

Systems Biology–Enabled Microbiome Research to Facilitate Predictions of Interactions and Behavior in the Environment

Summary of projects awarded in 2022 under Funding Opportunity Announcement DE-FOA-0002602

Genomic Science Program

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Funded Projects

- A Volatile Environment: How Volatile Mediated Plant and Microbial Interactions Extend the Rhizosphere and Enhance Soil Carbon Storage
- From Viromes to Virocells: Dissecting Viral Roles in Terrestrial Microbiomes and Nutrient Cycling
- Gatekeepers of Arctic Carbon Loss: Landscape-Scale Metabolites-to-Ecosystems Profiling to Mechanistically Map Climate Feedbacks
- Decoding the Unifying Microbial Metabolic Controllers on Soil Carbon Cycling Across Freshwater Wetlands
- Ecological and Molecular Controls of Metabolic Activity in Microbial Interactions Driving Ecosystem-Wide Methane Cycling
- Toward a Predictive Understanding of the Role of Plant-Derived Persistent Compounds in Peatland Soil Carbon Sequestration Under Climate Change: Revisiting the “Enzyme Latch” Hypothesis
- Predicting Post-Fire Nitrogen Cycling Through Traits and Cross-Kingdom Interactions
- Friends and Foes: Microbial Interactions and Soil Biogeochemistry After 23 Years of Experimental Warming
- How Microbes and Minerals Make Necromass that Persists
- Bacterial-Fungal Interactions: Bridging Soil Niches in Regulating Carbon and Nitrogen Processes



Researchers study how grassland bacterial-fungal interactions are shaped by the availability of different carbon sources. [Courtesy of University of Hawaii]

Environmental microbiomes consist of interconnected, dynamic, and highly diverse microbial populations. The scientific community has learned much about environmental microbiomes using omics-based technologies, such as genomics, transcriptomics, proteomics, and metabolomics. However, the mechanisms by which microbial interactions coordinate the exchange of nutrients, carbon, and energy in soil environments remain largely unknown. Also unclear is how such interactions translate to observed ecosystem properties across scales or how changes in microbial community composition and dynamics are controlled. To address these knowledge gaps, the U.S. Department of Energy’s Biological and Environmental Research (BER) program selected 10 projects in Fiscal Year 2022 aimed at enhancing the ability to understand—dynamically and predictively—microbial interactions in terrestrial ecosystems.

The awards are sponsored by BER’s Genomic Science program (GSP), which invests in crosscutting technologies and research aimed at achieving a mechanistic understanding of systems biology, biological community function, and environmental behavior. Specifically, GSP research includes efforts to discover the fundamental principles underlying (1) regulatory, metabolic, and signaling networks among microbes in microbe-plant interactions; (2) carbon cycling and its long-term storage or sequestration in soils; and (3) the biogeochemical conversion of other elements and nutrients, particularly as they relate to rapidly changing environmental conditions.

GSP’s interest in emergent system properties—such as net carbon sequestration, greenhouse gas emissions, nutrient cycling, carbon use efficiency, and



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water use efficiency—are tightly connected to its missions in energy security and environmental stewardship. By supporting research in this area, GSP seeks to understand microbial interactions in natural environments and how such interactions are shaped by environmental change. Understanding community responses to environmental perturbations and long-term change represents an important frontier for efforts to predict ecosystem behavior at different scales.

The new environmental microbiome projects align with these goals by seeking insights into the principles that control the microbial activity and interactions at play in these processes at scale. Moreover, the genetic potential of many environmental microbes is relevant to other GSP research priorities in bioenergy and biosystems design. As the projects advance fundamental understanding of environmental genomics, the research will likely have a broader impact across the BER mission space.

2022 Awards

A Volatile Environment: How Volatile Mediated Plant and Microbial Interactions Extend the Rhizosphere and Enhance Soil Carbon Storage

- **Principal Investigator:** Laura Meredith (University of Arizona)
- **Co-Investigators:** Malak Tfaily (University of Arizona); Jordan Krechmer (Aerodyne Research, Inc.); Eoin Brodie, Kolby Jardine, and Romy Chakraborty (Lawrence Berkeley National Laboratory)

