Systems Biology Enabled Research on the Roles of Microbiomes in Nutrient Cycling Processes

Summary of Projects Awarded in 2019 under the Funding Opportunity Announcement DE-FOA-0002059

Overview

The U.S. Department of Energy's (DOE) Genomic Science program (GSP), managed within the Office of Biological and Environment Research (BER), supports basic research aimed at identifying the foundational principles that drive biological systems. This basic research includes studies on the impact of microbiomes on processes in the environment, such as the global biogeochemical cycles of carbon and nutrients. The aim of BER-funded research is to generate a mechanistic, systems-level understanding of plant and microbial communities by leveraging genomic and other high-throughput omics-based technologies and tightly integrating them with computational and modeling approaches.

Since the realization that soil is a heterogeneous system teeming with life, much work has involved examining which organisms are there, the functions they are performing, the role each plays independently and within the community, and the key processes these microbial communities drive that contribute to higher-scale processes, including the movement and fate of nutrients in soils. These basic questions are still being asked today in terrestrial ecosystems and sedimentary environments due to the compositional heterogeneity of soils and the complexity of the microbial communities. Fortunately, increasingly sophisticated approaches for meta-omics (e.g., metagenomics, metatranscriptomics, metaproteomics, and metabolomics) coupled to high-resolution analytical technologies offers new tools for investigating the functional properties of microbial communities. Along with omics analyses, researchers have rapidly decreased the time it takes 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 facilitated more sophisticated studies on the roles played by individual microbes and the microbiomes they are a part of as key points of integration between major biogeochemical cycles (e.g., carbon, nitrogen, sulfur, and phosphorus) in experimentally challenging environments (e.g., soils, sediments, and permafrosts) resulting in new insights on the influence of microbial processes at larger, aggregate scales.

Systems-level research supported by GSP 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 biogeochemical cycles 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 application of data analytics and conceptual and numerical modeling to enable structural and functional understanding of complex systems that applies computational approaches across multiple spatial and temporal scales. These integrated studies provide opportunities to tease apart microbial network interactions as well as to assess species-specific and collective capabilities of the organisms contained within soil and sediment microbiomes.

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

- Systems biology studies on regulatory, metabolic, and signaling networks of microbes, microbial consortia, and microbe-plant interactions involved in biogeochemical cycling of nutrients. Proposed studies were asked to focus on systems biology research using model microbes or microbial consortia that are relevant to biogeochemical cycling in terrestrial ecosystems. Experimental systems were asked to be carefully chosen to facilitate development of microbial 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 processes involved in biogeochemical cycling in terrestrial and sedimentary ecosystems. Applications were asked to address the adaptation of genome-enabled techniques (e.g. metagenomics, metatranscriptomics, metaproteomics, and community-scale metabolomics) to interrogate relevant processes of microbes in terrestrial environments, either at field sites or using model micro- and mesocosms, and integrate resulting data into process understanding at the ecosystem scale. Applications were encouraged that target key microbe-mediated biogeochemical cycling processes in terrestrial systems to predict responses to shifts in temperature, precipitation, CO_2 concentration, or other environmental stressors.

2019 Awards

Biogeochemical Consequences of Microbial Evolution Under Drought

- **PI:** Steven Allison (University of California Irvine)
- **Collaborators:** Michael Goulden, Jennifer Martiny, Adam Martiny, Kathleen Treseder (University of California, Irvine); Eoin Brodie and Ulas Karaoz (Lawrence Berkeley National Laboratory)

This project aims to understand and predict how evolution in microbiomes affects carbon and nutrient cycling under environmental change. The following overarching question is addressed: How does evolutionary adaptation influence microbiome functioning in response to drought? The main hypothesis is that evolutionary change results in a significant shift in microbiome genetic composition and functioning under drought conditions.

To assess microbiome responses to drought, cutting-edge genomic, transcriptomic, and metabolomic methods will be combined with novel, field-based manipulations of microbiomes. Resulting data will be incorporated into new mathematical models of biogeochemical cycling. Due to changing global precipitation patterns, many ecosystems will experience more frequent and intense drought. It is known that microbiomes respond to drought through changes in genetic composition, and that these shifts have consequences for carbon and nutrient cycling. However, it is unknown to what degree evolutionary processes contribute to changes in genetic composition and biogeochemical functioning when microbiomes respond to drought. To address this gap, microbial evolution in the field under drought and ambient conditions will be evaluated. This will involve the application of omics approaches to quantify physiological, evolutionary, and demographic responses to drought and consequences for microbiome functioning.

The project consists of three main activities. The first activity will use genomic approaches and physiological assays to measure how much evolutionary processes, community shifts, and dispersal contribute to drought responses of microbiomes. Field experiments and laboratory assays will test drought responses of individual microbial populations, as well as whole communities. The second activity will use a suite of omics approaches, genetic manipulations, and advanced spectroscopy to determine the consequences of evolutionary and community responses for ecosystem benefits like carbon and nutrient cycling. It is expected that selection for improved microbiome performance under drought conditions will affect these benefits due to tradeoffs among drought tolerance and other aspects of microbial physiology. The third activity will incorporate findings into a predictive model. Current models of carbon and nutrient cycling omit evolution, thus results will be used to develop a new generation of microbial models that account for changes in microbiomes due to evolution. Measurements of tradeoffs between drought tolerance and other microbial characteristics will also improve predictions of carbon and nutrient cycling driven by evolution in microbiomes.

Microbiomes in grassland leaf litter shift with drought manipulation in the Loma Ridge Global Change Experiment in Irvine, California.

This project will determine how evolution in microbiomes influences rates of carbon and nutrient cycling, a topic that is not well understood scientifically. Although the work will focus on drought responses, the resulting conceptual advances can be applied to other problems that affect microbiomes, such as extreme weather events or nutrient pollution. By combining advanced molecular

techniques with physiological assays, connections among microbial genotypes and phenotypes that are evolving in response to environmental change will be established. Finally, a computer model that incorporates omics data and knowledge advances in microbial evolution to generate better predictions of ecosystem benefits under shifting environmental conditions will be established.

