## Soil Moisture Modulates Inter-kingdom Interactions as Visualized Using a Simulated Soil Core

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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.

**Abstract:** Understanding the basic biology that underpins soil microbiome interactions is required in order to predict the metaphenomic response to environmental shifts, such as changing moisture content. A significant knowledge gap is how such changes will affect microbial community structure and its metabolic landscape. Here, we visualized the metabolome of interacting organisms within the soil habitat by obtaining high resolution multidimensional maps of the compositional and functional state of soil microbial communities. This entails mapping the metabolic exchanges that occur within soil microbiomes using the mass spectrometry imaging (MSI) methodologies we recently developed.<sup>1,2</sup> Using a custombuilt Soil Box system, we demonstrate changes in microbial community growth and composition in different soil environments, contingent upon access to reservoirs of nutrient sources. The Soil Box, designed to press functionalized-glass slides against the soil surface at different depths from the top soil surface, emulates the probing depth of a common soil core. This enabled determining both the spatial organization of the microbial communities that form on the slides and their metabolites by using confocal microscopy in combination with matrix-assisted laser desorption ionization (MALDI)-MSI.

We found that increased adhesion of soil microbial biomass occurred on slides functionalized with chitin islands, with the seeding attachment event occurring as early as 2 h. The MALDI-MSI data showed a high abundance of bacterial-related lipid families on the chitin islands and low abundance on areas without chitin. Confocal microscopy measurements of these samples confirmed the increased growth of microbial biomass, and consumption of chitin during growth. The microbial growth and community dynamics were also sampled at different moisture regimes (i.e., 14%, 24%, and 34% of total soil weight). Fungal hyphal networks bridging different chitin islands over distances of 17 mm were observed only in the driest of conditions, indicating that such bridges may act as fungal highways during drought conditions. In all, through the use of multiple correlative imaging platforms, these results illustrate a system that provides unprecedented spatial information about interactions within soil microbial communities as a function of changing environments. We anticipate that further use of our Soil Box approach will be invaluable in probing specific intra- and inter-kingdom metabolic networks arising from a gradient of environmental stresses.

## References

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