

Biological Systems Research on the Role of Microbial Communities in Carbon Cycling

Summary of projects awarded in summer 2010 under Funding Opportunity Announcement DE-PS02-09ER09-25

Overview

One of the most daunting challenges facing science in the 21st Century is predicting the response of Earth's ecosystems to global climate change. To confront this and other challenges in energy and environment, the Genomic Science program within the U.S. Department of Energy's (DOE) Office of Biological and Environmental Research (BER) supports basic research aimed at achieving a systems-level understanding of plants, microbes, and microbial communities relevant to DOE missions.

Although the global carbon cycle plays a central role in regulating atmospheric carbon dioxide (CO₂) levels and thus Earth's climate, our understanding of the inter-linked biological processes that drive this cycle remains limited. Greater insight particularly is needed into the role microbial communities play in many critical carbon cycle processes. In many cases, these microbially mediated processes are only minimally represented in carbon cycle models, which may limit their predictive capability and scale of resolution. Elimination of these so-called "black boxes" will require innovative approaches aimed at linking structural and functional characterization of microbial communities with quantitative measurement of carbon cycle processes. In July 2009, BER solicited proposals for basic research on the contributions of microbes and microbial communities to carbon cycling processes in the following areas:

- **Systems-level studies on regulatory and metabolic networks of microbes and microbial consortia involved in biogeochemical cycling of carbon.** Proposed studies should focus on systems biology research using environmentally relevant model microbes or microbial consortia. Proposals are encouraged that examine the impacts of shifts in environmental variables, such as temperature, CO₂ concentration, and availability of water and nutrients,

on microbial carbon processing. Model systems should be carefully chosen to facilitate development of metabolic and regulatory network models that could ultimately inform larger-scale biogeochemical models of microbial processes in the environment. Interdisciplinary collaboration is encouraged to link laboratory studies of the chosen model system with environmentally relevant conditions.

- **Development of metatranscriptomic, metaproteomic, and other genome-enabled approaches to understand how shifts in environmental variables impact microbially mediated carbon cycling processes in terrestrial ecosystems.** Proposals should address the adaptation of genome-enabled techniques to the interrogation of terrestrial environments, either *in situ* or using model micro- or mesocosms. Proposals are encouraged that target key microbially mediated carbon cycling processes in terrestrial systems to predict responses to shifts in temperature, CO₂ concentration, and nutrient availability, among other things. Proposals also are sought that use genome-enabled techniques to identify and predict the impact of potentially beneficial associations between plants and microbes or microbial communities (both prokaryotic and eukaryotic) on overall ecosystem productivity.
- **Development of methods and techniques for imaging and analyzing microbially mediated carbon cycling processes in terrestrial ecosystems.** New approaches are needed for high-resolution characterization of microbial community structure and function in soils and other terrestrial environments. Proposals are encouraged that will enable *in situ* analysis of the functional processes of microbial communities and characterization of physical and chemical microenvironments at interfaces between microbes and biotic or abiotic surfaces (e.g., plant cells and soil aggregates).

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2010 Awards

Genes, Isotopes, and Ecosystem Biogeochemistry: Dissecting Methane Flux at the Leading Edge of Global Change

- **Principle Investigator:** Scott Saleska (University of Arizona)
- **Collaborators:** Virginia Rich (University of Arizona), Patrick Crill (Stockholm University), Gene Tyson (University of Queensland), Changsheng Li and Steve Frolking (University of New Hampshire), and Jeff Chanton (Florida State University)

This project aims to connect ecosystem-scale carbon flux to underlying microbial population dynamics and determine the impact of climate change variables on carbon cycling processes in a high-latitude permafrost site that is shifting to wetlands. Investigators will leverage a pre-existing, long-term manipulative field experiment in Sweden to examine how microbial community composition and function scale to ecosystem biogeochemistry of methane (CH₄) and CO₂. This study will involve a combination of *in situ* “omics” measurements on microbial community function, continuous collection of biogeochemical data, and computational modeling of processes on multiple scales. Successful completion of the proposed work would provide a new capability to integrate structural and functional measurements of microbial communities into ecosystem-scale biogeochemical process models.