Volatile organic compounds (VOCs) are a significant carbon pool produced via plant and microbial metabolism, but their contributions to soil organic carbon formation are not well understood. The team's primary goal is to quantify how plant and microbial VOCs directly and indirectly contribute to soil carbon stabilization, both in the region of soil adjacent to plant roots (i.e., rhizosphere) and throughout the soil profile. Researchers will determine the mechanisms that regulate VOC influences on soil carbon stabilization. The project's three main objectives are (1) to determine how root-released VOCs and VOC transformed by soil microbial communities directly influence soil carbon cycling and stabilization; (2) to determine how VOCs can influence soil carbon stabilization indirectly by acting as chemicals that carry information (i.e., infochemicals) needed for communication between organisms; and (3) to identify the role of VOC movement between otherwise separated regions in the soil (i.e., teleconnections) that most strongly enhance VOC conversion to stable carbon pools. The team will address these objectives using soil probes that nondestructively quantify *in situ* soil VOC consumption and

production, online soil and root measurement systems, unique field sites and infrastructure, and time-resolved carbon-VOC stable isotope labeling in laboratory-based artificial rhizosphere systems and growth chambers. Researchers believe this work will represent the first quantification of the potential impact of direct, indirect, and teleconnection roles of root VOCs on soil carbon pools. If VOC contributions are significant, this project will directly impact conceptual and numerical models that aim to enhance the understanding of soil carbon cycling and stabilization and contribute to a new frontier in microbial systems science that embraces VOCs as integral components of the suite of organic compounds present in an ecosystem. Furthermore, this project innovates approaches to nondestructively track microbial metabolism through real-time sensing that complements the destructive snapshots provided by most other omics-based techniques.

From Viromes to Virocells: Dissecting Viral Roles in Terrestrial Microbiomes and Nutrient Cycling

- **Principal Investigator:** Matthew Sullivan (The Ohio State University)
- **Co-Investigators:** Sarah Bagby (Case Western Reserve University); Paul Hyman (Ashland University); Sylvain Moineau (Université Laval); Malak Tfaily (University of Arizona); Robert Hettich (Oak Ridge National Laboratory); Vivek Mutalik (Lawrence Berkeley National Laboratory)

The activity of microbes in soil profoundly affects global energy and nutrient cycles. Consequently, whatever shapes soil microbial activity shapes the world. Substantial recent work in environmental microbiology reveals

that viruses are key drivers of microbial ecology in other systems, and researchers expect the same to hold true in soil. Soil is a complex milieu; it is highly structured, chemically and physically heterogeneous, and resistant to extrapolation. Thus, even as new methods have revolutionized scientific understanding of microbial and viral ecology in other systems, the understanding of soil microbial ecology has lagged, with soil viral ecology still further behind. Even less well understood are mobile genetic elements (MGEs), virtually unexplored in soils but known to be agents of rapid genomic change elsewhere.

The team's objective is to develop paradigms for understanding the role of viruses and MGEs in soil ecology via the genomics of natural communities and experiments of laboratory model systems. The team will also build tools (e.g., scalable new methods, new databases, and new model systems) to test these paradigms. In developing paradigms for soil viral ecology, researchers will focus intensively on one system—a permafrost thaw gradient at Stordalen Mire, Sweden. This system was the focus of the DOE-funded IsoGenie Consortium for nearly a decade, although only DNA viruses were studied (albeit using now outdated methods), and no investigation of RNA viruses or MGEs was attempted.

In Aim 1 of this project, researchers will leverage existing IsoGenie datasets and soil samples to (1) apply current virome approaches to identify, quantify, and characterize all types of viruses and MGEs from new data streams and in their microbiome context; (2) track carbon transformations and temperature responses of viruses and MGEs in experiments; and (3) apply such methods to two other DOE-funded sites to assess universalities.

Aim 2 of the project seeks to establish a mechanistic understanding of soil phage-microbe interactions. This will involve generating time-resolved multiomic datasets from the new soil phage-host model systems and using these to qualitatively assess metabolic reprogramming in virus-infected cells (Virocells), a major knowledge gap in the scientific understanding of viral ecosystem impacts. The team will also evaluate these soil phage-host model systems using scalable genetic methods to establish a large-scale understanding of how microbial cells become resistant to phages. Researchers will test hypotheses arising

from these genome-wide screens by phage-genome engineering to assess the lesser-studied phage components of the phage-host evolutionary arms race in soils.

