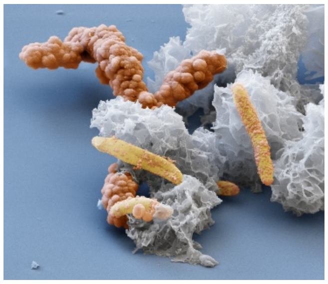

A report on the progress, future opportunities, and research needs in the field of Geomicrobiology and Microbial Geochemistry

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The field of Geomicrobiology and Microbial Geochemistry (GMG) is unified by its interdisciplinary focus on research questions at the nexus of Earth and microbial life sciences. Microorganisms have evolved to inhabit nearly all environments at and near Earth's surface, ranging from aerosols to the deep subsurface and from freshwater to hypersaline brines. Uncovering the dynamic interactions between microorganisms and their surroundings has extraordinary value to multiple scientific disciplines and to the strategic advancement of environmentally responsible technologies to address societally relevant problems. At an applied level, this includes identifying and exploiting the unique biomolecules that microorganisms synthesize for medical or engineering solutions and the application of target microorganisms to achieve desired environmental or biotechnological outcomes. At a more basic level, GMG investigations have led to new paradigms in our understanding of the interface between life and its physical and chemical surroundings across vast spatial and temporal scales. Determining an accurate and comprehensive understanding of biogeochemical interactions will be a cornerstone in implementing best strategies to protect ecosystems from predicted environmental changes. Broadly, findings from GMG have informed, advanced, and/or hold promise in the following areas:

- novel ways to produce nanomaterials, sustainable fuels, and biomaterials with target properties;
- new insights into antimicrobial and toxicological biochemistry;
- new insights into soil biogeochemistry to increase yields in agricultural crop production;
- integrating understanding of key microbial roles in carbon cycling to predict impacts and feedbacks of climate change
- efficient and economical ways to treat contaminated water and soils, including plastic degradation and transformation into value added products;
- cleaner, safer, and more economical methods to extract metal, metalloid, and organic materials from a variety of Earth materials;
- new insights and approaches to balancing element cycling (including, but not limited to, CO_2) in the atmosphere, ocean, and land through increased understanding of the role of activities of microorganisms in biogeochemical cycles;
- understanding the co-evolution of the biosphere and geosphere on Earth; and
- guiding the search for extraterrestrial life and the origins of life on Earth.

The seeds of the GMG field were sown by early work of environmental microbiologists and geochemists including Leeuwenhoek, Winogradsky, and Baas Becking, among others. These researchers developed the means to see and characterize microbes, allowing them to be studied within the context of their surroundings for the first time. Since this pioneering early work, the field of GMG has continued to evolve through technological advancement within the field itself and by incorporating knowledge gleaned from other fields. As an example, like many other fields, GMG was revolutionized by the discovery of DNA and the elucidation of its structure and chemistry. This discovery paved the way for theories of evolution to be tested and enabled an understanding of the informational basis underlying microbial function. DNA sequencing and bioinformatic technologies have continued to be developed owing in large part to the intellectual contributions of members of the GMG field. As Norman Pace, one of the pioneers of studying microbial genetic information in natural geobiological systems recently stated, "as we learn more about how to live with and make use of that still poorly understood microbial world. The Golden Age of microbiology is still ahead of us" (Pace, 2018). Indeed, the advent of advanced analytical techniques for identifying and characterizing microorganisms and their geological environments at relevant scales has enabled significant progress toward unraveling the key links between the microbial biosphere and the geosphere from the molecular to global scale.

Progress in the fundamental science of GMG is key to addressing societally relevant issues such as the impact of humans on Earth systems, the responsible use and development of energy and natural resources, the impact of climate change on Earth and ecosystem processes, and efforts to understand the evolution of life on Earth and search for life on other planets. As a relatively young field that has burgeoned in technological and intellectual prowess in its 20 years of formal existence, GMG is now poised to accelerate discovery, illuminate new thinking, and provide critical new insight into the links between the microbial world and the broader physical, chemical, and biological machinations of our planet. It is the understanding of our planet as an organism in its entirety that GMG can reveal and lend great insight toward; this is what makes GMG one of the most broadly impactful fields in all of science today and why it has been so successful over these past 20 years. Moving forward, Several key GMG themes are particularly well-positioned for advancement including:

- Resolving the microbial diversity of life on the planet and functional properties of uncultured lineages with promise for new technologies, molecules, and products for societal benefit.
- Developing understanding of how biologically induced environmental change shapes biogeochemical processes and influences biological evolution (i.e., geobiological feedbacks) in both natural and societally-impacted systems.
- Integrating "omics" approaches with geochemical techniques to develop next generation tools for monitoring, modeling and forecasting Earth systems.
- Modeling biogeochemical processes in deep time as a window to understand and predict Earth system response to shifts in climate and geochemistry.
- Identifying cryptic biogeochemical processes that involve short-lived reaction intermediates, interactions between organisms, and tightly coupled biological-chemical processes.