Molecular Mechanisms of Mycorrhizal-Decomposer Interactions and Impacts on Terrestrial Biogeochemistry

- **PI:** Jennifer Bhatnagar (Boston University)
- **Collaborators:** Rytas Vilgalys (Duke University), Colin Averill (Swiss Federal Institute of Technology Zurich), Hui-Ling Liao (University of Florida), Edward Brzostek (University of West Virginia)

Soil microorganisms make nutrients available for plants and control a large portion of greenhouse gas emissions from terrestrial ecosystems via respiratory release of carbon dioxide (CO_2) to the atmosphere. However, understanding of these processes is insufficient to predict the rate of carbon (C) and nutrient flow through soils and the ecosystems they support. A common and abundant type of soil microorganism that associates with the roots of live plants, known as ectomycorrhizal fungi (EMF), is often able to control the rate at which its plant host grows (i.e., how much CO_2 the plant captures through photosynthesis) and the amount of nutrients and C that are released from soil (e.g., as CO_2 respired to the atmosphere) during decomposition by microorganisms.

Model ecosystem microcosms for the study of plant-ectomycorrhizal-saprotroph interactions.

The goal of this project is to identify the specific ways in which EMF control these large fluxes of C and nutrients through plants and soils in terrestrial ecosystems. There is some evidence that EMF control the transformation of C and nutrients in soil by interacting with (e.g., supporting or inhibiting the activity of) free-living decomposers, such as saprotrophic microbes (SAPs) in soil, but there remains an intense debate about the mechanism and magnitude of these interactions in natural ecosystems.

This research project aims to determine how the combination of soil resources, plant growth, and the species composition of soil microbial communities shape plant-EMF-SAP interactions and their impacts on terrestrial biogeochemistry. Plants provide carbon in the form of sugar to their EMF symbionts in exchange for soil nutrients like nitrogen. This could cause strong interactions among EMF and SAPs, but recent studies have shown that this C transfer is controlled by the genetic compatibility of plants and the EMF species present in soil. This means that the interactions among plants, EMF, and SAPs may be critically dependent on the species composition of soil microbial communities and the environmental factors that disrupt it.

To understand plant-EMF-SAP interactions, this project leverages a model plant-EMF system: plants in the Pinaceae and their EMF symbionts in the genus *Suillus*. *Suillus* fungi and *Pinus* trees are a widespread ecological and economically important plant-EMF symbiosis. Previous collaborative research by the PIs with DOE national labs has generated full sequenced genomes of *Pinus* seedlings and their *Suillus* partners, which allow the quantification of biochemical networks of plant-EMF-SAP interactions under varying plant and soil resources in planned studies. To understand plant-EMF-SAP interactions effects on C and nutrient cycling, the research team is using a novel C3 plant - C4 soil system in the greenhouse. It allows researchers to manipulate the presence of EMF and trace the downstream effect on plant and soil nutrient cycling.

Researchers will apply experimental data from the model *Suillus-Pinus* system to develop and test the state-of-the-art ecosystem model FUN-CORPSE. This model represents the impacts of plants, EMF, and SAPs on plant growth and soil CO_2 emissions. Data on decomposition activity in soil, nutrient cycling, and soil CO_2 emissions from planned experiments will be used to create more mechanistically accurate versions of FUN-CORPSE, including soils that harbor EMF species with varying levels of genetic compatibility to their host plant. The FUN-CORPSE models will be compared to a large field dataset on soil microbial communities and biogeochemistry from the National Ecological Observatory Network (NEON). This groundbreaking new continental-scale dataset includes standardized, repeated measurements of plants and soils that span major Pinaceae-*Suillus* dominated forest biomes across the United States, allowing the project to evaluate how environmental variation shapes plant-EMF-SAP interactions and ecosystem biogeochemistry. This multi-scale ecological approach to studying plant-microbial-soil interactions will shed new light on one of the most direct, universal biological controls on terrestrial biogeochemical cycling across ecosystems.

Using a Systems Biology Approach to Describe the Role of Dissimilatory Phosphite Oxidation in the Global Phosphorus Cycle

- **PI:** John Coates (University of California, Berkeley)
- **Collaborator:** Andrew Jackson (Texas Technical University)

The project's objective is to investigate the role of microbial dissimilatory phosphite oxidation (DPO) in the global phosphorus and carbon biogeochemical cycles. The natural occurrence of DPO and phosphite (HPO_3^{2-}) in a broad range of geochemical environments, and to examine fundamental physiological and biochemical aspects of DPO will be investigated. The project will fill significant knowledge gaps in current understanding of the global phosphorus (P) cycle.

Phosphorus is essential for all life and predominantly exists as oxidized phosphate (P^{+5}) on Earth. The majority of this is trapped in mineral deposits, resulting in biological P limitation for most ecosystems. Models of the global P cycle consistently overlook alternative P redox states, although reduced P species have been identified in diverse environments and frequently serve as a biological P source when soluble orthophosphate is limited. Phosphite (P^{+3}) is a highly soluble, geochemically stable, reduced phosphorus compound that can account for up to 30% of total dissolved P in aqueous, sedimentary, and geothermal environments. Previous studies also suggest that the evolution of life on early Earth may have been dependent on phosphite. Furthermore, recent findings suggest there is a phosphorous redox cycle between phosphate and phosphite on contemporary Earth.

Currently, there is a gaping hole in understanding the underlying processes driving this cycle. While mechanisms of phosphate reduction to phosphite remain obscure, microbial oxidation is likely the principal mechanism by which localized phosphite is converted back to phosphate. Previous studies found that up to 67% of culturable bacteria from select environmental samples oxidized and assimilated phosphite as a P source. Further, over 1.5% of microbes in the IMG database are predicted to be capable of this metabolism based on gene content. More recently, two autotrophic dissimilatory phosphite oxidizing organisms have been described that use phosphite as an energy source through a largely uncharacterized metabolism, which is the focus of this study.

DPO may play a particularly important role in phosphite oxidation due to the substantially higher rates and quantities of phosphate production associated with this unique metabolism. Studies in the PI's lab have demonstrated that DPO is far more prevalent than previously recognized and appears to be dependent on carbon dioxide metabolism. If predictions of its prevalence are true, DPO-capable autotrophs could serve as a key driver of primary production in the deep biosphere where phosphite neogenesis occurs.