Aim 3 of the project seeks to provide foundational resources that will broadly help soil viral and microbial ecologists seeking to use systems biology approaches in these complex systems. First, researchers will attempt to adapt new ocean methods to soils to enable high-throughput virus capture and characterization (viral tag and grow) and targeted assessment of RNA viruses via RNA viral particle purification and sequencing. The team will also develop experimental systems specific to Stordalen Mire for future hypothesis testing and work with DOE's KBase for community organization efforts to build a centralized and curated online resource for viral genomics, proteomics, and taxonomy. Finally, the team will hold a soil virus workshop to cross-train others in the methods, which could benefit BER-relevant soil system studies. Together, this work should transform soil viral ecology as a field and advance the understanding of viruses, MGEs, and the soil microbiome as drivers of global biogeochemistry.

Gatekeepers of Arctic Carbon Loss: Landscape-Scale Metabolites-to-Ecosystems Profiling to Mechanistically Map Climate Feedbacks

- **Principal Investigator:** Ruth Varner (University of New Hampshire)
- **Co-Investigators:** Virginia Rich (The Ohio State University); Gene Tyson (Queensland University of Technology); Malak Tfaily and Scott Saleska (University of Arizona); Jeffrey Chanton and Rachel Younge (Florida State University); Eoin Brodie and William Riley (Lawrence Berkeley National Laboratory)

Permafrost systems are thawing rapidly and are consequential to carbon cycle dynamics under climate change. Thaw exposes permafrost soil carbon (roughly twice as much as is currently in the atmosphere) to decomposition with the potential to produce significant carbon dioxide (CO₂) and methane (CH₄). These, in turn, can accelerate climate warming. Discontinuous permafrost landscapes are at the leading edge of these changes, and they span a range of permafrost thaw stages as well as lesser-studied interconnected lakes. These lakes can be the exit point for

a significant fraction of CH₄ lost after thaw via ebullition (bubbling), and their emissions are projected to increase with ongoing climate warming. Simultaneously, thaw-initiated ecological succession of plant communities is increasing soil carbon storage and potentially also plant-derived inhibitory compounds that slow carbon processing. Accurate predictive understanding of the net effect of these simultaneously coupled loss, gain, and stabilization processes under increasing temperatures is urgently needed. To advance the grand challenge of understanding the fate of carbon in thawing permafrost, researchers focus on carbon cycle climate feedbacks to warming in landscapes with high CH₄ emissions in an Arctic mire ecosystem. The aims are to (1) identify and resolve key gaps in the understanding of microbial carbon processes consequential to carbon storage and CH₄ emission, (2) identify and resolve a mystery of microbial oxidation rates and controls consequential to emission mitigation, and (3) integrate resolved consequential unknowns into next-generation ecosystem models. Researchers will compare integrated substrate microbiome emissions in the two habitats (fens and lakes) that dominate climate feedbacks (via CH₄), quantify the rates and controls, and distill insights into an Earth system model to more accurately predict carbon cycling and climate feedbacks. Study sites will focus on Stordalen Mire, a model terrestrial ecosystem with site-specific genomes, metabolite spectra, carbon gas emissions and isotope profiles, and an interconnected lake sediment system with some of the highest observed natural abundance of anaerobic CH₄ oxidizers.

Decoding the Unifying Microbial Metabolic Controllers on Soil Carbon Cycling Across Freshwater Wetlands

- **Principal Investigator:** Kelly Wrighton (Colorado State University)
- **Co-Investigators:** Bailey Fosdick and Michael Wilkins (Colorado State University); Gil Bohrer (The Ohio State University); Jorge Villa (University of Louisiana); Yu-Ping Chin (University of Delaware); Sheel Bansal and Eric Ward (U.S. Geological Survey); William Riley (Lawrence Berkeley National Laboratory); Christopher Henry (Argonne National Laboratory)

Playing a key role in regulating climate, freshwater wetland soils have a disproportionately large impact

