.2	12.7
Unclassified	1.4	4.5	6.0	0.0	3.8
Actinobacteria	23.9	9.3	31.4	41.6	25.7
Firmicutes	16.2	52.6	5.8	2.2	1.1
Acidobacteria	0.0	1.1	14.4	3.6	17.3
Bacteroidetes	26.6	2.6	3.6	9.9	12.1
Chloroflexi	3.0	3.0	3.6	1.5	0.7
TM7	0.3	0.7	1.1	3.6	0.7
Unclassified bacteria	5.7	3.0	9.0	2.6	11.0

Adapted from Pepper et al. (2012).
Communities were characterized using 16S rRNA gene clone libraries. A natural desert soil is presented for comparison.
**Values listed for the classes of Proteobacteria (alpha, beta, etc.) represent the percentage of the total number of Proteobacteria belonging to each respective class.*

First, preexisting cells with the desired attributes may already exist within the soil but at low population numbers. In this case, metal-resistant cells with the ability to degrade the herbicide are at a competitive advantage, and their cell density will increase over time. In another scenario, horizontal gene transfer of genes encoding for metal resistance may occur via plasmid transfer to a cell which already has the necessary degradative genes, but previously lacked metal resistance. Following gene transfer, cell proliferation of the newly formed transconjugants can occur (Newby and Pepper, 2002). In either case, this process is termed "adaptation", which can occur within months, weeks or even days. Adaptation explains why repeated amendments of herbicide such as 2,4-dichlorophenoxyacetic acid (2,4-D) to soil result in enhanced areas of soil microbial degradation, relative to the first application. The time required for adaptation can also vary depending on the amount of metal added to soil, as shown in Figure 19.6. Here, as the amount of cadmium added to the soil increases, the adaptive time taken for degradation of 2,4-D increases. Without cadmium addition, no degradation of 2,4-D occurred during the first 7 days, whereas at the highest cadmium addition the adaptive time period was 21 days. Also, without cadmium, the 2,4-D degraded in 21 days, whereas with a cadmium amendment of 240 μg/g soil, degradation was only complete after 35 days.

In soil impacted by human activity, bacterial communities can be affected either adversely or beneficially due to the selective pressures imposed following anthropogenic inputs into soil. For example, Zerzghi *et al.* (2010) documented enhanced soil bacterial diversity following 20 years of continuous land application of biosolids. In contrast, metal additions to soil can reduce soil bacterial diversity (Kelly *et al.*, 1999).

19.3 MICROBIAL INTERACTIONS

19.3.1 Microbe–Microbe Interactions

Since environmental microorganisms often exist in close proximity to each other (e.g., as microcolonies or biofilms on soil particles), this increases the likelihood of microbial interactions occurring. These interactions may be positive, such as in commensalism and synergism, or negative, such as in competition, amensalism and predation (Table 19.5). In a commensal interaction, one population is benefited but another population(s) is not directly affected. This may include one population metabolizing a metabolic by-

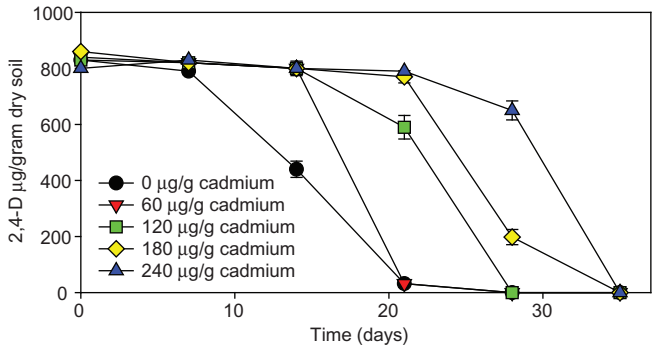


FIGURE 19.6 Biodegradation of 800 µg/g of 2,4-dichlorophenoxyacetic acid (2,4-D) in the presence of varying amounts of cadmium. Note that the onset of 2,4-D biodegradation is delayed as the cadmium concentration increases. Unpublished data, Pepper (2002).

TABLE 19.5 Types of Environmental Microbial Interactions

Interaction	Effect of Interaction		Example
	Population 1	Population 2	
Mutualism (symbiosis)	↑	↑	Relationship of leguminous plants with nitrogen-fixing rhizobia in which the plant provides the bacteria with organic carbon substrates and a growth habitat in exchange for fixed nitrogen.
Synergism	↑	↑	Syntrophic associations between fermentative bacteria and methanogenic archaeans in which bacteria hydrolyze organic compounds and ferment the products, resulting in generation of hydrogen, acetate, and formate. These materials are then consumed by archaeans, thus maintaining their concentrations at low levels and keeping the overall fermentation reactions energetically favorable.
Commensalism	↔	↑	Cometabolism of cyclohexane to cyclohexanol by one microbial population (which gains no energy from the process) followed by metabolism of the cyclohexanol by a second population.
Neutralism	↔	↔	Two spatially separated microbial populations that have no interaction.
Predation (parasitism)	↑	↓	Protozoa grazing on bacterial populations.
Amensalism	↔ or ↑	↓	Production of an antibiotic by *Streptomyces* spp. that inhibits the growth of fungi.
Competition (antagonism)	↓	↓	Two populations of heterotrophic bacteria (e.g., *Streptomyces* and *Pseudomonas* spp.) attempting to metabolize the same organic substrate.

