Determining key physiological and metabolic traits of soil microorganisms that regulate C assimilation and transformation

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Project Goals: Our project works towards a fundamental understanding of C cycling in soil as mediated by soil microorganisms and their interactions with plants. How do the interactions between roots and soil microorganisms affect transformations of root derived C, decomposition and loss as CO_2, as well as C sorption and stabilization in soil? We seek to gain a mechanistic understanding of the conversion of root-derived C to stabilized soil C, clarify the impacts of microbial activities on soil C sequestration, and substantially expand our understanding of molecular regulation of terrestrial C cycling.

Plant-soil-microbial interactions may strongly impact the stability of soil organic carbon (SOC). Plants exude variety of compounds, supplying rhizosphere microorganisms with readily assimilable substrates and driving microbial succession in response to developing plant roots. Uptake of root exudates may stimulate microorganisms to produce enzymes that degrade more complex SOC and potentially decrease the stability of older C in soil. Alternatively, it has been shown that root exudates may liberate C from protected mineral surfaces in soil making it more available for microbial mineralization. Together these contribute to the phenomenon known as “rhizosphere priming”. Although rhizosphere priming of SOM decomposition has been widely demonstrated, the mechanisms underlying this effect and our ability to accurately predict it remain uncertain. We combine modeling and experimentation to determine key traits of soil microorganisms relevant to their fitness in the rhizosphere and transformation of carbon.

To define putative microbial traits we used metagenomic and isolate sequences from a Mediterranean grassland soil to identify changes in microbial composition and function in response to plant growth. First, we carried out genome-centric analyses using 38 bacterial isolates representative of the dominant organisms identified in metagenomes of this Mediterranean grassland soil. To determine the trajectories of these bacterial heterotrophs during key developmental phases of our model plant (*Avena fatua*), we obtained DNA from rhizosphere and bulk soil sampled over 12 weeks of plant growth with ~5GB of metagenome reads obtained per time point. Reads were aligned to the isolate genomes and coverage of genomes and marker genes were used to track bacterial dynamics during the root growth. Second, a genome-centric approach was taken to reconstruct genomes from metagenomic reads obtained from field soil sampled from dry and wet seasons and mini-rhizotron bulk/rhizosphere soils sampled during root growth. A total of 851 GB of sequence were co-assembled and binned based on differential coverage and sequence composition. Curation of these bins resulted in 92 organismal bins (82 bacterial, 7 archaeal, and 3 fungal), each representing a reconstructed genome. These reconstructed genomes were classified based on their

response to root growth into general classes: positive responders (early, late, and gradual), and negative responders. The genomes were annotated with a specific focus on genes for degradation and transformation of complex carbohydrates, organic C-oxidation, C-fixation, and transporters; therefore linking the organisms and their response patterns with functional traits.

To test putative functional traits we used the sequenced heterotrophic isolates. Each genome was analyzed for key traits, such as minimum generation times, polymer degrading enzymes and transporters for different substrates (amino-, fatty-, organic-acids, sugars, nucleotides and plant hormones). Clustering of genomes according to these functional traits suggested that certain classes (e.g. α-proteobacteria) had a greater representation of rhizosphere fitness traits and may be expected to respond positively to root development.

We tested predicted traits using variety of functional analyses. Predicted minimum generation times (based on codon usage bias) were experimentally validated and demonstrated as a reliable approach for growth rate prediction in uncultivated microorganisms. Polymeric substrate preferences and enzymatic activities of different taxa were analyzed using nanostructure initiator mass spectrometry and fourier transform infrared spectroscopy. Based on these analyses Actinobacteria were shown to metabolize more recalcitrant C, such as lignin while α-proteobacteria demonstrated preferences towards cellulose when incubated with root litter. Enzymes involved in degradation of specific polymers were identified through secretome proteomic analysis.

The ability of rhizosphere microorganisms to utilize simple substrates and their metabolic response to plant root exudates was tested using a complex exudate medium. *Avena* exudates were collected, and identified my LC-MS/MS during plant development. Exudation composition showed significant differences both across vegetative stages and between vegetative and senescence stages. These patterns across plant developmental stages were associated with successional changes in rhizosphere microbial communities. Bacterial substrate preferences were tested by growing isolates on the exudate-derived medium and substrate uptake/release determined by LC-MS/MS. This approach provides an ability to link genome predictions of microbial substrate preferences with substrate utilization and niche specialization in the rhizosphere.

To predict the metabolic response of bacterial heterotrophs *in silico* to plant exudates and their impact on C turnover we developed genome-scale metabolic models of both positive and negative rhizosphere responders. The models were manually curated and gapfilled using BIOLOG and exometabolomics and are currently being adapted to represent extracellular enzyme production. Together these approaches are being used to predict and test how key traits of soil bacteria interact with root metabolism and soil organic matter to impact the phenomenon of rhizosphere priming. Our goal is to develop predictive metabolic and trait based models of the rhizosphere to simulate how climate driven changes in vegetation will feedback to soil C cycling and nutrient availability.

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