Approved Proposals FY23

Following are the approved user proposals for Annual CSP, FICUS JGI-EMSL, CSP Functional Genomics and CSP New Investigator calls.

FY 2023 Annual CSP Proposals

Proposer	Affiliation	Proposal Title	Proposal Description
Blonder, Benjamin	University of California at Berkeley	Identifying the genetic basis of complex phenotypes and climate adaptation in quaking aspen (Populus tremuloides)	Quaking aspen (Populus tremuloides) is the most widely-distributed tree species in North America, and is one of the iconic species of the West. The species has high economic, ecological, and bioenergy value. This project is a collaboration between scientists in the USA, Mexico, and Canada. It will generate a complete genome for the species, and will also generate sequencing data for thousands of individuals from across the species range, including relictual southern populations whose genetic information may be critical for climate

			adaptation and climate-smart management of this species.
Chen, Jay	Oak Ridge National Laboratory	Leveraging Natural Variations to Uncover Regulatory Mechanisms Governing Differential Biosynthesis of Terpenes in Populus	We propose to perform transcriptomics sequencing of leaf buds in 500 Populus trichocarpa natural variants to enable expression quantitative trait nucleotide mapping to identify genetic elements regulating terpene chemistry and bud phenology. The proposed research can provide new strategies for engineering terpene metabolism to produce terpenes as advanced biofuels and valuable bioproducts. The proposed research can also lead to new insights into the control of bud dormancy and enable new approaches for genetic engineering of dormancy-associated traits to enhance biomass production.

DeAngelis, Kristen	University of Massachusetts Amherst	Soil microbial stress-biogeochemistry metabolism adapts under climate change across seasons	Air temperatures are rising, winter snowpack is shrinking, and soil freeze/thaw events are increasing in high-latitude ecosystems. This research is designed to improve mechanistic understanding of how the combined stress of warming during the growing season and soil freeze/thaw cycles in winter impacts microbial biogeochemistry traits in northeastern forests. Our work integrates metagenomics, metatranscriptomics and metabolomics to study soil microbial communities at the Climate Change Across Seasons Experiment (CCASE) at the Hubbard Brook Experimental Forest (HBEF).

Dinneny, Jose	Stanford University	Understanding the mechanisms of metabolic exchange in the rhizosphere	The root system of plants represents a complex interface for microbial interactions. While much progress has been made in understanding specific symbiotic interactions between plants and microbes, much less is understood regarding the basic support functions that roots play in sustaining carbon delivery to the rhizosphere or in the functions that specific cell types in the root play in mediating such interactions. Here we utilize a genetic approach and a synthetic biology approach to manipulate the interfaces that root microbes interact with and the availability of key nutrients that likely determine the selective pressures that microbes experience within the rhizosphere. The use of JGI resources will allow us to better understand how these genetic manipulations ultimately affect the metabolic and transcriptional pathways that mediate such interactions.

Humphries, Jacqueline	Amyris, Inc.	A high-throughput, multi-omics approach to identifying new gene expression modules for industrial bioproduction in alternative fermentation hosts	Amyris is a company that engineers microbes for sustainable conversion of plant biomass into valuable molecules. We propose a high-throughput, sequencing-driven approach to designing new genetic tools for engineering less well-studied, yet industrially attractive microbial species. This work will, in alignment with the DOE mission, accelerate bioproduct development by expanding the tools we have to engineer a broader range of microbes and improve our understanding of physiology in these species of major interest to the scientific community.

Krasovec, Marc	National Center for Scientific Research (CNRS)	Phytoplankton spontaneous mutation rate	Mutations are the ultimate source of diversity and define the adaptive potential of species. The aim of this proposal is so to understand the evolution of the mutation rate and its variation with environmental changes in phytoplankton species. This will bring essential knowledge on phytolankton evolution and improve the genomic ressources by giving the map of genomes diversification.
Leewis, Mary-Cathrine	Agriculture and Agri-Food Canada	Life in Ancient Permafrost: using an isotope and ‘omics approach to determine how microorganisms survive and metabolize in subzero temperatures across geologic time	Permafrost is a layer of frozen soil that underlies about 25% of the Northern Hemisphere and contains almost half of the Earth’s soil carbon. Permafrost soils host a diverse microbial community however, we do not understand if and how those microorganisms survive and metabolize below freezing for millennia. We conducted a stable isotope probing experiment to track the activity of microorganisms that are metabolically active (18O-labeled) and/or involved in

			decomposition of labile carbon (13C-labeled) in permafrost from an age gradient of 5,000 to 33,000 years old.
Lofgren, Lotus	Duke University	Functional roles of secondary metabolism in ectomycorrhizal fungi	Despite the importance of Ectomycorrhizal fungi, we know little about how they interact with complex consortia to establish and maintain symbiosis. Using constructed communities of the Ectomycorrhizal genus Suillus, we are investigating the role of fungal secondary metabolites in 1) host-interfacing and mycorrhization, 2) competition via the production of antimicrobials and antifungals, and 3) the regulation of biotic and abiotic stress tolerance, using genome-informed transcriptomics and metabolomics.

Majumder, Erica	University of Wisconsin-Madison	Characterization of plastic deconstruction metabolic pathways in microbial communities derived from enrichments of plastic debris in soil and landfill samples	We propose the use of -omics to understand plastic-metabolizing microbial communities inhabiting soil and landfill debris samples. This project is related to the CSP FY2023 area of Biofuels, Biomaterials and Bioproducts because it implicates nutrient cycling in environmental microbiomes, i.e., plastic degradation in soil and landfill communities. This fits into the DOE mission for bioenergy by enabling sustainable processes for plastic degradation/upcycling and understanding plastic degradation in environmental microbiomes.

Nagy, Laszlo	Biological Research Centre of the Hungarian Academy of Sciences	A genome-wide view of the evolution of the most widely used lignocellulose-degrading Basidiomycota	Wood-decay fungi are primary contributors to the global carbon cycle by reintroducing organic carbon sequestered in plant biomass into the atmosphere. In this project we will analyse genomes and interrogate lignocellulose degradation of two of the most widely cultured groups of fungi worldwide, Agaricus spp. (button mushrooms) and Pleurotus spp. (oyster mushrooms). We anticipate that this project will contribute to a better understanding of plant biomass degradation by fungi and to harnessing this potential for transitioning to a greener and circular economy.

