Geomicrobiology and Microbial Geochemistry

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Geomicrobiology and Microbial Geochemistry (GMG) investigates the interaction between Earth and environmental systems and microbial life. Microbes shape their geochemical surroundings through their metabolism and growth needs, exerting control on every facet of redox, metal, organic, nutrient, and trace element geochemical and mineralogical components. Conversely, geochemical surroundings dictate what metabolisms are possible. Microbial evolution has occurred in concert with changing geosphere conditions – microbes have driven major shifts in the chemistry of oceans, continents, and atmosphere just as key changes in physical and chemical surroundings affects microbial communities (Knoll, 2003a,b). If one wishes to understand element cycling in any system containing water, including the role of microbes is critical to the story.

Development of Geomicrobiology and Microbial Geochemistry

As part of the broader disciplines of geobiology or biogeochemistry, the developing field of GMG has crucial overlaps with fields of biology addressing microbes, but is specifically focused on combined approaches to bring chemical, biological, AND geological perspectives together to characterize the role of microbes in environmental and geological processes (Fig. 1). Geobiochemistry turns this perspective toward establishing the role of geology in the development of organismal biochemical processes over time (Boyd and Shock, this issue).
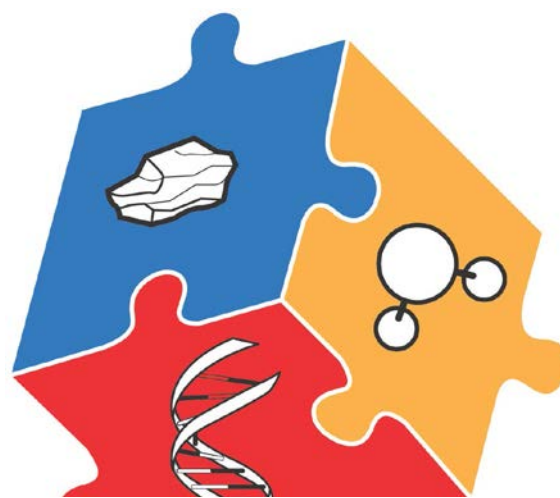

Figure 1: To understand biogeochemical cycles of elements and the evolution of life on Earth, the interplay of various aspects of biology, chemistry and geology must be considered.

Combined approaches to interrogate microbial activity (through physiology, genetics, culturing, microscopy) and geochemistry (aqueous, mineral, isotope geochemistry) have developed to address the important, and sometimes very complex, interactions between microbes and their surroundings.

This issue of Elements aims to illuminate the importance of microbes in natural systems and the revolution that is occurring in the rapidly growing GMG field. We show the rapid growth in our understanding that has come from characterizing links between microbes and their geochemical surroundings through time, and highlight emerging opportunities with the advent of new tools and paradigms.

The GMG field owes much to the early work of environmental microbiologists and geochemists. Environmental microbiology has a long history, arguably beginning with the invention and application of the microscope by Leeuwenhoek in the 17th century. However, the link between microorganisms and Earth processes was not fully recognized until Sergei Nicholaevitch Winogradsky's work formulated the concepts of chemolithotrophy and autotrophy after investigations of elemental sulfur transformations by *Beggiatoa spp*. (1887; as reviewed in Dworkin, 2011). Vladimir Vernadsky illustrated the temporal dimension of the interaction between life and Earth by arguing that the biosphere has shaped Earth's surface environment throughout geological time (Vernadsky, 1926). Lourens G.M. Baas Becking came up with the notion "everything is everywhere but the environment selects", a novel concept in biology that the community of organisms is determined by their physicochemical surroundings (see de Wit and Bouvier, 2006). Baas Becking also coined the term "Geobiology", as an effort to describe the relationships between organisms and the Earth (summarized in Becking, 1934; Quispell, 1998).

One of the first to bring together microbiology and geochemistry is Henry Ehrlich; his academic preparation as a microbiologist found a serendipitous union with geology when a colleague piqued his interest in the bacterial promotion of pyrite oxidation (Ehrlich, 2012). His long career has furthered our understanding of the role of microorganisms in a number of element cycles. Other geochemists also blazed the trail towards linking key physical and chemical concepts to microbial processes. The work of Robert Berner defined fundamental concepts of carbon cycling through deep time and the associated formation of metal sulfides and carbonate minerals (both processes that involve microbial activity). Professor Berner also steered much discussion towards the global, climatic implications of these small-scale processes. Additional work on chemical speciation, energetics, isotope fractionation, and kinetics fundamental to the geochemical underpinnings of microbial activity contributing to element cycling is based on the earlier work of geochemists that include Bob Garrels, Hal Helgeson, Sam Epstein, and Stanley Miller, among others.

