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Significance, History, and Challenges of Environmental Microbiology

This chapter is designed to instill in the reader a sense of the goals, scope, and excitement that permeate the discipline of environmental microbiology. We begin with five core concepts that unify the field. These are strengthened and expanded throughout the book. Next, an overview of the significance of environmental microbiology is presented, followed by a synopsis of key scholarly events contributing to environmental microbiology's rich heritage. The chapter closes by reminding the reader of the complexity of Earth's biogeochemical systems and that strategies integrating information from many scientific disciplines can improve our understanding of biosphere function.

Chapter 1 Outline

1.1 CORE CONCEPTS CAN UNIFY ENVIRONMENTAL MICROBIOLOGY

Environmental microbiology is inherently multidisciplinary. Its many disparate areas of science need to be presented coherently. To work toward that synthesis, this text uses five recurrent core concepts to bind and organize facts and ideas.

Core concept 1. Environmental microbiology is like a child's picture of a house – it has (at least) five sides (a floor, two vertical sides, and two sloping roof pieces). The floor is evolution. The walls are thermodynamics and habitat diversity. The roof pieces are ecology and physiology. To learn environmental microbiology we must master and unite all sides of the house.

Core concept 2. The prime directive for microbial life is survival, maintenance, generation of adenosine triphosphate (ATP), and sporadic growth (generation of new cells). To predict and understand microbial processes in real-world waters, soils, sediments, and other habitats, it is helpful to keep the prime directive in mind.

Environmental Microbiology: From Genomes to Biogeochemistry, Second Edition, Eugene L. Madsen.

Companion Website: www.wiley.com/go/madsen/enviromicrobio2e

Core concept 3. There is a mechanistic series of linkages between our planet's habitat diversity and what is recorded in the genomes of microorganisms found in the world today. Diversity in habitats is synonymous with diversity in selective pressures and resources. When operated upon by forces of evolution, the result is molecular, metabolic, and physiological diversity found in extant microorganisms and recorded in their genomes.

Core concept 4. Advancements in environmental microbiology depend upon convergent lines of independent evidence using many measurement procedures. These include microscopy, biomarkers, model cultivated microorganisms, molecular biology, and genomic techniques applied to laboratory- and field-based investigations.

Core concept 5. Environmental microbiology is a dynamic, methods-limited discipline. Each methodology used by environmental microbiologists has its own set of strengths, weaknesses, and potential artifacts. As new methodologies deliver new types of information to environmental microbiology, practitioners need a sound foundation that affords interpretation of the meaning and place of the incoming discoveries.

1.2 SYNOPSIS OF THE SIGNIFICANCE OF ENVIRONMENTAL MICROBIOLOGY

With the formation of planet Earth 4.6×10^9 years ago, an uncharted series of physical, chemical, biochemical, and (later) biological events began to unfold. Many of these events were slow or random or improbable. Regardless of the precise details of how life developed on Earth (see Sections 2.3 to 2.7), it is now clear that for ~70% of life's history, prokaryotes were the sole or dominant life forms. Prokaryotes (*Bacteria* and *Archaea*) were (and remain) not just witnesses of geologic, atmospheric, geochemical, and climatic changes that have occurred over the eons; prokaryotes are also active participants and causative agents of many geochemical reactions found in the geologic record. Admittedly, modern eukaryotes (especially land plants) have been major biogeochemical and ecological players on planet Earth during the most recent 1.4×10^9 years. Nonetheless, today, as always, prokaryotes remain the "hosts" of the planet. Prokaryotes comprise ~60% of the total biomass (Whitman et al., 1998; see Chapter 4), account for as much as 60% of total respiration of some terrestrial habitats (Velvis, 1997; Hanson et al., 2000), contribute to one half of global primary production via photosynthesis in marine habitats (Azam and Malfatti, 2007), and also colonize a variety of Earth's habitats devoid of eukaryotic life due to topographic, climatic, and geochemical extremes of elevation, depth, pressure, pH, salinity, heat, or light.

The Earth's habitats present complex gradients of environmental conditions that include variations in temperature, light, pH, pressure, salinity, and both inorganic and organic compounds. The inorganic materials range from elemental sulfur to ammonia, hydrogen gas, and methane and the organic materials range from cellulose to lignin, fats, proteins, lipids, nucleic acids, and

Table 1.1

Microorganisms' unique combination of traits and their broad impact on the biosphere

Traits of microorganisms	Ecological consequences of traits
Small size	Geochemical cycling of elements
Ubiquitous distribution throughout Earth's habitats	Detoxification of organic pollutants
High specific surface areas	Detoxification of inorganic pollutants
Potentially high rate of metabolic activity	Release of essential limiting nutrients from the biomass in one generation to the next
Physiological responsiveness	Maintaining the chemical composition of soil, sediment, water, and atmosphere required by other forms of life
Genetic malleability	
Potential rapid growth rate	
Unrivaled nutritional diversity	
Unrivaled enzymatic diversity	

humic substances (see Chapter 7). Each geochemical setting (e.g., anaerobic peatlands, oceanic hydrothermal vents, soil humus, deep subsurface sediments) features its own set of resources that can be physiologically exploited by microorganisms. The thermodynamically governed interactions between these resources, their settings, microorganisms themselves, and 3.6×10^9 years of evolution are probably the source of metabolic diversity of the microbial world.

