Marine Microorganisms, Biogeochemical Cycles, and Global Climate Change

Global environmental change demands a deeper understanding of how marine microbes drive global ecosystems

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The oceans maintain critical ecosystems that, ultimately, determine the habitability of the planet. Moreover, unicellular eukaryotes, bacteria, and archaea are the most abundant organisms within those ecosystems and thus the dominant biomass within the oceans, situated at the base of the food chain that feeds macroscopic invertebrates, fish, and mammals.

We are only beginning to uncover the extent of marine microbial genetic diversity and the complex roles that these microbes play in driving the biogeochemistry of global ocean ecosystems. Molecular biology, genomics, microscopy, mass spectrometry, and high-throughput cultivation are already providing useful information on the diversity and activities of marine microflora, including new biochemical pathways. However, in the face of global environmental change, we need to gain a deeper understanding of the distributions of marine microbes and their activities in maintaining ecosystem functions.

New Analytic Approaches Key for Assessing Marine Microbial Dynamics

Our inability to measure microbial activities over appropriate spatial and temporal scales limits our understanding of oceanic microbial communities and how they might affect global change. Thus, we need to devise better strategies for sampling or measuring these communities over the large spatial scales of the ocean. The recent International Census of Marine Microbes (ICOMM) and the Global Ocean Sampling program have assembled information on microbial diversity across marine habitats that provides the basis for identifying many microorganisms, ecotypes, and genes. However, such information is insufficient for assessing ecological functions. We need to learn how key microorganisms are likely to respond to various climate scenarios if we are to predict how ocean biology and biogeochemical cycles will change. Moreover, although the microorganisms that play key roles in marine ecosystems are composed of diverse taxa, we do not understand what this functional redundancy means for stability or resilience of their functions.

Marine microorganisms are not well represented in culture collections. Nevertheless, there is a high degree of genetic diversity even in those genera for which

Summary

• Marine microbes drive chemical reactions that contribute to Earth’s habitability.
• Although molecular techniques are used to monitor oceanic microbial communities, collecting intermittent samples for laboratory analysis limits the usefulness of this approach.
• Instruments that collect and analyze microbes in situ can provide unprecedented resolution of biogeochemical cycles over large spatial and temporal scales.
• We should combine knowledge of key genes with new instruments to obtain time-series information on key marine microbial biogeochemical transformations.
there are cultivated isolates, including, for example, the cyanobacteria *Prochlorococcus* and the heterotroph *Pelagibacter*, two of the most abundant microorganisms in the open ocean. Moreover, there is a high degree of genetic variability in natural assemblages of marine microorganisms, even within widespread genera such as *Prochlorococcus* and *Pelagibacter*, according to metagenomic and metatranscriptomic sequencing.

Much of the rest of the oceanic microorganisms, including most of the abundant archaea, are yet to be cultivated. Thus, our knowledge of them is based primarily on DNA sequencing. Moreover, the genomes of open-ocean microbial assemblages encode previously unknown proteins and protein families, whose functions are yet to be characterized. This uncharacterized diversity presents major challenges for anyone trying to determine how microbial communities might respond to climatic changes or other environmental stressors.

Part of this effort entails developing a better understanding of how microbial diversity currently affects ocean ecosystem activities. In addition to belonging to food webs, microorganisms catalyze reactions that cycle elements between oxidized and reduced forms, between solid and soluble forms, and between dissolved forms and gases.

Global environmental changes, including ocean acidification, warming, eutrophication, and pollution, are affecting ocean biology and microbiologically mediated biogeochemical cycles. However, the extent and consequences of such interactions are uncertain, as is the role that oceans and life within them play in damping or amplifying these perturbations. For example, we know that phosphorus concentrations changed strikingly at the ALOHA monitoring station in the North Pacific Ocean during recent decades (Fig. 1). Moreover, dissolved organic and inorganic carbon as well as pH changed profoundly over the same period. However, we still do not yet know to what extent microbiological changes drove those biogeochemical changes or how those changes affected microbiological community structures.
Jonathan Zehr recently rediscovered a drawing—depicting “a futuristic greenhouse with green things inside, which I labeled algae”—that he did while in elementary school. Now a professor of ocean sciences at the University of California, Santa Cruz (UCSC), he finds that drawing oddly prophetic. Years after completing that artwork, experiences at college led him closer to realizing his earlier abstract interests in aquatic microbes, he says, “beginning with being inspired to go on to graduate school in limnology,” the study of inland waters. Limnologist professor David T. Mason “intrigued me with the intricacies of ecosystem networks” and “forecast the future of my research interests by talking about information flow in ecological systems, long before anyone realized we would be tracking genes and genomes in the environment.”