Adapted from Atlas and Bartha (1998) and Bottomley (2005).
↑ = positive effect; ↔ = no effect; ↓ = negative effect.

product of another population. A classic example of this is the conversion of cyclohexane to cyclohexanol by one microbial population which gains no energy from the process. This conversion is an example of cometabolism or "fortuitous metabolism" reflecting the lack of benefit to the population initiating the transformation (Chapter 17). Other microbial populations (which cannot metabolize cyclohexane) can then metabolize the cyclohexanol. In the example above, if both microbial populations benefited from the interaction, these organisms would be classified as a consortium (pl. consortia) that worked together to metabolize the cyclohexane.

In synergistic interactions that benefit both (or all) microbial populations involved, the association may either be obligatory or optional, depending upon the organisms. An example of a generally optional synergistic interaction is syntrophy. Syntrophism, also known as cross-feeding, consists of multiple microbial populations

Information Box 19.3 Afla-Guard®—An Example of Competitive Exclusion

Production of toxins (e.g., aflatoxin) by environmental fungi such as *Aspergillus flavus* is a major problem for many crops. Recently, a biological control product has been developed and marketed for control of these fungi in crops such as corn. The basis for this control is use of a fungal strain that does not produce toxins. Spores of these atoxigenic fungi are added to fields on a carrier material, and following germination, the fungi colonize the developing ears of corn. The presence of these organisms serves to competitively prevent naturally occurring, toxin-producing fungal strains from colonizing the corn. Although this approach is subject to a variety of challenges, like any biological control mechanism, it has been shown to be capable of reducing aflatoxin levels by 85% or more!

Information Box 19.4 Fungistasis—An Example of Microbial Competition and Antagonism

It has long been recognized that adding organic amendments such as compost to soil can suppress many soil-borne plant pathogens. When this suppression involves the inhibition of fungal spore germination and hyphal growth, it is referred to as fungistasis. One possible explanation for this suppression is the presence of an active and diverse soil microbial community that competes with and inhibits proliferation of the plant pathogens. Although the exact mechanisms responsible for fungistasis are not clear, it appears that the process is at least partially due to decreased nutrient availability (due to competition from other microorganisms), and microbial production of inhibitory chemicals such as volatile organic compounds (VOCs) (Garbeva *et al.*, 2011). No matter what mechanism(s) is involved, this provides another example of the benefits of a diverse soil microbial community.

being involved in meeting each other's metabolic needs. One example of this is the association between fermentative bacteria and methanogenic archaea. The fermentative bacteria hydrolyze organic compounds and ferment the products resulting in generation of hydrogen, acetate and formate. These fermentation products are then consumed by the archaea, thus maintaining their concentrations at low levels and keeping the overall fermentation reactions energetically favorable. Some syntrophic interactions appear to involve the direct transfer of electrons between different populations through exchange of hydrogen, formate, cysteine or even through conductive nanowires (Sieber *et al.*, 2012).

If the synergistic interactions involve specific microorganisms (i.e., are species specific) or are obligatory, this is referred to as mutualism. Mutualism is commonly used synonymously with symbiosis. There are numerous examples of symbiotic relationships between different microorganisms (e.g., lichens), and between microorganisms and other organisms (e.g., *Rhizobium* spp.−legume interactions and mycorrhizal plants (Section 19.3.2.2)).

If two or more microorganisms are competing for the same resources or space, this will result in competition that will negatively affect one or more of the involved microorganisms. A good example of this is the use of atoxigenic strains of *Aspergillus flavus* to prevent the colonization of crops, such as corn, by strains of environmental fungi that produce mycotoxins (Information Box 19.3). Additionally, amensalism (i.e., antagonism) can occur due to the production of compounds that are toxic to other organisms. This includes antibiotics (Section 19.4), metabolic by-products such as alcohols and interference with cell−cell communication within a population (Chapter 20). Predation, or even parasitism, can also occur as microorganisms feed on other microorganisms. Examples of this include protozoa that graze on bacteria, fungi that trap nematodes and viruses that infect other types of microorganisms. In many cases, even though a negative interaction between microbial populations can be observed, it can be difficult to ascertain exactly which process (or processes) is responsible for inhibition of the other microbial groups (Information Box 19.4). If different microbial populations have no effect, either positive or negative, on each other, this is referred to as neutralism. This is easy to envision as a possibility for microorganisms that are spatially separated; however, in most cases, microorganisms that are spatially close will have some direct or indirect impact on each other as they metabolize and replicate.