on global carbon cycling. Despite occupying less than a tenth of the land surface, these soils hold upwards of 30% of carbon on Earth, which makes these habitats critical atmospheric CO₂ sinks. Yet, this carbon is not completely protected nor guaranteed to be stored in soils. In fact, much of it is destabilized by microorganisms. Soil microorganisms decompose stored carbon by breaking it down for energy and releasing CO₂, CH₄, and nitrous oxide. Climate warming is further exacerbated when these greenhouse gases are released from wetland soils. In fact, freshwater wetlands are recognized as the largest natural source of global CH₄. Ultimately, enhanced knowledge of soil microbiology in freshwater wetlands will be needed to develop climate-smart soil strategies that manage soil carbon gain from sequestration and loss through greenhouse gas emissions. Given their importance to climate, it is surprising that so little is known about the microorganisms or the microbial enzymes that control the conversion of soil carbon into CH₄. Fortunately, advances in genomic sciences have cracked the window on the soil carbon cycle, offering some of the first insights into microorganisms that contribute to and even reduce soil CH₄ emissions. While a promising start, these observations have been derived from site-specific studies, and thus any generalizable rules or understanding about the fundamental microbial controls on soil carbon cycling in freshwater wetlands are lacking. This knowledge gap hinders accurate predictions of greenhouse gas emissions from these climatically important soils. To address this knowledge deficit, researchers will sample more than 10 wetlands in the continental United States with varying CH₄ emissions ranging from low to some of the highest globally. The team seeks to identify the chemical and biological rules that explain why some wetlands emit high amounts of CH₄ whereas others do not, despite close geographic and climatic similarity. Researchers will use high-resolution methods for uncovering the molecular attributes of soil carbon and pair these with a first-of-its-kind microbial genome catalog, which will provide a blueprint for the cycling of carbon across, and unique to, different wetlands. This research will reveal the soil attributes that are conserved across high-CH₄-emitting wetlands and allow scientists to build a new era of climate models that are microbiologically informed. Key deliverables from the project will have value to scientific communities and more generally to society. Scientists

will benefit from the soil microorganism genome catalog, a community resource that inventories and describes the reactions catalyzed by hundreds of thousands of previously enigmatic soil microbes. This highly collaborative and multidisciplinary team will offer direction for increased realism in predictive process-oriented models of soil CH₄ flux. Beyond identifying climate-smart solutions, the microbial collection and models can be mined for new enzymes for biofuels or to guide precision therapeutics for enhanced bioremediation or carbon sequestration in soil.

Ecological and Molecular Controls of Metabolic Activity in Microbial Interactions Driving Ecosystem-Wide Methane Cycling

- **Principal Investigator:** Victoria Orphan (California Institute of Technology)
- **Co-Investigators:** Dipti Nayak, (University of California, Berkeley); Mark Ellisman (University of California, San Diego); Christof Meile (University of Georgia); Manuel Martínez García (Universidad de Alicante); Christopher Henry (Argonne National Laboratory); Robert Hettich (Oak Ridge National Laboratory)

Syntrophic anaerobic oxidation of CH₄ (AOM) among spatially organized consortia of anaerobic CH₄-oxidizing (ANME) archaea and partner bacteria exemplifies the power of interspecies cooperative metabolic interactions that lead to novel chemical transformations in the environment. Syntrophic AOM is now recognized as a significant biological CH₄ sink. Multiple groups of ANME from CH₄-rich anoxic environments worldwide have been described in highly ordered multi-celled consortia with one of several lineages of syntrophic bacterial partners. The pure culture isolation of these slow-growing ANME lineages has not yet been successful; however, culture-independent molecular, geochemical, and imaging approaches have elucidated aspects of their ecology and physiology, offering new insights into the potential mechanisms of syntrophic exchange in AOM. High-quality metagenome assembled genomes are now available for major ANME lineages and their syntrophic bacteria, providing the essential framework for hypothesis development and refinement with *in silico* metabolic modeling and experimentation. In parallel, molecular, isotopic, and chemical imaging methods have also been

developed or optimized for tracking active environmental microorganisms and viruses as well as assessing the specific metabolism of environmental microorganisms at the level of single cells within anoxic sediments. Additionally, archaeal genetics, including CRISPR-Cas9 gene editing, are now mature and offer an unprecedented opportunity to investigate the function of key ANME genes and their emergent phenotypes in a closely related host methanogen. With these advancements, the team of modelers and experimentalists are now uniquely positioned to advance a mechanistic and predictive framework to gain a systems-level understanding of CH₄-based sedimentary ecosystems and syntrophic AOM partnerships. The project's multidisciplinary research on AOM and CH₄-based sediment communities encompasses three main objectives for understanding AOM syntrophy and the broader interactions with associated microorganisms and viruses as well as their collective role as a CH₄ sink in anoxic sediments. These objectives, ranging in scale from intra-consortia to community-level to ecosystem, include (1) developing a molecular understanding of the mechanisms driving CH₄-fueled syntrophic interactions, (2) defining and characterizing the microbial communities associated with methanotrophic consortia, and (3) creating integrative modeling frameworks to explore the ecophysiology of AOM consortia.