The Impact of Global Warming on the Carbon Cycle of Arctic Permafrost: An Experimental and Field-Based Study

- **Principle Investigator:** Tullis Onstott (Princeton University)
- **Collaborators:** Satish Myneni (Princeton); Susan M. Pfiffner, John Sanseverino, A. Layton, and Tatiana Vishnivetskaya (University of Tennessee); Robert Hettich (Oak Ridge National Laboratory); Lyle White (McGill University); and Steve Elliott (Los Alamos National Laboratory)

This research will examine the impacts of elevated temperature on carbon processing by permafrost microbial communities. Investigators will use a combination of manipulative microcosm experiments on permafrost cores and correlated observations at a well-characterized field site. The project will employ a combination

of molecular techniques (including microbial community genomic, transcriptomic, and proteomic profiling); stable isotope probing; and measurements of biogeochemical fluxes such as CO₂, CH₄, and organic intermediates. These data will be integrated into a biogeochemical reaction/transport model that identifies specific pools of soil carbon most vulnerable to degradation and predicts CO₂ and CH₄ production as permafrost thaws.

Can Microbial Functional Traits Predict the Response and Resilience of Decomposition to Global Change?

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

This project will examine the impacts of altered water and nitrogen availability on carbon degradation by microbial communities in a California grassland ecosystem. Leveraging a previously established DOE experimental field site, investigators will use high-throughput DNA sequencing to monitor changes in microbial community composition and expression of genes mediating the processing of plant litter. These molecular approaches will be combined with direct measurement of carbon degradation rates and visualization of active community members. The information will be used to develop a predictive mathematical model of microbial community response to climate change variables; predictions then will be experimentally tested at the field site.

Metaomics Analysis of Microbial Carbon Cycling Responses to Altered Rainfall Inputs in Native Prairie Soils

- **Principle Investigator:** David Myrold (Oregon State University)
- **Collaborators:** Peter Bottomley (Oregon State University), Ari Jumpponen and Charles Rice (Kansas State University), Janet Jansson and Susannah Tringe (Lawrence Berkeley National Laboratory), and Robert Hettich and Nathan Verberkmoes (Oak Ridge National Laboratory)

The goal of this work is to optimize and apply multiple omics research approaches to understand key functional groups and metabolic pathways involved in the transformation of carbon by soil microbial communities in tall-grass prairie ecosystems. Investigators will leverage long-term rainfall manipulations at the Konza Prairie field research station in Kansas and the DOE Joint Genome Institute's ongoing soil metagenome project for this site. This study will analyze patterns of gene, protein, and metabolite expression by microbial communities in both field samples and controlled laboratory experiments to examine impacts of altered precipitation intervals on microbially driven carbon cycle processes in soil.

Systems-Level Insights into Alternate Methane Cycling Modes in a Freshwater Lake via Community Transcriptomics, Metabolomics, and NanoSIMS Analysis

- **Principle Investigator:** Mary Lidstrom (University of Washington)
- **Collaborators:** Ludmila Chistoserdova, Marina Kalyuzhnaya, and David Beck (University of Washington) and Victoria Orphan (California Institute of Technology)

Investigators will examine microbial consumption of methane as a carbon and energy substrate (i.e., methanotrophy), with an emphasis on identifying novel organisms capable of performing this metabolic process in the absence of oxygen. The project will leverage a pre-existing methanotroph-enriched metagenomic sequence library from lake sediments and will use a combination of community transcriptomics, stable isotope probing, mass spectrometry, and visualization techniques to follow gene expression and metabolite production and consumption relevant to methanotrophy in sediment microcosms. Results of the proposed work would provide important new information on a poorly understood aspect of the global carbon cycle that potentially could be impacted by global climate change.

Atmospheric Nitrogen Deposition and Microbial Mechanisms Enhancing Soil Carbon Storage

- **Principle Investigator:** Donald Zak (University of Michigan)
- **Collaborators:** Kurt Pregitzer (University of Nevada) and Cheryl Kuske (Los Alamos National Laboratory)

This project will increase understanding of the impacts of anthropogenic nitrogen deposition on microbially

mediated carbon cycling processes in soil. Specifically, the study will examine the observation that adding nitrogen results in decreased plant litter decay rates and increased carbon storage in soils in some forest ecosystems. Investigators will leverage a pre-existing, long-term field study of nitrogen levels in forest soils and use a combination of community transcriptomics to assess gene expression by prokaryotic and fungal community members. In parallel, the study will directly measure a number of soil carbon cycling processes. It also will link environmental factors affecting the regulation of microbial genes to biogeochemical processes in an attempt to understand specific mechanisms underlying the accumulation of soil carbon in response to nitrogen addition.