This interdisciplinary project will combine systems biology and omics approaches with physiological studies to elucidate the geochemical impact, the true environmental prevalence, and the metabolic machinery underlying DPO. A broad range of environmental settings will be surveyed to assess the natural occurrence and abundance of phosphite and its relation to DPO and other disparate environmental factors.

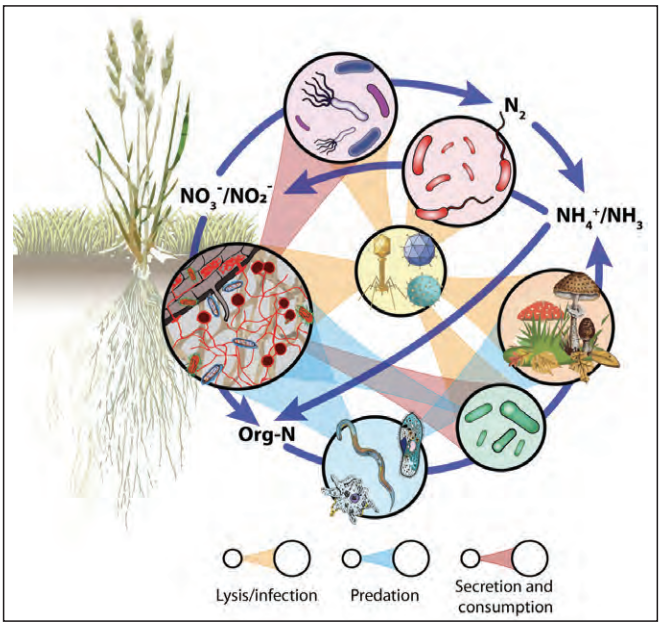
It is expected that this work will significantly influence current models of the global P cycle because DPO could additionally play an important, and heretofore unrecognized role in primary production and the global carbon cycle. The importance of this work is heightened by the fact that P is an essential and limiting nutrient in biological and agricultural systems, and is timely given current global pressures to identify novel carbon sinks.

Cross-Kingdom Interactions: The Foundation for Nutrient Cycling in Grassland Soils

- **PI:** Mary Firestone (University of California, Berkeley)
- **Collaborators:** Joanne Emerson (University of California, Davis); Nhu Nguyen (University of Hawai'i at Manoa); Jizhong Zhou (University of Oklahoma); Jennifer Pett-Ridge, Erin Nuccio (Lawrence Livermore National Laboratory); Eoin Brodie, Javier Ceja-Navarro, Trent Northen, Kateryna Zhalnina (Lawrence Berkeley National Laboratory); Graeme Nicol (Université de Lyon)

The belowground microbial world is a jungle: dominated by competition, predation, parasitism, cooperation, and mutualism. While the importance of ecological interactions is well understood for macro-organisms above ground, the roles of analogous microbial interactions in controlling soil nutrient cycling are largely unknown.

Decades of research has identified key microbial mediators of terrestrial nutrient cycling, their environmental sensitivities, and the functional genes and enzymes involved. Many aspects of bacterial, fungal, and microfaunal mediation of nutrient cycling are thoroughly documented. However, the organisms involved in nitrogen cycling interact in an extremely complex soil habitat. There is little understanding of how biotic interactions shape nutrient cycling in soil. This project asks how cross-kingdom and within-kingdom biotic interactions provide a functional framework for nitrogen (N) cycling in soil. Do greater complexities of biotic interactions result in higher rates of turnover and nutrient transformation? The project will also explore the effects on nitrogen and carbon (C) cycling of predation, competition, and



Belowground interactions among viruses, bacteria, archaea, arbuscular mycorrhizal fungi (AMF), saprotrophic fungi, microfauna, and roots mediate the cycling of nitrogen and its subsequent availability to plants.

cooperative/antagonistic interactions—among viruses, bacteria, archaea, arbuscular mycorrhizal fungi (AMF), saprotrophic fungi, microfauna, and roots. The research team's extensive past research on soil nutrient dynamics, pathways of root C-flow in soil, and exploration of biotic interactions associated with roots and decomposing litter provides a powerful foundation for the project.

The project will: (1) Determine how biotic interactions (among viruses, bacteria, fungi, and microfauna) control key N-cycle transformations including depolymerization of macromolecular organic N compounds, N-mineralization and immobilization, as well as nitrification and denitrification, and (2) assess how the spatial compartmentalization and transfer between soil compartments by fungal hyphae and mobile fauna determines the occurrence and rates of N-cycling processes. The multidisciplinary team involved in this project, with expertise in microbial ecology, soil viruses, microfauna, fungi, community systems biology (genomics, proteomics, metabolomics), stable isotope probing, network analysis, and food-web modeling will conduct greenhouse mesocosm experiments, small-scale viral and faunal lab experiments, and large-scale field experiments along with evaluation and synthesis of existing datasets in order to disentangle the importance of biotic interactions in controlling nutrient cycling. Combining multiple novel stable isotope techniques with current molecular methods will allow this project to explore, map, and quantify the complex web of biotic interactions that mediate and control N-cycling in soil.

Techniques will include stable isotope-based methods, metagenome and metatranscriptome sequencing, exometabolomics, as well as network analysis and system modeling that incorporate key microbial determinants. Without knowledge of the main viral, bacterial, fungal, faunal, and root interactions belowground, the team's attempts to model or predict terrestrial nutrient cycling will remain rudimentary.

From Viruses to Protists: Temperature Response of the Neglected Components of Microbial Controls on Peatland Nutrient Cycling

- **PI:** Jean Gibert (Duke University)
- **Collaborators:** Dale Pelletier, David Weston (Oak Ridge National Laboratory); Jonathan Shaw (Duke University); Steven W. Wilhelm (University of Tennessee)

Peatlands are one of the most highly threatened ecosystems on Earth. They occupy only 3% of the Earth's land surface but store approximately 25% of terrestrial carbon (C) as organic matter. The moss genus *Sphagnum* is responsible for much of the primary production in these harsh habitats, which are naturally low in mineral nitrogen (N) availability. As such, *Sphagnum* success and productivity depends on symbiotic interactions with microbial organisms: approximately 35% of the N used by *Sphagnum* is supplied by N_2-fixing bacteria (diazotrophs). N availability is a crucial determinant of *Sphagnum* growth, and thus, C sequestration in peatlands, which in turn impacts global biogeochemistry and climate.