Understanding controls on the mobility of elements is a cornerstone of geochemistry and underpins processes that have both societal benefit (*e.g.*, discovery and responsible use of energy and ore deposits) and detriment (*e.g.*, the release and spread of contaminants). GMG has made exceptional progress toward understanding the role of microorganisms as drivers of elemental mobility in natural systems. An emerging theme is that geochemical processes often blend microbially-mediated and abiotic reactions that are ‘cryptic’, i.e. difficult to detect, due to rapid cycling of reactive intermediates and products. Recent advances illuminate this coupling of microbiology and geochemistry, revealing the presence of cryptic reaction networks in the cycling of elements and explaining enigmatic microbial metabolisms and geochemical observations (e.g., Learman et al., 2011; Klueglein et al., 2014; Hansel et al., 2015). Only through tight integration of geochemical and biological approaches have such cryptic reactions been exposed as key drivers of element cycling. Key insights of the role of microorganisms in element cycling have also been gained by integrated use of isotopic variation, redox state, macromolecule composition (e.g., DNA, RNA, proteins and metabolic byproducts) and biological evolution to inform elemental metabolism through time, as reflected in the rock record, to show the co-evolution of both life and Earth.

Among the approaches that have transformed GMG over the past decade and that have revealed the presence of cryptic biogeochemical cycles is the application of molecular “omic” tools. “Omics” refers to a broad range of approaches that probe the massive information contained in various biological molecules used to encode information, direct cellular function, and produce proteins, metabolites, and other cellular materials. This includes techniques that investigate the DNAs (genomics and gene amplicons), RNAs (transcriptomics) and proteins (proteomics) expressed from a genome, and metabolites produced by the cell (metabolomics). Such approaches can be applied to pure or enrichment cultures or directly to complex communities containing many different species of microorganisms as they exist and behave in their natural environments (i.e., metagenomics, metatranscriptomics, metaproteomics). Recent results from such approaches have shown how biogeochemical processes are interconnected (Anantharaman et al., 2016), unveiled many new phyla (e.g. Baker et al., 2019), and raised new questions about fundamental principles that guide our understanding of life on the planet such as the structure and organization of the tree of life and the origin and evolution of eukaryotes (Hug et al., 2016; Zaremba-Niedzwiedzka et al., 2017; Parks et al., 2018). Moreover, long-standing knowledge about how geochemical processes are attributed to particular organisms has been rapidly expanded. For example, the number of phyla known to be involved in sulfate reduction (Anantharaman et al., 2018) and methanogenesis/methanotrophy (Evans et al., 2015), (Vanwonterghem et al., 2016; Colman et al., 2019) have greatly increased, not only in terms of ‘who’ is capable of these processes but where such metabolisms are possible. Findings like these underscore how much more there is to learn about the microbial contribution to Earth’s geochemical cycles and the power of -omic approaches to uncover the microorganisms and processes that are involved.

One area of GMG that has roots in the earliest of geologic thinking is studying the coupled evolution of life and the Earth’s chemical character through deep time. Foremost are questions regarding the major transitions in Earth history, including the timing and causes of the gradual oxygenation of Earth’s atmosphere and oceans and the expansion of eukaryotes during the Cambrian. Insights into such questions rely on understanding the behavior of elements and their isotopic ratios in the presence of O_2 and the sources of recalcitrant biomarkers, such as lipids. One of the key areas of advancement in GMG research over the last 20 years has been the development of new trace element, lipid, and isotopic proxies that have refined the pattern and timing of how oxygen first accumulated in the atmosphere (reviewed in Holland, 1984; 2006; Canfield, 2005; Lyons et al., 2014) (Anbar et al., 2007).

While the timing and causes of the major transitions in Earth history are increasingly coming into focus, estimates of the timing and causes of the emergence of biochemical pathways that drove such changes are less clear; but the cloud of the unknown has become smaller. For example, the classic organic molecular (biomarker) record of 2.7-billion-year-old oxygenic photosynthesis by cyanobacteria has been challenged and is now convincingly attributed to contamination by drilling fluids (Rasmussen et al., 2008; Brocks, 2011; French et al., 2013; 2015). This leaves the field of GMG with major uncertainties about the origins of this pathway and the subsequent redox evolution of the biosphere. Nevertheless, recent omics-based work suggests that oxygenic Cyanobacteria are derived from hydrogen dependent non-phototrophic organisms (Carnevali et al., 2019), with this diversification event likely to have taken place between 2.5 and 2.8 Ga (Shih et al., 2017; Magnabosco et al., 2018). If representatives of such lineages can be obtained in culture and their lipids characterized at a compositional and isotopic level, then it may be possible to identify new organic biomarkers for Cyanobacteria and to use these to further constrain their emergence by detection in the rock record.