19.3.2 Microbial Megacommunities

Microbes, particularly bacteria, like to adhere to surfaces, and surface-associated microbial communities normally have higher concentrations of microbes than microbial communities suspended in the water column, which are known as the plankton (see Chapter 6). Two such

microbial megacommunities are biofilm and rhizosphere communities. Biofilm communities range from 10^8 to 10^{10} CFU/cm^2 (Sjollema *et al*., 2011). Similarly, rhizosphere populations can be 10^8 to 10^9 CFU per gram of soil (Duineveld and Van Veen, 1999).

19.3.2.1 Biofilm Communities

Biofilm communities are complex microbial megapopulations consisting mostly of bacteria, but also other microbes including algae, protozoa and viruses (as bacteriophages). Biofilms form wherever there are water-associated surfaces, with moisture being a prerequisite for biofilm formation. Biofilms develop naturally on diverse surfaces including: water distribution pipes (see Chapter 28); rocks within rivers or lakes; and even our teeth. Sometimes biofilm formations are encouraged as in the case of the zooleal film which develops within the trickling filters of wastewater treatment plants (see Chapter 25). Microbial mats are specialized biofilms dominated by phototrophic prokaryotes, the cyanobacteria or blue–green algae. Lower layers of the mat can contain anaerobic sulfate-reducing bacteria (Risatti *et al*., 1994).

The formation and structures of biofilms are described in Chapter 6. Depth-dependent activities of biofilms are discussed in Chapter 7. Here we focus on biofilm communities and diversity. Biofilm communities are typically embedded within a complex mixture of macromolecules including both proteins and exopolysaccharides (EPS). EPS have been implicated as essential for biofilm architecture including the aggregation of bacterial cells, cell–cell recognition and communication, and gene transfer (Fleming and Wingender, 2010). Some bacteria including *Pseudomonas aeruginosa* also produce substantial amounts of extracellular DNA, or eDNA, and such eDNA may actually be a requirement for biofilm formation (Whitechurch *et al*., 2002). Overall, biofilm communities tend to be highly structured, with *Pseudomonas* spp. being particularly dominant members, and vital to the success of the biofilm community (Case Study 19.3)

19.3.2.2 Rhizosphere Communities

As we have seen, most normal soils do not contain abundant microbial substrates or nutrients, because microbial communities quickly utilize any substrates that are available. In contrast, the rhizosphere is a unique soil environment found in close proximity to plant roots, where substrates are more abundant because of the influence of the plant itself. Increased substrate availability in turn results in enhanced microbial activity, numbers and diversity. Thus, the rhizosphere exists because of complex soil–plant–microorganism interactions. Ultimately, microbial gene expression and diversity in the rhizosphere is controlled by these interactions, which in turn are influenced by environmental factors.

The term rhizosphere was coined by Hiltner in 1904 to describe the part of the soil that is influenced by plant roots. Originally, the rhizosphere was thought to extend 2 mm outward from the root surface. Now it is recognized that the rhizosphere can extend 5 mm or more as a series of gradients of organic substrate, pH, O_2, CO_2 and H_2O. Essentially two regions of the rhizosphere are now recognized: (1) the rhizosphere soil; and (2) the soil in direct contact with the plant root, which is the rhizoplane. Microorganisms also inhabit the root itself and are known as endophytes. The rhizosphere effect is caused by the release of organic and inorganic compounds from the plant roots, which can include root exudates, secretions, lysates or plant mucilages. It has also been shown that many families of plants release living root border cells through a programmed development process. These released cells synthesize novel compounds not produced while attached to the root, and can influence microbial behaviors adjacent to the root (Hawes *et al*., 2000).

Overall, a vast number of different kinds of microorganisms are found in the rhizosphere, and their numbers generally decrease from the rhizoplane outward toward bulk soil. The rhizosphere effect is often evaluated in terms of R/S ratios, where R = the number of microbes in the rhizosphere and S = the number of microbes in bulk soil. Thus, the greater the R/S ratio, the more pronounced the rhizosphere effect. R/S ratios vary for specific bacteria and fungi but numbers in the rhizosphere can be two to three orders of magnitude higher than in bulk soil.

Bacteria are the most numerous inhabitants of the rhizosphere and R/S ratios can typically be 20:1. In addition to increases in the overall bacterial population within the rhizosphere, specific soil–plant–microbe interactions have evolved that can either benefit or harm the plant. For example, *Agrobacterium* spp. are soil-borne bacteria which cause crown gall diseases (Section 20.2.3). In other cases, the abundant, diverse and active microbial populations in the rhizosphere can function as a "microbial buffer zone" that helps to protect the plant from soil-borne pathogens.