Nunn, Brook	University of Washington	Investigating the interactions of a phytoplankton community and its microbiome on a 4-hour timescale to reveal emerging and predictive properties across an algal bloom and bust cycle	Marine phytoplankton generate ~50% of the world’s oxygen. Understanding what initiates and terminates phytoplankton blooms is critical to generating accurate global carbon models for forecasting Earth’s future. Phytoplankton evolved in association with bacteria and their interactions potentially control and predict bloom dynamics. Here, we plan to leverage the first ever sample set of bacteria and phytoplankton collected every 4 hours across an entire phytoplankton bloom (from initiation to termination) to identify community interactions and molecular-level controls on algal bloom events.

Ohm, Robin	Utrecht University	Functional genomics of the lignocellulose-degrading fungus Schizophyllum commune: regulatory networks, sustainable fungal materials and fungal defense	Mushroom-forming fungi are among the most potent lignocellulose-degraders. In this project, we will study their regulatory network and the proteins they use to defend against competitors. Moreover, we use these fungi to make sustainable materials, such as leather-like and foam-like compounds. We will sequence several strains with properties that are beneficial to these fungal materials.
Pawlowska, Teresa	Cornell University	Unraveling the mechanisms behind the role of endosymbiotic bacteria in community structuring and evolution of Mucoromycota fungi	The goal of the project is to understand the mechanism allowing bacteria to live and persist inside cells of soil fungi over many generations. In addition, we propose to test several predictions concerning the roles of these symbiotic bacteria in: (1) diversification of their fungal host populations into new species and (2) structuring of ecological communities of their hosts. Our focal fungi include, among others, molds causing food spoilage and human diseases as well as

			beneficial associates of plant roots provisioning their hosts with mineral nutrients.
Saleska, Scott	University of Arizona	Primary succession of plant and microbial life: untangling inter-organismal interactions on a model early-successional landscape	We are investigating how biogeochemical cycles emerge from small-scale hydrological, geochemical, ecological and evolutionary processes interacting to create emergent landscape-scale terraformation. We focus on how microbial composition and function changes, in interaction with plants, as biological complexity increases: from simple microbial communities (including microbial crusts), to non-vascular plants (mosses without roots), to vascular plants with roots and sophisticated hydraulic architectures. This work thus illuminates pressing issues such as landscape restoration and carbon storage.

Wakao, Setsuko	Lawrence Berkeley National Laboratory	Evolutionary genomics of biomineralizing stramenopiles with impacts on global carbon cycling and biogeochemistry	Diatoms are an important group of oceanic algae that make intricately patterned silica cell walls and are responsible for 40% of the net photosynthesis that occur in the oceans making them a critical player not only in carbon cycling but also elemental cycling (Si) in the environment. In this work, we will sequence and compare the genomes and transcriptomes of algae from several related groups that produce silica biominerals to discover genes important for silica biomineralization.

Weimer, Bart	UC Davis	The role of carbohydrate and nitrogen fixation for sustainable plant microbiome interactions	The atmosphere is comprised of approximately 78% nitrogen, but crops cannot use this form of nitrogen directly. They require a microbial partner to convert atmospheric nitrogen into a biologically accessible form of nitrogen for use in growth. Legume crops, such as soybean and alfalfa, do this via an endo-symbiotic relationship with a bacterium. However, production of the major cereal crops relies on synthetic fertilizer. Identification of cereal crops with the ability to fix atmospheric N2 has been the “holy grail” of crop biologists for decades since this trait would potentially alleviate the need for synthetic fertilizers resulting in economic and environmental benefits.

Wilbanks, Elizabeth	University of California Santa Barbara	The role of population distributed immunity in the eco-evolutionary dynamics of bacteria and phage	In our proposal, we are looking how two different “bacterial immune systems” – known as CRISPR-Cas and diversity generating retroelements – evolve in natural populations of salt marsh bacteria. Our work will help us better understand how the battle between microbes and their phage unfolds, make better predictions about the future climate, and engineer new ways to produce renewable fuels and green chemicals.
Wilhelm, Steven	University of Tennessee, Knoxville	Direct resolution of virus-host interactions using bulk single-celled labeling and application to deep community metatranscriptomics	The project takes a recently developed approach that allows for single-cell resolution of hundreds to thousands of cells within a mixed community and uses it to ask which cells in nature are infected by viruses. Moreover, along with determining who is infected, the results will demonstrate which type of virus(es) are infecting which types of cells and provider insight into how the biochemistry and metabolism of all these different cell types change when infected. The end product of this

			research will provide new insight into how viruses shape the biogeochemistry of various ecosystems.
Wolfe, Marnin	Auburn University	Clover Genomics for Sustainable Bioenergy Mixtures	Adverse impacts of agriculture and climate change makes sustainable and economical bioenergy and food production urgently needed. Legumes offer a way to reduce fertilizer inputs because of their ability to biologically fix atmospheric nitrogen. In this project, we are developing genomic resources for clovers, genus Trifolium, one of the most important and broadly adapted legume genera. By bringing clover genomics up-to-par with bioenergy grasses, we seek a route forward for breeders and geneticists to develop bioenergy intercrops that produce more with less environmental input and expense.

FY 2023 Facilities Integrating Collaboration for User Science (FICUS) JGI-EMSL Proposals

Proposer	Affiliation	Proposal Title	Proposal Description
Bhatnagar, Jennifer	Boston University	Linking soil microbial stress metabolism to watershed biogeochemistry under climate change across seasons	With rising temperatures stemming from climate change, winter snowpack is shrinking and soil freeze and thaw events are increasing in high-latitude ecosystems. The project aims to improve understanding and model representation of the effects of warming during the growing season and soil freeze/thaw cycles in the winter for belowground biogeochemical cycles in northeastern forests.
Blazewicz, Steve	Lawrence Livermore National Laboratory	Parched: quantifying microbial ecophysiology and the fate of plant carbon during soil dry down	In seasonally dry grasslands, soil microbes bloom during the growing season, survive extremely dry periods, and rapidly mineralize soil carbon after the first rain in the wet season. This project will study the “dry-down” transition between seasons to determine how soil moisture shapes microbiome interactions, ecophysiological traits, and trait expression that affects the fate of cellular carbon.

