Deconstructing the Soil Microbiome into Reduced-Complexity Functional Modules

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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. 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: The soil microbiome is an invaluable component of ecosystem function. Improving our understanding of the soil microbiome will aid predictions of how microbially-mediated processes are affected by external perturbations. However, the soil microbiome's extensive taxonomic and functional diversity hinders comprehensive analysis of this system. One option for studying the soil microbiome in detail is to deconstruct it into discrete functional units for individual analysis. Here, we aimed to dissect the complex soil microbiome through targeted enrichments based on metabolic capacity, thereby obtaining reduced-complexity consortia we term 'functional modules'. We hypothesized that, through targeted enrichments of a starting soil inoculum, we can obtain functional module communities that are low-diversity, reproducible and predictable, and encapsulate a significant extent of soil phylogenetic diversity while also enriching for underrepresented soil taxa. Furthermore, we hypothesized that functional module communities are also distinct from one another with respect to gene expression patterns.

To generate functional module communities, we cultured a soil inoculum in minimal media with specific modifications for the module in question. Module categories included addition of simple carbon substrates (e.g. sugars, organic acids) or complex plant polysaccharides, supplementation with antibiotics, anaerobic modules with alternative redox acceptors, or imposition of alternative growing conditions reflective of common field stresses. In total, 324 communities were obtained across 66 distinct functional modules. Analyzing community composition via 16s rRNA amplicon sequencing revealed that all modules were significantly reduced in diversity and richness relative to control soil communities, with polysaccharide modules significantly more diverse than all remaining categories. With respect to reproducibility, anaerobic modules were the least predictable (highest variability between module replicates for community composition), followed by polysaccharide and stress modules.

Our approach isolated not only known soil microbiomes but also several that are not found in amplicon analysis of soil. We found that of the 241 unique taxa in the soil core microbiome, 90 were found in at least one functional module core, collectively encapsulating approximately 37.3% of soil phylogenetic diversity. While most major soil phyla were represented in module cores, there were several that were underrepresented (Verrucomicrobia) or not found (Planctomycetes) and likely require alternative strategies for enrichment. In addition, we were also able to obtain 481 taxa that were uniquely found in module cores, showing there is a significant extent of soil diversity that becomes measurable through our strategy. We also investigated functional trends across a subset of modules using a metatranscriptomics approach, to confirm that modules are significantly different at the functional as well as taxonomic level. Functional patterns varied by module: for example, pectin and xylan modules were elevated for transcripts involved in 'Glycan biosynthesis and metabolism' relative to the other three modules. Comparing patterns of enriched transcripts showed that each of these five modules has its own distinct pattern on the collective microbial metabolic map. These results highlight the potential for combining separate modules into functional patterns and ultimately reconstructing the full biochemical capacity of the soil microbiome.

The functional module approach used here has significant applications for microbiome analysis: module communities are more tractable for omics analysis while retaining interactions of the native parent community. When studying a particular metabolic niche or biochemical process, a reduced-complexity consortium of microbes implicated in this process can be obtained through this strategy. Furthermore, through investigation of functional trends, one can piece together the full functional potential of a microbiome system with higher detail than would be achievable through holistic analyses. Establishing this methodology will not only be beneficial for improving our understanding of the soil microbiome but could conceivably be applied to other complex microbiomes as well.

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