Toward a Predictive Understanding of the Role of Plant-Derived Persistent Compounds in Peatland Soil Carbon Sequestration Under Climate Change: Revisiting the “Enzyme Latch” Hypothesis

- **Principal Investigator:** Joel Kostka (Georgia Institute of Technology)
- **Co-Investigators:** Kostas Konstantinidis and Caitlin Petro (Georgia Institute of Technology); Malak Tfaily (University of Arizona); Rachel Wilson and Jeffrey Chanton (Florida State University); Christopher Schadt (Oak Ridge National Laboratory)

While peatlands are freshwater wetlands that make up just 3% of the Earth's landmass, they store about one-third of the planet's soil carbon as thick peat deposits. Most peatlands are found in cooler, wetter regions where the microbial breakdown of organic matter is relatively



Open-topped temperature control chambers operate in the Marcell Experimental Forest in Minnesota for climate change research. [Courtesy of Oak Ridge National Laboratory]

slow. As global temperatures rise, microbes could break into the peatland carbon bank, and the resulting decomposition of the ancient, combustible plant biomass would lead to increased levels of greenhouse gases, such as CO_2 and CH_4 , being released into the atmosphere and accelerating climate change. The overall goal is to uncover the mechanisms or biochemical pathways by which microbes, plants, and the environment interact to control the various steps in the microbial degradation of soil organic matter in peatlands. In particular, previous research indicates that phenolic compounds produced during the breakdown of lignocellulose found in the cell walls of certain plants may slow down microbial metabolism of soil organic matter. Understanding the mechanisms and controls of the microbial breakdown of organic matter with respect to the overlying vegetation will allow scientists to improve predictions of how the peatland soil carbon bank will be affected by climate change.

Hypotheses driving the new research include:

1. When peat soils are flooded with water, phenolic compounds derived from certain plants act as bottlenecks to microbial soil organic matter decomposition by binding and preventing enzymatic attack of organic matter polymers, thus the degradation of these polymers is the rate-limiting step in peatland soil organic matter decomposition (referred to as enzyme latch mechanism).

2. Since the enzymes that catalyze the breakdown of phenolic compounds are dependent upon oxygen, soil moisture content and oxygen availability largely determine which microbes and biochemical pathways are operative in the degradation of lignocellulose and lignin, thereby regulating soil organic matter storage (the opposite of decomposition) through the enzyme latch).
3. Climate change, which is expected to warm and dry out peatlands, will release the enzyme latch and cause microbes to break into the carbon bank by stimulating the enzymatic decomposition of phenolic compounds and organic polymers.
4. Conversely, changes in plant communities caused by climate change (i.e., the replacement of mosses by lignin-rich vascular plants and shrubs) will act to strengthen the enzyme latch by preventing microbial decomposition through the accumulation of plant-derived phenolic compounds.

Specific research objectives include a series of five tasks. Task 1 will be to test the enzyme latch hypothesis (prevention of soil organic matter decomposition by the accumulation of plant-derived phenolic compounds) in the field. Task 2 includes testing the enzyme latch hypothesis and its response to environmental conditions linked to climate change (e.g., temperature, redox, water content) under controlled conditions in the laboratory. Task 3 will determine the response of the enzyme latch to the manipulation of climate conditions (e.g., temperature, elevated CO_2 levels in the atmosphere) at the whole-ecosystem scale. Task 4 will use field and laboratory investigations to improve predictions of future carbon storage and develop quantitative indicators of the microbe-plant-environment interactions that control soil organic matter degradation in peatlands exposed to climate change. Task 5 will create a curated, comprehensive, and searchable genome and gene database that documents the microbial populations and processes mediating lignocellulose and lignin degradation in soils on a global scale, along with underlying physicochemical data geocoded via geographic information system mapping to reveal geographic distribution patterns of the populations.

The team's approach leverages the resources and expertise of DOE's Joint Genome Institute and the extensive

infrastructure and site characterization datasets of DOE's Spruce and Peatland Responses Under Changing Environments project located within the Marcell Experimental Forest in Minnesota. At this site, experiments are performed at the ecosystem scale using a design that allows for statistical testing of how environmental conditions that are impacted by climate change (e.g., temperature, elevated atmospheric CO₂, changes to plant communities) alter the flow of organic matter in peatlands.

