

Bridging the Soil Metagenome and Metaphenome Through Integrated Omics Analyses.

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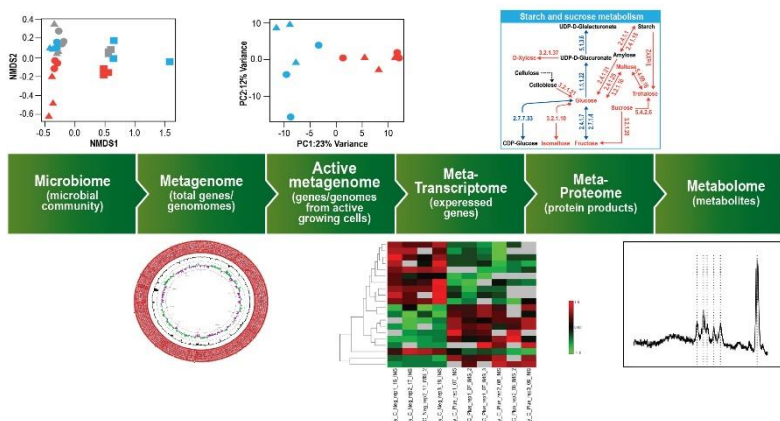
Project Goals:

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 will be 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 will be parameterized using experimental data, and predictions will be tested in soil to reveal spatially explicit microbial interactions. Discoveries from controlled experiments will be tested and validated in the field, using moisture gradient experiments at a new local field site. Data will be captured and shared through the establishment of a Soil Microbiome Knowledgebase (SMK). 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 microbiome responses to changing environmental conditions are manifested as shifts in community structure and/or modifications to microbial activity. These phenomena have traditionally been characterized through bulk scale measurements of respiration, nutrient cycling and soil organic carbon decomposition. However, bulk scale measurements mask the complexity of molecular interactions that occur between specific members of soil microbial communities. Here we aim to circumvent current limitations in understanding of the soil microbiome by employing multi-omics technologies to gain a molecular understanding of inter-kingdom species interactions and biochemical processes that occur in soil and how specific processes are impacted by environmental change; specifically drought. The multi-omics data will also serve to determine how the combined phenotypes of the soil microbiomes result in the soil metaphenome.^{1,2}

Currently, understanding of the soil metaphenome is hampered by the high microbial diversity and complexity of soil, experimental limitations, and incomplete genome-level understanding of biochemical pathways carried out by individual microbes and interacting community members in soil. Here, we demonstrate recent achievements in use of a multi-omics approach to link metaphenotypic observations to metagenome content. For this demonstration, we focused on a native prairie soil from Kansas where we have substantial existing metagenome data from the JGI Soil Metagenome Great Prairie pilot study.

First, we optimized a metaproteomics approach for soil. Limitations in mass spectrometric sensitivity and scan speed cycle can severely limit the number of peptides identified through this approach. However, the use of either on-line or off line 2 dimensional separations of the peptide mixture greatly increased the number of peptides identified as well as the molecular understanding of the metabolic and biochemical processes in the soil. In the future we aim to also apply specific activity based probes that are powerful tools to assess specific biochemical pathways that are active in soil microbiomes.



Potential Figure 1. A multi-omics approach enables deciphering different levels of soil microbiome information, ranging from community composition (microbiomes) to expression (metatranscriptomes) to protein production (metaproteomes) to metabolites (metabolomes). Together this multi-omics approach serves to elucidate the soil metaphenome; defined as the product of expressed genes (predicted from metagenomes) x environmental conditions.

We also used other steps in our multi-omics pipeline (Fig. 1) to assess key physiological traits expressed by a soil microbiome in response to specific nutrient and moisture perturbations³. Glycine was added as a mock root exudate and was found to have little effect on the community structure based on 16S measurements. However, there was a significant impact on the soil metaphenome as observed by significant changes in transcript expression and metabolite abundance. Not surprising, there was an increase in the metabolites that were products of glycine uptake and metabolism and an increase in the genes for glycine degradation and biosynthesis of serine and threonine, demonstrating the ability to discern these phenotypic responses in a completely untargeted manner in complex multi-omics datasets from highly diverse soils.

In contrast to the glycine addition, we found that moisture perturbation had an impact on the soil microbiome at all measured omics levels. In particular, soil desiccation caused a shift in community structure as well as a significant response in both the metatranscriptome and metabolome, including increases in metabolites and pathways for production of osmolytes, simple sugars, sugar alcohols and compatible solutes after drying. These results improve our understanding of the metabolic and biochemical processes occurring within soil microbial communities that ultimately lead to the soil metaphenome.

References:

1. Jansson, J.K. and Baker, E. S., (2016) *A multi-omic future for microbiome studies*. **Nat Microbiol.** 1:16049
2. Jansson, J.K. and Hofmockel K.S., (2017) The soil microbiome – From metagenomics to metaphenomics **Current Opinion in Microbiology (in press)**
3. Roy Chowdhury, T., et al., (2017) *Soil microbiome response to nutrient and moisture perturbations*. **Microbiome (submitted)**.

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