There are two examples of well-studied beneficial rhizosphere microorganisms. The first is bacteria capable of biological dinitrogen fixation, which is the process of converting atmospheric dinitrogen gas into ammonia. Free-living bacteria including species of *Azotobacter* and *Azospirillum* can be found within rhizosphere populations and provide associated plants with a source of fixed nitrogen (NH_3). In contrast to the free-living nitrogen-fixing organisms, the legume–rhizobia association involves a formal symbiosis in which both partners benefit (Information Boxes 16.2 and 16.3). Here, Gram-negative, heterotrophic bacteria originally classified within the genus *Rhizobium* interact with leguminous plants using an

Case Study 19.3 Biofilm Diversity and Community Structure

A recent study evaluated a new point-of-use (POU) device that relies on pathogen inactivation via biofilms developed within a newly manufactured 7-mm thick foam material termed "biofoam." Biofilms within the POUs were developed at three different locations in the U.S. (Montana, Michigan and North Carolina) using three different types of surface waters but under identical conditions. Following harvest, biofilms were analyzed utilizing 454 pyrosequencing of the 16S rRNA genes. Unexpectedly, it was found that there was a remarkable degree of shared community membership between the biofilms from the three locations. The similarity of class-level taxonomy of major biofilm operational taxonomic units (OTUs; ≥97% sequence identity cutoff) for three replicate POU biofilms from each location is shown in Figure 19.7. The top 100 shared OTUs represented 240,000 of the 306,000 raw sequences, and 25% of the shared sequences were classified within the genus *Pseudomonas* (class Gammaproteobacteria). In addition, members of the bacterial community found within the core microbiome of biofoam were closely associated with organisms commonly found in activated sludge, drinking water biofilms, rhizosphere, phyllosphere and soil ecosystems (Table 19.6).

The bacterial communities from each site were strikingly similar despite the fact that they were developed using different source waters. The OTUs classified to the genus level belong to many taxa that are well characterized for functions like the production of EPS, eDNA, quorum sensing molecules, proteases and chitinases, as well as numerous biochemical transformations such as biopolymer and PAH degradation. In addition, planctomycetes were prevalent, capable of the anaerobic oxidation of ammonium to nitrogen gas in the Annamox reaction, perhaps as a detoxifying mechanism.

Overall, this study suggests that unique microbial communities may self-assemble and that key members of the community are necessary for successful biofilm formation. Thus, similar to megacities, although all biofilms are unique, every biofilm may require fundamental structure and requirements in order to function.