Dalcin Martins, Paula	Radboud University (Netherlands)	Elucidating the impacts of viruses on soil organic carbon and greenhouse gas emissions from agricultural peat soils	Despite viruses being the most abundant biological entities on Earth, soil viruses are poorly characterized and their impacts on the biogeochemical cycles are vastly unquantified. The project aims to understand and quantify the impacts of viruses on soil organic carbon dynamics and carbon dioxide, methane, and nitrous oxide fluxes from agricultural peat soils.
Hug, Laura	University of Waterloo (Canada)	Microbial impacts on methane emission hot spots from municipal landfills	Landfills in Canada contribute 20% of methane emitted annually. Hot spots of methane emissions at landfills, associated with infrastructure that puncture cover soils, are unmonitored and not included in emissions models. This project aims to identify key factors and microbial populations that improve efficiency of methane oxidation to improve waste management protocols.

Hurley, Jennifer	Rensselaer Polytechnic Institute	Investigating the Effect of Negative Arm Protein Conformation on the Circadian Post-transcriptional Regulation of Cellulases	Fungal cellulases have the potential to be a major resource in biofuel production. Circadian timing over cellular metabolism could be tuned to maximize cellulase production. This project aims to map the clock “repressive complex” conformational shifts over the circadian day to analyze their effect on circadian regulation of biofuel production.
Kostka, Joel E	Georgia Institute of Technology	Metabolic exchange between *Spartina alterniflora* and sulfur chemosymbionts of the plant’s root microbiome	While root zones of coastal wetland plants are known hotspots for carbon and nutrient cycling, little is known about how plant–microbe interactions regulate coastal ecosystem function. The project aims to characterize the exchange of carbon and nitrogen that govern plant–microbe interactions in Spartina alterniflora—a dominant plant along the Gulf of Mexico and Atlantic coast.

Nelson, William C	Pacific Northwest National Laboratory	Environmental drivers of Inter-Kingdom metabolic interaction in marginal soil rhizosphere	Soils present a complex environment with numerous competing and cooperating populations that contribute to an overall ecosystem. The project aims to extend investigations into how soil moisture and seasonal changes affect carbon metabolism in marginal soils by quantifying and modeling the bacterial, archaeal, fungal, and viral community compositions and interactions in the rhizosphere.
O’Malley, Michelle	University of California, Santa Barbara	Deploying Advanced Molecular & Cell Free Expression Tools to Accelerate Characterization of Fungal Cellulosomes	Cellulosomes enable the breakdown of plant biomass into fermentable sugars. They are promising biotechnology tools to drive lignocellulosic conversion. While some sourced from fungi are known to host a wide diversity of enzymes, their diversity hampers structure determination via different methods. The project will pursue a two-pronged approach to enable structural characterization of fungal cellulosomes.

Umen, James	Donald Danforth Plant Science Center	Histone Methylomics and the Chromatin Landscape of Chlamydomonas reinhardtii	The genome for Chlamydromonas, a type of green algae, encodes 52 SET domain proteins, which are a family of lysine methyltransferases—enzymes that catalyze the transfer of methyl groups. Only two SET domain proteins have been biochemically characterized as Chlamydomonas. The project aims to identify and characterize methyltransferase domain enzymes in Chlamydomonas responsible for histone methylations.
van Munster, Jolanda	Scotland's Rural College	Elucidating temporal-spatial patterns in lignocellulose degradation by morphologically distinct anaerobe gut fungi	Effective mechanisms to disassemble raw lignocellulose into simple sugars are required to advance biotechnology for biofuels and biomaterials. This project aims to understand how the powerful degradative mechanisms of anaerobic gut fungi affect the composition and structure of crude lignocellulose from wheat straw, a common biofuel feedstock.

CSP Functional Genomics Proposals

Proposer	Affiliation	Proposal Title	Proposal Description
Blaudez, Damien	University of Lorraine	Leveraging functional genomics to expand the understanding of metal tolerance mechanisms in plant mutualistic fungi	The two families of Cation Diffusion Facilitators (CDF) and Metallothioneins (MT) are primarily known to be essential for metal tolerance in fungi and other eukaryotes. The CDF are the major class of metal transporters involved in the detoxification of Zn, Fe, Mn, Co in eukaryotes, while MT are involved in metal-chelation (essentially Cu, Cd) in cells, preventing their toxicity. In this project a functional genomics approach will be employed to better understand the gene function relationship of these two distinct gene families involved in metal tolerance. The broader impacts of this study include indispensable knowledge of the genetic basis of metal tolerance handling in these beneficial fungi for phytoremediation of metal-contaminated sites and for improving associated production of clean biomass from plants inoculated by highly tolerant fungal symbionts.

Booth, Thomas	DTU	DAP-sequencing for Plant-Streptomyces interactions	Streptomyces are soil bacteria that live in a range of environments, including among the roots of plants. Streptomyces living within these environments can serve as important partners and can influence the growth of plants. The interaction between the Streptomyces and the plant are mediated by a range of small molecules. The production of these molecules is controlled by proteins called transcription factors (TFs). TFs bind to regions of DNA, acting as switches to turn the production of specific molecules on or off. Therefore, where these transcription factors bind is important to understanding the ecological role of different molecules. This project will allow us to identify the binding sites of 92 TFs in 12 different strains of Streptomyces. This research will lay the foundation for understanding plant-Streptomyces interactions. In the future, we will be able to use this knowledge to develop technologies to increase crop yields (e.g. for biofuel feedstocks).

