Unraveling the Molecular Mechanisms Underlying the Microbiome Response to Soil Rewetting

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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 are designed to confront spatial challenges and inter-kingdom interactions among bacteria, fungi viruses and plants that regulate community functions. These 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. 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, such as drought, is increasing in frequency with unknown outcomes for soil microbiomes. Therefore, the mechanisms by which microbial communities respond to dehydration are very important to understand. This knowledge is particularly needed for arid, marginal lands, and for otherwise fertile lands that are experiencing reduced precipitation due to climate change. As soils dry we posit that microbes adapt by accumulation of compounds that offset changes in osmotic potential. Alternatively, they die and lyse releasing their intracellular compounds to the environment, thus creating different pools of organic carbon that are available to viable microbes upon rewetting. Of particular interest is the impact of shifts in soil moisture on soil lipids. Despite the central role that microbial lipids play in cellular homeostasis and phenotype, intact lipids have not been widely studied in the context of soil ecology.

Here, we aimed to understand how the physiology, metabolism, and interactions between soil microorganisms change in response to changes in soil moisture, and to use this understanding as a basis for predicting the soil metaphenome. Soil samples were collected from our irrigated field site that is managed by Washington State University, in Prosser, WA. The soil was used to establish soil microcosms that were desiccated to simulate drought. Using a series of incubation experiments, we evaluated the response of the soil microbiome to water addition, by measuring real-time respiration, microbial community shifts and metabolic and physiological adaptation through changes in microbial lipids.

Our results reveal that the soil lipidome is a robust indicator of the microbial community's functional response, even at short time-scales during which community composition may not undergo substantial change. A total of 838 unique lipids were identified from the soil samples across 6 timepoints, representing the largest number of lipids identified in soil to date[1]. Under dry conditions we observed an increase in lipids implicated in mediating

heat, osmotic and oxidative stress and nutrient deprivation. We also found an abundance of lipids containing fatty acid moieties that were characteristic of fungal metabolism. These included higher abundances of non-phosphorus membrane lipids (sulfoquinovosyl diacylglycerols, betaine lipids), ceramides and polyunsaturated fatty acids with longer chain lengths in glycerophospholipids and triacylglycerols. Shifts in the lipidome were not accompanied by significant changes in the fungal community composition. The bacterial community structure was, however, more sensitive to drought and rewetting, as indicated by significant increases in Firmicutes and decreases in Verromicrobia. This shift was accompanied by an increase in lipids with fatty acid characteristics typical of bacteria, such as unsaturated and monosaturated fatty acids with shorter chain lengths, suggesting rapid metabolic reactivation in the bacterial community.

Subsequent experiments targeted the impact of soluble vs solid substrates following rewetting and the relative importance of cellularly associated carbon compounds, or dehydrated DOC that becomes bioavailable after rewetting. By comparing the rate of CO_2 production from desiccated soils that were rewet with water (control) to those where ^{13}C labeled glucose was added in solution and solid forms, we observed the strong influence of bioavailability (soluble v solid) on catabolic metabolism using real-time mass spectrometry[1]. The rate of $^{12}CO_2$ production was 8x that of the $^{13}CO_2$ production in the soluble ^{13}C glucose amendment, indicating that the soil microorganisms were not immediately metabolizing extracellular compounds. The rate of $^{13}CO_2$ production was 3x faster under souble compared to soild ^{13}C glucose addition indicating that highly soluble carbon substrates were the first extracellular compounds metabolized followed by less soluble substrates. Our results suggest that soil microbes were initially accessing carbon native to the soils, followed by soluble, then solid ^{13}C glucose. These findings demonstrate that rewetting dry soil results in preference for easily accessible, perhaps intercellular C, followed by substrates that are dissolved or require enzymatic depolymerization for assimilation. In summary, this study illuminates physiological and metabolic responses to soil drying and-rewetting. In particular, the lipid data suggest that soil microbial communities can quickly react to changes in soil moisture by shifting their lipid compositions. This may have important implications for carbon allocation within and between organisms and the soil environment.

References:

1. Couvillion, S.Pl, Naylor, D., Smith, M.L., Paurus, P.L., Bloodsworth, K.J., Stratton, K.G., Metz,T.O., Lipton, M.S., Jansson, J.K., Hofmockel, K.S. Influence of soil moisture stress on the soil microbiome and lipidome (2021, in preparation).
2. Weitz, K.K., Smith, M.L., Hixson, K.K., Hill, E. A., Jansson, J.K., Hofmockel, K.S., Lipton, M.S. (2020) Real-Time Mass Spectrometry Measurements of Respiration Rates in Biological Systems. *Journal of the American Society for Mass Spectrometry*. Dec 1. https://doi.org/10.1021/jasms.0c00251

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