Discoveries that resolve fundamental links between microbes, the compounds they produce, and their interaction with their biotic and abiotic environment have implications that reach far beyond the field of GMG. For example, in 2018 an entirely new class of antibiotics was characterized by the development and application of culture independent methods to explore bacteriocidal activities of organic compounds and their biosynthetic pathways in soil bacteria (Hover et al., 2018). This study is particularly exciting given the recognition that the vast majority of genes continue to lack functional annotation, pointing to the large remaining reservoir of untapped but valuable biochemical novelty in natural microbial communities. Harnessing GMG tools such as new analytical power from high resolution spectroscopic methods (Ksionzek et al., 2016) that can resolve tens of thousands of specific organic molecules in a single water sample highlights the important role that GMG could play in this field. Antibiotic resistance in microbiota is omnipresent and a public health crisis of epic proportions sits on the near-horizon; yet, there is so much we don't know where GMG research may unlock whole new kinds of resources.

Infrastructure that is key to the continued scientific advancement of this field includes funding for large scale facilities to support multi-omics approaches, geochemical analyses at increasing powerful spatial/temporal resolution, and the computational infrastructure to integrate, analyze, archive, and disseminate the massive datasets now being produced. Moreover, larger scale (multi)institutional grants are needed to bring together critical mass in expertise that cross cuts disciplinary boundaries, including expertise in geochemical thermodynamics and kinetics, reactive transport modelling, microbial physiology and cultivation, omics (genomics, transcriptomics, and proteomics), biostatistics, statistical and modelling approaches for big data analysis, sedimentology, isotope geochemistry, hydrology, molecular biogeochemistry, and other areas to enable a holistic investigation and analysis describing how earth systems function interdependently. Finally, research is a social enterprise; major advances are often accomplished by interdisciplinary teams of people who work well together. A foundation for building such teams has been established in GMG by structures such as Geobiology Gordon Conference, the new Geobiology Society and its bi-annual meeting, and the International Geobiology Course, which has now trained almost 300 graduates in all aspects of GMG.

Future breakthroughs in GMG are likely to be facilitated by development through investment in one or more of the following emerging approaches:

- educational and computer pipelines and resources to more efficiently integrate and link data streams generated from existing and to be developed -omics platforms including genomics, transcriptomics, and proteomics,

- streamlined pipelines for the variable kinds of data represented by the all of the subset fields of GMG as a 'big data' statistical challenge to better inform analysis and understanding,
- developing and promoting training platforms that combine genetic, physiological, and/or biochemical approaches to define the functions of 'microbial dark matter',
- forming cross-cutting collaborations with engineers to develop and deploy next generation *in situ* sensors, analytical capabilities, and emerging methods for imaging single cells and materials at the nano- to micro- scales such that the physical and chemical underpinnings of biogeochemical cycles can be fully understood and realized,
- pursuing a recent paradigm shift to recognize 'cryptic cycles' in Earth and environmental processes where biotic and abiotic coupling is controlled via short lived, highly reactive intermediates,
- development of new computational approaches to bring together microbial and geochemical data in new ways and unravel the complexity of natural, modern and ancient, processes.

These naturally lead to a series of envisioned opportunities and grand challenges where these approaches may be applied to achieve the greatest advancements:

- Uncover the functionality and specific biochemistry associated with undefined sequences in microbial genetic code, the microbial dark matter
- Develop coupled biological-geochemical-mineralogical models of biogeochemical cycling that integrate enzyme-based kinetics with element cycling based on cell-specific and community-based approaches to describe element behavior
- Understand when and how energy is transduced from the geosphere to the biosphere in subsurface ecosystems
- Define fundamental principles that underpin the diversity of and limits to life
- Fully merge the genomic and geologic records to link the evolution of life with the evolution of Earth's oceans, atmosphere, and land.
- Define the temporal and spatial scales required to reveal links between microorganisms and their geochemical surroundings, and to synthesize and transfer our understanding of systems from global to molecular scales.

Specific recommendations to realize these opportunities include focus on strengthening the interface of microbiology, geochemistry, biochemistry, in terms of (i) funding structures to bring together interdisciplinary teams and stimulate collaboration across disciplines; (ii) investment in educational opportunities and resources (iii) moving GMG towards predictive capabilities through quantitative approaches and modeling. This includes calls to improve large-scale facilities used to gather genetic and geochemical information alongside a robust computational infrastructure to maximize use of these data. The continued development of a research structure that encourages training and research with teams bringing together the biological, chemical, and geological expertise and tools necessary to transform our understanding of GMG questions is central to continued success and advancement of scientific goals and discoveries.