Sphagnum peat moss from DOE's SPRUCE site growing in a petri dish within a temperature-controlled incubator with protist cultures in the back.

Preliminary data collected at the DOE Spruce and Peatland Responses Under Changing Environments (SPRUCE) site suggest that field warming drastically alters the *Sphagnum*-associated microbiome with decreased diazotroph abundance and reduced N_2-fixation. Yet, the mechanisms driving these changes in community composition and N_2-fixation remain unknown. Preliminary data further suggest that viruses and unicellular eukaryotes (protists)

are present and active in *Sphagnum* communities and may shape microbial community composition and biogeochemistry. Viruses are the most abundant bioactive agents on Earth, driving host evolution through infections and horizontal gene transfer. Similarly, protists are largely ignored in microbiome studies but are the most important consumers of prokaryotes (bacteria and archaea). Viral and protist responses to warming, as well as their impact on structuring the *Sphagnum* microbiome are, however, not known.

The project's primary objective is to provide a mechanistic understanding of how protist grazing and viral activity mediate *Sphagnum* responses to warming and to develop a model of *Sphagnum*-associated community response to predict changes in carbon and nitrogen biogeochemical cycles across sites and species of peatland mosses. To carry out these objectives, a combination of metagenomics and metatranscriptomics, paired with microcosm experiments and modeling will be used.

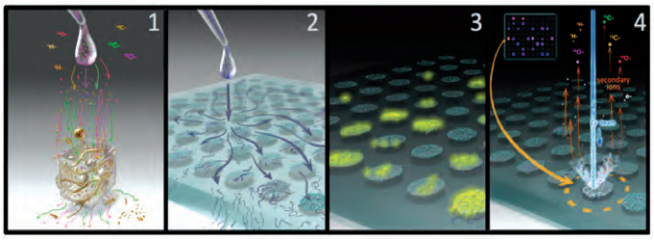
Briefly, Part 1 addresses the impacts of warming on *Sphagnum* communities at SPRUCE. Part 2 investigates the mechanisms underlying these community changes in a series of laboratory experiments involving *S. fallax*, bacteria, viruses, and protists. Part 3 will use lab-based mechanistic insights to parameterize a food web-virus model designed to better understand and predict warming impacts on community composition, the dynamics of *Sphagnum*-associated communities, and C sequestration in peatlands. This model will be integrated with current ELM-SPRUCE modeling efforts. Part 4 expands the sampling to include additional *Sphagnum* species, microhabitats (hummock versus hollow), and peatlands in different climatic regions to test community structure and ecosystem function model predictions.

Results of this research will dramatically impact the way researchers study the effects of warming, as the mechanisms uncovered via the planned experiments are likely to apply to other systems where protists and viruses are prevalent. This project will also provide a necessary framework that links metagenomics and metatranscriptomics "snapshot" data to the underlying mechanisms of change.

The GREEN Omics of Nutrient Feedbacks to Soil Warming

- **PI:** Bruce Hungate (Northern Arizona University)
- **Collaborators:** Ember Morrissey (West Virginia University); Jennifer Pett-Ridge, Steven Blazewicz, Xavier Mayali, Peter Weber (Lawrence Livermore National Laboratory); Kristen Hofmockel (Pacific Northwest National Laboratory); Egbert Schwartz, Paul Dijkstra, Michelle Mack, Benjamin Koch (Northern Arizona University)

This project will integrate genomics- and isotope-enabled measurements of Growth Rate, growth Efficiency, and the



Chip-SIP technology for exploring soil microbial nutrient cycling.

stoichiometry of Essential Nutrients during growth, or GREEN omics. The overarching objective is to develop and apply omics approaches to investigate microbial community processes involved in nutrient cycling. The specific project objectives are (1) to evaluate the microbial ecology of nutrient uptake, testing hypotheses about nutrient assimilation in response to temperature variation, (2) to evaluate the ecology of nutrient-use efficiency for soil microorganisms within a framework of ecological theory, and (3) to develop new isotope-enabled genomics and transcriptomics techniques that probe the microbial ecology of nutrient dissimilation. This work will push the frontier of isotope-enabled genomics by connecting quantitative stable-isotope probing to ecological theory about nutrient assimilation, nutrient-use efficiency, metabolic efficiency, and by applying these tools to understand the basic biology and ecology of soil microorganisms and how they transform nutrients in the environment.

This work will develop and apply new methods to understand the ecology of soil microorganisms using stable isotope tracers and genomics, which when combined offer powerful insights into the cycling of nutrients in the environment and the organisms that take up, use, and recycle those nutrients as they grow and die. This project will focus on particular soil microorganisms, bacteria and fungi, that make up the majority of life in soil, and which are responsible for most of the nutrient transformations in soil that are vital to ecosystems, and to people. This project will also evaluate how soil microorganisms and the nutrient cycling processes they catalyze are sensitive to shifts in temperature, a major driver of biological processes. This new suite of techniques will investigate and describe the microbial ecology of nutrient cycling in soil environments.

The work relies on four long-term field experiments where temperature-treated and control plots occur in Arctic, boreal, temperate, and tropical biomes. The systems biology approach will interrogate community and taxon-specific microbial controls over key biogeochemical processes in terrestrial environments, and test quantitative ecological and biogeochemical principles using genomics and isotope data, including theories of element limitation, growth efficiency, and nutrient use efficiency. The project

will therefore "form and test hypotheses on underlying ecological principles," and "facilitate scaling of concepts and data across multiple levels of biological organization," as outlined in the request for proposals. The project will occur in laboratory and field experiments, including a number of DOE-supported climate change experiments: the Merriam-Powell Climate Change Experiment (temperate), the Toolik Lake warming experiment (Arctic), the TRACE warming experiment at Luquillo Long Term Ecological Research program (tropical), and the SPRUCE experiment (boreal).

