

Determining the Soil Microbiome Community Phenotype "Metaphenome" in Response to Changing Soil Moisture and Carbon Content

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Project Goals: PNNL's Soil Microbiome SFA aims to achieve a systems-level understanding of the soil microbiome's phenotypic response to changing moisture through spatially explicit examination of the molecular and ecological interactions occurring within and between members of microbial consortia. Integrated experiments were designed to confront both the scaling challenges and inter-kingdom interactions that regulate networks of biochemical reactions. Individual- and population-based models for predicting interspecies and inter-kingdom interactions were parameterized using experimental data, and predictions were tested in soil to reveal spatially explicit microbial interactions. Discoveries from controlled experiments are planned to be cross validated in the field, using moisture gradient experiments at a new local field site. Data was captured and shared through an optimized data management pipeline. Knowledge gained will provide fundamental understanding of how soil microbes interact to decompose organic carbon and enable prediction of how biochemical reaction networks shift in response to changing moisture regimes.

Abstract: As climate changes, the mesic grassland ecosystems of the continental United States are predicted to experience increasing periods of drought. The influence of extended drought on functions carried out by interacting members of microbial communities across trophic scales is largely unknown but vital for understanding and predicting outcomes of future climate regimes on soil health and biofuel feedstock sustainability. PNNL's soil microbiome SFA has tackled this challenge using a cross-scale approach to decipher the molecular mechanisms underpinning interactions between soil microorganisms. Within this context, our team is working towards defining how water stress affects community functions, interkingdom interactions, and the decomposition rates of chitin, a highly abundant ubiquitous polysaccharide found in soil.

Starting at the field scale, we screened existing soil metagenomes to determine the global distribution of soil viruses and used this data to link viruses with their hosts. We identified thousands of novel soil viruses, including giant viruses, as well as novel auxiliary metabolic genes (AMGs), thus illuminating the potential roles of soil viruses. In addition, we deeply sequenced metagenomes (~6 Tb

total) from disparate grasslands (Kansas, Iowa, and Washington) to determine the functional potential of the different soils. To understand molecular mechanisms underlying signaling in the field, we identified novel siderophores^{1,2}. Concurrently, using novel metabolic modeling approaches we revealed that wet and dry grassland soils differ in key metabolic pathways. These findings will provide targets for future investigations at our local field site, where we established an irrigation field trial. Also, at our local field site, we planted a new bioenergy field stock (Tall Wheatgrass) to determine the influence of soil moisture and drought on the soil microbiome and plant-microbe interactions.

At the other end of the scale, we dissected the complex soil microbiome into tractable, stable, low complexity model consortia. Using our SFA consortia, we demonstrated the influence of species richness on community convergence and stability. These consortia provide a valuable resource for determining specific metabolic and species interactions which can be validated at our local field trial. Experiments with the SFA consortia also revealed the importance of spatial structuring. We visualized spatial interactions between specific soil populations and metabolic interactions during chitin decomposition, using newly developed soil microfluidic chips (Soil Chips) and Soil Boxes. We found that specific soil fungi help to bridge soil pores as soil dries down and that soil chemistry plays a vital role in this process. To determine which of the species synthesize and secrete chitinolytic enzymes we developed a suite of Activity-Based Probes (ABPs) to selectively label the active subset of chitinolytic enzymes. These data will be used to parameterize individual- and population-based models currently in development.

At the mesoscale, we examined the soil metaphenomic response of native soil communities in response to varying moisture regimes. Using real-time CO₂ measurements as the response variable, we investigated chitin degradation using multi-omics to determine the functional response of specific members of the soil microbiome. Together these studies are moving us towards gaining an understanding of the microbial community's phenotypic response "metaphenome"³ to soil moisture and drought.

References

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3. Jansson and Hofmockel. 2018. *Curr Op Microbiol.* 43:162-168.

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