Microorganisms are the primary agents of geochemical change. Their unique combination of traits (Table 1.1) cast microorganisms in the role of recycling agents for the biosphere. Enzymes accelerate reaction rates between thermodynamically unstable substances. Perhaps the most ecologically important types of enzymatic reactions are those that catalyze oxidation/reduction reactions between electron donors and electron acceptors. Complex mixtures of electron-rich (donors) and electron-poor (acceptors) occur across Earth's habitats (Chapter 3). Biochemical reactions between these pairs of resources are the basis for much physiological evolution. These biochemical reactions allow microorganisms to generate metabolic energy, survive, and grow. Microorganisms procreate by carrying out complex, genetically regulated sequences of biosynthetic and assimilative intracellular processes. Each daughter cell has essentially the same macromolecular and elemental composition as its parent. Thus, integrated metabolism of all nutrients (e.g., carbon, nitrogen, phosphorus, sulfur, oxygen, hydrogen, etc.) is implicit in microbial growth (Chapters 3 and 7). This growth and survival of microorganisms drives the geochemical cycling of the elements, detoxifies many contaminant organic and inorganic compounds, makes essential nutrients present in the biomass of one generation available to the next, and maintains the conditions required by other inhabitants of the biosphere (Table 1.1). Processes carried out by microorganisms in soils, sediments, oceans, lakes, and groundwaters have a major impact on environmental quality, agriculture, and global climate change. These processes are also the basis for current and emerging biotechnologies with industrial and environmental applications (see Chapter 8). Table 1.2

Table 1.2

Examples of nutrient cycling and physiological processes catalyzed by microorganisms in biosphere habitats. (Reproduced and modified with permission from *Nature Reviews Microbiology* from Madsen, E.L. 2005. Identifying microorganisms responsible for ecologically significant biogeochemical processes. *Nature Rev. Microbiol.* **3**: 439–446. Macmillan Magazines, www.nature.com/reviews)

Nutrient cycle	Process	Nature of process	Typical habitat	References
Carbon	Photosynthesis	Light-driven CO_2 fixation into biomass	Fw, FwS, Ow	Overmann and Garcia-Pichel, 2006; Falkowski, 2012
	Carbon respiration	Oxidation of organic C to CO_2	All habitats	Heemsbergen, 2004; Singh et al., 2010
	Cellulose decomposition	Depolymerization, respiration	Sl	Wilson, 2011
	Methanogenesis	Methane production	FwS, Os, Sw, S1	Hedderich and Whitman, 2006; Schink, 1997; Stams and Plugge, 2009; Schink and Stams, 2013
	Aerobic methane oxidation	Methane becomes CO_2	Fw, Ow, Sl	Smith et al., 2010
	Anaerobic methane oxidation	Methane becomes CO_2	Os, Gw	Boetius et al., 2000; Ettwig et al., 2010; Milucka et al., 2012
Biodegradation	Synthetic organic compounds	Decomposition, CO_2 formation	All habitats	Wackett, 2006; Boxall et al., 2004; Escher and Fenner, 2011; Singh, 2012
	Petroleum hydrocarbons	Decomposition, CO_2 formation	All habitats	Van Hamme et al., 2003; Jeon and Madsen, 2013; Timmis, 2010; Bombach et al., 2010
	Fuel additives (MTBE)	Decomposition, CO_2 formation	Gw, Sl, Sw	Deeb et al., 2003; Hyman, 2013
	Nitroaromatics	Decomposition	Gw, Sl, Sw	Spain et al., 2000; Esteve-Núñez et al., 2001; Ju and Parales, 2010
	Pharmaceuticals, personal care products	Decomposition	Gw, Sl, Sw	Alexander, 1999; Ternes et al., 2004; Ort et al., 2010
	Chlorinated solvents	Compounds are dechlorinated via respiration in anaerobic habitats	Gw, Sl, Sw	Maymo-Gatell et al., 1997; Adrian et al., 2000; Loeffler et al., 2013

Nitrogen	Nitrogen fixation	N_2 gas becomes ammonia	Fw, Ow, Sl	Karl et al., 2002; Martinez-Romero, 2006; Canfield et al., 2010; Martinez-Espinosa et al., 2011; Thamdrup, 2012; Zedr and Kudela, 2012
	Ammonium oxidation	Ammonia becomes nitrite and nitrate	Sl, Sw, Ow, Gw	Ward et al., 2011; Vajrala et al., 2012; Stahl and de la Torre, 2012; Hatzenpichler, 2012; Bock and Wagner, 2006
	Anaerobic ammonium oxidation	Nitrite and ammonia become N_2 gas	Os, Sw, Gw	van Niftrik et al., 2004; Jetten et al., 2009; Harhangi et al,. 2012
	Denitrification	Nitrate is used as an electron acceptor and converted to N_2 gas	Sl, Sw, Os, Fw, Gw	Zumft, 1997; van Breemen et al., 2002; Shapleigh, 2006; Bakken et al., 2012
Sulfur	Sulfur oxidation	Sulfide and sulfur become sulfate	Os, Sw, Gw	Sorokin et al., 2006; Dopson and Johnson, 2012
	Sulfate reduction	Sulfate is used as an electron acceptor and converted to sulfur and sulfide	Os, Gw	Rabus et al., 2006; Barton and Fauqu, 2009
Other elements	Hydrogen oxidation	Hydrogen is oxidized to H^+, electrons reduce other substances	Sl, Os, Sw,	Schink, 1997; Schwartz and Friedrich, 2006; Anantharaman et al., 2013
	Mercury methylation and reduction	Organic mercury is formed and mercury ion is converted to metallic mercury	FwS, Os	Sigel et al., 2005; Barkay et al., 2011
	(Per)chlorate reduction	Oxidants in rocket fuel and other sources are converted to chloride	Gw	Coates and Achenbach, 2004; Ettwig et al., 2012
	Uranium reduction	Uranium oxycation is used as an electron acceptor; hence immobilized	Gw	Lovley, 2003; Williams et al., 2012
	Arsenate reduction	Arsenic oxyanion is used as an electron acceptor; hence toxicity is diminished	FwS, Gw	Oremland and Stolz, 2003; Oremland et al., 2009
	Iron oxidation, acid mine drainage	Iron sulfide ores are oxidized, strong acidity is generated	FwS, Gw	Lovley, 2006; Emerson et al., 2010; Papirio et al., 2013

Fw, freshwater; FwS, freshwater sediment; Gw, groundwater; Os, ocean sediments; Ow, ocean waters; Sl, soil; Sw, sewage.

presents a sampling of the ecological and biogeochemical processes that microorganisms catalyze in aquatic or terrestrial habitats. Additional details of biogeochemical processes and ways to recognize and understand them are presented in Chapters 3 and 7.

1.3 A BRIEF HISTORY OF ENVIRONMENTAL MICROBIOLOGY

Early foundations of microbiology rest with microscopic observations of fungal sporulation (by Robert Hooke in 1665) and "wee animalcules" – true bacterial structures (by Antonie van Leeuwenhoek in 1684). In the latter half of the nineteenth century, Ferdinand Cohn, Louis Pasteur, and Robert Koch were responsible for methodological innovations in aseptic technique and isolation of microorganisms (Madigan et al., 2014). These, in turn, allowed major advances pertinent to spontaneous generation, disease causation, and germ theory.

