Probing the Relationship Between Osmolytes and Respiration in Soils - in Real Time

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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.

Soil is a highly diverse ecosystem with microbial and chemical dark matter that remains to be discovered. While this is an exciting frontier for discovery, it also represents an obstacle to deciphering predictable interactions that promote fertility and productivity of our nation's soils. Environmental stress from drought is increasing in frequencing 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 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, soluble organic molecules such as amino compounds (amino acids and glycine betaine) and simple sugars (trehalose). However, little is understood about the molecular response of the microbiome 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. We are testing the hypothesis that during dessication in arid and marginal soil collected from our local Washington field site, microbes will initially accumulate osmolytes only to rapidly metabolize them upon rewetting. In our initial experiment, we incubated soils at 10% moisture and harvested after 2 weels. The baseline GC-MS based analysis of the metabolomic extract from the soils revealed the presence of potential osmolytes including trehalose, glycine and other sugars and amino acids. We have also used the same GC-MS approaches to monitor the changes in the abundance of these metabolites over time during a rewetting event where after rewetting dried soil to 19% moisture was harvested at particular timepoints over a 3 hour period. The changes in the osmolytes reveal potential microbial mechanisms active after the rewetting event.

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. However, most analyses focused to date have studied this event by measuring respiration on the hour to day time frame, thus missing the immediate rewetting events. To provide information about the initial microbial response to wetting, we developed an atmospheric monitoring instrument that measures the levels of CO_2, O_2, N_2, H_2O and other relevant gases, simultaneously in real time, seconds, minutes and hours after a rewetting event (Figure 1). Using this apparatus, we have evaluated the effect of hydration level on the rate and amplitude of the initial respiration rate after rewetting revealing a linear relationship between the amount of water added and the rate and amount of CO_2 produced. Substrates (glucose, cellulose, chitin, plant exudates) can also be added to the reaction either by being dissolved in the H_2O that is added or directly mixed with the soil prior to the reaction initiation depending on the solubility of the substrate and experimental design. Additionally, O_2, H_2O, $^{12}CO_2$ and $^{13}CO_2$ production can be monitored simultaneously throughout the reaction for a more thorough understanding of the respiration effect.

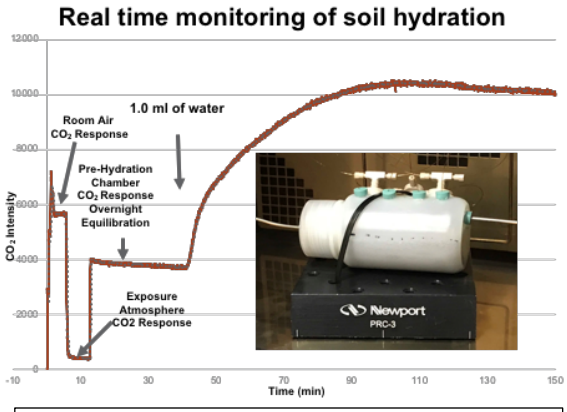

Figure 1: Picture of the gas monitoring reaction chamber (inset) and the rate and extent of CO_2 production over time as measuremed every 2 sec.

Combining data outputs from the standard baseline GC-MS approach to elucidate the metabolomic profile of the soils over time and the novel mass spectrometry based continuous gas monitoring system will further elucidate the relationship between osmolyte production, metabolism respiration rate in the soil after a rewetting event. These data were then used to better understand the metabolic pathways active in the soil microbiome and were linked with other omics data for the creation of more comprehensive models of microbial activity.

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