Despite these deep historical roots, only in the last 15 -20 years has GMG evolved into a robust discipline (Banfield and Nealson RiMG volume, 1997). It is not a coincidence that the first generation of truly interdisciplinary scientists trained at the intersection of microbiology, genetics, geochemistry, mineralogy, and computational approaches have demonstrated the critical roles of microbes in processes from a molecular to global scale. From the history of the early Earth to the mobility of elements, new insights are shifting paradigms for how the community thinks about key problems in the field. For example, Earth's deep history is now

interwoven with the evolution of microbes – the changes in iron and sulfur chemistry in ancient seas, the Great Oxidation Event, and the cycling of carbon in surface and diagenetic processes (Canfield, 2005; Lyons et al., 2014; Johnston, 2011). In modern environments, the importance of microbes in the mobility of metals, radionuclides, and organic contaminants is now rigorously documented as a key component for predicting mobility and mitigating contamination (Slater et al., 2008). Microbes are also critical for many processes affecting water quality, agriculture, and climate. The importance of microbes in N cycling for example is key in considering greenhouse gas levels, critical nutrients for crops, and important contaminants affecting water quality directly and indirectly via harmful algal blooms in marine and freshwater systems (Falkowski et al., 2008; Taylor and Townsend, 2010).

How Many Microbes are there and where do(?) we find them?

Microbes, representing the bacterial and archaeal domains of life, possibly represent more than 50% of the biomass carbon on the planet. Microbes are almost always too small to be seen with the naked eye, commonly about 1 micron in diameter, and usually shaped as a spherical (cocci) or rod (bacilli) forms. As small entities in large numbers (densities of cells in marine settings are around 10^6 cells ml^{-1} on average but can reach 10^{12} cells ml^{-1}, Amaral-Zettler et al., 2010), microbes have a very large surface area. In total, there are ~10^{30} microbes on Earth, occupying almost every conceivable corner of the surface and subsurface where water, nutrients and energy are found. This includes some locations one might imagine as exceeding hostile to life – deep within basalt fractures, within glaciers, in deep undersea thermal vents, in pH 0 waters with very high metal concentrations, and in the pores of rocks located in extremely dry deserts. In fact it has proven difficult to find a place on Earth that contains even the smallest amount of liquid water and no microbial life.

What are microbes made of?

As with all living things, microbes have the ability to grow and replicate. Acquiring the elements necessary for this growth is an important part of how microbes interact with their geochemical surroundings. This includes harvesting the carbon, oxygen, nitrogen, phosphorus and sulfur required for cellular materials and the metals (including iron, manganese, cobalt, nickel, zinc, and molybdenum) needed to form cofactors for specific enzymes and proteins. Microbial cells are mostly water (70-85%); the remaining biomass consists of a combination of 4 basic types of organic molecules – amino acids, nucleotides, fatty acids, and sugars which combine to produce the key macromolecules in cells - proteins, nucleic acids, lipids, and polysaccharides, respectively (Figure 2).

Metabolic function is centered on the production of specific proteins (enzymes) - large molecules composed of amino acids that catalyze reactions and can make up more than 50% of the biomass per cell. Proteins are complexly structured chains of amino acids called peptides, constructed at ribosomes (Figure 2 inset). Enzymes, as specific catalytic proteins often with a specific metal (or metal-sulfide) center, catalyze redox reactions in a specific 'lock-and-key' manner where the

compounds that are part of the reaction are selectively brought to and from the reaction center – i.e. a specific enzyme only interacts with specific reactants. Deoxyribonucleic acid (DNA) contains the blueprint for protein construction, carried to the ribosome by a specific type of ribonucleic acid (messenger RNA, mRNA) where amino acids are assembled on another form of RNA (transcription RNA, tRNA) into proteins (Figure 2 inset). Microbial DNA contains the possible instructions for what a specific microbe is capable of, i.e. its metabolic potential, but the RNA or protein production are indicators of what the cell is actually doing at any one point, i.e. indicators for their activity.

A specific microbe can be capable of multiple forms of metabolism and cellular function as encoded on DNA that is not necessarily used, or expressed, at any one time. Specific sections of DNA (a gene) encode for a specific cellular function that can be turned on, or expressed, in response to environmental variables. In many cases, this gene expression is correlated to the number of cells present and regulated by cell-cell communication via the secretion of signaling molecules (called quorum sensing).

All microbial cells include an envelope of lipids called the cell membrane that separates the inside and outside of the cell (Figure 2). Many microbial functions, including generation of adenosine triphosphate (ATP, a key energy compound important for biosynthesis and growth) and many metabolic reactions, occur at proteins bound to the cell membrane. Intracellular fluid pH is maintained near-neutral in most organisms and at a higher pressure (called the turgor pressure). A pH gradient is kept across all cell membranes (lower pH outside the membrane), called the proton motive force, driving ATP production.

Extracellular structures (outside the cell membrane) include peptidoglycan layers, additional lipid layers, and polysaccharide layers as part of a rigid cell wall or capsule of material. Microbes can also possess appendages that grow out of the outer cell wall that can facilitate cell movement. Shorter appendages called pili can cover the cell whereas flagella are typically much longer and include a complex molecular motor that can turn the flagella like a propeller. Movement can additionally be facilitated by intracellular structures some microbes possess called vacuoles that can be filled with gas to provide buoyancy.