Environmental microbiology also experienced major advancements in the nineteenth century; these extend through to the present. Environmental microbiology's roots span many continents and countries (Russia, Japan, Europe, and England) and a complex tapestry of contributions has developed. To a large degree, the challenges and discoveries in environmental microbiology have been habitat-specific. Thus, one approach for grasping the history and traditions of environmental microbiology is to recognize subdisciplines such as marine microbiology, soil microbiology, rumen microbiology, sediment microbiology, geomicrobiology, and subsurface microbiology. In addition, the contributions from various centers of training can also sometimes be easily discerned. These necessarily revolved around various investigators and the institutions where they were based.

As early as 1838 in Germany, C.G. Ehrenberg was developing theories about the influence of the bacterium, *Gallionella ferruginea*, on the generation of iron deposits in bogs (Ehrlich et al., 2015). Furthermore, early forays into marine microbiology by A. Certes (in 1882), H.L. Russell, P. Regnard, B. Fischer, and P. and G.C. Frankland allowed the completion of preliminary surveys of microorganisms from far-ranging oceanic waters and sediments (Litchfield, 1976).

At the University of Delft (the Netherlands) near the end of the nineteenth century, M.W. Beijerinck (Figure 1.1) founded the Delft School traditions of elective enrichment techniques (see Section 6.2) that allowed Beijerinck's crucial discoveries including microbiological transformations of nitrogen and carbon, and also other elements such as manganese (van Niel, 1967; Atlas and Bartha, 1998; Madigan *et al*., 2014). The helm of the Delft School changed hands from Beijerinck to A.J. Kluyver, and the traditions have been continued in the Netherlands, Germany, and other parts of Europe through to the present. After training in Delft with Beijerinck and Kluyver, C.B. van Niel was asked by L.G.M. Baas Becking to establish a research program at Stanford University's Hopkins Marine Station (done in 1929), where R.Y. Stainer, R. Hungate, M. Doudoroff, and many others were trained, later establishing their own research programs at other institutions in the United States (van Niel, 1967).

S. Winogradsky (Figure 1.2) is regarded by many as the founder of soil microbiology (Atlas and Bartha, 1998; Ackert, 2013). Working in the latter part of the nineteenth and early decades of the twentieth centuries, Winogradsky's career contributed immensely to our knowledge of soil and environmental microbiology, especially regarding microbial metabolism of sulfur, iron, nitrogen, and manganese. In 1949, much of Winogradsky's work was published as a major treatise entitled, *Microbiologie du Sol, Problémes et Methods: Cinquante Ans de Recherches. Oeuvres Complétes* (Winogradsky, 1949).

Many of the marine microbiologists in the early twentieth century focused their attention on photoluminescent bacteria (E. Pluger, E.W. Harvey, H. Molisch, W. Beneche, G.H. Drew, and J.W. Hastings). Later, transformations by marine microorganisms of carbon and nitrogen were explored, as well as adaptation to low-temperature habitats (S.A. Waksman, C.E. ZoBell, S.J. Niskin, O. Holm-Hansen, and N.V. and V.S. Butkevich). The mid-twentieth century marine studies continued exploration of the physiological and structural responses of microorganisms to salt, low temperature, and pressure (J.M. Shewan, H.W. Jannasch, R.Y. Morita, R.R. Colwell, E. Wada, A. Hattori, and N. Taga). Also, studies of nutrient uptake (J.E. Hobbie) and food chains constituting the "microbial loop" were conducted (L.R. Pomeroy).

Figure 1.1 Martinus Beijerinck (1851–1931). Founder of the Delft School of Microbiology, M. Beijerinck worked until the age of 70 at the University of Delft, the Netherlands. He made major discoveries in elective enrichment techniques and used them to advance the understanding of how microorganisms transform nitrogen, sulfur, and other elements. (Reproduced with permission from the American Society for Microbiology Archives, USA.)

At Rutgers University, Selman A. Waksman was perhaps the foremost American scholar in the discipline of soil microbiology. Many of the Rutgers traditions in soil microbiology were initiated by J. Lipman, Waksman's predecessor (R. Bartha, personal communication; Waksman, 1952). Waksman produced numerous treatises that summarized the history, status, and frontiers of soil microbiology, often in collaboration with R. Starkey. Among the

Figure 1.2 Sergei Winogradsky (1856–1953). A major contributor to knowledge of soil microbiology, S. Winogradsky described microbial cycling of sulfur and nitrogen compounds. He developed the "Winogradsky column" for growing diverse physiological types of aerobic and anaerobic, heterotrophic and photosynthetic bacteria across gradients of oxygen, sulfur, and light. (Reproduced with permission from the Smith College Archives, Smith College, USA.)

prominent works published by Waksman are "Soil microbiology in 1924: an attempt at an analysis and a synthesis" (Waksman, 1925), *Principles of Soil Microbiology* (Waksman, 1927), "Soil microbiology as a field of science" (Waksman, 1945), and *Soil Microbiology* (Waksman, 1952). A steady flow of Rutgers-based contributions to environmental microbiology continue to be published (e.g., Young and Cerniglia, 1995; Haggblom and Bossert, 2003).

In the 1920s and 1930s, at the University of Wisconsin, E.B. Fred and collaborators, I.L. Baldwin and E. McCoy, comprised a unique cluster of investigators whose interests focused on the *Rhizobium*–legume symbiosis. Several decades later, also at the University of Wisconsin, T.D. Brock and his students made important contributions to microbial ecology, thermophily, and general microbiology. Another graduate of the University of Wisconsin, H.L. Ehrlich earned a Ph.D. in 1951 and, after moving to Rensselaer Polytechnic Institute, carried out studies on the bacteriology of manganese nodules, among other topics. Author of six comprehensive editions of *Geomicrobiology*, H.L. Ehrlich is, for many, the founder of this discipline.

