control the host's motile behavior in relation to light intensity and ambient oxygen tension; this control is probably mediated by the rate of oxygen production by the symbionts (*32*).

The functional significance of syntrophic interactions is only understood in relatively few cases. For instance, sheaths of filamentous cyanobacteria are often covered by attached heterotrophic bacteria, but attempts to grow the cyanobacteria axenically (in the absence of any other microbial species) have so far failed. So the association must be vital to the cyanobacteria, but the nature of the interaction is unknown (*33*). A recent study has shown that in a mixed culture of two species of organotrophic bacteria (*Pseudomonas* sp. and *Burkholderia* sp.), each grew in separate species-specific colonies when provided with a certain organic substrate (citrate). But when citrate was replaced with 3-chlorobiphenyl, the cells only grew in mixed colonies, because the *Pseudomonas* cannot use 3-chlorobiphenyl directly but it can use a metabolite of the other species, thus engaging in a sort of syntrophic interaction (*34*).

The discovery that some bacterial species communicate by extracellular signals adds further complexity to the study of microbial motile behavior. Thus, in response to increased cell density (quorum sensing) or diminishing substrate supplies, bacteria may excrete signal molecules that induce swarming or change in colony morphology (*35*, *36*). The role of these types of mechanisms remains to be studied in natural communities.

The study of microbial motile behavior will lead to a deeper understanding of how microbial communities are assembled in nature. A particularly exciting aspect for the near future is the meeting of ecology and molecular biology, linking gene expression directly with the biological and the nonbiological environment. Such attempts have already been initiated with laboratory systems (*37*, *38*); transferring these techniques to the study of natural systems will not only provide increased ecological insight but also serve to show the unity of biological research.

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REVIEW

Geomicrobiology: How Molecular-Scale Interactions Underpin Biogeochemical Systems

Dianne K. Newman[1] and Jillian F. Banfield[2]

Microorganisms populate every habitable environment on Earth and, through their metabolic activity, affect the chemistry and physical properties of their surroundings. They have done this for billions of years. Over the past decade, genetic, biochemical, and genomic approaches have allowed us to document the diversity of microbial life in geologic systems without cultivation, as well as to begin to elucidate their function. With expansion of culture-independent analyses of microbial communities, it will be possible to quantify gene activity at the species level. Genome-enabled biogeochemical modeling may provide an opportunity to determine how communities function, and how they shape and are shaped by their environments.

Life and Earth have coevolved since their beginning. So intimate has this relation been that if one seeks to discover a part of Earth that has not been fundamentally affected by life, it may be necessary to penetrate hundreds of kilometers into the mantle. Yet even at these great depths, the chemistry of the mantle may have been modified by the slow but steady subduction of sediments with elemental and isotopic features arising from biological activity. Although multicellular organisms such as fungi, algae, higher plants, and humans have made a significant mark on Earth's geochemistry, averaged over geologic time, it is clear that the most important geochemical agents by far have been unicellular microorganisms (e.g., *Bacteria*, *Archaea*, and single-celled *Eucarya*).

Microbes have changed Earth in a number of ways. They have altered the chemistry of the atmosphere via oxygenic photosynthesis, nitrogen fixation, and carbon sequestration (*1*); they have modified the compositions of oceans, rivers, and pore fluids through control of mineral weathering rates or by inducing mineral precipitation; they have changed the speciation of metals and metalloids in water, soils, and sediments by releasing complexing agents and by enzymatically catalyzing redox reactions; they have shaped the physical world by binding sediments, precipitating ore deposits, and weathering rocks; and they have sustained communities of higher organisms through primary production and by remineralizing organic carbon. And most remarkably, they perform these functions in

every nook and cranny from the near surface to the depths, including even the most extreme environments (*2*).

If we are to begin to understand biogeochemical systems at a fundamental level, focus on single-celled organisms is warranted on the basis of their sheer abundance and metabolic potential (*3*, *4*). Microbes affect the chemistry and distribution of nearly all elements in the periodic table and, thus, directly influence their bioavailability. Because microorganisms are relatively simple, detailed analyses of how they work, in isolation and in communities, is a tractable problem. Here we revisit recent findings that have expanded our appreciation for the versatility and importance of microbial metabolisms in geochemistry and anticipate ways in which future geomicrobiological studies will create new understanding of the factors that define and regulate Earth's environments.

Metabolic Diversity

Virtually every month, new discoveries are made about surprising occurrences and modes of microbial life on Earth, ranging from proteorhodopsin-based phototrophy in the open ocean (*5*) to methanogenesis driven by geochemical reactions in Earth's interior (*6*). Niches once considered to be uninhabitable (such as those with pH of 0 in extremely rich metal solutions) have been found to harbor thriving microbial communities (*7*); compounds once thought to be refractory (such as kerogen, or long-chain alkanes under anaerobic conditions) are now known to serve as microbial growth substrates (*8*, *9*); organisms previously believed to be unculturable (such as anaerobic benzene oxidizers or soil bacteria that produce medically relevant natural products) have been brought into culture (*10*) and/or their genetic content has been expressed in recombinant strains (*11*); and enigmatic geochemical transformations (such as the anaerobic oxidation of methane or ammonium) are now attributed to the activity of consortia of bacteria and archaea (*12*, *13*). The importance and extent of microbial diversity have captured the attention of Earth scientists (*14*), and now the geosciences community is helping to define and explore interesting biogeochemical problems.

