Molecular basis of plant-microbe-soil interactions shaping microbial communities and ecosystem function in a Mediterranean grassland

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Project Goals: Our project addresses both a fundamental understanding of carbon (C) cycling as mediated by multi-trophic interactions in the rhizosphere and the potential impacts of altered precipitation regimes on these interactions. Specifically, our work employs the use of stable isotopes to identify and quantify pathways of C-flow through multi-trophic interactions in the rhizosphere. Primary goals are to broaden knowledge of the roles played by multi-trophic interactions in terrestrial C cycling and to discover if drought alters the interactions and/or C cycling. This research will substantially expand our knowledge of soil microbial ecology, belowground food web, and terrestrial C cycling under a changing climate.

Soil is the one of the most heterogeneous systems on earth where complex interactions exist between minerals, hydrology, chemistry and soil organisms. This heterogeneity creates diverse environments that often contrast in the type and availability of nutrients. Plants release substantial quantities of simple C molecules into the soil surrounding their roots, attracting bacteria with readily available resources. In contrast, the surrounding bulk soil represents an environment with significantly lower availability of small organic molecules. How microbes adapt to each of these environments and how these adaptations influence soil C transformations and microbial community assembly is complex and poorly understood.

To assess microbial adaptations and mechanisms of rhizosphere and bulk soil community assembly, we are using a common oat grass, *Avena spp.,* an abundant Mediterranean grassland species as a model system. We employ **(i)** exometabolomics to analyze microbial uptake of simple C molecules enriched in the rhizosphere; **(ii)** secretome analysis to identify mechanisms by which bacteria access root polymer carbon characteristic of the detritusphere; **(iii)** metatranscriptomic gene expression in the rhizosphere and bulk soil surrounding individual roots to determine how simple C compounds in root exudates influence complex soil organic matter degradation potential and community assembly.

(i) To investigate the ability of rhizosphere and bulk soil bacteria to metabolize simple carbon sources, we designed a medium to simulate previously identified plant exudates. We used LC-MS/MS based metabolomics to measure microbial consumption and release of metabolites and examine the substrate preferences of a diversity of rhizosphere and bulk soil bacterial isolates. We found that amino acids, sugars and nucleosides were consumed by all analyzed isolates. However, isolates that were preferentially stimulated by plant growth had substrate utilization preferences for aromatic organic acids, while bulk soil bacteria did not utilize these compounds. We used untargeted metabolomics to identify secondary metabolites released by rhizosphere

microorganisms that may be used to interact with the plant, influence its metabolism, and compete and cooperate with other organisms in rhizosphere.

(ii) Next we investigated the ability of soil bacteria to use complex polymeric carbon sources representative of the detritusphere. Carbon sources including cellulose, xylan, and a complex polymers contained within ground roots were provided as substrates and proteomics was used to identify extracellular proteins produced by rhizosphere and bulk soil isolates. We analyzed the activities of polymer degrading enzymes (β-glucosidase and β-xylosidase) with Matrix Assisted Laser Desorption/Ionization (MALDI) and labeled substrates. We determined that bulk soil adapted bacteria secrete a range of glycoside hydrolases (GH) and associated carbohydrate binding modules (CBMs) in the presence of plant polymers. We identified that bulk soil bacteria appear to have a preference for complex plant polymers, while rhizosphere bacteria appear metabolically adapted to use simple carbon sources that are commonly enriched in the rhizosphere. We propose that this substrate partitioning between rhizosphere and bulk soil organisms and release of secondary metabolites by bacteria are key mechanisms underlying rhizosphere community assembly and successional trajectories in observed soil.

(iii) To evaluate the hypothesized mechanisms of community assembly in the rhizosphere, we analyzed metatranscriptome gene expression of soil surrounding individual roots in microcosms experiments over 3 weeks of rhizosphere development. Half of the microcosms were also amended with root detritus to determine how simple and complex plant derived carbon influences community assembly and associated carbohydrate degradation potential. Population transcripts were binned using a unique reference database generated from soil metagenome-assembled genomes (MAGs), rhizosphere stable isotope probing (SIP)-MAGs, single amplified genomes (SAGs), and a panel of genomes from soil isolates. Both taxonomic and functional community composition rapidly diverged by 3 days, but functional composition underwent at major shift at 22 days that was undetectable by taxonomic community analyses. Bacterial niche differentiation during rhizosphere establishment was spatially and temporally regulated, where guilds of decomposers emerged that had distinct habitat preferences and timescales for decomposition. In particular, we identified three populations belonging to the Verrucomicrobiota, Fibrobacterota, and Burkholderiales that were synergistically upregulated in the rhizosphere amended with bulk litter. Roots stimulated the upregulation of genes coding for carbohydrate depolymerization, with the highest number of genes expressed in rhizospheres amended with root detritus. Together this work leads to a clear conceptual model where substrate preference and strategies for substrate utilization drive niche differentiation of bacteria in soil and provides a path to predictive models of rhizosphere microbial succession and its consequences for the fate of soil carbon.

References

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