The direction that a microbe travels is generally random as defined by Brownian motion. Some microbes however can point themselves, either through sensing a chemical gradient (of a particular compound, called chemotaxis), or by using elongated structures containing magnetic minerals such as magnetite or greigite (called a magnetosome) that functions as a compass needle in the Earth magnetic field.
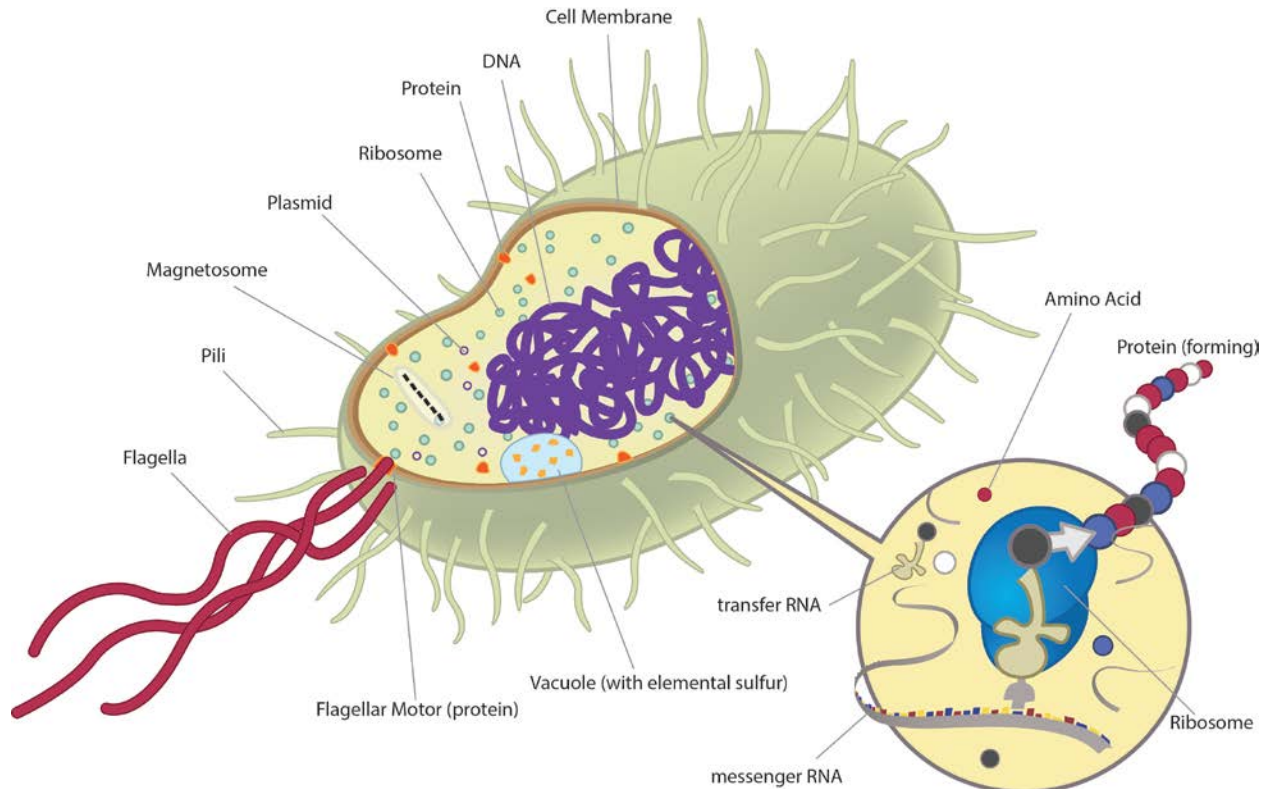
Figure 2 - Generalized structure of a typical microbe, inset details the process of basic protein construction at the ribosome.

How have microbes evolved?

The diversity of microbial life is both astounding in magnitude and far from completely defined. 99% of our knowledge of microbial diversity comes from applying molecular genetics techniques in a wide range of natural environments - from glaciers to hot springs to deep sea sediments (Pace et al., 2012; Quast et al., 2013). One gene that is present in all bacteria and archaea, but has mutated enough over time to delineate microbial species, is the gene encoding for part of the ribosome – the 16S rRNA. There are today over 4 million catalogued 16S rRNA gene sequences in databases (such as Genbank and SILVA; Yarza et al., 2014), a number that continues to increase rapidly.

In addition to a likely large number of undiscovered microbes on Earth, the DNA sequences of protein encoding genes from known microbes are often of unknown function (i.e. the gene's sequence is known, but not its function). This suggests there is also much left to discover in terms of new proteins, cofactors, and other molecular machinery associated with microbial function in many settings (popularly termed 'microbial dark matter'). GMG researchers are continuing to expand the genetic database with specific genes and full genomes, in addition to RNA and proteins, and link that information to microbial function in the environment (Dick and Lam, this issue).