For years, microbiologists and microbial ecologists have appreciated that microorganisms inhabit almost every environment where it is thermodynamically favorable for them to do so (*15*–*17*). Though clearly there must be limits to life, these limits seem ever more remote. For example, for many years it was believed that the minimum quantum of free energy that could be biochemically converted was –20 kJ mol^{-1} (*18*). A recent study with syntrophic microbial cultures, however, has brought this figure into question (*19*). Free energies as low as –4.5 kJ mol^{-1} were found to support the growth of butyrate-degrading organisms in co-culture with methanogens; values lower than –20 kJ mol^{-1} were calculated for different fermenters under different conditions (including sulfate-reducing and nitrate-reducing). Because these findings force our estimates of the thermodynamic constraints on life to drop, they increase the number of niches where we might expect microbial activity to occur. This may be particularly relevant for reservoirs on Earth where energy is limiting and microbial growth is expected to be slow, such as in the deep subsurface.

Of course, factors other than thermodynamics control microbial growth (such as pH, temperature, pressure, salinity, radiation, toxins, and trace metal supply). And it should not be forgotten that even if thermodynamics seem favorable, kinetic factors may preclude certain metabolisms from proceeding. How life responds (from the perspective of both single organisms and communities) in the face of environmental constraints and how it changes the environment in the process is perhaps the most fundamental topic in geomicrobiology. Although geomicrobiology can be defined broadly as the study of how microorganisms shape Earth's geochemistry, here we mainly limit our discussion to those processes that involve terrestrial systems. Within this, we focus still further on microbe-mineral interactions. Recent discoveries pertaining to the biogeochemical cycling of nitrogen (*20*) and phosphorus (*21*, *22*), microbial life in the oceans [e.g., trace metal limitation (*23*), iron acquisition (*24*), community analyses (*25*)], and metal metabolism by mycorrhizal associations (*26*, *27*) await a future integrative review.

[1]Division of Geological and Planetary Sciences, California Institute of Technology, Pasadena, CA 91125, USA. [2]Departments of Earth and Planetary Science and Environmental Science, Policy, and Management, University of California–Berkeley, Berkeley, CA 94720, USA.

*E-mail: dkn@caltech.edu (D.K.N); jill@seismo.berkeley.edu (J.F.B.)

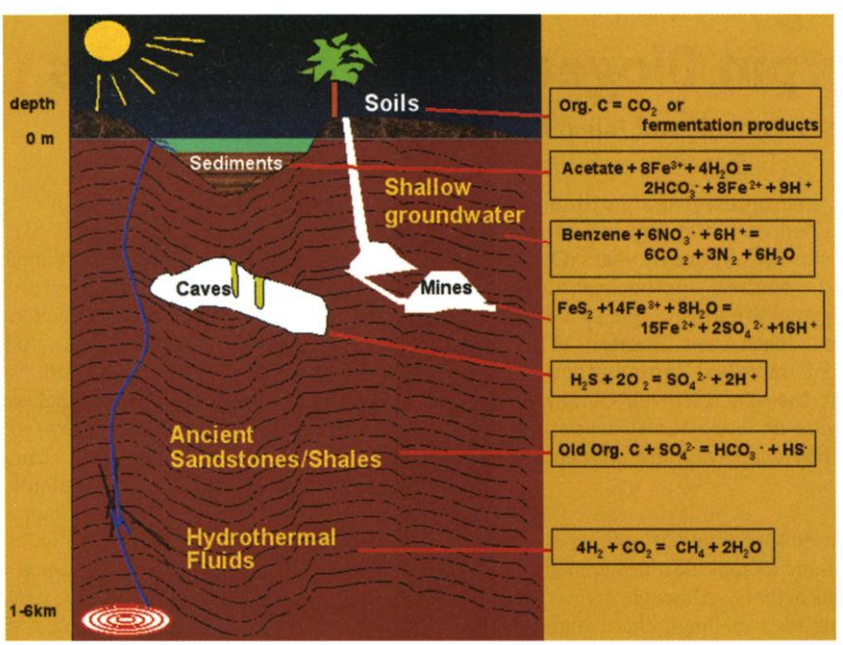

Fig. 1. We have only just begun to scratch the surface of recognizing important geomicrobial habitats, of which several representatives are shown in this illustration. Examples of metabolisms corresponding to each habitat are given at right. The depths at which the various habitats are drawn are not to scale, because the actual depths at which they occur can vary considerably.

