The interconnected rhizosphere: Roots stimulate high microbial network complexity and alter the expression of enzymes related to organic matter decomposition in soil

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Project Goals: The soil surrounding roots, the rhizosphere, is the primary nexus of belowground carbon cycling in terrestrial systems. Our project focuses on a fundamental understanding of C cycling in soil as mediated by soil microorganisms, their interactions, and how these interactions are changed in response to plants. In particular, our work investigates how the interactions between roots and soil microorganisms alter transformations of root derived C, and explores how roots alter the networks of microbe-microbe interactions in soil. The primary goals of this work are to determine how organic C decomposition is impacted by the interactions of the soil microbial community with living roots, and substantially expand our understanding of the microbial ecology and molecular regulation of terrestrial C cycling.

The soil surrounding plant roots, the rhizosphere, has long been recognized as a zone of great functional importance to plants and the terrestrial ecosystems they inhabit. Plants play a central role in transferring atmospheric CO_2 to belowground soil C pools, while microbes are primary mediators of C transformation and mineralization in the soil. However, the molecular mechanisms underlying soil-plant-microbial interactions are poorly understood. Rhizosphere microorganisms can alter the breakdown of plant tissues and root litter, and in many cases have been shown to accelerate the decomposition of detrital plant biomass. The soil microbial communities and carbohydrate and lignolytic gene transcripts mediating the decomposition of root litter in soil are largely unidentified.

We hypothesized that root exudates stimulate the expression of enzymes that are involved in decomposition of macromolecular C compounds. To assess how the abundance and diversity of decomposition enzymes differs in the rhizosphere relative to the surrounding bulk soil, we sequenced the metatranscriptomes of rhizosphere and bulk soil over time. Our results suggest that enzymes involved in the breakdown of plant polysaccharides were more highly expressed in the rhizosphere compared to the bulk soil. In addition, we are using genomic and proteomic approaches (including stable-isotope techniques) to identify key metabolic pathways responsible for C transformation and mineralization during root in-growth and root death/decay. By integrating stable isotopes as tracers of natural resource utilization (i.e. root litter), and analysis of the functional properties of the communities that respond to those resources, we can identify

the microbial communities that are stimulated in the soil microbiome in response to root litter, living roots, and their intersection.

While there has been much study of the interactions between plant roots and soil microorganisms, we know little about the interactions among the microbial members of the root microbiome and how these relationships change over time. To identify networks of potential microbe-microbe interactions within the rhizosphere, we examined the co-occurrence patterns of bacteria in the rhizosphere soil of wild oat (*Avena fatua*), and compared them to patterns in the surrounding bulk soil. Random matrix theory (RMT) based network analysis was employed to identify such interactions using rDNA gene sequencing over two growing seasons.

Our results revealed that bacterial networks in rhizosphere were substantially more complex than those in the surrounding bulk soils, and the complexity of the bacterial networks increased as the plants grew, even as univariate diversity decreased. In contrast, network patterns in the bulk soils remained relatively static over the experimental period. Increased network complexity coupled to decreased diversity highlights that interactions are a crucial dimension of community organization overlooked by univariate diversity metrics. Within the rhizosphere networks, groups of highly connected modules formed over time, which most likely represent both interactions as well as microbial niches developing in response to root-induced changes of the soil environment. Consistent with the hypothesis that extensive mutualistic interactions occur among rhizosphere bacteria, covariations were predominantly positive (>80%); we identified quorum-based signaling as one potential interaction strategy. Highly connected taxa identified as putative keystone species (module hubs and connectors) often had low relative abundance in the rhizosphere (< 0.1%), which suggests that focusing on abundant taxa may overlook organisms that could play important roles in maintaining rhizosphere community structure and function. Network complexity appears to be a defining characteristic of the rhizosphere microbiome, and is a previously undescribed property of this habitat.

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