

Profiling of the Microbiome Metabolic Response to Soil Rewetting

Mary Lipton^{1*}(mary.lipton@pnnl.gov), Montana Smith¹, Lisa Bramer¹, Sheryl Bell¹, Nikola Tolic¹, Sneha P. Couvillion¹, Vanessa Paurus¹, Emily Graham¹, Janet Jansson¹, **Kirsten Hofmockel¹**

¹Earth and Biological Sciences Directorate, Pacific Northwest National Laboratory, Richland WA, 99352

Website URL: <https://www.pnnl.gov/projects/soil-microbiome/research>

Project Goals: PNNL's Phenotypic Response of Soil Microbiomes SFA aims to achieve a systems-level understanding of the soil microbiome's phenotypic response to changing moisture. We perform multi-scale examinations of molecular and ecological interactions occurring within and between members of microbial consortia during organic carbon decomposition, using chitin as a model compound. Integrated experiments address spatial and inter-kingdom interactions among bacteria, fungi viruses and plants that regulate community functions throughout the soil profile. Data are used to parametrize individual- and population-based models for predicting interspecies and inter-kingdom interactions. Predictions are tested in lab and field experiments to reveal individual and community microbial phenotypes. Knowledge gained provides 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: Soil microbes are highly sensitive to changes in their environment, making rapid measurements essential for a comprehensive understanding of microbiome functions. For example, drought is one of the most common environmental stresses that soil microbiomes experience, with important implications for larger scale biogeochemical fluxes. Water drives microbial interactions because it influences everything from cell function to substrate fate within the soil system. Changing levels of soil moisture can affect the retention or loss of soil carbon. When soil organic carbon is rapidly mineralized from desiccated soils upon rewetting there is a rapid increase in the release of CO₂. It has also been observed that the release of CO₂ increases with the length of time that the soil has been exposed to the drought conditions. Understanding the molecular mechanisms behind this phenomenon is important for managing soil carbon, especially with the increased frequency of drought-rewet events due to climate change.

Here, we aimed to understand how the physiology, metabolism, and interactions between soil microorganisms change in response to desiccation and rewetting, and to use this understanding as a basis for predicting the soil metaphenome. Soil samples were collected from our irrigated tall wheatgrass field experiment, in Prosser, WA. This soil was used to in an incubation experiment where soils were exposed to dehydration, allowed to incubate in the desiccated state for 1 or 2 weeks and then rehydrated for 90 min. The metaphenome was evaluated by measuring real-time respiration, carbon and nitrogen contents, as well as metabolite, protein, and transcript profiles throughout the time course.

Our results revealed that microbial biomass carbon decreased through the dehydration period and additionally during the rewetting process, with similar results for both the 1 week and 2 week drought exposures. The microbial biomass nitrogen also decreased during the dehydration event. However, no significant changes were observed during rehydration suggesting that the microbial biomass C:N ratio decreased over the course of the experiment. Additionally, the reduction of microbial carbon during rewetting suggests that the microbes are not reassimilating the carbon immediately after rewetting (90 min). Instead, this carbon is being directed toward respiration. Real time mass spectrometry methods (Weitz et al., 2020) detected an immediate burst of CO₂ production within the first 10 minutes upon rewetting and a gradual increase during the subsequent 90 minutes. Total CO₂ respired from soil exposed to two weeks of drought was 75.6% higher than soil exposed to one week of drought, consistent with the Birch effect (Birch, 1958).

Metabolomic analysis was used to characterize the microbiome response to the dehydration and rehydration processes. GC-MS based metabolomics analysis identified 336 metabolites over the course of the experiment. While most metabolites did not change significantly throughout the incubation, 20 metabolites decreased, and 19 metabolites increased during dehydration. Conversely, 23 metabolites decreased and 10 increased during rehydration. These results illustrate changes that occurred in the soil metabolic profile in response to changing soil moisture levels. Specific metabolites changed during both phases of the experiment. For example, lyose, xylitol and other sugars accumulated during dehydration and decreased after rehydration, although mannitol increased throughout the experiment. These observations support the hypothesis that soil microbes accumulate sugars and other compounds during dehydration to protect against osmotic stress. The metabolites of interest are currently being mapped to metabolic pathways to identify potential metabolic strategies that are active during the dehydration and rehydration response.

These results indicate that soil microbial communities react rapidly to changing moisture conditions. Understanding the mechanisms underlying these changes will have important implications for carbon allocation within and between organisms in the soil environment.

References/Publications

1. Weitz K.K., Real-Time Mass Spectrometry Measurements of Respiration Rates in Biological Systems. *J. Am. Soc. Mass Spectrom.* **2021**, 32, 3, 648–652
2. Birch, H. F., Pattern of Humus Decomposition in East African Soils. *Nature* **1958**, 181 (4611), 788-788.

Funding Statement: PNNL is a multi-program national laboratory operated by Battelle for the DOE under Contract DE-AC05-76RLO 1830. This program is supported by the U. S. Department of Energy, Office of Science, through the Genomic Science Program, Office of Biological and Environmental Research, under FWP 70880. A portion of this work was performed in the William R. Wiley Environmental Molecular Sciences Laboratory (EMSL), a national scientific user facility sponsored by Office of Biological and Environmental Research and located at PNNL.