Activity in the Environment

In this brief survey, we journey from the activities of microorganisms in sedimentary environments to hydrothermal fluids several kilometers below the surface of Earth (Fig. 1). Beginning near the surface, we note that sedimentary microorganisms play important roles in the cycling of several elements, including carbon, iron, and sulfur. These include heterotrophs that are sustained by organic carbon originating from primary production in surface waters, as well as chemolithotrophs who get their energy from the inorganic products of heterotrophic metabolism. Examples of the latter include the extensive sulfur-oxidizing mats of *Thioploca* off the Chilean and Peruvian coasts, as well as the largest bacterium known to date, *Thiomargarita namibiensis* (some cells reach three-quarters of a millimeter in diameter), which grows by coupling sulfide oxidation to nitrate reduction (*28*). Neutrophilic iron-oxidizing bacteria have been found in association with iron plaque on the roots of wetland plants (*29*, *30*), and bacteria with the ability to oxidize iron under nitrate-reducing conditions have been detected in a variety of freshwater sediments (*31*).

As oxygen is used in near-surface sediments, anaerobic microorganisms take over as the primary degraders of organic matter. Recently, a specific enrichment of bacteria of the family *Geobacteraceae* [anaerobic heterotrophs that can couple the oxidation of organic compounds to the reduction of insoluble Fe(III) oxides] was shown to grow by oxidizing organics with a graphite electrode as the sole electron acceptor (*32*). This type of activity may hold promise for the bioremediation of organic contaminants and/or energy harvesting for instruments, as members of this family are known to represent a sizable fraction of the microbial population in diverse sedimentary environments. In addition to the *Geobacteraceae*, many guilds couple organic matter oxidation to the reduction of inorganic compounds such as selenate, nitrate, manganese oxides, arsenate, sulfate, and carbonate for energy generation. Collectively, their metabolism is versatile, and they may help immobilize inorganic contaminants such as technetium and uranium (by catalyzing mineral precipitation) (*33*) or degrade organic contaminants such as benzene and chlorinated hydrocarbons through either oxidative or reductive reactions (*34*). For example, microorganisms able to use (per)chlorate as a terminal electron acceptor are ubiquitous in pristine and hydrocarbon-contaminated soils and sediments. They have been shown to oxidize a variety of monoaromatic compounds (including benzene) to CO_2 under anaerobic conditions (*10*). Elucidation of the metabolic pathways that govern these transformations is in progress, and identification of the genes involved will soon be aided by the completion of the genomic sequence of *Dechloromonas aromatica* strain RCB, a representative from this group.

The exciting concept that microorganisms have contributed to the formation of certain ore deposits over geologic time stems from the recognition that they can precipitate metals from solution. Though most ore deposits are thought to relate to transport and deposition by high-temperature fluids and magmas, in some cases low temperature origins may be possible. For example, it has been suggested that iron-oxidizing phototrophs may have played a role in the deposition of Banded Iron Formations over 2.5 billion years ago (*35*). New evidence has revealed that natural communities of sulfate-reducing bacteria (SRB) can generate essentially pure ZnS deposits from dilute groundwater solutions, providing support for a biogenic origin of many low-temperature metal sulfide ore deposits (*36*). In addition, recent evidence indicates that hyperthermophilic and mesophilic dissimilatory Fe(III)-reducing bacteria and archaea can couple oxidation of hydrogen to reduction of Au^{3+}, leading to Au^0 precipitation (*37*). Hyperthermophilic microorganisms can couple oxidation of hydrogen or organics to the reduction of metals in hydrothermal solutions, leading to the formation of magnetite (Fe_3O_4) and uraninite (UO_2) ore deposits at ~100°C (*38*).

Given their potential importance to ore deposit formation, how might we go about determining the distribution of metal-precipitating microorganisms? Let us consider the case of SRB. Devereux *et al.* (*39*) used probes that bind specifically to the 16S ribosomal RNA (rRNA) of target organisms to quantify the abundance of SRB in a sediment and to identify the predominant group in a zone characterized by mercury methylation (an activity attributed to SRB). The 16S rDNA gene has been widely used to expand our appreciation for microbial diversity in the environment (*4*). However, in microbial ecology, species identification does not always correlate with metabolic function. Accordingly, an alternative to assessing microbial activity in the environment is to target functional genes. In the case of SRB, most of these investigations have focused on analysis of sequences for dissimilatory sulfite reductase (DSR). This approach was used to identify SRB in a hypersaline microbial mat and to evaluate their distributions relative to oxygen gradients (*40*). Phylogenetic analysis of partial dissimilatory sulfite reductase gene sequences indicates that *Archaeoglobus*, an archaeal sulfate reducer, acquired the ability to reduce sulfate via an ancient lateral transfer from a bacterial donor (*41*). These studies show that it is possible to determine which species are controlling key geochemical transformations as well as to resolve details of the evolution of a pathway central to biogeochemical sulfur cycling over much of Earth's history.

