

Systems Biology–Enabled Research on the Roles of Microbial Communities in Carbon Cycling Processes

Summary of Projects Awarded in 2016 Under Funding Opportunity Announcement DE-FOA-0001458

The Genomic Science program within the U.S. Department of Energy’s (DOE) Office of Biological and Environmental Research (BER) supports fundamental systems biology research to advance understanding of plants, microbes, and multi-species communities in energy and the environment. Exploring the translation of organisms’ genomes into catalytic proteins, regulatory systems, and metabolic pathways can shed new light on the mechanistic basis of biological processes and how these processes change in response to community interactions and shifting environmental variables. By better understanding relationships between systems biology properties of microbes and environmental processes, Genomic Science program research can help bridge biogeochemical cycle understanding from molecular- to ecosystem-scales.

The global carbon cycle plays a central role in regulating atmospheric carbon dioxide levels and thus Earth’s climate, but understanding of many key processes driven by microbial communities at the most foundational levels of the carbon cycle remains limited and contributes to uncertainties at higher-scale processes. This has been particularly true for terrestrial ecosystems and sedimentary environments, which present formidable experimental challenges due to their compositional heterogeneity and complex microbial communities. Fortunately, increasingly sophisticated approaches for “metaomics” (i.e., metagenomics, metatranscriptomics, and metaproteomics) coupled to high-resolution analytical technologies have offered new tools for the investigation of functional properties of microbial communities. There has also been a rapid acceleration in researchers’ ability to isolate and analyze microbes and consortia from the environment, build genetic tools for their experimental manipulation, and construct predictive models of their metabolic and regulatory processes. These advances have allowed much more sophisticated studies on the roles played

by microbial communities as key points of integration between major biogeochemical cycles (e.g., carbon, nitrogen, sulfur, and phosphorus) and how these interconnections impact the rate and magnitude of carbon cycle processes in changing ecosystems. Improved metaomics tools have also allowed researchers to expand their work on microbes in experimentally challenging environments vulnerable to climate change variables (e.g., soils, sediments, and permafrost), resulting in new insights with important implications for understanding the roles of microbes in the climate change processes and the global carbon cycle.

Systems-level research emphasizes studies on the underlying principles of complex systems and facilitates scaling of concepts and data across multiple levels of biological organization. Applying this approach to understanding the roles of microbial communities in carbon cycle processes provides opportunities to not only examine their functional attributes in the environment, but also to form and test hypotheses on underlying ecological principles. Such studies rely heavily on integrated multidisciplinary research that incorporates experimentation on model organisms and systems, collection of observational data on communities and ecosystems, and computational biology approaches for integration and modeling of data across multiple spatial and temporal scales.

BER solicited applications for omics-driven basic research on the contribution of prokaryotic and eukaryotic microbes and microbial communities to carbon cycling processes in terrestrial soil and sedimentary ecosystems in the following areas:

- **Systems biology studies on regulatory and metabolic networks of microbes, microbial consortia, and microbe-plant interactions involved in biogeochemical cycling of carbon.** A particular focus is on systems biology research using model microbes or microbial consortia that are relevant to carbon cycling

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processes in terrestrial ecosystems. Model systems should be carefully chosen to facilitate development of community-scale metabolic and regulatory models, examine fundamental ecological principles, and inform understanding of larger-scale biogeochemical processes in the environment of interest.

- **Development and application of omics approaches to investigate microbial community functional processes involved in carbon cycling in terrestrial ecosystems.** There is a need to adapt genome-enabled techniques (e.g., metagenomics, metatranscriptomics, metaproteomics, and community-scale metabolomics) to interrogate relevant

functional processes of microbes in terrestrial environments, either at field sites or using model micro/mesocosms, and integrate resulting data into process understanding at the ecosystem scale. Applicants were encouraged to target key microbially mediated carbon cycling processes in terrestrial systems to predict responses to shifts in temperature, precipitation, carbon dioxide concentration, or other climate change variables.