Castrillo, Gabriel	BBSRC-UKRI	Metabolic barriers controlling root microbiota	The ability of microbes to establish beneficial interactions with plants is of critical importance for plant to adapt to different environments. Still we don’t know the details of the “filtering” mechanisms plants used to maintain optimal combinations of microbes in the roots. With the proposed project we will study what is the contribution of changes in duckweed root metabolic profiles on the structure of the microbiota and estimate the impact of certain root metabolites on the root microbial composition. This project will open new avenues for the design of microbial-based strategies aim to optimise root function under stressful environmental conditions.

Li, Han	UC Irvine	Enabling Scalable Redox Reactions in Biomanufacturing	Microbes with an engineered metabolism can convert renewable resources such as CO2 and biomass to biofuels and chemicals, curtailing the societal demand for fossil fuels. This project aims to establish a toolkit to augment Nature's capability of producing biofuels and biochemicals via introducing artificial driving forces. We have demonstrated the proof-of-concept of this approach. Now we aim to drastically expand the application of this technology towards the production of a wider range of fuels and commodities with unprecedented carbon efficiency. Therefore, the outcome of this project will have a broad impact on the metabolic engineering and synthetic biology community.

Liu, Yi	University of Texas Southwestern Medical Center	Synthesis of reporter gene variant libraries to determine functions of codon usage in gene expression	Proteins are made of defined sequences of different amino acids, which are encoded by different genetic codons. Most amino acids can be encoded by two to six different genetic codons called synonymous codons. Synonymous codons are not used with equal frequencies in all organisms and different organisms have different preferences to use certain synonymous codons, a phenomenon called codon usage bias. In this proposal, we plan to synthesize large random libraries of reporter genes that encode for the same protein but with different synonymous codon profiles. Our study will allow the determination of gene design principles that can optimize production of any protein. This knowledge will play an important role in future design of human vaccines, production of protein therapeutic drugs and any protein-based products need to biofuel production, and the understanding of gene regulatory networks from micro-organisms to human cells.

Marchand, Jorge	University of Washington	Function beyond Nature: Discovery of xenonucleotide kinases for emerging synthetic biology applications	Researchers seek to add additional letters to life's instruction book by using chemically synthesized DNA bases, opening an opportunity for a 12-letter DNA alphabet (A, T, G, C, B, S, P, Z, X, K, J, V). These synthetic bases are colloquially known as XNAs (Xenonucleic Acids), and they can endow biotechnology with new functions, and work alongside the standard nucleic acids. To integrate XNAs into our biotechnology landscape, we must therefore discover nucleotide kinases that can activate XNAs. We believe that undiscovered nucleoside/tide kinases that can activate XNAs exist in Nature. Research previously conducted on nucleoside/tide kinases has shown that they are capable of working with many different modifications in the chemical structure of nucleic acids. Motivated by these previous observations, we are proposing to screen many nucleoside/tide kinases from different organisms (viral, bacteria, archaea, and eukaryote) to find ones that are compatible with XNAs.

Marsh, Neil	University of Michigan	Surveying the diversity of prenyl-FMN-dependent (de)carboxylase enzymes	This proposal aims to discover new enzyme-catalyzed reactions that may be useful in the production of biofuels and chemical feedstocks from renewable resources. Because enzymes are proteins that work in water, at neutral pH and at ambient temperatures they are inherently more environmentally friendly than most of the chemical processes currently in use. The family of enzymes we are investigating are known as prenylated-flavin-dependent decarboxylases (these are often known by their abbreviation as UbiD enzymes). These are a very recently discovered family of enzymes found in bacteria and fungi. Depending on the conditions, UbiD enzymes can attach carboxyl groups to otherwise unreactive aromatic molecules – this activity makes them useful for chemical synthesis applications. They can also remove carboxyl groups from various common aromatic carboxylic acids, which makes them useful for the synthesis many commodity chemicals and biofuels.
Özdemir, Emre	Center for Biosustainability	Enabling design and development of platform Streptomyces strains for bioproduction through transcription factor mapping	We want to improve our methods of getting the chemicals we want from the bacteria we have. We work with Streptomyces; they are very good at making different chemicals, but they also have complex genomes. We want to understand how these genomes control what chemicals the bacteria make, and how we can change them to make new chemicals at a high yield. We want to find out how these factors work together and influence the chemical production. We have two goals: one is to make new or improved chemicals for agriculture, and

			the other is to make improve our capabilities for biomanufacturing.
Wong, Fong Tian	Agency for Science, Technology and Research	New Characterization and Engineering of Bacterial Transcriptional Activation Domains for Metabolite Regulation	This proposal capitalizes on our profound expertise in microbial engineering, backed by cutting-edge computational power and multiplex DAP-seq (DNA affinity purification sequencing) technology. Our primary goal is to predict and characterize transcription factors (TFs) across genomes, enabling a rapid and comprehensive understanding of TF regulation within microbes. This valuable knowledge will pave the way for innovative hypotheses in microbial engineering, propelling a revolutionary wave of sustainable biomanufacturing applications. By delving into the intricate interactions within biological systems, particularly for biomanufacturing, valuable insights can be uncovered to optimize the capabilities of actinobacteria and other microbial cell factories.

CSP New Investigator Proposals

Name	**Affiliation**	**Proposal Title**	Proposal Description

Bell, Emma	KTH Royal Institute of Technology (Sweden)	Long-term dynamics of virus-host interactions in a brackish coastal ecosystem, the Baltic Sea	Coastal ecosystems are of high importance to society supporting both industry and recreation and they contribute significantly to carbon and nitrogen cycling globally. These ecosystems are under increasing pressure from human activity (nutrient loading from agriculture and industry) and climate change (rising sea temperatures and acidification). In the Baltic Sea, this has led to an increase in the size and frequency of phytoplankton blooms that can be harmful to water quality, marine biodiversity, and human health. We aim to understand the extent to which viruses contribute to the collapse of these blooms compared to other factors (e.g., temperature, light, or nutrient limitation) and the role of viruses in the coastal ecosystem. To do this it is important to link viruses to the microbes they infect, we will therefore explore both microbial and viral datasets spanning several years of regular sampling in the Baltic Sea. The results are important for understanding the importance of viruses in global element and nutrient cycles and are of use for future ecosystem management decisions affecting coastal waters.