Another University of Wisconsin graduate, M. Alexander, moved to Cornell University in 1955. For four decades prior to Alexander's arrival, soil microbiological research was conducted at Cornell by J.K. Wilson and F. Broadbent. From 1955 to ~2000 Alexander's contributions to soil microbiology examined a broad diversity of phenomena, which included various transformations of nitrogen, predator–prey relations, microbial metabolism of pesticides and environmental pollutants, and advancements in environmental toxicology. Many environmental microbiologists have received training with M. Alexander and become prominent investigators, including J.M. Tiedje.

In Europe (especially in the Netherlands and Germany) Beijerinck's "Delft school" has continued to have a high impact upon the discipline of microbiology, well into the twenty-first century. Key subdisciplines advanced in critically important ways include: taxonomy/systematics (e.g., E. Stackebrandt, K.-H. Schleifler, and W. Ludwig), anaerobic physiology (e.g., R. Thauer, J.G. Kuenen, B. Schink, M.S.M. Jetten, M. Straus, F. Widdel, A. Stams, and W. Zumft), and microbial ecology (e.g., R. Conrad, R. Amann, and G. Muyzer).

Other schools and individuals in Britain, Italy, France, Belgium, and other parts of Europe, Japan, Russia, and other parts of Asia, Africa, Australia, the United States, and other parts of the Americas certainly have contributed in significant ways to advancements in environmental microbiology. An insightful review of the history of soil microbiology, with special emphasis on eastern European and Russian developments, was written by Macura (1974).

The many historical milestones in the development of environmental microbiology (most of which are shared with broader fields of biology and microbiology) have been reviewed by Atlas and Bartha (1998), Brock (1961), Lechevalier and Solotorovsky (1965), Macura (1974), Madigan et al. (2014), van Niel (1967), Waksman (1925, 1927, 1952), Vernadsky et al. (1998), and others. Some of the highlights are listed in Table 1.3.

Table 1.3

Selected landmark events in the history of environmental microbiology

- The first visualization of microscopic life by van Leeuwenhoek in 1684
- The role of microorganisms as causative agents of fermentations discovered by Pasteur in 1857
- The use of gelatin plates for enumeration of soil microorganisms by Koch in 1881
- Nitrogen fixation by nodules on the roots of legumes discovered by Hellriegel and Wilfarth in 1885
- The use of elective enrichment methods, by Beijerinck and Winogradsky, in the isolation of single organisms able to carry out ammonification, nitrification, and both symbiotic and nonsymbiotic nitrogen fixation
- Recognition of the diverse populations in soil (e.g., bacteria, fungi, algae, protozoa, nematodes, insect larvae)
- Documentation of anaerobic cellulose decomposition by Omelianskii in 1902
- The study of sulfur-utilizing phototrophic bacteria by van Niel and others
- The specificity of legume-nodulating bacteria (Fred et al., 1932)
- The discovery and development of antibiotics
- Direct microscopic methods of examining environmental microorganisms via staining and contact-slide procedures
- The development of radiotracer techniques, leading to metabolic activity assays
- A diversity of advancements in analytical chemistry for detecting and quantifying biochemically and environmentally relevant compounds
- Developments in molecular phylogeny (Woese, 1987, 1992; Pace, 1997, 2009)
- The application of molecular methods to environmental microbiology (Olsen et al., 1986; Pace et al., 1986; Amann et al., 1991, 1995; Ward et al., 1993; White, 1994; van Elsas et al., 1997; de Bruijn, 2011a, 2011b; Liu and Jansson, 2010)

As this historical treatment reaches into the twenty-first century, the branches and traditions in environmental microbiology become so complex that patterns of individual contributions become difficult to discern. A complete list of schools, individual investigators, and their respective discoveries is beyond the scope of this section. The author apologizes for his biases, limited education, and any and all inadvertent omissions that readers may notice in this brief historical overview.

1.4 COMPLEXITY OF OUR WORLD

Although we humans are capable of developing ideas or concepts or models that partially describe the biosphere we live in, real-world complexity of ecological systems and subsystems remains generally beyond full scientific description. Figures 1.3 and 1.4 are designed to begin to develop for the reader a sense of the complexity of real-world ecosystems – in this case a temperate forested watershed. The watershed depicted in Figure 1.3 is open (energy and materials flow through it) and features dynamic changes in time and space. The watershed system contains many components ranging from the site geology and soils to both large and small creatures, including microorganisms. Climate-related influences are major variables that, in turn, cause variations in how the creatures and their habitat interact. Biogeochemical processes are manifestations of such interactions. These processes include chemical and physical reactions, as well as the diverse physiological reactions and behavior (Table 1.4). The physical, chemical, nutritional, and ecological conditions for watershed inhabitants vary from the scale of micrometers to kilometers. Regarding temporal variability, in situ processes that

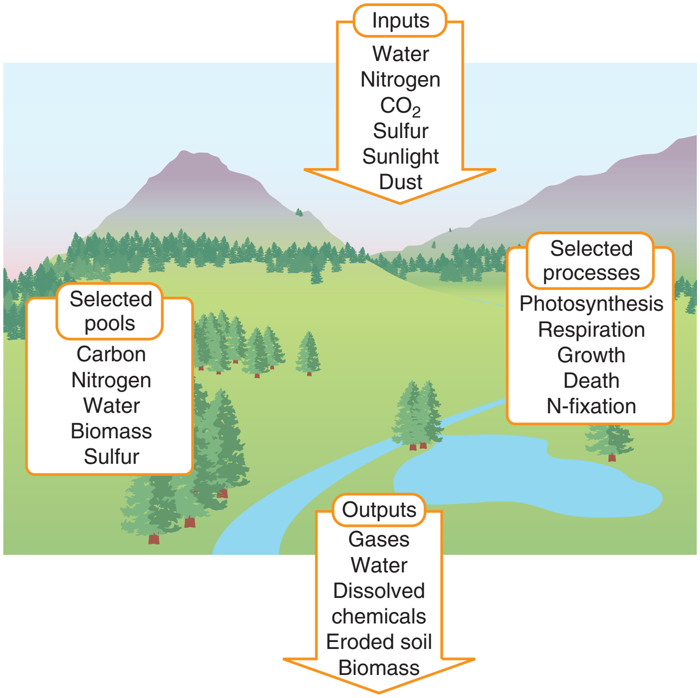


Figure 1.3 Watershed in a temperate forest ecosystem. Arrows show the inputs and outflows for the system. Reservoirs for carbon, nitrogen, and other nutrients include biomass, soil litter layer, soil mineral layer, subsoil, snow, streams, and lakes. Dominant physiological processes carried out by biota include photosynthesis, grazing, decomposition, respiration, nitrogen fixation, ammonification, and nitrification. Key abiotic processes include insolation (sunlight), transport, precipitation, runoff, infiltration, dissolution, and acid/base and oxidation/reduction reactions (see Table 1.4). Net budgets can be constructed for ecosystems; when inputs match outputs, the systems are said to be "steady state".