In addition to promoting mineral formation, microorganisms also catalyze mineral dissolution. In aerobic environments, microbes may dissolve minerals through the excretion of various organic compounds. For example, because iron in aerobic soils is sequestered into minerals, organisms produce a class of biomolecules called siderophores that strongly bind iron and shuttle it to the cell surface, thereby increasing mineral dissolution rates (*42*). Kinetic data for the dissolution of goethite ($\alpha \cdot FeOOH$) by trihydroxamate siderophores suggest that these molecules adsorb iron via a single hydroxamate group in bidentate ligation with an Fe(III) center (*43*). A synergistic effect in the presence of an additional ligand, oxalate, was attributed to coupling of oxalate-promoted dissolution with complexation of Fe(III) by the siderophore (*44*). In anaerobic environments, anaerobic respiration may also promote mineral dissolution (or mineral transformation, depending on the geochemical conditions). An example of this is the reductive dissolution of Fe(III) oxides, which liberates metalloids such as arsenic that are adsorbed to the oxides (*45*). Culture-independent methods are now being used to identify the genes that encode the production of organic molecules in the soil (*11*) that likely participate in this type of reaction.

Traveling deeper into Earth, we turn our attention to caves and mines, which provide direct access to microbial communities kilometers beneath Earth's surface. Cave habitats may support a wide variety of species, including invertebrates that are sustained by chemoautotrophic microorganisms that oxidize hydrogen sulfide (*46*, *47*). A microbial role in the formation of cave deposits (such as speleothems) has been proposed based on microstructural, morphological, and isotopic data (*48*). However, at the present time, the role of organisms in speleothem formation is controversial (*49*, *50*). Subsurface mine systems are populated by a diversity of archaeal, bacterial, and eucaryal species. Metal sulfide ore deposits dominated by pyrite (FeS_2) are widespread and are of great interest because they are the source of environmentally damaging metal-rich sulfuric acid solutions (acid mine drainage). In contrast to some cave systems (*46*), acid mine drainage populations are typically dominated by only a small number of species belonging to diverse phylogenetic groups (Fig. 2). The simple community structure is probably due to the small number of electron donors and acceptors, extremely low pH, and high concentrations of toxic metals (*51*). Because these communities are essentially isolated from fixed carbon and nitrogen compounds formed at Earth's surface, acid mine drainage may provide ideal environments for detailed studies of how microbial communities work.

Acid mine drainage sites provide perhaps the best known example of microbially controlled mineral solubilization. Ferric iron, the primary sulfide oxidant at low pH, is regenerated slowly in abiotic systems by reaction of ferrous iron released by pyrite dissolution with oxygen. Some bacteria and archaea are able to enzymatically oxidize iron, which provides them with energy to sustain their growth (*52*). The ferrous iron enzymatic oxidation rate is up to six orders of magnitude faster than the inorganic reaction (*53*). However, this does not mean that inorganic oxidation is completely insignificant. Evaluation of the microbial effect requires consideration of the number of active iron-oxidizing cells and the rate at which each cell oxidizes iron. Edwards *et al.* (*54*) quantified these rates for individual microbial species and consortia of Fe(II)-oxidizing prokaryotes at low pH. A preliminary estimate indicated that the microbial populations in one sampled region of an acid mine drainage site account for ~70% of Fe(II) released by pyrite dissolution.

Subsurface microbiology is not limited to caves and mines, however. Deep drilling projects in many parts of the world have revealed evidence for microbial life in rocks at great depth (*55*). Examples include sulfate re-

duction occurring between sandstone and shales that were deposited during the Cretaceous period (90 to 93 million years ago) (*56*) and methanogenesis driven by geothermal waters rich in hydrogen (*6*). In both of these cases, microbial activity was detected at least 200 m below land surface. Even deeper environments have been sampled for signs of microbial life. Notable examples of this are the gold mines of South Africa, which represent the deepest accessible excavations in the world. Some fissure water samples from these mines are thought to originate from anaerobic, saline groundwater at a depth of 5 to 6 km, where temperatures are in the hyperthermophic range (*57*). Through culture-independent analysis, novel archaeal sequences have been retrieved from these samples, including a clone that is related to known hypothermophilic species of *Pyrococcus* from a marine vent system (*58*). One question that remains open is whether the organisms that inhabit these environments are metabolically active in situ. The recent finding that *Shewanella oneidensis* and *Escherichia coli* can survive and oxidize formate at pressures greater than 1000 megapascals (corresponding to pressures ~50 km below Earth's crust) suggests that metabolic activity may occur at depth (*59*).

Mechanistic Details

Many of the above studies were performed using culture-independent methods, and such approaches exemplify an exciting new frontier in geomicrobiology. Nevertheless, if we are to understand the mechanisms of important geomicrobiological processes at the level of genes and proteins, work with and establishment of model systems are still essential. Here, we provide a few examples where classical genetics and biochemistry have provided insights into how life responds to and affects its environment.

A topic that has inspired several studies at the molecular level is how bacteria respire minerals. Most terminal electron acceptors that bacteria use for respiration, such as oxygen, nitrate, and sulfate, are soluble. This means they can freely diffuse to the cell to receive electrons from the membrane-bound molecules of the respiratory chain. How bacteria transfer electrons to solids like hematite (α-Fe_2O_3) and goethite presents a real problem. Because these minerals are effectively insoluble under environmentally relevant conditions, simple dissolution and diffusion of ferric iron to the cell cannot be the answer.

