Unraveling the Molecular Mechanisms Underlying the Microbiome Response to Soil Rewetting

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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. We focus on a multi-scale examination of the molecular and ecological interactions occurring within and between members of microbial consortia. Integrated experiments are designed to confront spatial challenges and inter-kingdom interactions that regulate networks of biochemical reactions. The exchange among bacteria, fungi, viruses and plants are being characterized in the context of microbial metabolism and community function. These experimental data have been 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. Our cross-scale experiments are coordinated together to investigate the influence of moisture on the interkingdom-interactions. Data is 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.

Environmental stress from drought is increasing in frequency with unknown outcomes for soil microbiomes. The mechanisms in which the microbial communities respond to dehydration is very important, particularly in arid and marginal lands. As soils dry, the water potential across the cell wall decreases leading to osmotic stress which is compounded by limited diffusion. To address environmental changes caused by differences in moisture level, microbes must shift physiology and metabolic interactions to survive. One mechanism microbes employ to offset decreases in moisture levels is the production of osmolytes to reduce their internal water potential and maintain fluid balance. Mostly, microbes use simple organic molecules with good solubility such as amino compounds (amino acids and glycine betaine) and simple sugars (trehalose). However, the synthesis and storage of these compounds is energetically costly to the microbe and little is understood about the molecular response to the changes in soil moisture, and how that affects the phenotype.

Here, we aimed to understand how the physiology, metabolism, and interactions of soil microbes change in response to moisture, and to use this understanding as a basis for predicting the soil metaphenome. While microbes respond to drought by different mechanisms, a common phenomenon is the rapid mineralization of soil organic matter and increased rate of CO_2 release upon rewetting dry soil, termed the 'Birch Effect'. We are testing the hypothesis that during desiccation in an arid, marginal soil, microbes will initially accumulate osmolytes only to rapidly metabolize them upon rewetting. One mechanism for the disposal of the osmolytes is the rapid release of CO_2, DOC and nutrients, arising from intracellular material and varies in magnitude based on the biomass. Most analyses measure respiration on the hour to daytime frame, thus missing the immediate rewetting events. To provide information about the initial microbial response to wetting, we developed and an atmospheric monitoring instrument (RTMS) that measures the levels of CO_2, O_2, N_2, H_2O and other relevant gases, simultaneously in real time. In these

experiments, desiccated soil was rewet, and gas levels were measured in seconds, minutes and hours after rewetting.

After rewetting desiccated soil, we observed a rapid production of CO_2 in the first 90 minutes after which the rate stabilized. In order to determine the origin of the carbon, we amended the water used in the rewetting step with glucose to act as a surrogate for extracellular carbon. In these experiments, we observed a biphasic CO_2 response; a rapid release of CO_2 that leveled off at 90 minutes, and a larger production of CO_2 over the next 4 hours. We hypothesized that this initial burst was due to the metabolism of intracellular compounds and the secondary burst was due to the metabolism of extracellular compounds. To test this hypothesis, we used ^{13}C labeled glucose in the experiment to differentiate between $^{12}CO_2$ and $^{13}CO_2$ production. The $^{12}CO_2$ portion of the glucose addition resulted in a respiration response that was identical in rate and amplitude to that observed in a water-only control. The $^{13}CO_2$ production was identical to 4-hour response, leading to the supposition that internal metabolites are respired before external ones after rewetting. In the same experiment, we were also able to detect O_2, N_2 and H_2O over the same time scale. By taking measurements of all the gasses in real time, we were able to deconvolve the order and rate in which the microbial communities were producing or consuming the different gasses in the first 90 seconds after the addition of water.

Evaluation of the soil metabolomes at specified time points within 3 hours after wetting were also examined. GC-MS based analysis revealed 119 metabolites, including trehalose, glycine and other sugars, and amino acids. Of these metabolites, 58 were confidently identified, and 61 spectral features represent opportunities to discover new metabolite identifications. GC-MS based lipidomic analysis revealed changes in fatty acid profiles and related molecules in response to rewetting dry soil. LC-MS/MS based lipidomic analysis revealed significant changes across multiple lipid classes including glycerophospholipids, glycerolipids and sphingolipids. For example, triglycerides, which can potentially function as storage molecules and regulate cellular fluidity, were found to increase in rewet soil and also showed a higher degree of saturation. Together our multi-omic analysis is contributing towards a deeper understanding of the soil microbiome metabolic and metaphenomic responses to drought.

Funding statement. This research was supported by the U.S. Department of Energy (DOE), Office of Biological and Environmental Research (OBER), as part of BER's Genomic Science Program (GSP), and is a contribution of the Pacific Northwest National Laboratory (PNNL) Soil Microbiome Scientific Focus Area "Phenotypic Response of the Soil Microbiome to Environmental Perturbations." A portion of this work was performed in the William R. Wiley Environmental Molecular Sciences Laboratory (EMSL), a national scientific user facility sponsored by OBER and located at PNNL. PNNL is a multi-program national laboratory operated by Battelle for the DOE under Contract DE-AC05-76RLO 1830.