At UCSC, Zehr studies marine microorganisms involved in biogeochemical cycles—a tapestry of chemical reactions that maintain nutrients, trace elements, and gasses on Earth. “Nitrogen fixation and nitrogen cycling in general are key to the cycling of materials that support the productivity of life in the oceans,” he says. “Understanding how and when these processes occur, and, more importantly, how they are controlled by factors such as temperature, will be key to predicting what will happen to the habitability of the planet under future scenarios.” Moreover, many of the microbes of the oceans have not been cultivated. Some of them have unusual metabolisms, which may be useful for biotechnological applications.

Zehr, 54, fell in love with the oceans as a teenager during a visit to Oregon. Soon after, he moved to coastal Washington, where he earned a B.S. degree in biology from Western Washington University in Bellingham in 1981. From there, he moved to graduate school at the University of California, Davis, where he completed his Ph.D. in 1985. “I realized the significance and intrigues of microbes as I pondered the biogeochemical cycles of my research site, Castle Lake, California, a subalpine glacial lake near Mt. Shasta,” he says. That experience led him to admire microbes and their roles in ecosystem processes. “The next major shift in my interests came while working on nutrient cycling under the guidance of Drs. Doug Capone and Paul Falkowski, doing experiments with the radioactive tracer $^{15}$N,” he adds. “Doug and Paul influenced my shift in interest to nitrogen fixation and the molecular biology of organisms in the environment.”

Zehr was born in Champaign, Ill., and grew up in Indiana and Pennsylvania, the son of a minister. He has two brothers and a sister. “I was always interested in science,” he says. “I liked reading about science, and my second-grade teacher used to let me have extra library time. One of my brothers influenced my interest in wildlife and biology in general.”

Apart from that early interest in science, Zehr started riding motorcycles as a teenager, and still owns a Harley Davidson. “I like to ride the California coast and inland,” he says. “My realization that the organism we study lacked the photosystem II genes came to me while riding the Harley in the coast hills with a friend. I texted my lab research manager to do the penultimate experiment when I got off the bike.”

After finishing his Ph.D., he worked as a National Research Council-U.S. Geological Survey research assistant in Menlo Park, Calif. Between 1986 and 1988, he was a postdoctoral research associate at Brookhaven National Laboratory and the State University of New York at Stony Brook. From 1988–1990, he was a postdoctoral research associate at New England Biolabs Inc., in Beverly, Mass. The Biolabs experience provided “a unique opportunity to learn molecular biology techniques and changed the way I look at microbiology, ecology, and science in general,” he says. He also spent seven years, beginning in 1992, at Rensselaer Polytechnic Institute before moving to UCSC in 1999.

In addition to motorcycles, he enjoys photography and the company of Darwin, his 11-year-old, blue-and-gold Macaw. “I used to live with a lot of dogs and cats too, but I’ve just always liked tropical birds,” he says of the parrot. “Darwin speaks, when he isn’t squawking. He has also picked up a few words of Spanish, French, and English. And he’s usually the first to answer the phone with ‘hello.’ I named him Darwin because he is always a vibrant reminder of biological diversity and evolution.” However, Darwin hasn’t learned to ride the motorcycle... yet.

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Microbial Biogeochemical Cycles Determine Earth’s Habitability

Microbially mediated biogeochemical cycles began controlling the habitability of Earth when life arose more than 3 billion years ago. However, several major biogeochemical pathways and novel microorganisms were discovered only within the past decade. Although some taxa are not very abundant, they play critical roles in ecosystems, such as replenishing elements or nutrients needed by other, more abundant life forms. Thus, repeatedly measuring the collective biogeochemical activities of marine microbial communities is central to understanding how global climate change will affect marine ecosystems.