Different mechanisms for electron transfer to minerals during respiration have been proposed. The first is that bacteria solubilize the minerals by producing chelators. Although the addition of synthetic chelators has been shown to stimulate microbial electron transfer to iron minerals, to date no evidence has been found that bacteria use this mechanism in respiration (*60*). The second is that they use soluble shuttles, such as organic compounds with quinone moieties, to transfer electrons from the cell to the mineral (*61*). These shuttles may be exogenous substances or may be produced by the organisms themselves (*62*). Recent results from genetic screens with the iron-reducing organism *S. oneidensis* strain MR-1 suggest that these shuttles may share structural and functional properties with redox active antibiotics (*63*, *64*). The third mechanism is that bacteria directly transfer electrons from the cell surface to the mineral. A variety of biomolecules (including cytochromes, quinones, dehydrogenases, and secretory proteins) have been identified as participating in this electron transfer pathway (*65–68*). Of these, several are located on the outer membrane of the cell and presumably make contact with the mineral directly (*69*).

Another topic that has received molecular attention is the mechanism of precipitation of manganese oxides by diverse *Bacillus* species. Dormant spores produced by these organisms enzymatically oxidize soluble Mn(II) to insoluble Mn(IV) oxides. A representative of this group, *Bacillus* sp. strain SG-1, is believed to catalyze this process by a multicopper oxidase, MnxG. Recently, phylogenetic analysis based on 16S rRNA and *mnxG* sequences obtained from 15 different Mn(II)-oxidizing spore formers (including SG-1) revealed extensive diversity within the genus *Bacillus*, with organisms falling into several distinct clusters and lineages (*70*). In addition, active Mn(II)-oxidizing proteins of various sizes were recovered from the outer layers of purified dormant spores of the isolates (*70*).

Mechanistic details of important biogeochemical activities may also be identified from uncultured organisms. An outstanding example of this is the recent finding that proteorhodopsin-based phototrophy occurs globally in the marine environment. This discovery was made by expressing select DNA fragments from uncultured marine organisms in *E. coli* and observing the production of functional proteorhodopsin (*71*). Biophysical techniques were then used to show that native organisms expressing proteorhodopsin are widespread (*5*). Whether these organisms can fix CO_2 is currently unknown. But given their global distribution, it seems likely that they may have a significant impact on carbon and energy flux in the oceans.

Lastly, an important step forward in the mechanistic analysis of natural samples has been the combination of gene probes with molecular-level isotopic measurements. This approach has permitted the direct correlation of geochemical activities (such as anaerobic methane oxidation) with likely source organisms (methanogenic archaea and sulfate-reducing bacteria), even though the latter may have never before been cultured (*12*, *72*).

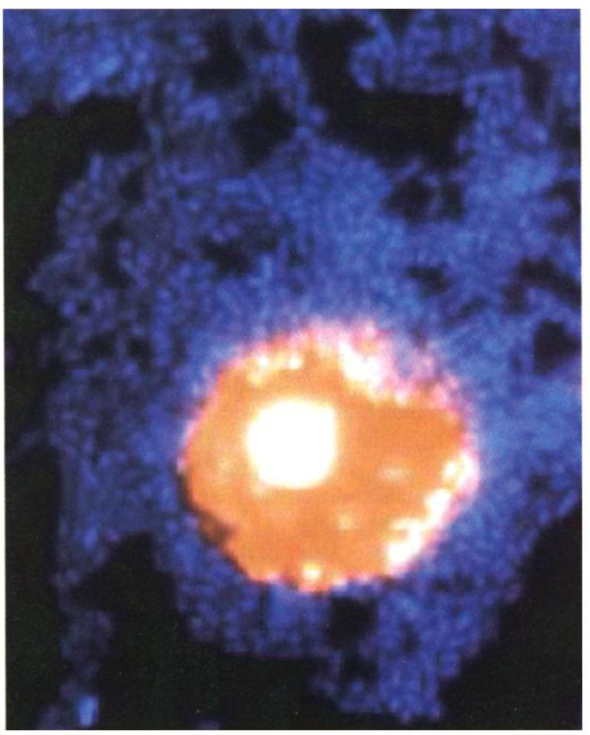

Fig. 2. Epifluorescence image of a protist and associated bacteria in a pH 1.3 acid mine drainage community from Iron Mountain Mine, California. DNA is stained blue by 4′,6′-diamidino-2-phenylindole (DAPI). Protist cells (orange) have been labeled by in situ hybridization (FISH) with a probe (Euk502) that binds specifically to eukaryote RNA. The protist nucleus appears white because both DNA and RNA are stained. The bacteria are approximately 1 to 2 μm in length. Image courtesy of Brett Baker.