Predicting Post-Fire Nitrogen Cycling Through Traits and Cross-Kingdom Interactions

- **Principal Investigator:** Sydney Glassman (University of California, Riverside)
- **Co-Investigators:** Steven D. Allison (University of California, Irvine); Joanne B. Emerson (University of California, Davis); Peter M. Homyak (University of California, Riverside); Michael J. Wilkins (Colorado State University)

Wildfires are increasing in frequency, size, and severity across the globe. Unlike ecosystem disturbances that primarily impact vegetation, wildfires kill microbes, thereby dramatically altering the composition, function, and abundance of post-fire soil microbiomes with downstream impacts on soil nitrogen cycling. Post-fire environments favor pyrophilous (or fire-loving) microbes that are undetectable pre-fire but vastly increase in abundance post-fire. While researchers know that fire can select for increased abundance of pyrophilous microbes, their traits and impacts on soil nutrient cycling remain largely unknown. In this project, the team will focus on how wildfire severity and pyrophilous microbial interactions regulate nitrogen cycling and the emission of greenhouse gases. In particular, researchers will examine impacts on nitrous oxide, a powerful greenhouse gas with 300 times the warming potential of CO₂, and its implications for long-term ecosystem recovery and Earth's climate. Understanding how microbial interactions and their traits govern nitrogen cycling is critical to predict post-fire nitrogen behavior and ecosystem recovery and has led researchers to ask the overarching question: Do conserved microbial traits and cross-kingdom interactions drive post-fire nitrogen cycling across ecosystems? The team asks this question across scales, from individual genomes to more complex microbiomes, allowing

researchers to predict the impacts of wildfire disturbance on ecosystem nitrogen cycling with a modeling approach that incorporates microbial traits. Additional questions will be asked across three ecosystems (Mediterranean grasslands, chaparral shrublands, and montane coniferous forests) that are experiencing increased fire frequency and are abundant across western North America and many other parts of the globe. Researchers ask: (1) How do microbial traits change during post-fire succession? (2) How does fire severity influence microbial succession and gene expression of nitrogen cycling functions? (3) How do cross-kingdom interactions change during post-fire succession? and (4) How do traits and interactions affect ecosystem nitrogen fate and cycling? To answer these questions, the team will (1) identify traits and interactions among archaea, bacteria, fungi, and viruses that affect nitrogen cycling genes and biogeochemistry in all three ecosystems using DNA sequencing datasets and laboratory experiments; (2) test and refine these insights through experiments with highly controlled and replicable fires in pyrocosms to simulate soil heating; and (3) scale up microbial traits and interactions to the ecosystem level by integrating the measurements with a computational model that accounts for microbial behaviors that impact nitrogen cycling.

Microbiological insights assessed via advanced genomics sequencing techniques of both DNA (to examine who is there) and RNA (to examine their functions) will be paired with biogeochemical approaches that combine laboratory assays with isotopic measurements to track the production and consumption of compounds involved in greenhouse gas emissions. Experimental approaches and modeling will span microbial kingdoms and even domains (archaea, bacteria, fungi, and viruses) and diverse fire-impacted ecosystems to assess how these results can be extrapolated at broad spatial and temporal scales. Recent catastrophic wildfires have alarmed scientists, policy makers, land managers, and the public, and there is growing public awareness of the role of microbiomes in human and environmental systems. The need to understand how soil microbiomes respond to fire severity and change over time has never been more urgent.

This project will train early career scientists to provide critical information on the traits of pyrophilous microbes and improve predictions of fire impacts on nitrogen cycling and greenhouse gas emissions while simultaneously identifying unifying controls of microbial responses to wildfire. Project results can be leveraged to improve management of post-fire landscapes, which are increasingly common with environmental change.

Friends and Foes: Microbial Interactions and Soil Biogeochemistry After 23 Years of Experimental Warming

- **Principal Investigator:** Bruce Hungate (Northern Arizona University)
- **Co-Investigators:** Egbert Schwartz, Paul Dijkstra, Benjamin Koch, and Michelle Mack (Northern Arizona University); Ember Morrissey (West Virginia University); Jennifer Pett-Ridge and Steven J. Blazewicz (Lawrence Livermore National Laboratory); Kirsten Hofmockel (Pacific Northwest National Laboratory); Javier A. Cejas-Navarro (Lawrence Berkeley National Laboratory)

This project will take a systems-level approach to quantify how microbial community interactions, particularly predation and cooperation, affect both soil carbon responses to warming and energy flow in soil. The specific objectives of the project are (1) to test how 23 years of climate change alters microbial interactions in the field and affects the fate and formation of soil carbon by integrating quantitative stable isotope probing, omics, and a carbon tracer; (2) to quantify microbiome interactions that change the biochemical and assemblage-scale efficiency of carbon use and its fate in soil using experiments that manipulate temperature, predation, and water availability; and (3) to infer ecological interactions using multilayer network analysis, machine learning (ML), and ecological models. This project will investigate the role of ecological interactions within the soil microbiome and its role in soil carbon cycling and persistence. The work is set in the context of a long-term warming experiment in which a final soil harvest is planned for 2024, 23 years after the experiment began.