Evolutionary relationships between organisms can be defined by the degree of dissimilarity between the genes derived from a common ancestor, represented graphically as a tree (Figure

3A). Trees can be constructed from any common fragment of RNA or DNA (even the full genome), as a means of comparing similarity between any set of species. Life evolved from a last common ancestor, the deepest branch of the tree. Closest to this are thermophilic microorganisms (Figure 3A); Evolution of microbes occurred for a couple billion years before even the simplest eukaryotes (Figure 3B), and the genetic variability in the microbial domains (bacteria & archaea) is extremely broad. Genetic variation occurs in two primary ways – random mutation of a specific DNA base pair, or transfer of a genetic fragment from either a plasmid or virus (termed horizontal gene transfer). On the tree of life, random mutation occurs in small steps, resulting in a branching, dendritic pattern. Horizontal gene transfer, on the other hand, occurs by tying together branches that may have evolved apart for a long time, by sharing a chunk of genetic material at one point in time.
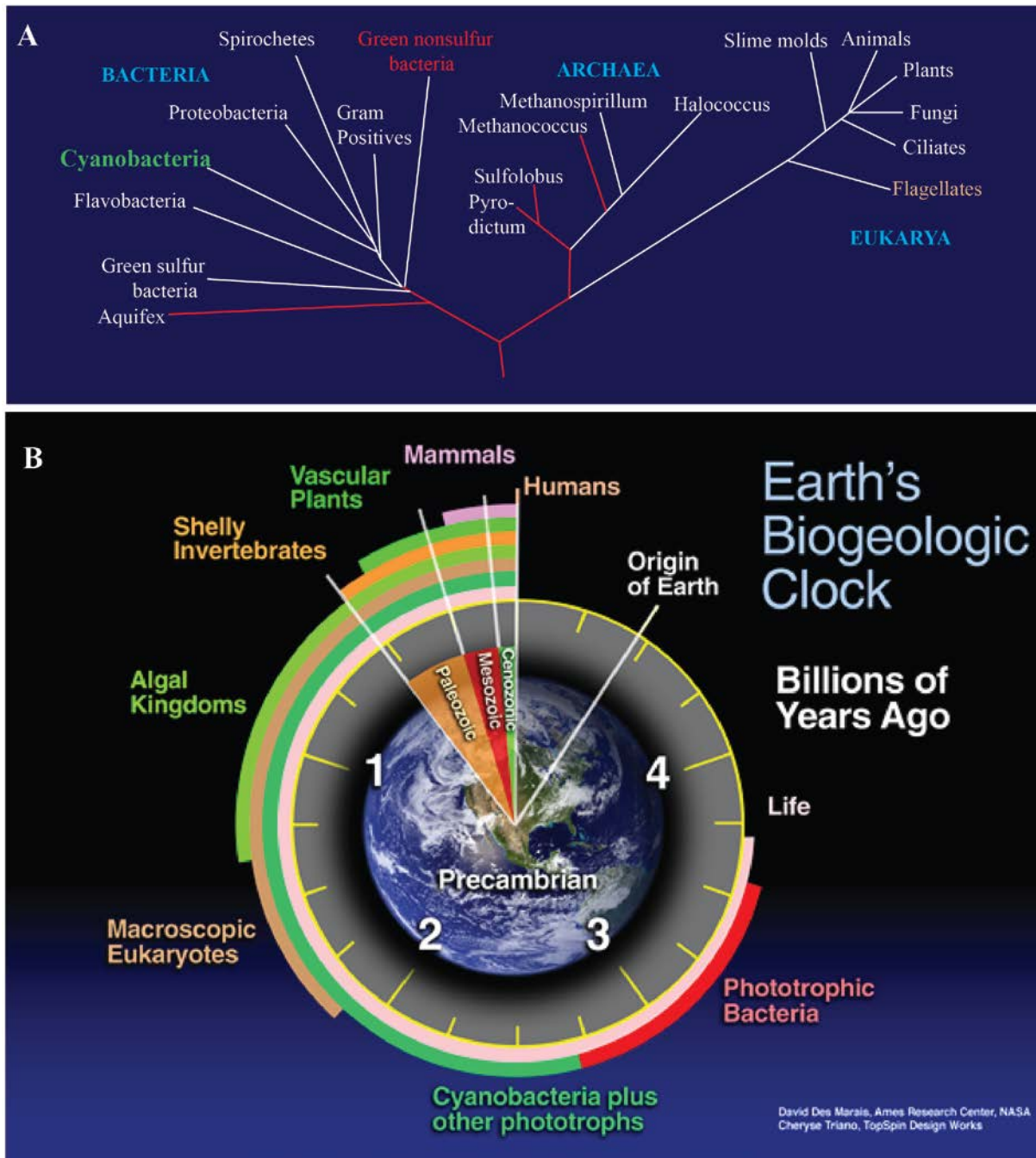
Figure 3 – A. Representation of a simple phylogenetic tree based on rDNA gene data.   Distance is a quantitative measurement of genetic similarity; red lines are associated with thermophilic groups. Image adapted from Woese et al. 1990. B. Representation of the timing of organismal appearance on Earth separated into major groups, color coded groups link some key groups between Figures 3A and 3B (B by permission of D. Des Marais).