2016 Awards

A Trait-Based Framework for Linking Microbial Communities with Carbon Transformations Under Precipitation Change

- **Principal Investigator:** Steve Allison (University of California, Irvine)
- **Collaborators:** Michael Goulden, Adam Martiny, Jennifer Martiny, Kathleen Treseder (University of California, Irvine), Eoin Brodie, Trent Northen (Lawrence Berkeley National Laboratory)

Global climate change is predicted to have broad effects on precipitation patterns across the world, and the potential for droughts of increased length and severity are a particular source of concern. There has been a great deal of study on the ecological implications of drought for plant communities, but impacts on complex microbial communities in the underlying soil and the biogeochemical processes they perform are not nearly as well understood. This project will examine the effects of drought conditions on carbon decomposition processes performed by soil microbial communities in southern California grasslands. The investigators will focus on key uncertainties revolving around “tradeoffs” made by important classes of microbes in balancing expression of traits associated with drought tolerance, resource acquisition, and growth yield. These traits are thought to play important roles in structuring microbial communities and determining overall rates of carbon decomposition under different water availability conditions. The team will use a coordinated approach integrating omics-driven systems biology techniques, high-throughput

analytical technologies, and trait-based modeling frameworks. By understanding the ecological tradeoffs that influence microbial community structure and function under these conditions, the investigators hope to better predict the effects of drought on carbon cycling processes in grasslands ecosystems impacted by global climate change.

Determination of the Roles of Pyrophilous Microbes in Breakdown and Sequestration of Pyrolyzed Forms of Soil Organic Matter

- **Principal Investigator:** Thomas Bruns (University of California, Berkeley)
- **Collaborators:** Matthew Traxler, Anthony Iavarone (University of California, Berkeley), Thea Whitman (University of Wisconsin), Igor Gregoriev (Lawrence Berkeley National Laboratory)

Wildfires are expected to become increasingly prominent in the western United States due to shifts in temperature and water availability associated with global climate change, but their impacts on molecular-scale biogeochemical processes in soil remain poorly understood. Certain types of pyrophilous—or fire-loving—fungi and bacteria are known to become more prominent in soils in the aftermath of fires, but community interactions of these organisms and their ability to consume carbon compounds that have been chemically altered by fire (i.e., pyrolyzed) are not well characterized. This project will examine the effects of wildfire on the structure and function of microbial communities and their subsequent decomposition of pyrolyzed soil

carbon pools. To examine these processes under laboratory conditions, the study team will construct “pyrocosms” that allow controlled burning of experimental mesocosms representative of western forest soils. The investigators will then use metagenomic and metatranscriptomic analysis to track changes in microbial community composition and functional gene expression after a fire event. In parallel, stable isotope studies will be used to track consumption of pyrolyzed soil carbon and its subsequent movement through the microbial food web. This study will provide the first detailed analysis of the effects of wildfires on carbon cycling activities of soil microbial communities and advance understanding of molecular-scale consequences of global climate change.

Microbial Metabolic Dependency and Its Impacts on the Soil Carbon Cycle

- **Principal Investigator:** Daniel Buckley (Cornell University)
- **Collaborators:** Johannes Lehmann (Cornell University), Will Wieder (University of Colorado Boulder), Trent Northen (Lawrence Berkeley National Laboratory), Mary Lipton (Pacific Northwest National Laboratory)

Soils act as the critical foundation of most terrestrial ecosystems, but surprising gaps remain in our basic understanding of the biological processes that mediate decomposition of soil organic matter. The interplay of these processes with site-specific physical and chemical conditions ultimately determine whether soil carbon is stabilized in mineral complexes or released to the atmosphere. The degree to which microbial community structure and coordinated metabolic activities influence overall rates of decomposition and carbon storage and release in soil ecosystems remains an especially contentious question. This project aims to map and measure the complex web of carbon transformations carried out by microbial communities in soil environments. The technical approach developed by this team, which involves coupling of metaomics analysis of microbial community attributes and stable isotope tracking of carbon movement through multiple trophic levels, permits a more quantitative evaluation of these processes than would be possible using more traditional microbial ecology techniques. This approach will allow investigators to examine metabolic interdependencies between key community members, which are hypothesized to play a greatly underestimated role in determining

higher-order rates of decomposition. These processes have been primarily considered in terms of enzyme kinetics, so the results of this study could significantly alter understanding of the basic forces governing carbon cycling in soils.

The “Who” and “How” of Microbial Control over Soil Carbon Dynamics: A Multi-Omics, Stable Isotope Probing, and Modeling Approach

- **Principal Investigator:** Kristen DeAngelis (University of Massachusetts Amherst)
- **Collaborators:** Seeta Sistla (Hampshire College), Erin Conlon (University of Massachusetts Amherst), Stuart Grandy, Serita Frey (University of New Hampshire)

Soils of North American forests host incredibly diverse communities of fungi and bacteria, but the functional implications of this diversity and its relevance to broader ecosystem processes is extremely difficult to assess. In this project, a team of researchers will explore carbon decomposition processes performed by fungal and bacterial communities of Harvard Forest, a long-term ecological research site in Massachusetts. The goal of the study is to evaluate which biological and environmental factors are most important in determining carbon use efficiency (CUE) in populations of microbes that employ differing ecological strategies. This information will allow the team to examine how overall community CUE is impacted by changes in key environmental variables and begin to link microbial CUE measurements to broader carbon cycle processes at the scale of the forest ecosystem. The project will use a nested experimental strategy incorporating physiological studies of organisms in pure culture, experimental microcosm systems of intermediate complexity, and field studies at Harvard Forest. A multiscaled modeling approach will be developed to incorporate data from the different lines of research; this will allow examination of carbon cycling processes at the levels of molecular decomposition mechanisms, integrated functional activities of microbial communities, and biogeochemical cycles of the forest soil ecosystem.