Within the microbial realm, viruses and other microorganisms range over several orders of magnitude—from large diatoms that can almost be visualized with the unaided eye to viral particles less than 1 μm in diameter. Detecting microorganisms is difficult enough. However, determining their identity, their biogeochemical functions, and what they are doing at any given moment cannot be obtained by microscopy.

Thus, analyses based on molecular biology are key to characterizing how microbial diversity affects ecosystem biogeochemistry and detecting the impact of both short-term perturbations and long-term climate change. Molecular biology techniques provide the means to detect, quantify, and characterize microbes as well as their genes and gene expression in seawater samples. Advances in molecular biology techniques such as high-throughput sequencing, proteomics, and high-density microarrays for analyzing community composition and gene expression provide tools for studying various biogeochemical processes, including nitrogen and carbon fixation and anaerobic ammonium oxidation. The appearance of specific gene transcripts and proteins can provide valuable information regarding the nutritional status of microbial populations, while also providing information on how nutrient availability can change in natural populations (Fig. 2).

Molecular biology techniques can be used to interrogate natural populations for changes in species composition, nutrient cycling, and nutrient availability (Fig. 2). However, sampling vast oceans remains a challenge against the backdrop of complex ocean hydrodynam-
ics. Understanding microbial patterns is critical for scaling the effects of global change across ocean basins, and between oceanic and coastal systems.

Challenges in Analyzing Ocean-Scale, Microbiologically Driven Processes

The oceans are not uniform. They are physically and chemically diverse, and vary in stratification, salinity, chemical composition, nutrient availability, and temperature. Phenomena such as blooms of microorganisms occur on unpredictable time scales that routine sampling programs can miss. Sampling by ship limits data resolution in space and time, while also undercutting efforts to build a fuller understanding of how patterns are linked to physical processes. Mathematical modeling can help, but predictions made through modeling need to be tested against observations.

Thus, one of the major challenges in understanding the effects of global change on marine microbes is the vast time and space scales of the oceans, and the dynamic nature of ocean currents, stratification, and turbulence. Put another way, the challenge is to understand what happens in oceans at microbial scales, while extrapolating across meters, kilometers, and ocean basin scales.

Remote sensing from satellites and from networks of moored instruments and those on autonomous vehicles helps to address the problem of measuring both physical and chemical processes across oceanic spaces. Such systems are now providing long-term observations of physical, chemical, and biological parameters. For example, instruments mounted on more than 3,000 floats provide 100,000 temperature/salinity (T/S) profiles and velocity measurements per year; they are distributed at about a 3-degree spacing across major ocean basins (http://www.argo.ucsd.edu/FRAbout_argo.html). Moreover, nitrate and oxygen sensors on Argo floats are estimating primary production rates on the same scale (http://www.mbari.org/chemsensor/APEXISUS.htm).

Other regionally and globally distributed systems provide data from a multitude of sources (e.g., http://www.ioos.gov/, http://www.interactiveoceans.washington.edu/, http://www.ioc-goos.org/). If relevant microbial data could be acquired in parallel with such measurements, it would be possible to link biogeochemical changes with underlying microbiological functions.

Recently, molecularly based analytic instruments have been deployed to remote sea stations, making it possible to detect specific genes, enzymes, and enzyme activities along with environmental parameters that govern microbial communities (Fig. 3). Without such instruments, the resolution from sampling microbial populations is too low to determine what factors control their distributions and activities throughout the ocean. However, the develop-
ment of technologies to assay genes and to measure gene expression in situ is catching up with systems that monitor ocean chemistry and physics worldwide.

For example, autonomous profilers can detect bulk chemical changes as a way to infer large-scale, microbi ally mediated biogeochemical transformations in the ocean. However, those observations cannot yet be linked with instruments that measure the abundance and activity of specific microorganisms and genes. By merging remote-sensing instruments with others that track genetics and genomics, it should soon be possible to overcome this limitation.