Table 1.4

Types of biogeochemical processes that typically occur and interact in real-world habitats

Type	Processes
Physical	Insolation (sunlight), atmospheric precipitation, water infiltration, water evaporation, transport, erosion, runoff, dilution, advection, dispersion, volatilization, sorption
Chemical	Dissolution of minerals and organic compounds, precipitation, formation of secondary minerals, photolysis, acid/base reactions, reactions catalyzed by clay-mineral surfaces, reduction, oxidation, organic equilibria, inorganic equilibria
Biological	Growth, death, excretion, differentiation, food webs, grazing, migration, predation, competition, parasitism, symbiosis, decomposition of high molecular weight biopolymers to low molecular weight monomers, respiration, photosynthesis, nitrogen fixation, nitrification, denitrification, ammonification, sulfate reduction, sulfur oxidation, iron oxidation/reduction, manganese oxidation/reduction, anaerobic oxidation of methane, anaerobic oxidation of ammonia, acetogenesis, methanogenesis

directly and indirectly influence fluxes of materials into, out of, and within the system are also dynamic.

At the scale of ~1 m, humans are able to survey habitats and map the occurrence of both abiotic (rocks, soils, gases, water) and biotic (plants, animals) components of the watershed. At this scale, much progress has been made toward understanding ecosystems. Biogeochemical ecosystem ecologists have gained far-reaching insights into how such systems work by performing a variety of measurements in basins whose sealed bedrock foundations allow ecosystem budgets to be constructed (Figure 1.3). When integrated over time and space, the chemical constituents (water, carbon, nitrogen, sulfur, etc.) measured in incoming precipitation, in outflowing waters, and in storage reservoirs (lakes, soil, the biota) can provide a rigorous basis for understanding how watersheds work and how they respond to perturbations (Likens and Bormann, 1995). Understanding watershed (as well as global) biogeochemical cycles relies upon rigorous data sets and well-defined physical and conceptual boundaries. For a given system, regardless of its size, if it is in steady state, the inputs must equal the outputs (Figure 1.3). By the same token, if input and output terms for a given system are not in balance, key biogeochemical parameters of interest may be changing with time. Net loss or gain is dependent on relative rates of consumption and production. Biogeochemical data sets provide a means for answering crucial ecological questions such as: Is the system in steady state? Are carbon and nitrogen accruing or diminishing? Does input of atmospheric pollutants impact ecosystem function? What goods and services do intact watersheds provide in terms of water and soil quality? More details on measuring and modeling biogeochemical cycles are presented in Chapter 7.

Large-scale watershed data capture net changes in complex, open systems. Though profound and insightful, this approach leaves mechanistic microscale cause-and-effect linkages unaddressed. Measures of net change do not address dynamic controls on rates of processes that generate (versus those that consume) components of a given nutrient pool. Indeed, the intricate microscale interactions between biotic and abiotic field processes are often masked in data gathered in large-scale systems. Thus, ecosystem-level biogeochemical data may often fail to satisfy the scientific need for details of the processes of interest. An example of steps toward a mechanistic understanding of the ecosystem process is shown in Figure 1.4. This model shows a partial synthesis of ecosystem processes that govern the fate of nitrogen in a watershed. Inputs, flows, nutrient pools, biological players,

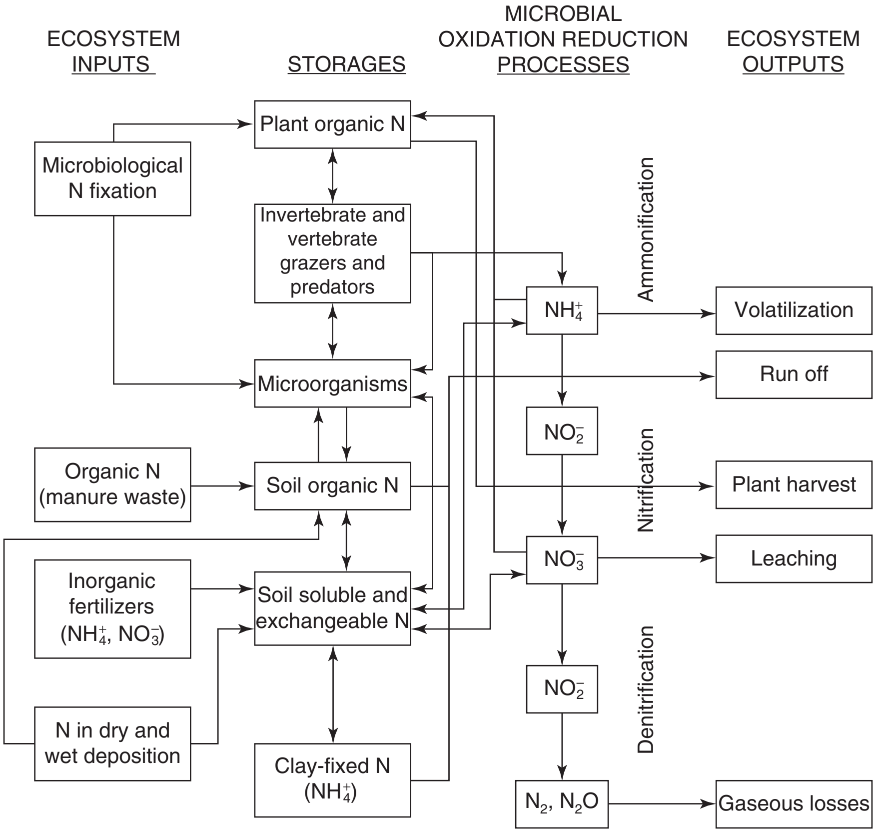


Figure 1.4 Flow model of nitrogen (N) cycling in terrestrial ecosystems. Shown are basic inputs, storages, microbial processes, outputs, and both biotic and abiotic interactions. (Reprinted and modified with permission from Madsen, E.L. 1998. Epistemology of environmental microbiology. *Environ. Sci. Technol.* **32**:429–439. Copyright 1998, American Chemical Society.)