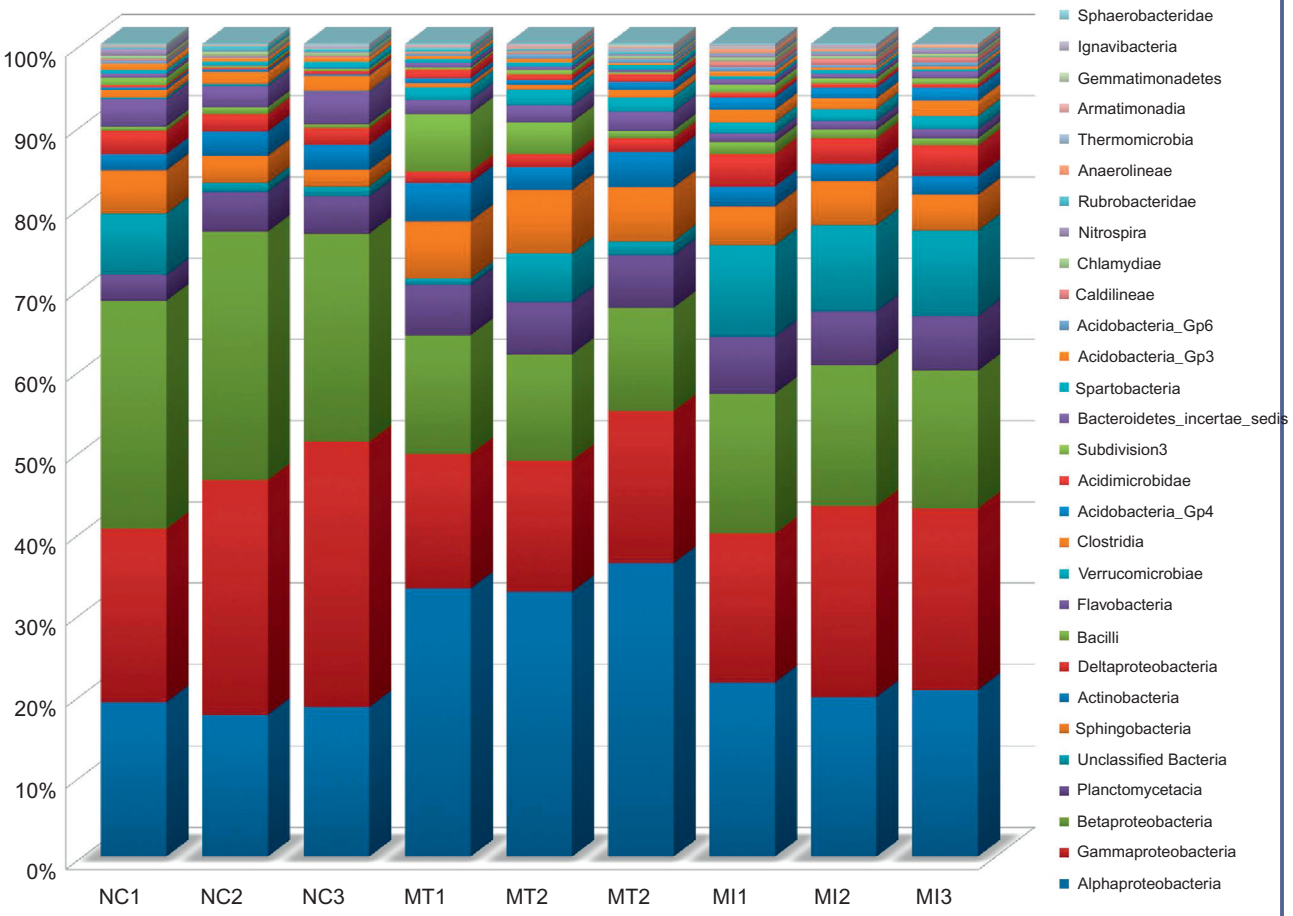


FIGURE 19.7 Bacterial composition (class level) of biofilms in point-of-use (POU) devices used to treat drinking water at three locations in the U.S. NC = North Carolina; MT = Montana; and MI = Michigan. Three replicates were analyzed at each site. From Iker *et al.*, (2013).

TABLE 19.6 Summary of Shared Bacteria in Point-of-Use (POU) Devices Used to Treat Drinking Water at Three Locations in the U.S.

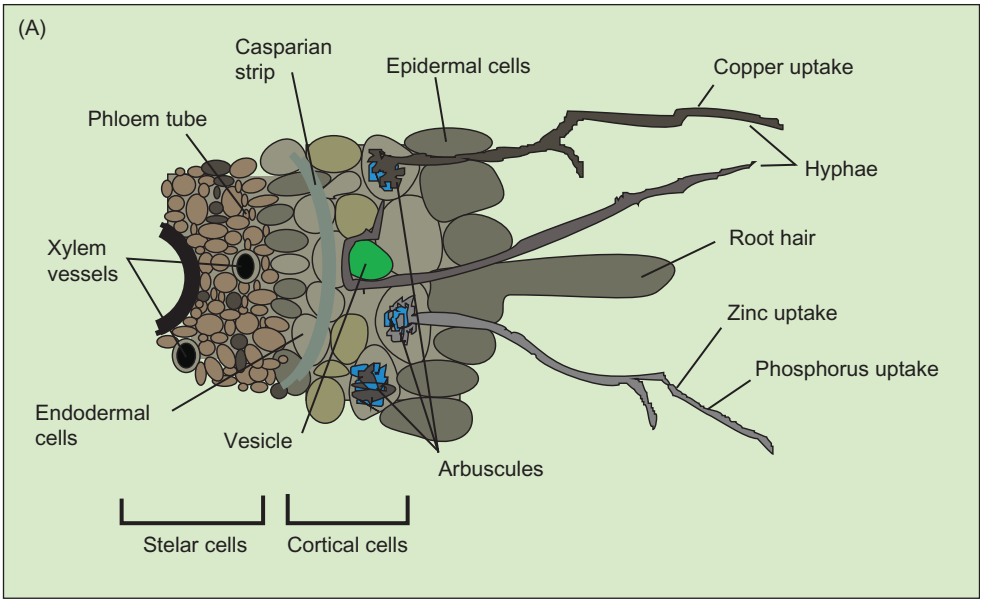
OTU	# of Sequences	Classification	Environment(s) With Similar Organisms
001	66281	*Pseudomonas*	Biofilms (plants, environment, engineered water)
002	20032	Unclassified	Soil, water
003	18065	*Sphingomonas*	Soil, water
004	12951	*Sporosarcina*	Soil, water
005	12596	*Arenimonas*	Drinking water
006	11078	*Rhodobacterium*	Soil, water
007	10126	*Janthinobacterium*	Water biofilms
008	8073	*Rhizobiales*	Rhizosphere
009	7426	*Flavobacterium*	Soil, water
010	6104	*Massilia*	Rhizosphere
011	5934	*Bacteroidetes*	Water
012	5776	*Phyllobacteriaceae*	Phyllosphere