What do Microbes Eat?

Microbes interact with their geochemical surroundings to harness energy from redox compounds (edibles and breathables) that are out of equilibrium and to harvest the elements required for growth and reproduction (Nealson, 2001). One consequence of these processes is that microbes often catalyze reactions that have a lot of energy but are kinetically very slow as abiotic reactions. One example is the reaction of H_2 with O_2. This is a fundamental microbial metabolism, but if you put these things together in a sterile vessel they generally do not react. If you add a flame, however, the mixture explodes– thus demonstrating the tremendous potential energy available for the microbes to utilize in a more controlled fashion with enzymes.

The metabolism of microbes is remarkably varied and organisms are known to bring together almost every thermodynamically viable combination of possible electron donor-acceptor pairs that exists in water. Much like a battery, coupling of redox compounds harnesses a flow of electrons through the machinery of a microbe to drive all cellular processes; this energy is equivalent to the specific amount of free energy available from the reaction of the electron donor-acceptor pair (Figure 4A, B). Microbes can gather energy via 3 general processes: chemotrophy, phototrophy, and disproportionation (Figure 4 A and C).
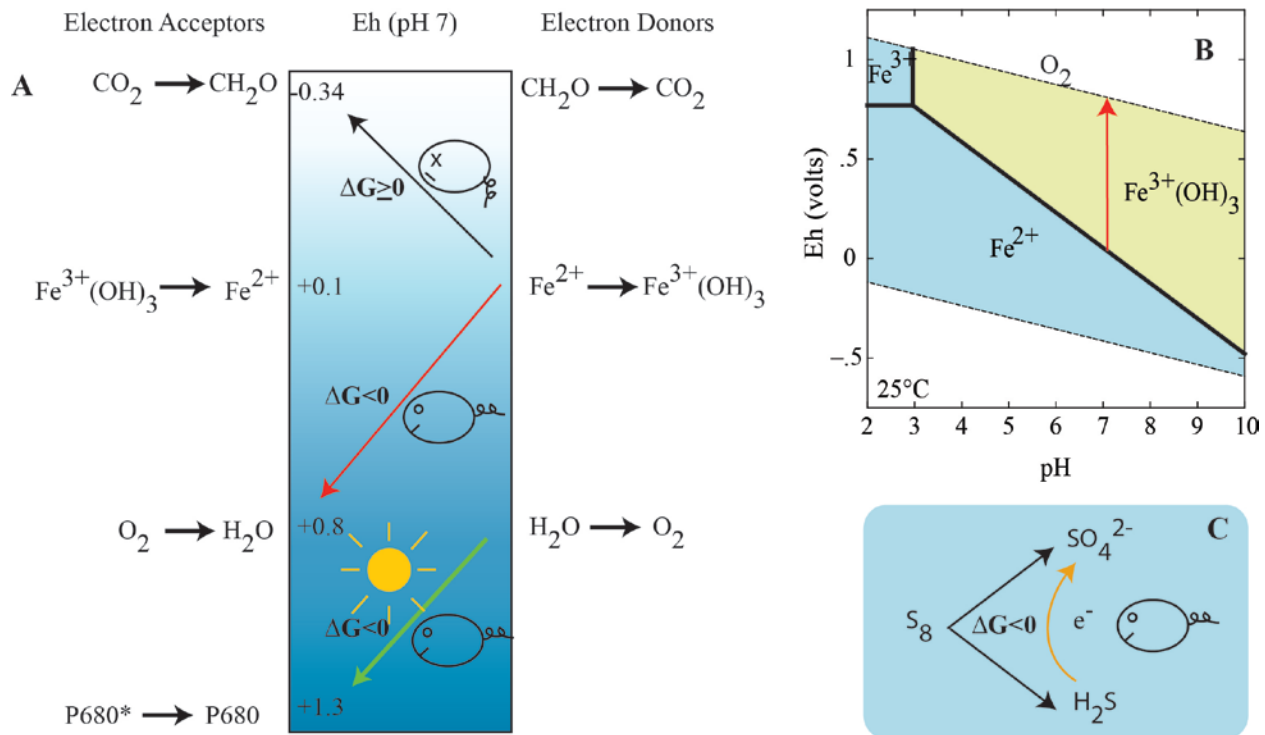

Figure 4 – A. Relative positions, expressed as electropotential (Eh) of electron donors ('edibles'; right side) and electron acceptors ('breathables'; left side) at pH 7. The energy for cellular function comes from the energy separation of these redox couples, much like connecting opposite terminals of a battery ($\Delta G_R < 0$ is energetically possible). B. Eh-pH diagram showing how geochemists have viewed the same information with pH as a variable; Note the red arrow coupling Fe^{2+} oxidation with oxygen reduction in the thermodynamically favorable direction in both A and B panels. Three types of general metabolism are associated with redox reactions: Chemotrophy, coupling energetically favorable redox donor/acceptor pairs; Phototrophy, coupling an energetically favorable e-

donor with an e- acceptor at a photoactive enzyme site in a high energy state due to interaction with photons; and Disproportionation (C), splitting of a single source molecule into a more reduced and a more oxidized form (also known as fermentation, the same type of reaction that creates alcohol from sugar).