physiological reactions, and transport processes are depicted. Understanding and measuring the sizes of nitrogenous pools, their transformations, rates, fluxes, and the active biotic agents represents a major challenge for both biogeochemists and microbiologists. Yet Figure 1.4 considerably simplifies the processes that actually occur in real-world watersheds because many details are missing and comparably complex reactions and interactions apply simultaneously to other nutrient elements (C, S, P, O, H, etc.). Consider a data set in which concentrations of ammonium (a key form of nitrogen) are found to fluctuate in stream sediments. Interpreting such field measurements is very difficult because the ammonium pool at any given moment is controlled by processes of production (e.g., ammonification or dissimilatory reduction of nitrate to ammonia by microorganisms), consumption (e.g., aerobic and anaerobic ammonia-oxidizing microorganisms, nutrient uptake by plants and many microorganisms), and transport (e.g., entrainment in flowing water, diffusion, dilution, physical disturbance of sediment). Clearly, the many compounded intricacies of nutrient cycling and trophic and biochemical interactions in a field habitat make biogeochemical processes, especially those catalyzed by microorganisms, difficult to decipher.

1.5 MANY DISCIPLINES AND THEIR INTEGRATION

Given the complexity of real-world habitats that are home to microorganisms (see above), what is to be done?

- **How can we contend with complexity?**
- **What approaches can productively yield clear information that enhances our understanding of the role of microorganisms in maintaining our world?**
- **How do microorganisms carry out specific transformations on specific compounds in soils, sediments, and waters?**

Answer: The optimistic answer to these questions is simple. We use the many tools on hand to twenty-first century science.

The principles are sound, the insights are broad, and the sophisticated technologies are ever expanding. To counterbalance the challenges of ecosystem complexity, we can utilize: (i) robust, predictable rules of chemical thermodynamics, geochemical reactions, physiology, and biochemistry; (ii) measurement techniques from analytical chemistry, hydrogeology, physiology, microbiology, molecular biology, omics; and (iii) compound-specific properties such as solubility, volatility, toxicity, and susceptibility to biotic and abiotic reactions. A partial listing of the many areas of science that contribute to advancements in environmental microbiology, with accompanying synopses and references, appears in Table 1.5.

Table 1.5

Disciplines that contribute to environmental microbiology

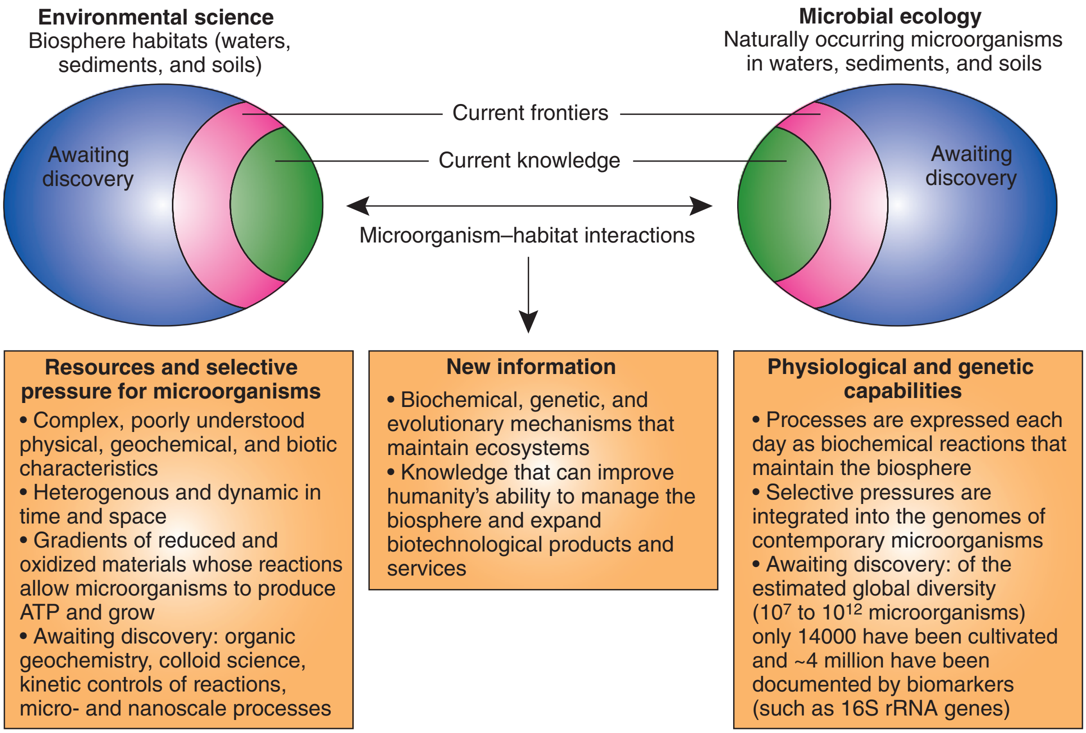
Discipline	Subject matter and contribution to environmental microbiology	References
Environmental microbiology	The study of microorganisms that inhabit the Earth and their roles in carrying out processes in both natural and human-made systems; emphasis is on interfaces between environmental sciences and microbial diversity	Pepper et al., 2014; Liu and Jansson, 2010; Mitchell and Gu, 2010
Microbial ecology	The study of interrelationships between microorganisms and their biotic and abiotic surroundings	Kirchman, 2008, 2012; Ogilvie and Hirsch, 2012; de Bruijn, 2011a, 2011b; McArthur, 2006
Soil microbiology	Environmental microbiology and microbial ecology of the soil habitat; with emphasis on nutrient cycling, plant and animal life, and terrestrial ecosystems	Paul, 2007; Varma and Oelmüller, 2007
Aquatic microbiology	Environmental microbiology and microbial ecology of aquatic habitats (oceans, lakes, streams, groundwaters)	Canfield et al., 2005; Kirchman, 2008
Microbiology	Holistic study of the function of microbial cells and their impact on medicine, industry, environment, and technology	Madigan et al., 2014
Microbial physiology	Integrated mechanistic examination of bacterially mediated processes, especially growth and metabolism	White et al., 2012; Lengeler et al., 1999; Ljungdahl et al., 2010; Schmitz et al., 2013
Public Health microbiology	Relationships between microbes, environment, and human disease	Burlage, 2012
Geomicrobiology	Interactions between geological and microbiological processes	Ehrlich et al., 2015; Barton et al., 2010
Microscopy	The use of optics, lenses, microscopes, imaging devices, and image analysis systems to visualize small structures	Mertz, 2010; Morris et al., 2010
Biochemistry	Molecular examination of the structure and function of subcellular processes, especially ATP generation, organelles, biopolymers, enzymes, and membranes	Nelson et al., 2008; Berg, et al., 2012
Biotechnology	The integrated use of biochemistry, molecular biology, genetics, microbiology, plant and animal science, and chemical engineering to achieve industrial goods and services	Glick et al., 2009; Vallero, 2010
Biogeochemisty	Systems approach to the chemical reactions between biological, geological, and atmospheric components of the Earth	Schlesinger, 2005; Fenchel et al., 2012; Vernadsky et al., 1998