The Genomics Revolution

The most rapid recent advances in our understanding of cultured microorganisms can be attributed to the advent of genomics. The first microbial genome sequencing projects focused almost entirely on pathogenic strains, but within the past few years biogeochemically relevant microorganisms have joined their ranks. The ease of genomic sequencing has increased dramatically,

and this is nowhere better seen than in the ever-rising number of microbial genome projects that are listed on the Web site at the National Center for Bioinformatics (www.ncbi.nlm.nih.gov/cgi-bin/Entrez/genom_table_cgi). We note, however, that in the majority of geologic settings, only a few isolated microbial strains have been characterized, and they do not necessarily represent the dominant organisms in that environment. A desirable goal for future sequencing projects will be to identify the major players in any given environment and bring them into culture so that their physiology and biochemistry may be studied with the aid of genomic information. It will also be important to determine the degree to which gene content, genome organization, and gene regulation vary between strains in a given environment.

Despite the limited data set, much has already been learned from genome sequencing projects that is causing us to reevaluate the history of life. Comparative genomics (e.g., comparisons of coding regions from different organisms for similar biomolecules) has revealed phylogenetic incongruities that span the universal tree. For example, the vast majority of gene products from the *Archaea* most resemble counterparts among the *Bacteria* and not the *Eucarya*, yet the rooted phylogenetic tree [based on small subunit rRNA (SSU rRNA)] clearly places the *Archaea* as specific relatives of the *Eucarya* (*73*). Although reexamination of the universal tree on the basis of alignments of 23 orthologous proteins conserved across 45 species from all domains has resulted in trees that support the SSU rRNA trees with respect to the separate monophyly of domains (*74*), differences in GC content and codon usage patterns within genomes suggest that lateral gene transfer has been a primary evolutionary mechanism throughout Earth's history (*75*, *76*). The jury is still out regarding which cell types emerged first and how, but as more genome sequences from evolutionarily interesting microbes (such as anaerobic protists) become available, we will be in a better position to use DNA as a fossil in speculating about evolution. A profound advance will come by linking the emergence of particular genes to major events in Earth's history. This is a difficult task, but some intriguing work has already been done in this area (*77*).

A powerful new tool that has been made possible by whole-genome sequencing is DNA microarray technology. This involves the simultaneous monitoring of gene expression patterns for all messenger RNAs (mRNAs) of an organism. DNA microarray technology makes it possible to determine how (and how rapidly) organisms respond to changes in the geochemistry or biology of their habitats (e.g., to an increase in concentration of a toxic metal; decrease in temperature, variations in ionic strength and pH; or additions or deletions of microbial species). An example of this is a recent microarray study that was performed with *S. oneidensis* (*78*). In that work, it was possible to identify the networks of genes that were up- or down-regulated depending on the type of metabolism in which the cells were engaged (e.g., growth on alternative electron acceptors, including iron). Inspired by studies where microarrays have been used to study microbial development [e.g., studies of the life cycle of the stalked bacterium *Caulobacter crecentus* (*79*) or biofilm formation by *Pseudomonas aeruginosa* (*80*)], we anticipate microarrays will play an increasingly important role in investigating geomicrobiological problems. For example, once enzymes are identified (such as those involved in metal oxidation or reduction) it may be possible to monitor the activity of genes that encode these enzymes with organism-specific resolution in mixed communities. As more information is amassed about important gene sequences from the environment, it will be feasible to extend array technology to monitor expression patterns of genes from uncultured organisms.

Future Perspectives: Modeling Microbial Communities

Microbial ecology offers a logical framework for analysis of the feedbacks and interconnections between the physical, chemical, and biological features of an environment. The extent to which the rules and/or theories of macro ecology apply to microbial ecology is an intriguing open question. Detailed understanding will require documentation of the makeup of biological communities, determination of the ways in which community members interact at the biochemical level, the nature of system inputs (e.g., sunlight, nutrients) and outputs (e.g., CO_2, solutes), and the ways in which organisms modify and respond to changes in their environments. For example, cells may compete for limited resources with the other members of their community; they may supply nutrients to the system by breaking down a substrate that then becomes available to other organisms; they may generate toxic metabolic byproducts that restrict the growth of other members of their community; and they may influence the genetic program of their neighbors by sending out molecules that control gene expression. In modeling ecological networks, it is not only important to characterize these interactions, but it is essential to have a metric for monitoring the overall state of the system.

To date, it has not been possible to take apart a natural system, analyze it, and model it at this level. In many systems, this may never be possible, but "simple" microbial systems, such as biofilms, may provide an opportunity. Biofilms are surface-attached microbial communities, with members that are largely cooperative and genetically capable of communication through chemical signaling (*81*). They are ubiquitous, resilient, responsive to their environment, and amenable to laboratory analysis. Though much has been learned regarding the genetic pathways taken by a variety of organisms when transitioning from the planktonic to the sessile phase (*82*, *83*), little is known about how these pathways change in response to changes in the environment. Clearly, at the scale most relevant to bacteria (the microscale), an important environmental factor that affects biofilm development by a given species is the presence of other organisms.