In order for a microbe to utilize these redox compounds for energy generation, the compounds must be brought inside the cell membrane (See Figure 2). Many redox compounds cannot diffuse across the lipid membrane, and require additional molecular transporters that facilitate transport of specific molecules across the membrane. Once inside the membrane, redox compounds interact with the specific proteins that catalyze the metabolic reaction and act to harness the energy from the flow of electrons. This energy drives biosynthesis reactions that construct organic molecules that form macromolecules for cell growth and replication. Microbes can build these molecules from the most basic carbon building block, carbon dioxide (this is termed autotrophy), or from more complex molecules the organism can source from the external environment (termed heterotrophy).

GMG studies have revolutionized our understanding of how mineral dissolution and precipitation can be significantly controlled by microbial metabolisms (Gadd, 2010). Microbes additionally are able to utilize mineral forms as electron donors or acceptors, minerals that can be many orders of magnitude larger than the cell. The problem of utilizing mineral forms as electron donors or acceptors is solved by microbes via production of molecules to either solubilize minerals (chelators) or via production of molecules that can themselves transport the electrons out of the cells (electron shuttles).

Microbes are capable of dissolving minerals as part of their metabolism, they also produce minerals as a direct or indirect consequence of their metabolism. This can occur by production of chemical species that lead to direct precipitation (e.g. sulfide or poorly soluble metal ions) or by changing the surrounding conditions in ways that favor mineral precipitation (e.g. pH). This 'induced biomineralization' is a critical process in the formation of many mineral groups including carbonates, metal oxides, metal sulfides, phosphates, elemental forms, and silica among others. These biominerals can differ from chemically identical/similar minerals produced abiotically (in size, crystallinity, and incorporation of organics for example), and are often dispersed or complex aggregates of nanoparticles with very high surface areas.

Direct metabolic and growth interactions are not the only way microbes interact with their surroundings. Coupled with the presence of key reactive sites as part of their extracellular material, microbes exhibit a huge surface area and have the potential to play a large part in the mobility of metals, ions, and organics through sorption processes (e.g.: Fein et al., 1997). These same reactive sites at the microbe surfaces can additionally influence the spatial positioning of specific ions coming together to make mineral forms, effectively templating specific mineral forms via interaction with extracellular microbial materials like polysaccharides.

Redox transformations driven by microbial metabolism can have a significant impact on the cycling of many environmentally and geologic significant materials, including carbon, oxygen, iron, nitrogen, sulfur, selenium, manganese, arsenic, and uranium. Falkowski et al. (2008) developed a model for global geochemical cycling that illustrates the importance of biologic element cycling and ties to abiotic atmospheric and deep geological element fluxes associated

with tectonics, mountain building, erosion, geothermal activity, and sediment diagenesis). It is evident that if one wishes to interpret element cycling on the surface of Earth or potentially of any planet, the microbial transformations based on protein-catalyzed metabolic reactions must be a key part of the task.

Combining abiotic and biotic reactions is another consideration in characterizing the role of microbes in element cycling. This is because the products of specific metabolisms may interact with other compounds abiotically in key ways. These reactions are often very fast and thus do not present themselves in ways that are easily detected through chemical analyses. These reactions (sometimes called cryptic reactions) are nonetheless often governing reaction pathways, isotopic fractionation, and the availability of other compounds necessary for metabolism (see Hansel et al., this issue).

How do we characterize microbes and their link to geochemistry?

While the discovery of microbes was made along with the invention of the microscope, characterization of microbes and their metabolism has advanced significantly with new genetic and chemical techniques. Prior to the availability of genetic information, microbes were characterized based on pure cultures, the isolation of individual microbial species using specific electron donor-acceptor combinations under controlled physicochemical settings. Culturing continues to be a critical part of identifying, and characterizing, microbial function; interrogation of genetic and protein materials have revolutionized this practice in the last 30 years. Today we utilize techniques to quantify the DNA, RNA, and proteins from microbes to identify and characterize microbes and gain new insights into their functions in the environment. Such molecular-based studies are termed genetics, transcriptomics, and proteomics, respectively— and collectively these techniques are termed 'omics' (see Dick and Lam, this issue). Microscopy and omics information has also been integrated. With the use of techniques to attach tags to genetic fragments that bind to specific RNA sequences, we can 'illuminate' specific microbes under the microscope (including optical and electron microscopes). Another part of the microbe that is also used for identification is a portion of the membrane lipids that comprise the cell membrane. These lipids are important indicators of microbial presence, or 'biomarkers', because the long chain carbon 'skeleton' of the lipid is very resistant to change and the specific structure of many lipids is unique to specific groups of organisms. Indeed these compounds may serve as molecular fossils long after the organism dies.