Adapted from Iker et al., (2013).
Table includes the number of community sequences belonging to each OTU (operational taxonomic unit; ≥ 97% similarity cutoff), their closest taxonomic identification, and other environments that have been found to harbor similar organisms.

elaborate cell−cell signaling process (Chapter 20) that results in profound physiological changes in both organisms. These bacteria are known colloquially as rhizobia and are characterized as fixing nitrogen for the plant host in return for carbon sources supplied by the plant as photosynthates. The symbiosis occurs within newly formed root organs called root nodules that develop in response to the presence of specific soil-borne rhizobia. During nodulation the bacteria and the plant contribute to the production of leghemoglobin, which maintains low internal O_2 levels so nitrogenase does not become inactivated. The rhizobia themselves undergo physiological changes, are known as bacteroids, and actually conduct the process of nitrogen fixation. As the plant host matures, ultimately the root nodules senesce as the bacteria become less active and new nodules are formed. Another group of bacteria, *Frankia* spp., form symbiotic relationships with over 200 species of woody plants and shrubs. Since *Frankia* spp. are members of Actinomycetes, plants in these associations are often referred to as actinorhizal plants.

A second important group of rhizosphere microorganisms is the mycorrhizal fungi, which also form symbioses with plants. These fungi act as an extension of the plant root system which aids in the uptake of almost all plant nutrients, but in particular phosphorus, which typically has low solubility and therefore availability in the soil solution. Such fungi assist in the plant uptake of nutrients from dilute solutions by scavenging soil nutrients, utilizing active transport mechanisms to concentrate nutrients against steep concentration gradients. They appear to increase the bioavailability of these compounds for the plant. When released from fungal hyphae, such nutrients can be taken up by plant roots. In addition, when nutrients are stored within the fungus, the fungus can act as a reservoir of nutrients for future plant utilization. The mechanisms that cause the fungus to release its nutrients are not well understood. The plant supplying the fungus with carbon compounds, mostly as hexose sugars, completes the mutualistic association. Thus, each symbiont aids the other in terms of required nutrients.

Mycorrizal fungi become endemic in most soils and form extensive networks of fungal hyphae that can connect different plant species. In addition, on larger root systems, different fungi can infect the same root system. Mycorrhizal fungi naturally infect most plants, but in some commercial cropping systems such as the establishment of pine seedlings in pots, plants can be infected with known highly effective strains of fungi. There are several different types of mycorrhizal fungi. The vesicular−arbuscular mycorrhizae (VAM) are also known as endomycorrhizal fungi, which as the name implies are found mostly within the internal tissues of the root. This type of fungus is frequently found in fertile soils, and is characterized by the presence of smooth vesicles and branched arbuscules that are involved in the storage and transfer of nutrients between the fungus and the plant (Figure 19.8). About 90% of all vascular plants are



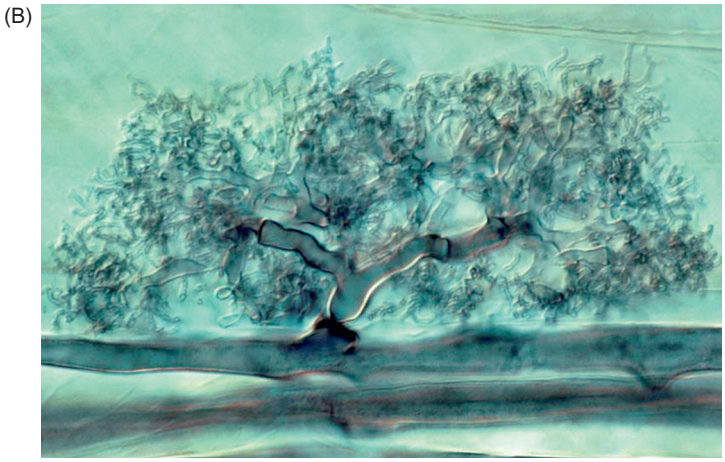


FIGURE 19.8 (A) A typical endomycorrhiza showing hyphae extending beyond the root epidermis into the rhizosphere. Intracellular arbuscules are also visible. (B) Highly magnified picture of a *Glomus* sp. arbuscule. Photo courtesy Mark Brundrett.

associated with such fungal symbionts. The VAMs are classified into several genera, notably *Glomus* and *Gigaspora* spp., within the phylum *Glomeromycota*. The orchidaceous mycorrhizae fungi (Basidiomycetes) are much more specific than other VAMs, and infect only plants of the orchid family, which contains thousands of species, most of which are tropical. The physiological relationship between the orchid and these fungi is different because in this association, it is the fungus that supplies the plant with a source of carbon. This is the only type of mycorrhizal association in which the carbon flow is into the plant from the fungus. In some cases, mature orchids can therefore live without conducting photosynthesis. It is also of interest that many orchids are associated with *Rhizoctonia* spp., including *R. solani*, which are common plant pathogens.