Directing Traffic in the Rhizosphere: How Phage and Fauna Shape the Flow and Fate of Root Carbon Through Microbial Pathways

- **Principal Investigator:** Mary Firestone (University of California, Berkeley)
- **Collaborators:** Rosemary Gillespie (University of California, Berkeley); Neo Martinez (University of Arizona); Jizhong Zhou, Zhili He (University of Oklahoma); Jennifer Pett-Ridge (Lawrence Livermore National Laboratory); Eoin Brodie, Javier Ceja-Navarro, Trent Northen (Lawrence Berkeley National Laboratory); Mary Lipton (Pacific Northwest National Laboratory); Angela Hodge (University of York)

When grasslands experience drought conditions, the amount of carbon entering soil via grass roots and related carbon decomposition processes are quickly affected. However, the roles of specific microorganisms involved in these changes—in particular understudied classes of organisms such as viruses and eukaryotic metazoans—remain unclear. This project will examine the roles of microbial communities in carbon cycle processes occurring in the rhizosphere of the common grass *Avena barbata* during experimental shifts in water availability. The team aims to dissect interactions at the root–microbe–soil interface and investigate particularly significant microbe-microbe interactions occurring between fungi, bacteria, archaea, viruses, and eukaryotic microbes. This work will involve a series of experimental systems escalating in relative complexity: a “root chip” to examine fine-scale relationships, mesocosms in controlled growth chambers to explore the microbe–plant–soil interface, and field studies at a grassland ecological research site. Systems biology techniques, stable isotope-enabled high-resolution imaging mass spectrometry (NanoSIMS), and computational modeling will be employed to measure functional processes; characterize relationships; and understand their influence on broader carbon cycle processes. This study should significantly advance understanding of biological processes driving carbon cycling in terrestrial grasslands impacted by droughts and provide a clearer view of the interactive roles of all members of these complex communities.

Scaling the Microbial Ecology of Soil Carbon

- **Principal Investigator:** Bruce Hungate (Northern Arizona University)
- **Collaborators:** Egbert Schwartz, George Koch, Paul Dijkstra, Benjamin Koch, Michelle Mack (Northern Arizona University); Ember Morrissey (West Virginia University); Jennifer Pett-Ridge, Steven J. Blazewicz, Peter Weber (Lawrence Livermore National Laboratory); Kirsten Hofmockel (Pacific Northwest National Laboratory)

While molecular systems biology techniques to assess the diversity and functional potential of microbial communities have grown increasingly sophisticated, linking the resulting information to more quantitative process measurements and relating it to higher-scale biogeochemical cycles remains a major challenge. The goal of this project is to develop a quantitative, omics-enabled stable isotope probing (SIP) methodology that will allow measurement of microbial substrate utilization and growth rates at the level of defined taxa. The approach will involve the combination of metagenomics and transcriptomic community characterization with several analytical technologies that allow more quantitative functional analysis (e.g., NanoSIMS and Chip-SIP) and development of new modeling approaches that permit scaling of data from microscopic to ecosystem levels. This technique will be used to measure soil microbial community carbon cycle processes in laboratory microcosms and soil warming experiments at an ecological field research site, allowing the team to estimate carbon use efficiency and predict carbon dioxide production by defined taxa within complex microbial communities. By integrating these results with broader biogeochemical process measurements, this project aims to quantitatively examine the roles of specific subsets of microorganisms in ecosystem-scale carbon cycle processes and determine their functional responses to rising soil temperatures, which are predicted to accompany global climate change.