Microbial metabolism often plays a significant part in controlling the details of geochemical cycling of specific elements. Consideration of the appropriate spatial or temporal scale over which to sample is critical. This is a challenge because sampling at too course a scale may group microbes with geochemical processes that are not necessarily related to each other. Investigation of these processes therefore requires different tools that facilitate analysis at appropriate scales. The application of *in situ* tools for analysis of redox species in aquatic systems has thus been a key advancement for understanding the role of microbes in element cycling, as has the development of techniques to stabilize components to minimize changes between sampling and analysis. Nanoscale and molecular characterization of microbe-geochemical interactions have

also proven critical to the advancement of the GMG field, with synchrotron-based EXAFS/XANES, STXM, (nano)SIMS, and electron microscopy, diffraction, spectroscopy, electrochemical, and chromatographic instrumentation being important. As with other geochemical lines of inquiry, isotopes, especially of elements involved in metabolic reactions, are a critical tool for investigating microbial processes. Especially powerful with the analysis of isotopes in this context is quantifying fractionation associated with interaction of inorganic electron acceptor-donor forms with specific enzymes.

Observation of particular chemical components in specific settings can indicate control by microbial processes when abiotic processes known to drive the same chemistry are too slow to be realized. For example the reduction of sulfate to sulfide at low temperatures abiotically is a very slow chemical process – the observation of significant fluxes of sulfide at lower temperatures is thus strong evidence for microbial sulfate reduction metabolisms. Sulfur isotopes are strongly fractionated by sulfur reducing bacteria (Fike and Grotzinger, 2008; Johnston et al., 2007), providing another line of chemical evidence for this role of microbes in a specific geochemical process. This idea of a chemical or mineralogical biomarker for life has seen significant research (e.g. Wacey et al., 2015) – especially as proof of life requires it would be impossible for a certain chemical signature to be made without life. While this may sound like a simple task, it has proven not to be so easy, especially when we are looking for proof of life in ancient rocks, meteorite fragments, and utilizing robotic spacecraft on and orbiting other planetary bodies.

Studies of microbial roles in specific element cycles and the use of biomarker evidence in modern systems have provided context to decipher the record of microbial processes recorded in ancient rocks. Conversely, the ancient rock record provides us with an opportunity to see how microbial processes affect large scale element cycling over time as other conditions (for example climate) have changed. Lyons et al. (this issue) provides a detailed look into each viewpoint towards understanding the ways chemical indicators of microbial processes may be preserved in rocks (rocks that have gone through at least diagenesis if not metamorphism), and what the rock record may tell us about how Earth and life have changed over time. Our picture of early Earth, including the conditions of the atmosphere and oceans, weathering of ancient craton material, and preservation of ancient sediments, is interwoven with the evolution of microbial life. Chemical and isotopic evidence for biotic and abiotic processes is linked to the evolutionary patterns observed in the genetic tree of life. In particular, early Earth was a place devoid of appreciable oxygen, and the earliest microbial metabolisms that evolved were almost certainly anaerobic. In the absence of oxygen, metabolisms involving hydrogen, iron, and sulfur where particularly important in the evolution of early Earth. The most 'deeply branching' (at the base of the genetic tree of life) microbes are most similar to microbes found in anoxic environments today. However, it is also thought that microbes capable of oxygenic photosynthesis (generating O_2), especially cyanobacterial species, evolved relatively quickly thereafter. This shift helped to shape the chemical changes on early Earth, leading to the Great Oxidation Event when O_2 in the atmosphere became significant (Lyons et al., 2014). Earth processes also have shaped microbial evolution, and ideas about the role of metal availability, the intensity of UV radiation at Earth's surface, and element fluxes from mineral weathering all have likely influenced evolution of microbes and their specific, protein-based functionality.

Emerging topics in GMG

This issue of Elements will highlight key areas of emerging thinking in the field of Geomicrobiology and Microbial Geochemistry:

- The principles and origins of geobiochemistry – interplay of Earth processes and the evolution of microbial metabolisms.
- Applications of omics to Earth processes – new capabilities for sequencing the macromolecules that compose microbial cells and communities offer insights into GMG.
- Reactive intermediates as a key to understanding microbial roles in element cycling – deciphering cryptic cycles through genetics and detailed geochemical interrogations.
- Emerging geochemical views of Earth's ancient microbial worlds – basis for the new paradigms refining our picture of Earth's coupled geochemical and microbial evolution.
- Emerging frontiers – examples of how GMG as an inherently interdisciplinary field is forging ahead with other allied fields to advance scientific understanding.

From a practical standpoint, GMG tackles fundamental science with significant societal relevance - supporting the recovery and use of oil, coal, and natural gas, the responsible utilization of Earth materials used for electronics, batteries, food production, and building materials of all kinds, the supply of clean, fresh water, and how climate change will impact all manner of Earth systems supporting civilization. Considering deep time GMG processes can help us also understand how the biogeosphere adapts to global changes in time, using the past as a key to the future.