Table 1.5 *Continued*

Discipline	Subject matter and contribution to environmental microbiology	References
Microbial genetics	Molecular mechanistic basis of heredity, evolution, mutation in prokaryotes, and their biotechnological application	Snyder et al., 2013
Omics	Umbrella term that encompasses bioinfomatics-based systematic analysis of genes (genomics), proteins (proteomics), mRNA (transcriptomics), metabolites (metabolomics), etc.	Schmidt, 2012; Shah and Gharbia, 2010; Mayer, 2011; Marco, 2010; Kraj and Silberring, 2008
Aquatic and soil chemistry	Fundamental reactions of aqueous inorganic and organic chemistry and their quantification based on thermodynamics, equilibrium, and kinetics	Stumm and Morgan, 1996; Tratnyek et al., 2011; Hites and Raff, 2012; Bleam, 2012
Geochemistry	Chemical basis for rock–water interactions involving thermodynamics, mineral equilibria, and solid-, liquid-, and vapor-phase reactions	Drever, 2005; Albaréde, 2009; Holland and Turekian, 2010
Soil science	Study of the intrinsic properties of soils and examination of physical, chemical, and biotic processes that lead to soil formation; the crucial role of soils in agriculture and ecosystems	Brady and Weil, 2007; Shukla and Varma, 2011; Buol et al., 2011; Huang et al., 2012
Limnology	The study of freshwater ecosystems, especially lakes and streams	Wetzel and Likens, 2010
Hydrogeology	The study of the physical flow and migration of water in geological systems	Brooks et al., 2013; Wilderer, 2011
Analytical chemistry	Methods and technologies for detecting, separating, and identifying molecular structures of organic and inorganic compounds	Harris, 2010; Patnaik, 2010; Hites and Raff, 2012
Civil and environmental engineering	Physical, chemical, hydraulic, and biological principles applied to the quantitative design of water supply, wastewater, and other engineering needs	Rittmann and McCarty, 2001; Mihelcik and Zimmerman, 2010
Ecology	Integration of relationships between the biosphere and its inhabitants, with emphases on evolution, trophic dynamics, and emergent properties	Krebs, 2008; Chapin et al., 2011
Environmental science	Multidisciplinary study of how the Earth functions, with emphasis on human influences on life support systems	Miller and Spoolman, 2012; Chiras, 2010

Conceptually, environmental microbiology resides at the interface between two vigorously expanding disciplines: environmental science and microbial ecology (Figure 1.5). Both disciplines (spheres in Figure 1.5) seek to understand highly complex and underexplored systems. Each discipline currently consists of a significant body of facts and principles (green inner areas of spheres in Figure 1.5), with expanding zones of research (pink bands). But the chances are high that information awaiting discovery (blue areas) greatly exceeds current knowledge. For example, nearly all current information about prokaryotic microorganisms is based upon measurements performed on about 14,000 isolated species. These cultivated species represent approximately 0.1% (or less) of the total estimated diversity of microorganism in the biosphere (estimates range from ~10^7 to 10^{12}, Yarza et al., 2014; see Sections 5.1 to 5.7). The exciting new discoveries in environmental microbiology emerge by examining how microorganisms interact with their habitats (central downward arrow in Figure 1.5).

Thus, the path toward progress in environmental microbiology involves multidisciplinary approaches, assembling convergent lines of independent



Resources and selective pressure for microorganisms

- Complex, poorly understood physical, geochemical, and biotic characteristics
- Heterogenous and dynamic in time and space
- Gradients of reduced and oxidized materials whose reactions allow microorganisms to produce ATP and grow
- Awaiting discovery: organic geochemistry, colloid science, kinetic controls of reactions, micro- and nanoscale processes

New information

- Biochemical, genetic, and evolutionary mechanisms that maintain ecosystems
- Knowledge that can improve humanity's ability to manage the biosphere and expand biotechnological products and services

Physiological and genetic capabilities

- Processes are expressed each day as biochemical reactions that maintain the biosphere
- Selective pressures are integrated into the genomes of contemporary microorganisms
- Awaiting discovery: of the estimated global diversity (10^7 to 10^{12} microorganisms) only 14000 have been cultivated and ~4 million have been documented by biomarkers (such as 16S rRNA genes)

Figure 1.5 Conceptual representation of how the disciplines of environmental science (left sphere) and microbial ecology (right sphere) interact to allow new discoveries at the interface between microorganisms and their habitats. Information in each discipline is depicted as a combination of current knowledge, current frontiers, and knowledge awaiting discovery. Microbial Ecology and Environmental Microbiology have considerable disciplinary overlap (see Table 1.5); nonetheless, advancements in the latter are represented by the central, downward arrow. (Reproduced and modified with permission from *Nature Reviews Microbiology*, from Madsen, E.L. 2005. Identifying microorganisms responsible for ecologically significant biogeochemical processes. *Nature Rev. Microbiol*. **3**:439–446. Macmillan Magazines Ltd, www.nature.com/reviews.)

evidence, and testing alternative hypotheses. Ongoing integration of new methodologies (e.g., from environmental science, microbial ecology, and other disciplines listed in Table 1.5) into environmental microbiology ensures that the number of lines of evidence and the robustness of both their convergence and their tests will increase. A conceptual paradigm that graphically depicts the synergistic relationship between microbiological processes in field sites, reductionistic biological disciplines, and iterative methodological linkages between these disciplines is presented in Figure 1.6.