Microorganisms are the engines of nature's nutrient cycles, responsible for making nutrients available to plants, for creating soil organic matter which supports natural systems, and for influencing the composition and radiative properties of the atmosphere. Yet, there is virtually no quantitative understanding of how microbial rates of nutrient transformation are distributed among the tens of thousands of microorganisms in soil. This work has the potential to advance the field by providing a means to ascribe element fluxes with taxonomic resolution, and to provide the first tests of the sensitivities of these processes to temperature.

Using Culture-Independent Methods to Link Active Compound-Specific Carbon Degradation to Greenhouse Gas Production and Recycling in Natural Populations of Permafrost Microbes

- **PI:** Karen G. Lloyd (University of Tennessee)
- **Collaborators:** John Cliff (Pacific Northwest National Laboratory); Robert Hettich (Oak Ridge National Laboratory); Tullis Onstott (Princeton University); Andrew Steen, Tatiana Vishnivetskaya (University of Tennessee)

Permafrost is one of Earth's largest reservoirs of soil organic carbon (SOC). As permafrost thaws, heterotrophic microbial communities degrade the newly available SOC, often resulting in large and variable fluxes of the greenhouse gases carbon dioxide (CO_2), methane (CH_4), and possibly nitrous oxide (N_2O) to the atmosphere.

This project addresses three questions that are fundamental for understanding how permafrost thaw affects greenhouse gas emissions. (1) What are the relative contributions of CO_2-consuming (autotrophic) versus CO_2-producing (heterotrophic) processes in thawing permafrost? (2) As SOC degrades, what are the relative production rates of CO_2, CH_4, and N_2O? (3) Which metabolic pathways and microbes drive SOC degradation in thawing permafrost? These questions will be addressed with intact core incubations from Bayelva, Svalbard, Norway, the site of a 20-year permafrost monitoring program that has tracked physical, hydrological, and geochemical data, without any explicit inclusion of microbiology so far. Svalbard is also one of the most rapidly thawing areas of the Arctic and is experiencing extreme weather events, making it a canary in the coal mine for Arctic permafrost shifts. Large-scale slumping and collapse of permafrost exposes deeper layers to the surface, and different layers have different contributions to the production and consumption of greenhouse gases.

Active layer above permafrost at the Bayelva long-term monitoring station in Ny Ålesund, Svalbard, Norway.

To determine how these factors impact SOC-degrading microbial communities in intact core incubations, pore gas composition and surface fluxes, pore water chemistry and isotopic compositions of dissolved inorganic carbon, dissolved organic carbon, CO_2, CH_4, and N_2O will be monitored. Subsamples for microbiological analyses before and two weeks after permafrost thaw, after core slumping, and after 12 and 18 months, from four depths in triplicate cores, will be taken. Injection of 13C-labeled compounds at different depths will be used to map the temporal and spatial effects of carbon cycling. Measuring microbial community structure alone is inadequate for addressing microbial responses to permafrost thawing for the following three reasons: (1) Many microbial groups in permafrost have no cultured representatives, so their functions cannot be inferred from their identities, (2) even well-characterized microbial clades may act differently in a natural environment than they do in pure culture, and (3) microbial communities often alter their activity levels or metabolic pathways without changing in cell abundance. Therefore, in addition to microbial community structure assessments with 16S rRNA gene amplicon libraries, metagenomes will be obtained to assess community-wide gene functions and single-cell amplified genomes (SAGs) to show gene functions of intact cells. The activity of the natural microbial population will be directly assessed through a combination of metatranscriptomes, metaproteomes, (meta)-metabolomes, custom-designed enzyme activity assays, and nanoSIMS substrate incorporation assays. Data from these varied microbial omics analyses will be combined

into a geochemical model to achieve predictive capabilities for linking microbial ecosystem activities to gas fluxes from surface permafrost, as well as newly exposed permafrost layers.

Cell to Ecosystem: Understanding Methane and Associated Nutrient Cycling by Sediment Hosted Syntrophic Consortia and Their Viral Predators

- **PI:** Victoria Orphan
- **Collaborators:** Long Cai (California Institute of Technology), Mark Ellisman (University of California, San Diego), Christof Meile (University of Georgia), Manuel Martinez Garcia (University of Alicante, Spain), Robert L. Hettich (Oak Ridge National Laboratory), Christopher Henry (Argonne National Laboratory)

The anaerobic oxidation of methane (AOM) is a significant worldwide microbial process in anoxic lake and ocean sediments, responsible for sequestering up to 80% of this greenhouse gas. Often considered a metabolism on the edge of thermodynamic probability, the impact of ANaerobic MEthane-oxidizing (ANME) archaea on carbon and nutrient cycling in sediment ecosystems is far reaching. They not only serve as a sink for methane coupled to diverse electron acceptors, but they also catalyze the transformation of many important nutrients including nitrogen, phosphate, and iron.

While information about the potential mechanisms supporting metabolism in AOM is now available, remarkably little has been learned about their nutritional requirements, their dependencies on bacterial partners, and consequentially, their ultimate impact on nutrient transformation and bioavailability within sedimentary ecosystems, and beyond. Further, the role of viruses within sediment ecosystems represents an essential but vastly understudied aspect of the transformation of carbon (C) and nutrients by AOM.

Viruses are now widely appreciated as central players in biogeochemical cycles across diverse ecosystems. These nanoscale predators have been shown to enhance the turnover of essential nutrients, thus stimulating microbial growth and environmental viruses themselves may constitute an important reservoir of nitrogen and phosphorous. Little is known about the role of viruses in methane-impacted sedimentary ecosystems, however prior genomic and microscopy evidence suggests that AOM consortia are susceptible to phage infection.