To reach these goals, the GMG field embraces opportunities for future advances. Coupling of microbial (especially the data-rich field of omics) and geochemical data will require advanced computational approaches to uncover new details of interactions between microbes and their surroundings. Merging the ancient rock record and the record of evolutionary changes contained in the DNA of microbes can yield key information on how Earth systems respond to changes on a global scale and how we may look for evidence of life on other planets. Considering the key spatial and temporal scales across which linked microbial and geochemical parameters change may help unravel the role of microbes in processes from local to global scales.

As more is learned about links between microbes and their surroundings, the fundamental scientific advances of GMG can also be applied in concert with other fields and modes of inquiry. Details about the function of microbes can yield key insights on human health – both in terms of the interaction of our own flora of microbes and in understanding how specific protein catalyzed reactions function, are triggered, and may be utilized or inhibited towards better health. Microbes play key roles in formation of many natural and synthetic biomaterials (that may have fundamentally different properties), and in the development of biogeobatteries as an alternative energy source. Additionally, microbial roles in the recycling of critical materials may yield insight on enhancing reuse and recovery, and even on the preservation of fossils of multicellular life. Lastly, GMG applications are closely linked to goals of astrobiology and the search for life on other planets (see Templeton and Benzerara, this issue).

Acknowledgements

References:

Amaral-Zettler L, Artigas LP, Baross J, Bharathi PAL, Boetius A, Chandramohan D, Herndl G, Kogure K, Neal P, Pedros-Alio C, Ramette A, Schouten, S, Stal L, Thessen A, Leeuw J, and Sogin M (2010). A global concensus of marine microbes. IN: McINtrye A (ed.) Life in the World's Oceans. Chapter 12.

Banfield, J and Nealson, K (1997) Geomicrobiology: Interactions between microbes and minerals. Reviews in Mineralogy, V. 35, 448 p.

Canfield DE (2005) The early history of atmospheric oxygen: Homage to Robert M. Garrels. *Annual Review of Earth and Planetary Sciences* 33, 1-36.

Dworkin, M. (2011) Sergei Winogradsky: A founder of modern microbiology and the first microbial ecologist. *FEMS Microbiology Reviews* 36, 364-379.

Falkowski, PG, Fenchel, T, and Delong, EF (2008) The microbial engines that drive earth's biogeochemical cycles. *Science*, 320:1034-1039

Fein JB, Daughney CJ, Yee N, and Davis T (1997) A chemical equilibrium model for metal adsorption onto bacterial surfaces. *Geochimica et Cosmochimica Acta,*61, 3319-3328.

Fike DA, Grotzinger JP (2008) A paired sulfate-pyrite δ34S approach to understanding the evolution of the Ediacaran-Cambrian sulfur cycle. *Geochimica et Cosmochimica Acta* 72, 2636-2648.

Gadd GM (2010) Metals and microbes: Geomicrobiology and bioremediation. *Microbiology-SGM* 156, 609-643.

Johnston DT, Farquhar J, Canfield DE (2007) Sulfur isotope insights into microbial sulfate reduction: When microbes meet models. *Geochimica et Cosmochimica Acta* 71, 3929–3947.

Johnston DT (2011) Multiple sulfur isotopes and the evolution of Earth's surface sulfur cycle. *Earth-Science Reviews* 106, 161–183.

Knoll AH (2003a) The geological consequences of evolution. *Geobiology* 1, 3-14.

Knoll AH (2003b) Life on a Young Planet: The First Three Billion Years of Evolution on Earth. Princeton University Press, Princeton and Oxford, 277 pp

Lyons, TW, Reinhard, CT, and Planavsky, NJ (2014) The rise of oxygen in Earth's early ocean and atmosphere. *Nature* 506: 307-315.

Nealson, KH (2001) Harnessing microbial appetites for remediation. *Nature Biotechnology* 21: 243 - 244 doi:10.1038/nbt0303-243

Nealson, K and Ghiorse, WA (2001) Geobiology: Exploring the interface between the biosphere and the geosphere. American Academy of Microbiology, Washington, DC. 23 p.

Pace, NR, Sapp, J, and Goldenfield, N (2012) Phylogeny and beyond: Scientific, historical, and conceptual significance of the first tree of life. *Proceedings of the National Academy of Sciences* 109, 1011-1018.

Slater, G.F, Cowie, B., Harper, N., Droppo, I.G. (2008) Variation in PAH inputs and microbial community in surface sediments of Hamilton Harbour: Implications to Remediation and Monitoring *Environmental Pollution* pp 60-70. doi10.1016/j.envpol.2007.08.009

Taylor, PG and Townsend, AR, (2010) Stoichiometric control of organic carbon-nitrate relationships from soils to sea. *Nature*, 464: 1178-1181.

Vernadsky, VL (1926) *The Biosphere*. English translation by D.B. Langmuir, Copernicus, New York, 1998.