Tracking Down Cheaters: Molecular Analysis of Carbon Consumption by Organisms that do not Contribute to Extracellular Enzyme Pools

- **Principle Investigator:** Chris Blackwood (Kent State University)
- **Collaborators:** L. Leff and X. Mou (Kent State University) and Gail Rosen (Drexel University)

The proposed work investigates the role of microbial “cheaters” in the degradation of plant matter in soils. The majority of plant biomass is composed of long, complex carbon polymers (e.g., lignocellulose) that are too large for microbes to directly import. Instead, many soil microbes invest energy in the production of extracellular enzymes that break down plant matter outside the cell and consume the resulting carbohydrate fragments. However, rather than producing their own extracellular enzymes, some soil microbes “cheat” by taking up carbohydrates liberated by those of other organisms. This study would use a combination of molecular approaches and stable isotope probing to identify cheating microbes, investigate the regulation of this metabolic strategy, and examine the impact of cheating on carbon cycling in soil environments.

Connecting Genomes to Physiology and Response in Marine Photosynthetic Eukaryotes: Systems Biology of the Green Alga *Micromonas*

- **Principle Investigator:** Alexandra Worden (Monterey Bay Aquarium Research Institute)
- **Collaborators:** Sebastian Sudek and Zbigniew Kolber (Monterey Bay Aquarium Research Institute), David Haussler (University of California, Santa Cruz), and Richard Smith and Stephen Callister (Pacific Northwest National Laboratory)

Investigators seek to better understand the physiology of the globally distributed photosynthetic alga *Micromonas* and its responses to climate change variables. Researchers will perform manipulative chemostat experiments to examine shifts in the functional processes of two *Micromonas* strains (both alone and in mixed cultures) in response to changes in pH, CO₂ concentration, temperature, and nutrient availability. These functional shifts will be assessed by comparing transcriptomic and proteomic expression patterns over time, developing metabolic network models, and identifying pathway interactions and control points. Investigators cite several factors in selecting this organism as a model system for this study. In addition to its role in global carbon cycling processes and potential for expansion in climate change scenarios, *Micromonas* also has much shorter generation times and a smaller genome than many other algae and plants, making systems biology analyses less ambiguous and more cost effective. Results of this study would provide a better sense of how carbon cycling processes in marine algae may be affected by climate change.

Methanogenic Archaea and the Global Carbon Cycle: A Systems Biology Approach to the Study of *Methanosarcina* Species

- **Principle Investigator:** William Metcalf (University of Illinois, Urbana-Champaign)
- **Collaborators:** Nathan Price and Rachel Whitaker (University of Illinois, Urbana-Champaign)

This research will develop systems biology knowledge of methanogenic microbes capable of converting the organic acid acetate to methane (the archaeal class *Methanosarcina*). Since this process is a major source of methane production from global wetlands, understanding the function and regulation of the underlying metabolic processes would allow better prediction of potential responses to climate change variables. The proposed work involves sequencing the genomes of 40 representative species, performing comparative genomic analysis to identify patterns in metabolic and regulatory circuits, assembling transcriptional networks, and developing an integrative metabolic network model of this class of organism.

Development and Integration of Genome-Enabled Techniques to Track and Predict the Cycling of Carbon in Model Microbial Communities

- **Principle Investigator:** Jill Banfield (University of California, Berkeley)
- **Collaborators:** Trent Northen and Steve Singer (Lawrence Berkeley National Laboratory) and Pan Chongle, Bob Hettich, and Nathan VerBerkmoes (Oak Ridge National Laboratory)

The objective of the proposed work is to integrate omics (i.e., genomic, transcriptomic, proteomic, and metabolomic) datasets and stable isotope probing to develop a computational model of carbon cycling by a microbial community. The project will extend ongoing studies of a well-characterized microbial biofilm system composed of bacteria, archaea, fungi, and viruses. Investigators will track the flux of both organic carbon and CO₂, as well as nitrogen sources, through the community and examine how they change in response to perturbation by elevated temperature. The major advantage of this community, originally sampled from acid mine drainage, is that it represents a defined, self-contained, and experimentally tractable ecosystem. This presents an attractive model system for developing new techniques (in this case, community-scale metabolomics) not yet ready for use in more complex ecosystems.

Microbial Food Web Mapping: Linking Carbon Cycling and Community Structure in Soils Through Pyrosequencing-Enabled Stable Isotope Probing

- **Principle Investigator:** Dan Buckley (Cornell University)

The proposed work aims to develop a new method that will couple pyrosequencing of soil microbial community DNA and RNA to an improved stable isotope probing method using labeled carbon compounds. This novel combination of two powerful techniques will allow direct, cultivation-free identification of microbial community members while simultaneously mapping the microbial food web involved in consumption of carbon compounds representative of biomass inputs. Researchers will then use this methodology to compare microbial community structure and carbon cycling properties in a set of varying soil types collected from a well-established field site.