A promising approach for studying ecological complexity, therefore, might be to model a simple mixed species biofilm in the laboratory, where species composition and environmental conditions may be strictly controlled. A starting point for such an analysis might be to study the interactions of a mixed community of iron and sulfate reducers that attach to a solid substrate with respect to their assembly, organization, and metabolic activity within the biofilm under different environmental conditions (e.g., varying electron acceptors, carbon limitation, flow rate, and composition of the surface). The dynamic response of the system to chemical changes induced by the metabolism of the cells could be monitored, with the aid of microelectrodes and microscopy, over time during biofilm development. In the case where steel serves as the surface for attachment, corrosion could be used as a general system metric because iron and sulfate reducers are known to affect corrosion in different ways (*84*). Even modeling this relatively simple and controllable system poses a real challenge, as definition of all the parameters that control the organization of the cells (biological, chemical, and physical) is nontrivial. Assuming that this can be done and networks can be defined, in time different microorganisms (such as iron- or sulfur-oxidizing bacteria) could be sequentially added to this reductionist system to more accurately capture the complexity of corrosion communities in nature.

This approach has obvious limitations. It is also necessary to consider where, in the real world, we may encounter relatively simple microbial ecosystems that might be tractable. The extreme environments found within reasonably isolated, subsurface acid mine drainage systems provide a target. Here, the biological complexity of communities is reduced to a small number of organisms, and the biological and geochemical states can be completely described. A starting point for development of methods for field analyses is to use laboratory-scale bioreactors to create and test biogeochemical models for acid mine drainage systems. In these systems, the kinetics of the key inorganic and biologically me-

diated mineral dissolution and redox reactions are known, and the geochemistry of the input and outflow solutions can be measured over time. Changes in the community size, structure, and composition and levels of expression of genes (selected using community genomic information) as a result of system perturbation can be monitored with the use of oligonucleotide probes (*85*) and microarrays.

Studies performed with these systems should allow us to address fundamental ecological questions, such as: how do organisms self-organize in response to changes in their environment (both biological and chemical)? In turn, how does their organization affect the chemistry of their environment? What are the metabolic and genetic networks that link the members of the community to one another? And how robust are these networks in the face of environmental perturbations? The results of these analyses may generate new understanding of relations between substrate diversity, species diversity, partitioning of function, and energy and materials flow.

As we begin to sample the genomes of natural populations, we must confront the question of species- and subspecies-level genome diversity. Given the current genome sequencing capabilities, we anticipate that it will be possible in the near future to simultaneously analyze the gene content of all organisms within simple microbial communities. It will be interesting to see how gene content and genome organization vary within "species" in natural communities (and perhaps why).

Documentation of the gene content of microbial populations in natural systems via genome sequencing or other methods raises at least as many questions as it solves. An important challenge will be to determine the function of genes or groups of genes that have not previously been identified. *In silico* prediction, analysis of homology with previously characterized genes, genetic manipulations, and biogeochemical experiments are critical tools needed to meet this challenge. Laboratory- or field-based studies that correlate changes in environmental conditions or metabolism with gene expression levels may also help constrain the roles of genes whose functions are uncertain (Fig. 3).

The challenges associated with genome-enabled microbial ecology are considerable, and the extent to which the task is tractable remains controversial. However, detailed understanding of all the pathways responsible for biogeochemical reactions and the interactions between these pathways are important long-term goals. Integration of molecularly resolved geochemical and biological data will require the development of models with appropriate structure and complexity (*86*). When these models become available, it should be possible to decipher species-environment interactions and species-species interactions (competitive and synergistic); the partitioning of biosynthetic, biodegradation, and other pathways; and the flow of energy and elements (such as carbon, nitrogen, sulfur, and phosphorus) between community members. Once we understand the design principles for microbial communities, comparison with studies of gene expression in multicellular organisms may reveal why only a small fraction of Earth's inhabitants (e.g., most eukaryotes) abandoned the ability to use the vast majority of available energy sources and populated only a tiny subset of Earth's habitable environments.