**Genomics Data from Remote Sensors Will Lead to Global Insights**

With advances in remote-sensing technologies, microbiologists no longer need to rely exclusively on ship-based expeditions to acquire genomic data from ocean-dwelling microorganisms. Obtaining detailed information by remote sensing not only makes it possible to better understand biogeochemical cycles but, more importantly, provides a baseline and means for detecting biological shifts corresponding to global environmental changes. Information detailing the spatial and temporal dynamics of these microbial populations will also help to refine mathematical models to more accurately predict the fate of ocean ecosystems.

Despite considerable recent progress, developing biosensors remains a challenge. One persistent problem is keeping pace with rapid advances in molecular and microbiological technologies. For an instrument to remain useful for environmental microbiologists, it must be adaptable to the latest molecular techniques. To this end, developers of ocean-sensing technology must remain forward-thinking and pragmatic with respect to instrument development.

Another major challenge lies in identifying the best suite of biological signatures to monitor when tracking biogeochemical cycles and then incorporating them into “ecogenomic” sensors to detect them at sea. Specific genes can be targeted as proxies for estimating the abundance of specific organisms, and also to establish the presence of genes whose products are related to basic biogeochemical transformations (Fig. 1).

Detecting specific RNAs, proteins, or enzymatic activities also can provide useful information on biogeochemical activities because such molecules reflect gene expression and physiological status. RNA or protein targets can also serve as measures of ecosystem function, reflecting species growth rates, nutrient limitation, cell stress, or death.

Several specific molecular probes have proved useful at the lab bench for studying biogeochemical activities that occur in marine environments. For instance, DNA hybridization arrays can provide a phylogenetic fingerprint of a microbial community at a particular site, while quantitative polymerase chain reaction (PCR) is used to quantify the abundance of ecologically important genes (such as 16S-rRNA, rbcL, rpoC) in specific microorganisms from a site, and reverse-transcription PCR is used to interrogate gene expression activities or physiology of microorganisms (such as ftsZ for cell cycle status, nifH for nitrogen fixation, ntcA for nitrogen limitation, and phoX for phosphorus stress). Such assays can be tuned to a phylum, class, genus, or species. This approach can be extended to measure a variety of components within marine biogeochemical cycles, including ammonia oxidation, iron metabolism and limitation, and usage of different phosphorus sources (e.g., phosphonate vs. inorganic phosphate).

Once appropriate targets are identified, in situ sensors and high-throughput methods can be engineered based on sampling and analytical needs. Such networks should be implemented with cross-system standard operation procedures for sampling, data analysis, and data communication. These downstream issues need to be considered early during development, and should complement data collection by ocean observing systems and at ocean time-series stations.

We can apply what we know about marine microbiology to learn more about how global environmental change is affecting microbiological communities and biogeochemical functions. For instance, biogeochemistry data collected at long-term monitoring sites should be linked to underlying microbial community functions. They can be characterized by measuring key genes whose transcription patterns are linked to biogeochemical transformations (Fig. 1).

Using metagenome-level expression tools effectively will depend on developing exten-
sive knowledge about many different organisms and their genes as well as metagenomes from uncultivated microorganisms. This effort can best be accomplished with broad participation across the microbiological community. One such program was organized following the workshop, “Microbiological Targets for Ocean Observing Laboratories” (MicroTOOLs) (http://es.ucsc.edu/~wwwzehr/mtools/). Implementing remote sensing and instrumentation to measure microbial processes is one of the objectives of the National Science Foundation Science and Technology Center C-MORE (Center for Microbial Oceanography: Research and Education).

Once engaged in such a broad-based collaborative effort, members of the scientific community can evaluate phylogenetic and gene expression targets for cycling key nutrients such as nitrogen, phosphorus, and iron, while concurrently identifying key clades of major taxa to serve as indicators of marine microbes carrying out critical biogeochemical functions. Such a collaborative approach would not only provide benchmarks across ecosystems but would also be a new way of doing marine microbiology. More importantly, it would enable us to determine how marine microbial communities change in space and time, and hopefully, how they are responding to global environmental change.

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SUGGESTED READING