Plant Stimulation of Soil Microbial Community Succession: How Sequential Expression Mediates Soil Carbon Stabilization and Turnover

- **Principle Investigator:** Mary Firestone (University of California, Berkeley)
- **Collaborators:** Eoin Brodie (Lawrence Berkeley National Laboratory), Jennifer Pett-Ridge and Patrik D’Haeseleer (Lawrence Livermore National Laboratory), and Jizhong Zhou (Oklahoma University)

This project focuses on the role of soil microbial communities in degradation and transformation of carbon released from the roots of grasses and will examine the impact of elevated CO₂ concentrations on these processes. Investigators will establish microcosms using soil samples and seedlings of *Avena barbata* (a grass common to the western United States) labeled with ¹³CO₂ and then use a combination of stable isotope probing (SIP) and metatranscriptomic sequencing to identify metabolic pathways of soil microbes responsible for carbon processing. Fluorescent in situ hybridization (FISH) probing and NanoSIMS analysis will be used to identify active community members and visualize spatial associations between microbes and plant roots. Also, functional gene microarrays will be validated and used to increase sample throughput at later stages. These data will be used to generate a computational model representing shifts in microbial community processes over time and under elevated CO₂ conditions.

From Community Structure to Functions: Metagenomics-Enabled Predictive Understanding of Temperature Sensitivity of Soil Carbon Decomposition to Climate Warming

- **Principle Investigator:** Jizhong Zhou (University of Oklahoma)
- **Collaborators:** James M. Tiedje (Michigan State University), Yiqi Luo and Zhili He (University of Oklahoma), Edward Schuur (University of Florida), and Konstantinos Konstantinidis (Georgia Institute of Technology)

The goal of the proposed research is to use community-scale omics techniques to examine microbial community processes affecting the temperature sensitivity of soil carbon decomposition to climate warming. Investigators predict that patterns of temperature sensitivity of soil respiration will differ among contrasting ecosystems, specifically temperate grassland soils in Oklahoma and tundra soils in Alaska, and that these differ-

ences will primarily depend on variations in microbial community structure and functional properties. This hypothesis will be tested by determining the temperature sensitivity of soil organic matter in laboratory and field experiments at two long-term research sites. Concurrently, the team will analyze changes in microbial community structure and function in response to warming using omics-based molecular approaches. Researchers then will integrate these datasets to develop a random matrix theory model that will enable prediction of how key microbial processes controlling ecosystem carbon storage respond to climate change.

Systems-Level Analysis of Function and Adaptive Responses of Methanogenic Consortia

- **Principle Investigator:** Derek Lovley (University of Massachusetts, Amherst)
- **Collaborators:** Bernhard Palsson and Karsten Zengler (University of California, San Diego)

Researchers hypothesize that conversion of organic compounds to methane by microbial consortia can occur via direct transfer of electrons between methanogenic archaea and syntrophic bacteria. This mode of metabolism would be more energetically favorable than the currently favored hydrogen transfer hypothesis. Investigators will test their hypothesis using a combination of physiological experiments on well-characterized model organisms, omics techniques to monitor changes in gene expression during co-culture, and computational modeling to generate genome-scale metabolic flux models of the partner organisms and paired processes. If proven correct, this hypothesis would fundamentally alter our understanding of the mechanisms involved in biological methane production, a globally significant process in environments such as sediments and wetlands.

Syntrophic Interactions and Mechanisms Underpinning Anaerobic Methane Oxidation: Targeted Metaproteogenomics, Single-Cell Protein Detection, and Quantitative Isotope Imaging of Microbial Consortia

- **Principle Investigator:** Victoria Orphan (California Institute of Technology)
- **Collaborators:** Chris House (Pennsylvania State University), Mark Ellisman and Jeff Grethe (University of California, San Diego), and Bob Hettich (Oak Ridge National Laboratory)

This project will optimize techniques for characterizing microbial processes involved in carbon cycling in anaerobic environments. The project will focus on model syntrophic consortia of methane-consuming archaea and sulfate-reducing bacteria, organisms that play a major role in the global methane cycle. Investigators will develop advanced visualization, isotopic, and proteogenomic methods with submicron-scale resolu-

tions to examine structural and functional properties of these consortia maintained in laboratory microcosms. The major emphasis of this work is to understand key physiological and ecological factors that affect rates and magnitudes of methane consumption, with a particular focus on studying the ability of the consortia to shift their metabolic pathways in response to changing environmental conditions.

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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