The project will harness the power of isotope tracers and omics (e.g., metagenomics, metatranscriptomics, metabolomics) to test the long-term impacts of climate change (temperature × precipitation) on ecological interactions and soil carbon cycling and persistence. Objective 1 will be met by conducting field measurements as part of a long-term climate change study (temperature × precipitation) in a montane meadow that was initiated in 2002. In this experiment, massive loss of soil carbon and functional changes in the microbiome suggest that both cooperative (between friends) and antagonistic (between foes) microbial interactions mediate soil carbon cycling and persistence. Objective 2 will involve focused laboratory experiments to test hypotheses about the short- and long-term effects of climate change as mediated by the direct effects of temperature and water availability as well as indirect effects mediated by changes in microbial interactions. The focus on short- and long-term effects addresses the interpretive challenge of warming impacts that change over time. This project will advance the field by clarifying the roles of microbial interactions in such sequential responses. Objective 3 focuses on synthesizing multiple data streams simultaneously using existing multilayer analytical tools to infer (1) interactions in the microbiome, (2) the strong threads through the complex web of the microbiome that indicate biological pathways, and (3) the controls over energy and carbon flow. Objective 3 will also develop new approaches that support combining ecological models with ML.

Microorganisms are major engines of the land carbon cycle, influencing the composition and radiative properties of the atmosphere by both creating and consuming soil organic carbon at a global scale. Soil organic carbon provides multiple ecosystem services, and when respired, exacerbates climate change. This work investigates the interactions in microbial communities that underpin these dual roles of microorganisms in creating and consuming soil carbon. The project will push the frontier of isotope-enabled genomics by connecting isotope-informed omics to theory about interactions in eco-

logical communities and their effects on soil carbon cycling and persistence.

How Microbes and Minerals Make Necromass that Persists

- **Principal Investigator:** Kristen DeAngelis (University of Massachusetts, Amherst)
- **Co-Investigators:** S. Emil Ruff (Marine Biological Laboratory); Manuel Kleiner (North Carolina State University); Melanie Mayes (Oak Ridge National Laboratory)

Sometimes soil carbon persists for a long time, but scientists don't know enough about this process to predict the long-term storage of carbon. Microbial necromass is generated when new organic matter enters soil through plant detritus, and similar amounts enter soil through the continuous recycling of nutrients by microbial turnover. This microbial necromass represents a steady stream of new organic matter to soil, but new necromass is very sensitive to decomposition and loss as CO₂. Surface attachment to minerals and other organic matter is the prevailing theory on the mechanism of soil carbon stabilization, but reactive surfaces and soil pores reduce bioavailability of nutrients that drive microbial turnover. The big question is: How do microbes and minerals make necromass that persists? This team will take a trait-based approach to test an overarching hypothesis that necromass biomolecules are sensitive to decomposition depending on microbiome traits, nutrient bioavailability, and soil types.

This project includes three laboratory experiments designed to test the team's hypothesis. All three experiments use four biomolecules (carbohydrates, lipids, nucleic acids, and proteins) as substrates that represent the majority of necromass composition and vary in nutrient (nitrogen and phosphorus) content. By studying necromass biomolecules in combination with ¹³C and/or ¹⁸O labels, researchers can identify the distinct microbiomes active in transforming these four major classes of necromass. Objective 1 is to define the microbial populations capable of degrading necromass macromolecules. Researchers will selectively enrich soil microbiomes with carbohydrates, lipids, nucleic acids, and proteins as the sole carbon source. By reconstructing genomes from the enrichment cultures, the team can identify the pathways used to degrade the macromolecules under oxic or fer-

mentative conditions. Metaproteomics will describe how these pathways vary by substrate or energy source, and enzyme kinetics will help to model nutrient-specific pathways. Objective 2 is to define the mineral and microbial traits that drive necromass decomposition. To do this, researchers will generate mature model soils that vary in pore size or clay activity and then add macromolecules (carbohydrates, lipids, nucleic acids, and proteins) and use ¹⁸O-H₂O for stable isotope probing-metaproteomics. This will define the pathways and populations active across soil and macromolecule types as well as substrate-specific carbon use efficiency. Objective 3 is to define the food webs and fate of necromass macromolecules over time. To do this, the team will study natural soils from a temperate deciduous forest in New England where long-term experimental warming has induced alternative nutrient availabilities compared to their controls. ¹³C-labeled substrates will be traced through food webs, into soil compartments, and into stable, microbial, or mineral (CO₂) fates.

