15. Mapping soil carbon from cradle to grave #2: Multi-level omics analyses for parameterization of trait-based models of rhizosphere microbial community function

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Project goals: Our primary objective is to determine how organic C decomposition and stabilization processes in soil are impacted by the interactions between plant roots and the soil microbial community (bacteria, archaea, fungi, microfauna). To accomplish this requires information on the key features or traits of grassland soil microorganisms that impact their fitness as members of the rhizosphere and/or detritosphere. Through the identification of these traits, we propose to develop and parameterize trait-based models of microbial community function to interpret and predict carbon stabilization and turnover in the rhizosphere.

Theoretical approaches to understand the links between community diversity and ecosystem processes have become important tools in ecology. In particular, the application of trait-based modeling to explain complex patterns in taxonomic distribution across spatial and temporal scales and environmental gradients is increasingly common within studies of plant ecology. These approaches also show promise for improving hypothesis testing in microbial ecology, by developing frameworks linking microbial functional guilds (metabolically diverse organisms with a common function like lignocellulosic biomass decomposition) with the physiological and ecological traits that govern fitness independent of their phylogeny. We are adopting a Dynamic Energy Budget-based heterotrophic framework to incorporate metabolic theory into our ecological framework. When used to describe grassland soil microbial communities, this model has the potential to reproduce the diversity of organic molecules (e.g., exudates and polymer pools) and different exudate input rates and stoichiometry - thus selecting for different combinations of ecophysiological traits that maximize fitness under specific conditions resulting in a dynamic emergent community.

To begin to dissect the grassland soil microbial communities into its functional guilds we are using a range of 'omics approaches to characterize metabolic potential and niche preference. We have sequenced soil metagenomes taken at two physiologically relevant time points (peak plant activity and prior to wet-up) and are reconstructing genomes. Predicted genes relevant to soil biogeochemical cycling are being functionally annotated using a comprehensive suite of HMM models. We have also generated a large library of isolated bacterial heterotrophs and have sequenced 40 of those to date. Niche preference of bacteria, archaea and eukarya is being determined using non-targeted metatranscriptomic sequencing and phylogenetic marker reconstruction.

For our two soil metagenomes, between 250-400 Gb of high quality sequence data was obtained. Assembly and binning are ongoing, but initial classification shows both metagenomes are dominated by Actinobacteria and Alpha-proteobacteria. We have developed a functional gene based database and pipeline for metagenome sequence data analysis and are testing this for annotation of unassembled and assembled data. 290 bacterial isolates were obtained from multiple dilute media formulations incubated over 2.5 months. At the 97% homology level, the majority of OTUs were unique to one media type. Comparison with estimates of OTU abundance reconstructed from metagenomes using Emirge shows that

the isolates represented between 8 and 13% of the soil bacteria by relative abundance with a similar phylogenetic distribution to the complete bacterial community. Preliminary analysis of 14 bacterial isolate genomes (Actinobacteria, Alpha-, Beta-, Gamma-Proteobacteria, Firmicutes, Bactreroidetes) demonstrates a varied repertoire of carbohydrate active enzymes with little phylogenetic signal. Analysis of codon usage bias suggests differential minimum generation times that may relate to growth strategy.

In order to determine whether specific members of the soil microbial and microfaunal communities showed niche preference for litter-containing rhizosphere versus bulk soil, we extracted and sequenced total RNA from appropriate regions of greenhouse-maintained mini-rhizotrons cultivated with the annual grass *Avena fatua*. SSU rRNA genes were reconstructed using Emirge. Using this PCR-independent approach we determined that the litter in the presence of living roots selects for numerous Actinobacteria and Chloroflexi as well as fungi, while litter in bulk soil selected for Firmicutes and Bacteroidetes in addition to protists from the Amoebozoa and Alveolata amongst others.

Together these data are being used to determine the ecophysiological traits associated with rhizosphere enrichment and litter decomposition in grassland soils. Our overall goal is to use these and other information to assign functional roles to soil microorganisms and to develop mathematical models to predict their dynamics and contributions to soil carbon transformation.