Boehm, Alexandria	Stanford University	Diversity and activity of chemoautotrophic nitrifier communities across physicochemical gradients in the subterranean estuary	Subterranean estuaries (STEs) within coastal aquifers play a critical role at the interface of terrestrial and coastal ecosystems. The mixing zone of freshwater and seawater within the subsurface of sandy beaches is a natural “biogeochemical reactor” where the oxidation state and form of nutrients, trace metals, and carbon (C) are readily transformed along the steep gradient in salinity. The groundwater salinity can transition from freshwater to marine in <5-10 m, which has important ramifications for geochemistry and microbiology. However, there is a scarcity of knowledge pertaining to the microbial ecology and biogeochemistry of STEs. No study to date has used modern meta-omic approaches to characterize the STE microbiome. Previous studies have been limited to 16S rRNA gene amplicon sequencing or targeted sequencing of specific functional genes for denitrification (nirK and nirS) or nitrification (amoA). There is a critical need to understand the microbiome (microbial diversity and metabolic function) of the STE; this will lead to a better understanding of the overall contribution of beach aquifers to biogeochemical cycling on the planet, and how their disappearance via sea level rise might affect global elemental cycling.

Bunbury, Freddy	Carnegie Institution for Science	Photosynthesis at high temperatures: genetic and phenotypic underpinnings of thermotolerance in cyanobacteria	
Chaput, Gina	University of California at Davis	The assembly rules of the seagrass microbiome: Host, priority and priming effects on Zostera marina microbiome as a comparative system for terrestrial and aquatic plants	Our overarching goal is to advance the understanding of plant-microbe interactions during seedling development and microbiome establishment. We propose three EcoFAB pilot experiments that will enable us to study the assembly dynamics of the seagrass microbiome. Using the seagrass species, Zostera marina as a model system, we aim to define how plants recruit and retain their microbiome in diverse environmental habitats.

Choudoir, Mallory	North Carolina State University	Linking soil microbial metagenomic responses and carbon fluxes across seasonal variation and between long-term land use regimes	Climate resilient land management is critical for maintaining productive and sustainable agroecosystems. Diverse microbial communities moderate the exchange of carbon between the soil and the atmosphere, and climate change and increasing extreme weather events threaten the capacity of soils to act as a natural climate change mitigation resource. This project will compare the functional genomic potential of microbial communities from agricultural fields managed differently for over two decades and across a time series capturing a heavy rainfall event. We will link microbial community genomic features with carbon dioxide emission rate measurements to identify and quantify microbial traits predictive of greenhouse gas emissions and the global carbon cycle. The goal of this project is to develop a predictive framework for understanding microbial drivers of soil nutrient loss in managed agroecosystems in a changing world.

Couvillion, Sneha	Pacific Northwest National Laboratory	Uncovering the Small Molecule-Mediated Interactions in the Rhizosphere: Investigating the Impact of Plant Exudates on Microbial Community Dynamics and Metabolism	The rhizosphere hotspot for diverse and dynamic microbial communities. These microorganisms include bacteria, fungi, and other tiny creatures that live in and around plant roots. Many of these microorganisms are beneficial to the plant, helping it absorb nutrients from the soil or protect it from harmful pathogens. In turn, the plant provides these microorganisms with a source of food and shelter. This mutualistic relationship between plants and microorganisms is essential for healthy soil and plant growth, and studying the rhizosphere can help us understand how to better support sustainable agriculture and ecosystem health.. We know that plant release small molecule compounds through their roots and these molecules can impact microbial metabolism in the soil. On the other hand, microorganisms can also release their own molecules that help the plant grow and be resilient to environmental stress and disease. However, we still don’t know exactly which molecules are involved and how they work together. Our research aims to figure out how these small molecules facilitate interactions and how they impact the activity of microorganisms in the soil around plants. Studying plant-microbe interactions can help us better understand how plants and microorganisms interact with each other and with their environment. This knowledge can be applied in

many ways, such as developing more sustainable agricultural practices, improving plant productivity, and mitigating climate change. This research fits with the mission of the Department of Energy (DOE) Biological and Environmental Research (BER) program, which aims to advance our understanding of complex biological and environmental systems and develop new technologies to address energy and environmental challenges. The study of plant-microbe interactions is relevant to several of the BER program's research areas, such as terrestrial ecosystems, biogeochemistry, and microbial genomics. By studying these interactions, we can gain insights into how living systems function and develop new tools and strategies to improve sustainability and resilience in the face of environmental change.

Fletcher, Jessica	University of Colorado at Denver	Zinc tolerance in the Pinus contorta-Suillus tomentosus ectomycorrhizal system	We propose to study metal tolerance in the Suillus-pine ectomycorrhizal system using a combination of genomic and metatranscriptomic sequencing approaches. We hypothesize that metal tolerance in Suillus species arises by convergent evolution and that American S. tomentosus displays independent but similar metal tolerance patterns as European S. luteus. We also expect metal tolerant S. tomentosus to shield Pinus contorta from zinc stress. Our goal is to unveil the genetic basis of zinc tolerance in S. tomentosus and its role in protecting P. contorta from metal toxicity. This project is part of our long-term goal of investigating environmental adaptation in mutualistic associations.

