Title: Visually Mapping Phenotypes and Community Interactions at the Microbial Scale

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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 address spatial and inter-kingdom interactions among bacteria, fungi viruses and plants that regulate community functions throughout the soil profile. Data are used to parameterize 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. Knowledge gained provides 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 Text: The physical and biochemical interactions between microbial community members drive the rate and extent of soil organic matter (SOM) decomposition. However, soil is a heterogeneous and porous environment in which microbial connectivity to other cells and nutrients is largely dependent on moisture levels. An important but challenging scientific objective is gaining a predictive understanding of how the dynamic interplay between key microbial features (e.g., motility and enzyme/metabolite production strategies) and soil aqueous phase variability affect SOM decomposition rates. The challenges associated with this objective are extensive due to the inherent chemical, physical, and biological complexity of soil. To address these challenges, we have developed a suite of tools that can generate spatially explicit cellular and molecular level insights into the microbial phenotypes and interactions involved in SOM degradation in a soil-like environment. The newly developed tools include 1) fluorescent protein (FP) expressing bacterial strains isolated from a naturally evolved soil microbial community (McClure et al. 2020) allowing for tracking of species abundance and co-aggregation in real time, 2) a porous and transparent artificial soil habitat, or Soil Chip, that is well suited for fluorescence based and MS imaging.

Using these tools, we generate empirical data that can be used to test and parameterize microbial explicit models (agent-based and reaction-diffusion models) and to test hypotheses. In our Soil Chips we hypothesize that both microbial functionality (motility and metabolic capacity) and C substrate complexity and solubility dictate strain growth profiles and assembly over time.

Specifically, motile strains will have greater biomass than non-motile strains when a C substrate is insoluble, and producer strains that synthesize C substrate degrading enzymes will have lower biomass over time than cheater strains. Additionally, competing strains will assemble to form larger aggregate patches in an attempt to privatize resources. To test these hypotheses, we conducted experiments designed to map strain level assembly patterns, growth and motility of a microbial consortium treated with insoluble chitin or freely diffuse chitin products. To do so, phylogenetically diverse FP tagged bacteria including *Rhodococcus sp. S2-17::mTagBFP2*, *Variovorax paradoxus::mScarlet-I*, and *Sphingopyxis fribergensis::mClover3,* were incubated for 4 days in saturated Soil Chips treated with either chitin, chitopentaose (chitin oligomer), or n-acetyl glucosamine (NAG; chitin monomer). Unlike NAG, chitin and chitopentaose require extracellular enzymes to degrade the substrates into products that can be taken up by cells and utilized for growth. Furthermore, chitin was localized to an area ~700 μm away from cell inoculum port, whereas NAG and chitopentaose diffused freely throughout the Soil Chips. Soil Chips were imaged regularly by confocal laser scanning microscopy.

Our results indicate that when treated with NAG, the consortium generated greater unmixed strain aggregate patches than when treated with chitopentaose and chitin. This suggests competitive interactions as all strains can assimilate NAG. When treated with chitopentaose, the chitinolytic strains, non-motile *Rhodococcus* and motile *V. paradoxus,* started to grow by day 1, whereas the non-chitinolytic and motile *S. fribergensis* grew to a high density and likely outcompeted *V. paradoxus* growth by day 4. With chitin, we see the most even strain distribution and the smallest strain patch sizes by day 4, evidence of more cooperative than competitive strain interactions. Our initial hypothesis is supported by these preliminary results and will be more rigorously tested through the comparison with simulation results from predictive spatiotemporal interaction models that are being developed. This research will serve as our benchmark for future studies designed to test moisture effects on microbial phenotypes and chitin decomposition rates.

References

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