This work also includes novel biochemical extraction, quantification, and identification of the extracellular enzymes involved in decomposition. The final objective is to use statistical and mechanistic modeling to integrate microbiome dynamics in soil carbon dynamics. Statistical modeling using path analysis will define latent variables for yield (growth), resource acquisition (extracellular enzymes), and stress (biofilms and surface association) as well as test the strength of these traits in driving necromass decomposition and the extent to which they are linked to necromass persistence. Mechanistic modeling will incorporate proteomic and biogeochemical data into the Continuum Microbial Enzyme Decomposition (CoMEND) model, a carbon, nitrogen, phosphorus (CNP) model derived from the MEND model that applies the principles of soil organic matter decomposition to CNP-associated substrates in a defined enzyme cascade. This model will predict systems-level carbon cycling (e.g., the fate of organic carbon over time). Incorporating data from simple to complex soil microbiomes will help define how carbon, nitrogen, and phosphorus availability contribute to the microbes and minerals that make necromass persist. Key deliverables include gene-centric and genome-centric analyses of soil populations involved in necromass biomolecule degradation; enzyme

kinetics specific to soil carbohydrate, lipid, nucleic acid, and protein degradation; a novel approach for quantifying and characterizing intracellular versus extracellular enzymes in soils using selective extractions coupled to soil metaproteomics; and trait-based predictive statistical and mechanistic models to define the microbial, metabolic, and mineral processes controlling necromass stabilization and long-term persistence.

Bacterial-Fungal Interactions: Bridging Soil Niches in Regulating Carbon and Nitrogen Processes

- **Principal Investigator:** Nhu Nguyen (University of Hawaii)
- **Co-Investigators:** Mengting Yuan (University of California, Berkeley); Jennifer Pett-Ridge (Lawrence Livermore National Laboratory)

Life in the soil is all about interactions. Fungi and bacteria are the two dominant groups of soil organisms that consume, process, and translocate plant-derived organic matter and thus are critical to global nutrient cycling. Fungal hyphal networks are important gateways for carbon and nutrient exchanges between plants and soils, and there is an increasing recognition that such processes are co-mediated by their interactions with bacteria. Yet, the understanding of these interactions has generally been correlative, and mechanisms of these interactions in the context of nutrient cycling are far from understood. In this project, researchers aim to build a quantitative and mechanistic framework of how bacterial-fungal interactions (BFIs) can change the availability and fate

of carbon and nitrogen across the complexity of soil niches in different soil types and abiotic conditions. The team will address three objectives: (1) Characterize how grassland BFIs are shaped by the availability of different carbon sources; (2) understand whether and how BFIs mediate carbon stabilization and destabilization in soils of different mineralogies; and (3) quantify how drought interplays with carbon sources, carbon availability, and soil mineralogy in influencing BFIs and BFI-mediated soil processes. Researchers will use an iterative approach (scaling from field to mesocosm to simplified microcosm experiments) to study the influence of BFIs on soil carbon and nitrogen mineralization, stabilization, and fluxes under the context of different soil mineralogy and drought. ^{13}C and ^{15}N will be utilized as stable isotope tracers to characterize the fungal-bacterial interactome that is relevant to soil carbon and nitrogen processes. The team will follow these processes through isotope-assisted metagenomics, metatranscriptomics, metabolomics, and quantitative and data integration tools using network analysis and omics-informed, niche-identified ecosystem modeling. The project will address the gap resulting from the over-simplified culture-based BFI studies and the mainly correlative field surveys of fungal-bacterial co-occurrence. Leveraging the above methodologies, researchers aim to identify the mechanisms important to BFIs across broad grassland ecosystems, quantify carbon and nitrogen dynamics, model interactions in natural soil environments, and use this powerful dataset to better predict terrestrial carbon and nitrogen cycling under climate change.



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