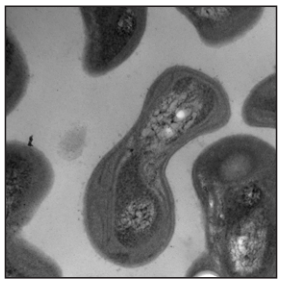
The overarching scientific goal of this multidisciplinary research project is to build on these recent discoveries and expand understanding of interactions and fundamental activities involved in cycling of carbon and nutrients by syntrophic methanotrophic archaeal-bacterial consortia and associated viruses in anoxic sedimentary environments. Specific objectives are to (1) quantify energy and nutrient exchange [e.g., nitrogen (N), phosphorus (P), iron (Fe) and vitamins] within AOM consortia and between ANME-bacterial partners; (2) identify virus-host interactions associated with AOM and assess C and N transfer through viruses in methane-impacted sediment ecosystems; (3) model energy and nutrient exchange in AOM consortia and viral-host interactions (i.e., viral activity), and their environmental distribution patterns. The project's experimental emphasis cuts across scales that are important for understanding microbial and viral interactions and activities within their habitats, as well as community wide biogeochemical transformations.

These research goals will be accomplished through the application of novel molecular techniques targeting DNA, RNA, proteins, and metabolites combined with a unique multimodal analytical imaging pipeline. The ecophysiological capabilities of diverse sediment-hosted methanotrophic consortia will subsequently be modeled to develop a more comprehensive understanding of the energetic and nutritional interactions between different AOM partner couplings that occur in sediments.

Microbial Competition for Copper: Impacts on Carbon and Nitrogen Cycling

- **PI:** Jeremy D. Semrau (University of Michigan)
- **Collaborator:** Alan DiSpirito (Iowa State University)

Methanotrophs, or methane-oxidizing bacteria, are a group of microbes that play a key role in controlling the emission of methane, a potent greenhouse gas. Methanotrophic activity, however, is strongly affected by a number of environmental parameters, most notably copper. That is, methanotrophs have a well-known "copper-switch" where the form and activity of the enzyme responsible for the oxidation of methane—the methane monooxygenase—is controlled by copper.

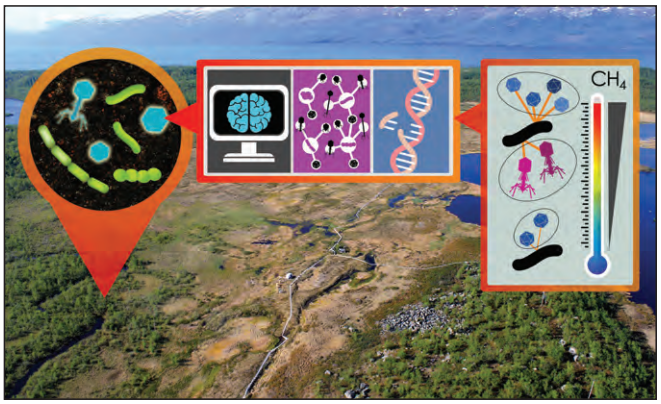


Transmission electron micrograph of *Methylocystis* strain SB2, a methanotroph capable of producing a chalkophore, or high affinity copper chelating agent.

It has previously been demonstrated that some methanotrophs produce a copper chelator termed methanobactin (MB) that binds copper with extremely high affinity. As a result, MB provides these methanotrophs a "copper monopoly" where other microbes no longer can collect copper. Specifically, it was found that MB prevents many denitrifiers from

completely converting nitrate to dinitrogen gas. Rather, nitrous oxide is the terminal product as the nitrous oxide reductase (responsible for converting nitrous oxide to dinitrogen) is also copper-dependent, and the ability of denitrifiers to collect copper substantially decreases in the presence of either MB or MB-producing methanotrophs. Nitrous oxide is a much more potent greenhouse gas than methane, so efforts to stimulate methanotrophs to reduce methane emissions may inadvertently increase nitrous oxide emissions. Not all methanotrophs, however, produce MB. Rather, some methanotrophs produce an alternative copper uptake system called MopE/MopE*, and the ability of MB to steal copper from MopE/MopE*-producing methanotrophs is unknown. Thus, copper competition may not only exist between methanotrophs and denitrifying bacteria, but also among different methanotrophs, affecting overall methanotrophic community activity.

It is the goal of this project to determine how significant microbial competition for copper is, particularly how such competition affects greenhouse gas emissions in different environments. In addition, there are other copper-containing enzymes that are widely distributed in nature (e.g., the ammonia monooxygenase and many oxidases). Thus, the planned work is also a critical first step in understanding how microbial competition for copper affects much more broadly ecosystem functioning. The work will also enable a determination to what extent copper uptake systems are public versus private goods and how these systems may affect microbial community evolution.



Arctic soils are thawing at unprecedented rates and represent a large reservoir of carbon that could drastically impact climate.

Virus in Soils: Key Modulators of Microbiomes and Nutrient Cycling?

- **PI:** Matthew Sullivan (Ohio State University)
- **Collaborators:** Sarah Bagby (Case Western Reserve University); Paul Hyman (Ashland University); Sanggu Kim (Ohio State University); Sylvian Moineau (Université Laval, Canada); Vivek Mutalik, Trent Northen (Lawrence Berkeley National Laboratory); Tfaily Malak (University of Arizona); Ben Temperton (University of Exeter)

The activity of microbes in soil profoundly affects global energy and nutrient cycles. In consequence, whatever shapes soil microbial activity shapes the world. Substantial recent work in environmental microbiology has revealed that viruses are a key driver of microbial ecology in other systems, and it might be expected that the same holds true in soil. But soil is a complex milieu—highly structured, chemically and physically heterogenous, and resistant to extrapolation. Thus, even as new methods have revolutionized understanding of microbial and viral ecology in other systems, understanding of soil microbial ecology has lagged, and understanding of soil viral ecology is still further behind.

The project's objective is to develop paradigms for understanding the role of viruses in soil ecology, and to build the tools—scalable new methods, new databases, and new model systems—to test the validity of these paradigms. In developing paradigms for soil viral ecology, the project will focus intensively on one system, a permafrost thaw gradient at Stordalen Mire in Sweden that has been the focus of the DOE-funded IsoGenie Consortium for nearly a decade. Despite the rich ecological picture emerging from IsoGenie's work, the technical challenges in developing soil viral methods have left Stordalen's viruses virtually unstudied. Recent advances by PI Matthew Sullivan within IsoGenie have laid the groundwork for this new project to broadly and transformatively impact soil viral ecology.