The ericaceous mycorrhizae are fungi characterized by association with a specific group of plants known as the Ericaceae, which form important plant communities in moors, swamps and peat. The plants involved include heathers, rhododendrons and azaleas, which are often found on nutrient-poor, acidic soil at high altitudes and at colder latitudes. The fungi involved are typical of the endomycorrhizal fungi in that they have intracellular hyphae, but they do not form arbuscules. In this association, the fungus supplies the plant with nutrients, and the plant supplies the fungus with carbon substrate. The fungi also seem to be able to make the plants more tolerant of heavy metals and other soil contaminants. Most of the fungi involved seem to be members of the Ascomycetes.

The Ectomycorrhizae form associations that are characterized by intercellular (between cell) hyphae as opposed to the intracellular (within cell) penetration of the VAMs. These mycorrhizas are formed on the roots of woody plants, with a thick fungal sheath developing around the terminal lateral branches of roots (Figure 19.9). This is also known as the mantle and is connected to the network of intercellular hyphae found in the root cortex

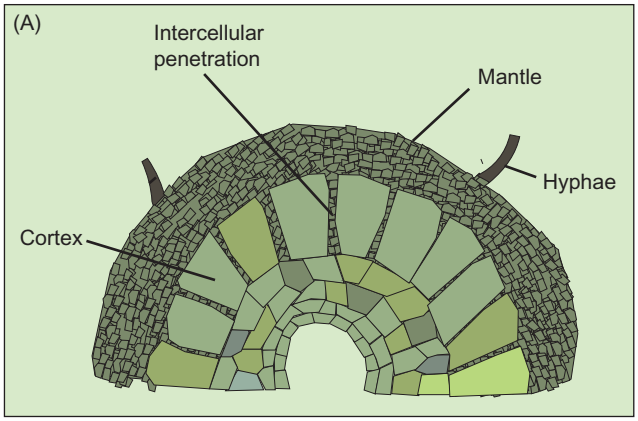


FIGURE 19.9 (A) Cross-section of ectomycorrhizal rootlet showing the exterior fungal sheath or mantle and intercellular penetration. (B) White ectomycorrhizal mantle on a tree root, courtesy Terry W. Henkel.

known as the Hartig net. The plants involved with these mycorrhizas are all trees or shrubs, whereas the fungi involved are often Basidiomycetes or Ascomycetes. Carbon substrate is supplied by the plant to the fungus, and minerals, in particular phosphates, are supplied by the fungus to the plant. Ectomycorrhizal fungi are commonly used as inoculants for pine seedlings, and other plants, in containers prior to planting in reforestation projects.

19.3.3 Microbial Interactions Impacting Animal and Human Pathogens

Many environments, such as soil and aquatic systems, contain microbial pathogens of animals and humans. With the exception of geo-indigenous pathogens (naturally occurring and capable of metabolism, growth and reproduction in soil), such as *Coccidioides immitis* and *Naegleria fowleri*, these pathogens generally do not regrow in the environment, but instead exist transiently while in between hosts. However, while in the environment, these organisms are negatively affected by microbial interactions such as competition and predation. These effects from indigenous, antagonistic microorganisms are commonly referred to as "biological factors" when discussing the environmental fate of pathogens (Chapter 22), and play critical roles in water and wastewater treatment processes. For example, biofilms of complex microbial communities, known as zooleal film (Chapter 25), are vital to the secondary treatment of wastewater and function to degrade organic materials and also decrease levels of pathogens. In slow sand-filtration point-of-use devices, biofilms referred to as schmutzdecke are known to inactivate pathogens, including viruses (Bauer *et al.*, 2011). Even if the pathogenic organisms are "native" to the soil, they are subject to the same plethora of inhibitory microbial interactions that affect introduced organisms.

While most of these microbe−microbe interactions are considered to be negative with respective to population levels of pathogens, in some case these interactions can actually result in increased pathogen levels. For example, although *Bacillus anthracis* (the causative agent of anthrax) is widely found in soil, there has been some debate about whether it actively grows in soil, or just exists in the soil as spores while in between host organisms (Pepper and Gentry, 2002). However, recent evidence (Dey *et al.*, 2012) has demonstrated that after *B. anthracis* spores are ingested by soil-dwelling amoeba they can germinate and multiply (under environmental conditions) within the amoeba, ultimately resulting in the death of the amoeba and the release of the additional *B. anthracis* cells into the environment.