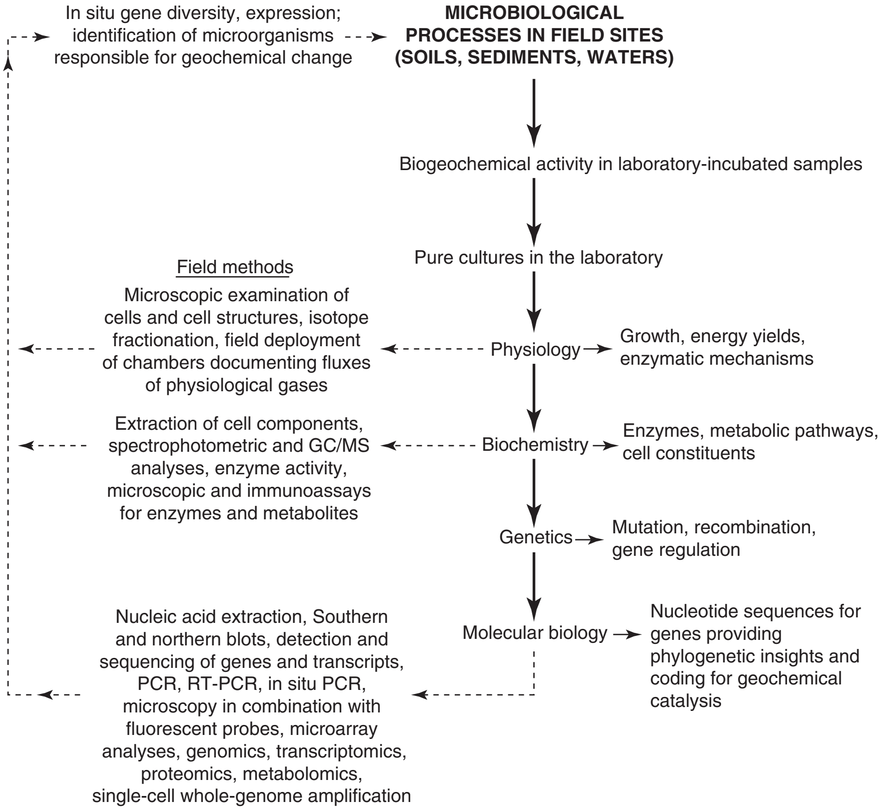


Figure 1.6 Paradigm for how the integration of disciplines and their respective methodologies can extend knowledge of environmental microbiology. Relationships between microorganisms responsible for field biogeochemical processes, reductionistic disciplines, and their application to microorganisms in field sites are depicted. The three different types of arrows indicate sequential refinements in biological disciplines (large downward-pointing solid arrows), resultant information (small arrows pointing to the right), and innovative methodological applications to naturally occurring microbial communities (dashed arrows). GC/MS, gas chromatography/mass spectrometry; PCR, polymerase chain reaction; RT, reverse transcriptase. (Reprinted and modified with permission from Madsen, E.L. 1998. Epistemology of environmental microbiology. *Environ. Sci. Technol.* **32**:429–439. Copyright 1998, American Chemical Society.)

Observations of microorganisms in natural settings instigate a series of procedures progressing through mixed cultures, isolation/cultivation of pure cultures, and physiological, biochemical, genetic, and molecular biological inquiries that each stand alone scientifically. Appreciable new knowledge of naturally occurring microorganisms is gained when advancements from the pure biological sciences are directed back to microorganisms in their field habitats. These methodological advancements (shown as dashed arrows in Figure 1.6; see Chapter 6 for methodologies and their impacts) and the knowledge they generate accrue with each new cycle from field observations to molecular biology and back. Thus, integration of many disciplines is the path forward in environmental microbiology.

STUDY QUESTIONS

1. Core concept 1 presumes a two-dimensional house like that drawn on paper by school children. If you were to expand the concept to three dimensions, then two more walls would be required to keep the "house of environmental microbiology" from falling down. What two disciplines would you add and why? (Hint: for suggestions see Table 1.5.)
2. Core concept 3 uses the phrase "mechanistic series of linkages between our planet's habitat diversity and what is recorded in the genomes of microorganisms found in the world today". This is a hypothesis. If you wanted to test the hypothesis by completing measurements and assembling a data set, what would you do? Specifically, what experimental design would readily test the hypothesis? And what would you measure? What methodological barriers might hamper assembling a useful data set? How might these be overcome? (Hint: Sections 3.2 and 3.3 discusses genomic tools. Answer this question before *and* after reading Chapter 3.)
3. Many names of microorganisms are designed to recognize individual microbiologists who have contributed to the discipline. For instance, the genera *Pasteurella*, *Thauera*, and *Shewanella* are named after people. Similarly, the species designations in *Vibrio harveyii*, *Desulfomonile tiedjei*, *Thermotoga jannaschii*, *Nitrobacter winogradkyi*, and *Acetobacterium woodii* are also named for people. Use the world wide web or a resource like *Bergey's Manual of Systematic Bacteriology* or the *International Journal of Systematic and Evolutionary Microbiology* to discover the legacy of at least one person memorialized in the name of a microorganism.
4. Go for a walk outside to visit a forest, agricultural field, garden, or pond, stream or other body of water. Sit down and examine (literally, and aided by your imagination) the biotic and abiotic components of a cubic meter of water, sediment, or soil. This cubic meter defines a study system. What do you see? Divide a piece of paper into six columns with the headings "Materials and energy entering and leaving", "Inorganic materials", "Organic materials", "Organisms", "Interactions between system components", and "Biological processes". Add at least five entries under each column heading. Then imagine how each entry would change over the course of a year. Compare and contrast what you compiled in your listing with information in Figures 1.3 to 1.6 and Tables 1.2 and 1.4.

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