In Aim 1, the project will seek to leverage existing IsoGenie datasets and soil samples to (1) apply and improve the analytics and machine-learning methods the research team established for viral ecogenomics in soils, (2) track carbon transformations and microbial host dynamics over the course of viral incubations, and (3) use microscopy to ask the fundamental question of what the baseline rate of viral infection is and how it varies across environmental gradients.

Aim 2 seeks to broadly optimize viral ecogenomic tools for use in soils, including developing several new critically needed methods that do not yet exist for any environment. First, the project will improve capture of DNA and RNA viruses in soils. Through the VirION method for low-input, long-read sequencing, improved recovery of intact dsDNA viral genomes will be achieved, while improved analytics and physical particle capture methods will allow better detection of RNA viral sequences in

IsoGenie bulk soil metagenomes and give us access to a particularly understudied region of the viral world. Second, several methods will be adapted to establish high-throughput virus-host linkages—critically needed with the pace of new viral discovery that is vastly outpacing characterization and classification. Third, the project will establish biochemistry-informed informatics to identify mobile genetic elements in the soil virome, which likely drastically impact the soil microbiome and geochemistry but remain virtually unknown. Fourth, the project will develop and deploy scalable forward genetics methods to establish a large-scale understanding of phage resistance in a model system, and apply CRISPR-Cas9 engineering to test resultant hypotheses from these genome-wide screens and assess phage components of the virus-host arms race.

Finally, this aim seeks to establish baseline paradigms in the understanding of viral infection dynamics and the resultant biogeochemical impacts via metabolite profiling using novel instrumentation and analytics. Aim 3 looks to provide foundational resources that will broadly help soil viral and microbial ecologists seeking to use systems biology approaches in these complex systems. First, hundreds of microbial strains isolated from a range of soils will be screened to isolate viruses to provide approximately 15 new genome-sequenced and host-range-characterized virus-host model systems for future study. Second, a centralized and curated online resource for viral genomics, proteomics, and taxonomy, will be built, and a workshop will be held to train colleagues in the newly developed methods. Together, this project will transform soil viral ecology as a field, and with it understanding of viruses and the soil microbiome as drivers of global biogeochemistry.

Corrinoids as Model Nutrients to Probe Microbial Interactions in a Soil Ecosystem

- **PI:** Michiko E. Taga (University of California, Berkeley)

Earth's soils are habitats for communities of microorganisms that promote plant growth, store carbon, and cycle nutrients. The thousands of microbial species living in soil form an intricate web of nutrient-sharing interactions in which some species produce nutrients that other members cannot make.

The overall goal of this research is to understand how these molecular exchanges occur and how they shape microbial communities. Understanding soil nutritional interactions in detail may lead to new methods to improve soil health, resulting in increased agricultural efficiency and carbon capture. However, the high level of diversity in soil microbiomes makes it challenging to achieve this goal using current microbiology methods. This research leverages a group of key nutrients related to vitamin B12, known as corrinoids, to investigate soil microbial interactions. Different bacterial species must share corrinoids, because they are made by only a subset of the bacteria that use them. Corrinoids are used for a variety of metabolic processes critical for bacteria in soil and other environments, such as synthesizing DNA and breaking down various carbon and energy sources. Importantly, corrinoids are structurally diverse, and bacteria in isolation can use only some of the corrinoid forms that are available in their environment. Whether this specificity impacts bacterial growth and metabolism on a community scale has not been studied.

Because different bacteria require different corrinoids, it is hypothesized that corrinoids are keystone nutrients that can shape soil microbial communities. To test this hypothesis, this project will examine the effects of corrinoid addition on bacterial populations across multiple levels of complexity using custom-synthesized corrinoids for those that are not commercially available. The project will use DNA sequencing–based approaches to determine which bacteria are present in soil and soil-derived enrichments and measure how they respond to different corrinoids. The project will also use the sequencing data to predict which species produce and use particular corrinoids and test these predictions by measuring corrinoid dependence of individual bacterial species isolated from soil.

By investigating a key nutrient at levels spanning the whole community to individual isolates, this work will provide an unprecedented and multifaceted view of metabolic interactions in a soil microbial community. Determining how key nutrients impact microbial abundance and activity could lead to new strategies for managing soils for agricultural and environmental applications.

Dissection of Carbon and Nitrogen Cycling in Post-Fire Soil Environments using a Genome-Informed Experimental Community

- **PI:** Thea Whitman (University of Wisconsin, Madison)
- **Collaborators:** Thomas Bruns, Matthew Traxler (University of California, Berkeley); Igor Grigoriev, Ben Bowen (Lawrence Berkeley National Laboratory)

Wildfires are a natural part of many forest ecosystems, with globally important carbon (C) storage and nutrient cycling consequences. Wildfires are increasing in frequency and severity in western North America. Forest fires affect soil C stocks in complex ways. Some C is released into the atmosphere through combustion, while a large percentage of the C is added to the soil in the form of pyrogenic (fire-affected) organic matter (PyOM). Worldwide, it is estimated that 16% of soil organic

matter (SOM) is pyrogenic, while in areas with frequent fires, this number may be as high as 80%.

A prescribed burn in a jack pine barrens in 2016.

Understanding how wildfires affect SOM cycling requires understanding how microbes respond to PyOM and other post-fire soil conditions. However, understanding of microbial interactions with post-fire soil is in its infancy. Outstanding questions include: Which microbes are capable of degrading PyOM? What are the relevant genes and metabolites associated with this degradation? How does the presence of nutrients such as nitrogen (N) affect post-fire PyOM and SOM degradation? What are the key interactions among post-fire microbes?

The PI has developed resources during a previous DOE grant (DE-SC0016365) that enabled the dissection of the post-fire environment. These resources include: a 13 CO_2 labeling growth chamber, a "charcoalator" for producing "pyrocosms" (PyOM) for simulating depth-resolved effects of fire, and a collection of genomic resources including 12 genome-sequenced pyrophilous fungi and more than 80 pyrophilous bacterial isolates (20 of which are about to be sequenced). The project will use these in combination with genomics, transcriptomics, metabolomics, and stable isotope techniques to address the following specific objectives: (1) determine how dominant post-fire soil microbes affect the fate of PyOM; (2) assess the interaction between N availability and PyOM mineralization by post-fire microbial communities and individual pyrophilous microbes; (3) define the network of microbial interactions that facilitate PyOM breakdown over time and the key genes involved.