Fresnedo Ramirez, Jonathan	Ohio State University	Biopolymer prediction in Taraxacum kok-saghyz to support inulin-derived biofuels	The goal of this proposal is to develop a predictive model for the final concentration and yield of inulin in Taraxacum kok-saghyz root biomass from sampling aerial mass (i.e., leaves) at the early stages of development (two months). This prediction will be enabled by gathering information about the relative abundance of metabolites, which we can potentially also associate with gene expression in aerial (leaves) and root tissues, as well as with polymorphisms in the sequences of differentially expressed genes. Our model will rely on a vast and robust database of relative abundances of metabolites in leaf and root tissues at several stages of development during the crop cycle (six months), contrasted with the actual concentration of inulin at those stages, as well as with final concentration and yield.
Gajigan, Andrian	University of Hawaiʻi at Mānoa	Genomics and metatranscriptomics profiling of a novel dinoflagellate-giant virus system	

Hammer, Tobin	University of California, Irvine	Exploring functional diversity in wild bee gut microbiomes	Bees have been making a living on a strict pollen and nectar diet for millions of years. They are able to digest this plant material in part because of their gut microbiome. Bacteria in the bee gut encode enzymes that break down complex molecules, especially those in the walls of pollen grains. These enzymes could be useful in efforts to develop biofuels and bioproducts from plant biomass. However, research exploring the plant-metabolizing functions provided by bee microbiomes has almost exclusively focused on one bee species, the Western honey bee (Apis mellifera). Expanding the scope of microbiome exploration to other bee species is likely to yield novel functions. In this work, we will comprehensively characterize gut microbiomes in 17 species of wild bumble bees. We will mine bacterial genomes present in these bees for useful products, such as carbohydrate-active enzymes, or biosynthetic genes involved in production of antibiotics or other novel metabolites. Finally, this research may aid in efforts to understand, and potentially prevent, bee declines. Bees are critical pollinators, but are sensitive to environmental stressors such as agrochemicals and heat. The bacterial data generated through this project may help explain why some bee species are more resilient to stress than others, and

			could ultimately inform development of bee probiotics.

Hazard, Christina	Ecole Centrale de Lyon, University of Lyon (France)	Determining in situ rates of host-virus evolution in soil	This research project aims to address the DOE’s mission of understanding organismal controls on terrestrial biogeochemical cycling and gaining an understanding of the role of microbes in controlling biogeochemical processes and key elements in the environment. Specifically, it aims to understand the consequences of virus infection on microorganisms that perform critical processes in the global carbon cycle. While we understand the consequences of virus infection of plants and animals in causing disease, the impact of viruses infecting microorganisms, including bacteria and archaea (prokaryotes), are much less known. In the marine environment, we recognise that virus infection results in cell death and lysis of approximately 20% of prokaryotes, strongly influencing fluxes of nutrients and biogeochemical elemental cycling. In comparison, the impact of bacterial infection on prokaryotes in soil is largely unknown. This is, in part, due to the structural complexity and vast diversity of prokaryote communities, with one gram of soil containing up to ten billion cells that represent tens of thousands of different individual populations. Metagenomic sequencing of soil microbial communities enables characterization of this vast diversity of microorganisms (including viruses) and identification of infection linkages

			between viruses and hosts at a broad level. To enable a detailed analysis of active virus-host interactions between individual populations over time, we will focus on the critical biogeochemical process of methane oxidation, which is performed by a taxonomically restricted group of bacteria. This analysis will examine the response of individual bacterial populations, at the genetic level, to virus infection by characterizing how the ‘bacterial immune system’ responds and evolves in the presence of active virus infection. This will allow us to determine how fast both virus and host population dynamics change, how quickly they evolve as a result of interaction, and ultimately how important viruses are in influencing critical microbially-mediated biogeochemical cycles in soil.
Kaçar, Betül	University of Wisconsin at Madison	High-throughput resurrection of ancestral nitrogenase enzymes	

Kuhn, McKenzie	University of New Hampshire	Identifying the genetic basis of complex phenotypes and climate adaptation in quaking aspen (Populus tremuloides)	Northern lakes are expected to warm by 4-12 C over the next 80 years. Models are only starting to consider how microbial genotypes drive ecosystem-level CH4 emissions. However, it’s really the phenotypic manifestation (metabolic activity) of genotype/environment interactions that is ultimately driving ecosystem outputs. Understanding the composition and abundance of methanogens in northern lake sediments is of growing interest, but transcriptome data characterizing the activity of methanogens is rare. Through research and the sequencing data requested here we aim to build upon the foundations of this previous work by characterizing methanogen activity in situ and in potential methane production incubations under average current in situ temperatures (12 C) and predicted sediment temperatures under warming scenarios (22 C).

Mackelprang, Rachel	California State University, Northridge	Determining in situ rates of host-virus evolution in soil	Permafrost (permanently frozen soil) underlies approximately one quarter of Northern Hemisphere terrestrial surfaces and contains enormous amounts of carbon. This carbon is protected from microbial decomposition by frozen conditions. However, climate change threatens to induce large-scale permafrost thaw. As permafrost thaws, the stored carbon becomes available to soil microbes, who decompose it and produce globally significant quantities of carbon dioxide and methane. Permafrost can vary substantially in age. The formal definition of permafrost is soil that has been frozen for more than two consecutive years. However, it can be much older. For example, much of the permafrost in interior Alaska is tens of thousands of years old. Despite the frozen conditions, microbial communities are active in permafrost, slowly metabolizing carbon. Over geologic timescales, these processes can influence how readily it is converted to greenhouse gasses when thawed. Therefore, understanding the microbial carbon processing in permafrost may inform the contribution of permafrost of different ages to climate change when thawed.

McDonald, Tami	St. Catherine University	Extremophile ecophysiology: How algae are adapted to life in the world's driest desert	The Atacama lies on a narrow strip of land between the Pacific to the west and the Andes to the east. When water-laden air passing west over the Andes cools as it rises, rain falls on the eastern slope. This creates on the western slope a rain shadow—the Atacama desert—that runs all the way to the ocean. Concomitantly, the cool ocean current on the west coast keeps almost all moisture from being absorbed into the air from the oceanic side. The little moisture held in the ocean air condenses as night falls, forming in the middle altitudes a narrow band of unique biological communities called fog oases. Exploiting the dramatic moisture gradient in the Atacama allows us to understand the combination of physical and biotic factors that determine when and how a symbiotic organism like Trentepohlia can survive as a free-living alga, vs the costs (i.e donating photosynthate to a symbiont) and tradeoffs (such as reduced sexual reproduction) of the ecological niche expansion associated with forming a lichen symbiosis as a survival strategy. This in turn will help us understand carbon cycling in dryland habitats, which will be increasingly important as desertification increases.