Systems-Level Understanding of Interconnected Methane and Nitrate Cycles Using Complex Synthetic Communities

- **Principal Investigator:** Mary Lidstrom (University of Washington)
- **Collaborators:** Ludmila Chistoserdova, David Beck (University of Washington)

In sedimentary environments found in lakes, wetlands, and oceans, limited availability of oxygen results in carbon cycle processes featuring methane—a potent greenhouse gas—as a prominent intermediate or endproduct. Methane-oxidizing microbes occupying the interface between anoxic and oxic zones play a particularly significant role in these ecosystems because they often determine the balance of carbon dioxide versus methane that will be released as the final product of organic matter decomposition. Recent studies of microbial communities inhabiting the sediments of freshwater lakes have suggested factors such as fermentative metabolism, growth on minimal oxygen concentrations, and collaborative metabolic processes all play more important roles in methane cycle processes than previously appreciated. This project will further examine these factors and their influence on carbon cycle processes using constructed model communities of the relevant microorganisms cultivated from freshwater lake sediments. This approach will allow the team to rigorously test hypotheses in controlled experiments, map metabolic networks controlling key processes, and construct predictive computational models of methane cycling. The results of these studies should considerably advance understanding of the underlying mechanisms that control methane cycling in sedimentary ecosystems and help predict how these communities would respond to changing environmental variables.

Identification and Ecophysiological Understanding of New Microbial Players, Processes, and Multiscale Interactions in the Global Methane Cycle

- **Principal Investigator:** Victoria Orphan (California Institute of Technology)
- **Collaborators:** Gene Tyson (The University of Queensland), Mark Ellisman (University of California, San Diego), Christof Meile (University of Georgia), Chris Kempes (Santa Fe Institute), Bob Hettich (Oak Ridge National Laboratory)

In some deep sea sediments, methane is abundantly available as a potential carbon and energy source. However, the absence of oxygen in these environments makes it extremely difficult to eke out enough energy from

methane consumption to enable microbial growth. In these environments, consortia of methane-consuming Archaea and sulfur-reducing bacteria have evolved an intimate partnership known as syntrophy that allows them to collaboratively convert methane to carbon dioxide, gaining energy in the process. Recent findings have revealed a much wider diversity of microbes involved in these processes than previously suspected, and new evidence demonstrates that extracellular electron transfer between the partners plays a critical role in mediating syntrophic methane consumption. This project aims to identify new organisms capable of anaerobic methane oxidation, determine the molecular factors facilitating metabolic coupling and energy transfer, and link their functions to broader biogeochemical processes occurring in sedimentary environments. The team will use a combination of omics-enabled systems biology approaches, stable isotope-enabled high-resolution imaging mass spectrometry (NanoSIMS), and computational modeling approaches that establish spatial patterns of activity in an ecosystem context. This strategy will advance systems biology understanding of the role of microbes in mediating methane-oxidation under anoxic conditions and more clearly establish their linkage to broader biogeochemical processes that compose the global carbon cycle.

Illuminating Pathways to Carbon Liberation: A Systems Approach to Characterizing the Consequential Unknowns of Carbon Transformation and Loss from Thawing Permafrost Peatlands

- **Principal Investigator:** Virginia Rich (Ohio State University)
- **Collaborators:** Matt Sullivan (Ohio State University); Scott Saleska, Bonnie Hurwitz (University of Arizona); Gene Tyson (The University of Queensland); Ruth Varner, Steve Frolking (University of New Hampshire); Jeff Chanton, Bill Cooper (Florida State University); Patrick Crill (Stockholm University); Eoin Brodie, William Riley (Lawrence Berkeley National Laboratory); Malak Tfaily (Pacific Northwest National Laboratory)

Rising temperatures associated with climate change are rapidly thawing permafrost environments across North America, Europe, and Asia, releasing massive amounts of previously frozen soil organic matter to microbial decomposition. In these newly formed wetlands, it will be critical to understand the role of microbial communities in carbon cycling and determine whether carbon dioxide or methane will be the dominant endproduct. This project will examine microbial community dynamics at

an ecological research site in northern Sweden. The site spans a natural temperature gradient, where northern areas remain frozen as permafrost and more southern portions are progressively transitioning into thawed wetlands. Building on previous efforts that established a sense of the microbial community structure and functional processes along the thaw gradient, investigators will focus on key questions that most directly address scaling and integration of microbial carbon cycle processes—including the poorly understood viral community

component—into ecosystem-level biogeochemical process understanding. The team will use a combination of omics-enabled systems biology approaches, analytical chemistry, experimental manipulation of mesocosms, and computational modeling frameworks to map omics data to biogeochemical process understanding. The results of this study will advance the understanding of the role of microbes in organic matter decomposition in thawing permafrost and help inform predictions of subsequent impacts on the global climate system.

Further information on BER objectives in this area of research can be found in the DOE report *Carbon Cycling and Biosequestration: Integrating Biology and Climate Through Systems Science*. The report, along with a listing of the funded projects discussed in this document, is available at genomicscience.energy.gov/carboncycle/.

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October 2016