

## Development and Analysis of Reduced Complexity Microbial Consortia Emerging from Native Grassland Soil Systems

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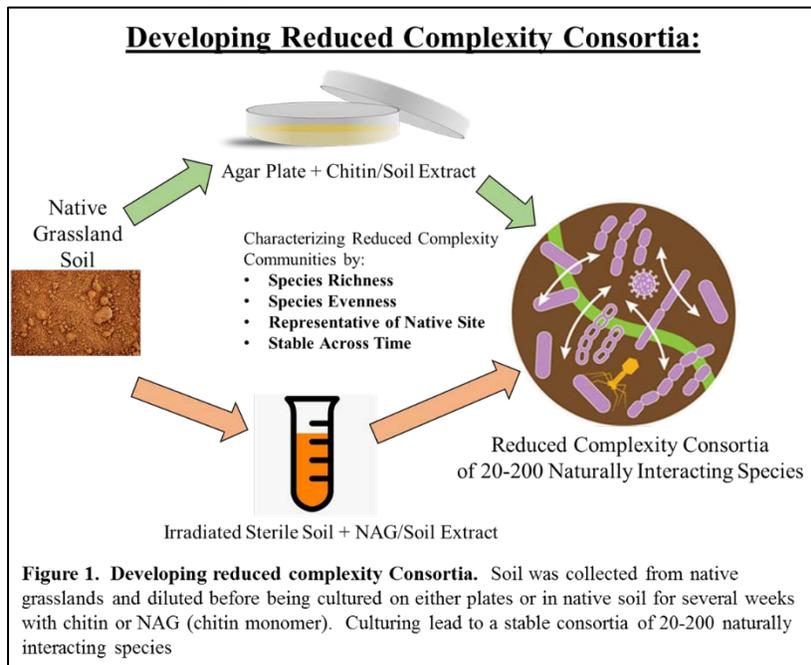
**Project Goals: PNNL's Soil Microbiome SFA aims to achieve a systems-level understanding of the soil microbiome's phenotypic response to changing moisture through spatially explicit examination of the molecular and ecological interactions occurring within and between members of microbial consortia. 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:

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 (**Figure 1**). We hypothesize that predictable reaction modules exist and that analysis of these model consortia can identify these reaction modules, and other fundamental aspects of soil microbiome interactions. These interactions and aspects can then be tested in a specific, robust manner in the native soil sites, greatly expanding our knowledge of the functioning of soil microbiomes.

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  $10^{-1}$  to  $10^{-4}$  and cultured in irradiated sterile native soil. Cultivation was carried out with the monomer of chitin, N-acetyl-glucosamine (NAG), exogenously added at 100 ppm. We confirmed that initial dilutions decreased the richness and complexity of our native soil and found that culturing these dilutions in soil for 15 weeks lead to stable microbial consortia containing both bacteria and fungi. We also found that emerging communities were highly representative of the native soil site. These stable consortia contained significantly reduced membership with between 250-300 OTUs (compared to the native soil containing ~2000 OTUs), while still containing a number of phyla that are characteristic of soil such as

Verrucomicrobia and Actinobacteria. In addition to cultivation in soil, we also developed consortia on plates using chitin itself as a major source of carbon and nitrogen. These plate communities represented microbiomes that were even further reduced in complexity while again containing a diverse community of several different phyla. Plate communities contained between 20-70 OTUs, richness levels that are far



easier to interrogate with –omics approaches, showed significant bioactivity when moved back into a soil environment as measured by CO<sub>2</sub> production and also showed stable levels of taxa across time.

This series of reduced complexity consortia that are representative of native soil sites 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 metaphenome of soil systems, especially as they respond to critical perturbations including drought.

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