Mei, Ran	National Institute of Advanced Industrial Science and Technology (Japan)	Unraveling the interplay between sediment microorganisms in biomass consumption	All over the planet, numerous microorganisms are involved in the cycling of nutrients stored in the biomass. The advent of sequencing technology has brought far more microorganisms within reach. However, detailed understanding of how they interact and respond to changes in living conditions requires experiments in a laboratory using active samples, which is still challenging for certain ecosystems. This study proposes to utilize a unique bioreactor that has been operated for 16 years and effectively maintains the activity of sediment microbial samples. Because the pool of organic carbon bound up in microbial biomass in the subsurface is so large and microbes there have distinct lifestyles compared to their counterparts in other ecosystems, understanding how that organic carbon is processed in the sediment would clarify a major and still poorly understood component of the global carbon cycle. By coupling well-controlled lab tests with targeted sequencing analysis, we expect to tease apart the complex interplay between sediment microorganisms in biomass consumption. This will provide a unique perspective on evolution of nutrient cycling genes and enzymes, using terrestrial freshwater and estuary samples as comparators. The carbon and nitrogen-cycling enzymes they find could be valuable for

			understanding their contribution to the biogeochemical cycles across the globe, including greenhouse gas emission, and discovering new strategies for processing of terrestrial biomass for biofuel or biomanufacturing uses.
Michaud, Alex	Bigelow Laboratory for Ocean Sciences	Ecology and adaptation of microorganisms immured in the West Antarctic ice sheet	Particulates from the oceans and continents is mobilized, transported, then deposited onto the West Antarctic Ice Sheet (WAIS) and buried through glaciological time, while atmospheric gases are trapped within the ice. Glacial ice forms a stratigraphic record of these impurities that has led to our current understanding of paleoclimate conditions and trends. Microorganisms are part of this impurity record within the ice sheet. In fact, the stratigraphic record of microbial cell abundance shows potential links to paleoclimate conditions. While these results are promising, we do not yet have the data to determine if ice sheets reliably record microbial cells deposited from the ice sheet surface or if environmental filters in the ice sheet alter the deposited assemblage of microorganisms. Our current knowledge of microorganisms in glacial ice comes from culture-dependent methods and taxonomic marker genes isolated from bulk DNA extracts. Microorganisms have been grown in culture media from Tibetan Plateau glaciers and the

			Greenland and Antarctic ice sheets. Our cell-specific analyses will provide information on the total microbial community and the viable fraction.
Moyers, Brook	University of Massachusetts, Boston	Genome annotation of a bioremediating salt marsh plant	Salt marshes are coastal ecosystems that provide important services to the communities that live in and around them. Coastal cities and towns all over the world depend on these services, which include flood protection, pollutant filtration, and carbon storage as well as recreational activities like fishing, walking, and birding. Salt marshes are impacted by human activities, such as nitrogen or heavy metal run-off from urban, agricultural, and other sources. Pickleweeds (Salicornia) are an understudied group of plants that play important roles in marsh health and restoration. Pickleweeds can tolerate high levels of heavy metal contamination, and will take up heavy metals at high concentrations. We are studying how these plants provide remediation services to marshes and the role they play in nitrogen cycling and nitrogen run-off responses of their ecosystems. To do this, we are investigating how different sources of nitrogen affect the ways that genes are expressed by pickleweeds.

Peterson, Benjamin	University of California, Davis	Influence of labile permafrost dissolved organic matter on mercury-methylating microorganisms	Mercury is a global pollutant that can accumulate in aquatic food webs, resulting in consumption advisories for fish and other aquatic creatures. Before it accumulates in tissues, however, it must be converted to methylmercury by microorganisms. Understanding the activity of these microorganisms is critical to understanding patterns in mercury contamination. Permafrost in the Arctic holds massive stores of both mercury and organic carbon, both of which are released when permafrost thaws. The organic carbon is highly accessible to microorganisms living in the soils and sediments, including the ones that can produce methylmercury. However, the response of the microbial community mediating methylmercury production to the permafrost-derived organic carbon has not been studied. This project aims to identify the relationship between permafrost-derived organic carbon and the microbial production of methylmercury. First, we will conduct experiments that identify the microorganisms and chemical conditions that lead to methylmercury production across a depth gradient in permafrost-impacted soils. Second, we will use freshly leached organic carbon from intact permafrost in experiments to simultaneously measure the effect of the labile organic carbon on methylmercury production and identify the

			microorganisms activated in response to the amendment. This novel approach will provide new information on how thawing permafrost is likely to impact methylmercury contamination of downstream food webs.

Ren, Dacheng	Syracuse University	Genetic basis of bacterial persistence	This proposal seeks to understand how stress tolerance allows bacteria to survive in harsh environments and what genes and pathways are important to persistence. Persistence is a state of extreme tolerance to stress in a subpopulation of cells, and it is not well understood. It is correlated with reduced metabolism and thus cellular activities. Understanding the mechanism of persistence will help engineer better strains and appropriate process design, which are important for improving tolerance to toxic bioprocess products while maintaining productivity of the process. Bacterial biofilms arise when bacteria attach to a surface and embed themselves in a self-produced matrix. Although persistence is a low frequency event in common cases, it increases significantly in biofilms and under harsh conditions that are commonly also encountered in biofuel production. This new information will uncover how persister cells survive despite being exposed to stressors that are lethal to other cells in the same population. These findings will help engineer better bacterial strains for bioprocesses. In addition to tolerating toxic biofuel end-products, persister cells can tolerate other stresses such as heavy metals (bioremediation by environmentally friendly bacteria) and antibiotic treatment (persistent

infections caused by pathogens). Therefore, investigating persisters will allow us to promote tolerance for beneficial applications while reducing undesired persistence of pathogens. Overall, understanding persistence at the genetic level will bridge important knowledge gaps in bacterial physiology, and pave the path for engineering better processes towards sustainable energy production and beyond.