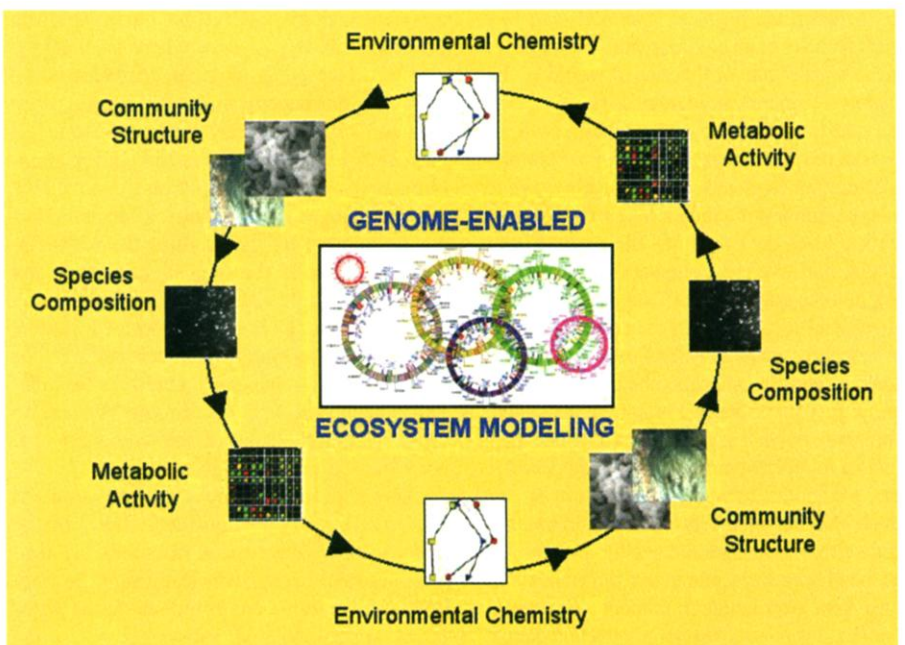

Fig. 3. Representation of the iterative cycle required for genome-enabled ecosystem modeling. In a given physical environment (be it a biofilm in the laboratory or an acid mine drainage system), the chemistry of that system is measured at a given point in time. The microbial community is described (e.g., with respect to species number and spatial organization) and members of the community are identified (species composition). For systems where the species and/or their respective genomes are known, samples may be processed and analyzed with microarrays to determine metabolic activity at that time point. Because microorganisms both sense and respond to their environment, and in turn change their environment, genome-enabled ecosystem modeling will be aimed at identifying the networks that govern these systems and how they change over time. The rings in the center that resemble those of the Olympics are meant to convey the overlap and/or codependencies between microbial genomes in a given community.

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REVIEW

Merging Genomes with Geochemistry in Hydrothermal Ecosystems

Anna-Louise Reysenbach[1]* and Everett Shock[2]*†

Thermophilic microbial inhabitants of active seafloor and continental hot springs populate the deepest branches of the universal phylogenetic tree, making hydrothermal ecosystems the most ancient continuously inhabited ecosystems on Earth. Geochemical consequences of hot water-rock interactions render these environments habitable and supply a diverse array of energy sources. Clues to the strategies for how life thrives in these dynamic ecosystems are beginning to be elucidated through a confluence of biogeochemistry, microbiology, ecology, molecular biology, and genomics. These efforts have the potential to reveal how ecosystems originate, the extent of the subsurface biosphere, and the driving forces of evolution.

In 1897, Davis (*1*) published a paper in *Science* that described the "vegetation" of hot springs at Yellowstone National Park, including observations of life at 85°C, and 6 years later Setchell (*2*) carefully extended these observations to 89°C. Despite the contributions to thermophile microbiology by Brock (*3*) and others, high-temperature organisms remained curiosities until the molecular, phylogenetic, and genomic revolutions of the past two decades moved them to the center of debates about the mechanisms of evolution, the depths of the biosphere, mineral-microbe relations, the origins of ecosystems, the emergence of life, and the potential for life on other planets.

Many of the questions driving current research perplexed the pioneers as well. Davis speculated that "Perhaps . . . these organisms resemble more closely the primitive first forms of life than any other living types" and wondered about their evolution, dispersal, and ecology. Setchell too posed a problem that still plagues biochemists: "What is it that enables the protoplasm of the thermal organisms to withstand a temperature which coagulates, and consequently kills, the protoplasm of the majority of organisms." Here, we examine how these ideas with roots in the 19th century are being tested today with the modern methods of molecular biology and theoretical biogeochemistry. In particular, we attempt to reconcile recent molecular and genomic data with ecological and geochemical observations of hydrothermal systems. Both genomic and geochemical information are records of evolutionary changes that have occurred in hydrothermal systems (Fig. 1). Genomes of several thermophiles (we use the general term "thermophile" to refer to all organisms that grow optimally above 45°C, including hyperthermophiles that only grow optimally above 80°C) are now sequenced, and geochemical evidence of ecosystem evolution is available in nearly 4 billion years of history of hydrothermally altered rocks, as well as in active hydrothermal ecosystems. Once decoded and integrated, these genomic and geochemical clues can reveal the geologic and evolutionary history of how biogeochemical interactions turn hot water and rocks into habitats.

Diversity of Geochemical Energy Sources

The vast majority of hydrothermal systems operate in the subsurface, without necessarily manifesting any surface expression. Never-

[1]Department of Biology, Portland State University, Portland, OR 97201, USA. [2]Group Exploring Organic Processes in Geochemistry, Department of Earth and Planetary Sciences, Washington University, St. Louis, MO 63130, USA.

*To whom correspondence should be addressed. E-mail: reysenbacha@pdx.edu (A.-L.R); shock@zonvark.wustl.edu (E.S.)

†Address after 1 July 2002: Department of Geological Sciences and Department of Chemistry and Biochemistry, Arizona State University, Tempe, AZ 85287, USA