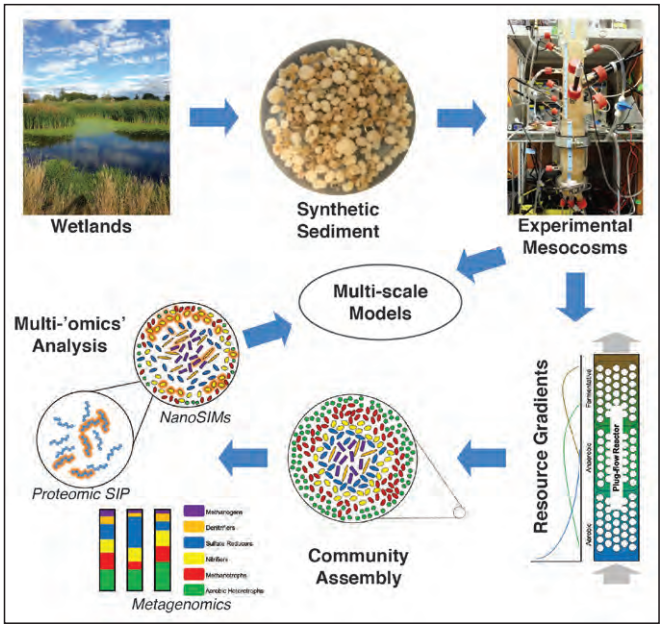
The project directly addresses the Genomic Science program's goals of achieving a predictive, systems-level understanding of plants, microbes, and biological communities, by improving understanding of mechanisms and processes controlling the impacts of wildfire on C and N cycling in soils. The approach incorporates hypothesis-driven observations, experimental manipulations, and ecological theory, with the tools of genomics, transcriptomics, and metabolomics at isolate and community scales. The project's findings will be relevant for ecosystem management, post-fire recovery, and fundamental microbial genomics, ecology, and biogeochemistry.

Integrating Single-Cell Wetland Microbiome Structure, Function, and Activity to Ecosystem-Scale Biogeochemical Fluxes

- **PI:** Mari Winkler (University of Washington, Seattle)
- **Collaborators:** Xavier Mayali (Lawrence Livermore National Laboratory); Chongle Pan (University of Oklahoma); Ludmilla Chistoserdova, David Stahl, Samuel Bryson (University of Washington)

This collaborative project takes advantage of expertise in four disciplines (biological process engineering, mathematical modeling, sediment microbiology, and omics-driven science) to implement a game-changing technological approach to link the physiology of individual wetland microbial cells to larger mesoscale ecosystem processes. The encapsulation of naturally resident microbiota in hydrogel particles (called biogranules) of different sizes provides a format that closely replicates natural diffusion-limited processes, and the entrapment allows the system to experience extremes of imposed environmental conditions without community washout. Native communities entrapped in a novel biogranule format (i.e., biogranule column mesocosms) will be used to examine how anticipated climate-related changes in wetland habitats will impact biogeochemical activities in different redox zones at different scales of resolution, from the functional roles of individual cells, to interacting populations, and to systems-level processes. Specifically, the project will quantify the impact of climate change stressors on model wetland microbial communities and the ecosystem processes they sustain through the integration of mathematical modeling and highly controlled hydrogel column experiments.

This experimental design provides for both a systems-level analysis (by continuous sampling of net fixation and release of gaseous microbial excretion products) and a coincident fine-scale analysis of the supporting microbiology using NanoSIMS paired with FISH, metagenomics, and Proteomic Stable Isotope Probing. This research will use highly automated column mesocosms to mimic natural wetland conditions by closely simulating their chemical, thermal, and physical gradients. The project will specifically investigate how predicted climate change-associated stressors impact the microbial populations of two wetland systems. Lacustrine wetlands are known to be impacted by lower rainfall and warmer temperature, leading to more frequent drought events and therefore to lower above-ground productivity and higher belowground oxygen exposure. Planned experiments will mimic this scenario by changing the availability of oxygen to test the impact on anaerobic respiratory processes, alterations of the microbial community structure, net metabolic rates, and transformations/partitioning of carbon and nitrogen



Overview of experimental approach for examining potential impacts of climate change on wetland microbiomes.

including the stepwise conversion of intermediates (NO_2-NO- N_2O -N_2) in the denitrification pathway.

Estuarine wetlands are expected to be impacted by saltwater intrusion due to sea level rise, which will shift the microbial community from methanogenesis towards sulfate reduction at a specific salt concentration, which will be experimentally altered. In both wet lands, the team will trace how carbon mineralization rates will vary as a function of the different redox zones, local microbial functional guild structures, and the forms of available organic carbon. Hydrogel biogranule communities will be infused with a mixture of organic carbon polymers designed to approximate plant fiber composition (cellulose, xylan, lignin, and pectin), providing for the controlled and stable microbial contact required for hydrolysis and metabolism of these complex carbon sources. Stable isotope labeling with 15N-labeled ammonium in combination with NanoSIMS will be used to assess changing activities with depth in the individual hydrogel grains, and in relation to varying redox zones of the mesocosm system. Organism-specific activity profiles will be inferred by combined metagenomic and proteomic SIP analyses, and validated by more selective NanoSIMS analysis of carbon mineralization using 13C-labeled intermediates of polymer degradation (coniferyl alcohol, glucose, acetate, CH_4, and CO_2) to resolve patterns of activity distribution and possible metabolic interactions among individual organisms and populations.

This experimental format offers a unique way to bridge different biological scales, linking single-cell scale measurements (with NanoSIMS and FISH) to population scale (with metagenomics and Proteomic SIP), to the ecosystem scale (with biogeochemical flux measurements) to inform global scale mathematical models. The system response to imposed perturbations will be used for iterative modeling. Particle-level transformations will serve to both model and predict net C and N conversions, which should be consistent with measured net capture and release of CO_2 and CH_4 from the column system and activities identified by proteomic SIP. These microscale models may contribute to more robust large-scale models and to project future climate-related changes in wetland processes.

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