Rober, Allison	Ball State University	Using metatransciptomics to link aquatic biofilm microbial diversity and function	Aquatic microbial biofilms are comprised of autotrophic (algae) and heterotrophic (bacteria and fungi) microorganisms that play an essential role in the structure and functioning of aquatic ecosystems. Biofilm composition and metabolism are strongly influenced by differences in hydrologically mediated environmental conditions with consequences for net CO2 emissions. Conditions that promote a higher proportion of autotrophic (algae) biofilm results in greater CO2 uptake from the atmosphere, whereas a biofilm dominated by heterotrophic microorganisms (bacteria and fungi) promotes greater CO2 emissions. The composition of autotrophic and heterotrophic components of the biofilm are intricately linked and perturbations to one portion of the biofilm community can cascade through the rest. Therefore, it is anticipated that changes in gene expression that control metabolic functions within the autotrophic component of the biofilm will be reflected in the make-up and functioning of the heterotrophic component of the biofilm and vice versa. This project is expected to reveal the influence of environmental conditions on gene expression within the autotrophic and heterotrophic components of the biofilm. Further, this research is likely to facilitate the discovery of correlated patterns of abundance between certain eukaryotic and prokaryotic microbes, and link

			trait-mediated metabolic functions at the community level. Using coupled metagenomic and metatranscriptomic approaches to evaluate how abiotic and biotic interactions shape microbial communities and microbial-mediated biogeochemical processes addresses a critical knowledge gap in the field of aquatic microbiology and will provide a better understanding of how aquatic microbes participate in biogeochemical cycling within peatlands.

Scott, Neal	Queen's University (Canada)	Multi-scale variation in permafrost soil microbial community composition across the Arctic	Permafrost soils contain a disproportionate amount of organic material (i.e. carbon) compared to global soil carbon stocks. The Arctic is experiencing some of the most rapid warming compared to any place on earth. The ultimate fate of the carbon and nutrients in Arctic soils will play an important role in the regulation of future climate. This warming could also influence biodiversity patterns in the Arctic by altering soil microbial communities. In order to understand the potential impact of climate change on the Arctic, we need critical information on the soil microbial community, and how it might interact with the environment to alter key processes that might influence future climate. Microorganisms play a critical role in the regulation of carbon and nitrogen cycles in soils. The ultimate consequence of this regulation ranges from production of greenhouse gases that influence the climate system, to influencing nutrient availability that affects plant (and other organisms) growth. Yet our knowledge of these organisms in Arctic environments is limited. Using a unique set of permafrost soil cores collected at several sites in the Arctic as part of the ADAPT project, we have the opportunity to assess variability in soil microbial communities across gradients ranging from less than one meter to multiple kilometers. We propose to obtain shotgun metagenomic sequences from 92

			soils cores representing 23 sites and 2 soil depths covering a significant part of the Canadian Arctic. Results from this work will provide key insights on variation in microbial community structure at a range of spatial scales, and pave the way for future work that can more directly address connections between soil microbial community structure and biogeochemical processes.

Tatsumi, Chikae	Boston University	Soil fungal community function in biogeochemical cycling and plant symbiosis under increasing urban green space disturbance intensity	This project investigates the function of soil fungal communities in various urban green spaces with different disturbance intensities across Boston through RNA sequencing. Urban areas have expanded worldwide in the past few decades and are predicted to increase further in the next decades. There are various types of landscapes where urban trees live, such as urban forests, parks, lawns, and streets, which are increasingly affected by human activity. Our study has revealed that soil physicochemical properties largely change with the increasing disturbance intensity in urban green spaces, which would strongly affect soil microbial activity. Nevertheless, the variation in soil microbial activity between different urban landscapes is poorly understood. Soil fungi have important roles in supporting the growth and health of urban trees, which are necessary for the mitigation of urban extreme heat, drought, and air pollution. This study would determine how the disturbance intensity in urban green spaces shapes soil microbial communities and determines their function using comprehensive RNA- and DNA- sequencing datasets, soil physicochemical measures, tree health data, and biogeochemistry. By summarizing these results, we will identify the best green space management to

			maximize urban tree health and C sequestration in cities.
Tlaskal, Vojtech	Biology Centre CAS (Czech Republic)	Uncovering the functional potential and interactions of carbon and nitrogen cycling microorganisms adapted to an extreme habitat	The proposed project will provide new knowledge on the environmentally relevant microbes from an understudied system of saline-alkaline lakes and on the interaction between these microbes. Given the key function of microorganisms in the global carbon and nitrogen cycles and their importance in emissions of greenhouse gases, understanding of their physiology and diversity patterns can contribute to mitigation of climate change. It can also help to develop novel biotechnological approaches within the framework of green chemistry.
Torralbo, Fernando	University of Cordoba (Spain)	Influence of rhizobia association on transcriptome of common bean to drought recovery	This pilot project proposes to characterize the influence of the root-associated rhizobia on expression of gene clusters involved in drought tolerance and recovery responses and growth rate of roots of two genotypes of Phaseolus vulgaris with contrasting resistance to drought stress. Common beans are an important part of human diet, but also have essential roles in the uptake and recycling of nutrients, a process critically dependent on plant’s interaction with its rhizoplane. The proposed pilot project will contribute to elucidate gene clusters involved in plant-microbe interaction in the context of

			environmental factors that alter plant source-sink balance.
Valdez Nuñez, Luis Felipe	National University of Cajamarca (Peru)	Bioprospecting for acidophilic microorganisms in high-altitude mining tunnels in Hualgayoc, Peru using omics tools.	Mining tunnels are usually abandoned after their profitable period of life being cataloged as open sources of pollution, especially for releasing metals and acidic drainage. Despite these conditions, these places represent the habitat for well-adapted microbial communities that could be essential in the industry and environmental care sectors. These microorganisms contain the machinery to mobilize/immobilize many chemical elements (such as metals), something that can be useful in biomining and bioremediation technologies, respectively, contributing to building more sustainable processes and solving environmental problems. However, microorganisms from Peruvian mining tunnels are not well understood yet and need to be better investigated to know more about how they live and transform chemical compounds in their environment. We propose to characterize microorganisms that naturally occur in abandoned acidic and high-altitude mining tunnels from Peru using nucleic acids analyses. Focusing on different environmental samples we are expecting to get new insights into the biotechnological potential of these special microorganisms.