

Generation and Analysis of Reduced Complexity Model Soil Consortia

Ryan McClure,^{1*} (Ryan.McClure@pnnl.gov), Dan Naylor¹, Yuliya Farris¹, Sarah Fansler¹, Michelle Davison¹, **Kirsten S. Hofmockel¹ and Janet K. Jansson¹**

¹Earth and Biological Sciences Directorate, Pacific Northwest National Laboratory, Richland, WA

Project Goals:

PNNL's Soil Microbiome SFA aims to achieve a systems-level understanding of the soil microbiome's phenotypic response to changing moisture. We focus on a multi-scale examination of the molecular and ecological interactions occurring within and between members of microbial consortia. Integrated experiments are designed to confront spatial challenges and inter-kingdom interactions that regulate networks of biochemical reactions. The exchange among bacteria, fungi, viruses and plants are being characterized in the context of microbial metabolism and community function. These experimental data have been 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. Our cross-scale experiments are coordinated together to investigate the influence of moisture on the interkingdom-interactions. 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.

Abstract:

Soil microbial communities are critical to the overall carbon cycle and to the decomposition of complex biopolymers such as chitin and cellulose. Despite the critical nature of these microbiomes, a detailed understanding of how the interactions between members lead to emergence of community functions is lacking. This is due, in part, to the complex nature of the soil microbiome with thousands of species across several kingdoms contributing to the overall response of soil. In order to gain a more detailed view of the soil environment, we took an approach based on developing and analyzing reduced complexity microbial consortia that contain fewer species than the native soil but are still representative of this site and are more experimentally tractable. We hypothesize that analysis of these model consortia can identify reaction modules (sets of interacting species or enriched metabolic functions), and other fundamental aspects of soil microbiome interactions. These microbial relationships can serve as functional predictions for future tests to be carried out at our native field site, greatly expanding our knowledge of the functioning of soil microbiomes. We expect that such consortia will serve as valuable tools for the research community at large to identify metaphenomes and microbial traits.

To generate reduced complexity soil microbial consortia, we collected samples from our native field site containing a grassland silt loam soil. This native soil was then diluted to various levels, ranging from 10^{-2} to 10^{-3} and cultured on agar plates containing soil extract (water soluble soil nutrients) as well as chitin (100 ppm). Cultivation was carried out for several months with replating performed once per week to maintain the community. We found that species richness rapidly fell when soil microbial communities were cultured on plates, reaching levels of between 20-70 OTUs in only 2-3 weeks. In addition, several of the communities examined via 16s amplicon analysis still represented a fairly diverse community with

several different phyla, orders and genera represented. Very few of the communities were completed dominated by only a few bacterial or fungal species.

We also found that consortia could be stored as glycerol stocks or lyophilized stocks and reconstituted with a high degree of reproducibility. Most of the reconstituted consortia tested were very similar to the parent consortia on plates and there was consistent similarity across replicates of reconstituted stocks showing that regrowth from a stock on a plate is a deterministic process. We were also able to isolate many of the constituent species from these consortia and carry out pair wise incubations to begin to explore their interspecies interactions. These data, combined with co-abundance networks of these consortia during growth in soil, show that *Rhodococcus*, *Streptomyces* and *Rhizobiales* species found in our consortia are likely critical to the functioning of this community. These species occupy high central positions in our species co-abundance network and co-cultivation experiments show that, especially in the case of *Rhodococcus*, there are likely beneficial interactions with other species of these consortia.

This series of representative and reduced complexity are powerful tools that can be used by the soil community at large to interrogate the response of soil microbiomes to a number of perturbations and to confirm critical interactions between microbial species, particularly inter-kingdom interactions that characterize the emergent behavior of soil microbiomes. Constructed reduced complexity consortia provide a means to more powerfully leverage high-throughput, multi-omic techniques to better characterize these interactions and the major constituent players that are a part of them. Further knowledge of these interactions will help us better understand the overall metapenome of soil systems, especially as they respond to critical perturbations including drought.

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