19.4 MICROBIAL DIVERSITY AND NATURAL PRODUCTS

Terrestrial and aquatic environments are home for billions of microorganisms including bacteria and fungi. In this chapter we have focused primarily on bacterial diversity, and as we have seen, the diversity of environmental bacteria is enormous. Although not as diverse as bacteria, fungal populations are also extremely diverse with one million different species estimated to exist (Gunatilaka, 2006). Overall, from the less than 1% of bacterial species and 5% of fungal species that have been identified, a treasure chest of natural products critical to maintaining human health and welfare have been discovered (Table 19.7). These compounds represent a small portion of the elaborate compounds that environmental microorganisms produce in order to communicate with, stimulate and/or inhibit other microorganisms.

Actinomycetes and fungi are particularly rich sources of metabolites with novel biological activities including antibiotics. Antibiotics are compounds produced by microorganisms that kill or inhibit other microorganisms. Thus, they are a class of chemicotherapeutic agents that

TABLE 19.7 Products Derived from Environmental Microorganisms and Other Natural Sources

Item	Extent %	Reference
Prescription drugs	40	Strobel and Daisy, 2003
New chemical products registered by U.S. Food and Drug Administration	49	Brewer, 2000
Approved drugs between 1989 and 1995	60	Grabley and Thiericke, 1999
Approved cancer drugs between 1983 and 1994	60	Concepcion *et al.*, 2001
Approved antibacterial agents between 1983 and 1994	78	Concepcion *et al.*, 2001

Source: Pepper et al. (2009).

can be used to control infectious disease. Since antibiotics are natural products obtained largely from environmental microorganisms, soils and similarly diverse environments are the ultimate source of antibiotics. The first and perhaps most effective antibiotic discovered was penicillin, isolated by Sir Alexander Fleming in 1929 from the soil-borne fungus *Penicillium*. This antibiotic has proved to be highly effective in treating many bacterial infections, including staphylococcal and pneumonococcal infections. Later in 1943, another potent antibiotic was discovered by Selman Waksman, a feat for which he later received the Nobel Prize. This antibiotic, streptomycin, was isolated from the actinomycete *Streptomyces griseus*. Since then, soil actinomycetes have been shown to be the source of numerous antibiotics. In fact, over 50% of all known antibiotics are derived from the genus *Streptomyces* (Kieser *et al.*, 2000).

However, bacteria are prokaryotic organisms with the ability to metabolize and replicate quickly. They are also very adaptable genetically. Hence, when confronted with an antibiotic, a genetic or mutational change may confer resistance to the antibiotic. Thus, the more that antibiotics are used, the more likely it is that antibiotic-resistant strains will develop. This is of great concern since several human pathogenic bacteria are becoming resistant to popular antibiotics (see Section 31.4).

More recently, interest has centered on rhizosphere bacteria and endophytic microbes as a new source of natural products including antibiotics. Endophytes are bacteria or fungi that live within plants (e.g., in roots) without pathogenic effects. In contrast, rhizosphere organisms reside in soil adjacent to, and under the influence of, plant roots. In both cases, the microbes receive plant metabolites or exudates as a source of nutrition. In return, many of the microbes, especially the endophytes, provide metabolites that protect the plants. They are proving to be a source of natural products effective in controlling a wide variety of human pathogenic microbes, and new source of antibiotics. For example, the endophytic *Streptomyces* sp. strain NRRL 30562 produces wide spectrum antibiotics known as munumbicins capable of controlling multi drug-resistant strains of *M. tuberculosis* (Castillo *et al.*, 2002).

Endophytes are also proving to be useful as anticancer agents. Paclitaxel, the world's first billion-dollar anticancer drug, is produced by many endophytic fungi associated with the yew (*Taxus*) species (Strobel and Daisy, 2003). Other beneficial endophytic natural products include: pestacin with antioxidant activity (Harper *et al.*, 2003), bioinsecticides (Findlay *et al.*, 1997) and insect repellents (Daisy *et al.*, 2002). Other public health benefits are derived from antidiabetic agents that act as an insulin mimetic (Zhang *et al.*, 1999) and immunosuppressive drugs (Lee *et al.*, 1995). New cultural techniques (see Chapter 10) and cloning of community DNA extracted from soil (see Section 13.6) are further enhancing the discovery of beneficial natural products from the vast diversity of environmental microorganisms (Daniel, 2004).

QUESTIONS AND PROBLEMS

1. Calculate the number of soil bacteria that surround a typical homeowner on a quarter acre lot. Assume that an acre furrow slice (one acre to a depth of one foot) weighs 2 million pounds and contains the vast majority of the soil-borne bacteria.
2. What is the bacterial diversity in the quarter acre lot from question 1, assuming it is situated in Minnesota?
3. How could you increase microbial diversity in the soil from questions 1 and 2?
4. Is it always beneficial for an ecosystem to have a diverse microbial community? Explain your reasoning. Compare your answer to the way that most crops are currently grown.
5. Why does the cloning of community DNA extracted from soil potentially increase the availability of beneficial natural products that were previously unknown?
6. What is the largest impact of soil microorganisms on human health?

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