

## **Roots stimulate expression of decomposition transcripts in the soil microbiome**

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**Project Goals: Our project (*Mapping soil carbon from cradle to grave: drafting a molecular blueprint for C transformation from roots to stabilized soil organic C*) focuses on a fundamental understanding of C cycling in soil as mediated by soil microorganisms and their interactions with plants. We are measuring how organic C decomposition is altered when soil microbial communities interact with living roots, and want to better understand how interactions between soil minerals and microorganisms affect C stabilization processes, particularly in the rhizosphere. Through our research we seek to provide 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.**

The soil surrounding roots, the rhizosphere, has long been recognized as a zone of great functional importance to plants and is a hotspot of belowground carbon cycling in terrestrial systems. Plants transfer atmospheric CO<sub>2</sub> to belowground soil C pools, while microbes are the primary mediators of C transformation and mineralization in soils. The rhizosphere environment alters the microbial breakdown of plant tissues and root litter, and can accelerate the decomposition of detrital plant biomass, a process commonly termed as “priming”. However, the molecular mechanisms underlying rhizosphere C cycling are poorly understood, and the hydrolytic and lignolytic proteins mediating the decomposition of root litter in soil are largely unknown

We hypothesized that root exudates stimulate the expression of enzymes that are involved in decomposition of macromolecular C compounds. To assess how enzyme-mediated decomposition differs in the rhizosphere relative to the surrounding bulk soil, we analyzed community gene expression (metatranscriptomes) and single cell genomes of rhizosphere and bulk soil associated with wild oat (*Avena fatua*) over time (3, 6, 12, and 22 days). To isolate roots of a defined age in a mature plant, we used microcosms with a transparent experimental sidecar to track roots as they grew. Half of the microcosms were amended with dried *A. fatua* root litter, which was added to the experimental sidecar immediately prior to the start of the experiment. After harvesting rhizosphere and bulk soils, RNA was extracted, and ribosomal RNA was depleted to enrich for mRNA. In total, we sequenced 48 soil metatranscriptomes, which contained approximately 40 million high-quality, paired-end mRNA reads per library. Transcripts were mapped to 96 metagenomic genome bins, 35 single-cell genomes, and 39 isolate genomes; all derived from soil collected at the same location in Hopland, CA, USA. Differential expression analysis showed significant changes in gene expression between rhizosphere and bulk soils at all time points.

Our results indicate that a large number of C decomposition transcripts were more highly expressed in the rhizosphere compared to bulk soil. Of the Carbohydrate Active Enzyme (CAZyme) transcripts that significantly differed between rhizosphere and bulk soil, 96% of were significantly elevated in the rhizosphere. These included transcripts for cellulose and hemicellulose degradation genes, including beta-glucanases, beta-glucosidases, and xylanases. Gene transcripts potentially involved in decomposing microbial necromass were also elevated in the rhizosphere (e.g., chitinases, lytic murein transglycosylases, peptidoglycan/xylan/chitin deacetylases). While we found that many of our reference genomes had the genetic capacity to decompose plant polymers (ca. 33%) and all of these organisms had detectable gene expression, we measured significant transcription of plant decomposition genes in only a small subset of these organisms (ca. 4%). Three genome bins derived from the soil metagenome were particularly active in the rhizosphere compared to bulk soil, and altered their expression of CAZymes depending on the age of the root. *Janthinobacterium* (Oxalobacteriaceae) had higher expression of cellulases and xylanases near young roots (3-6 days old), while *Rhizobacter* (Burkholderiales) and an unclassified Streptomycetaceae (Actinobacteria) had higher expression of cellulases and xylanases in more mature roots (12-22 days old).

This work identifies potential molecular mechanisms that underpin the ‘priming effect’ in rhizosphere soil. We found that transcription of genes involved in decomposition was stimulated in the rhizosphere at all time points, which supports the hypothesis that roots stimulate enzymes for the decomposition of macromolecular carbon in soil. The expression of decomposition genes was dynamic and changed as the root grew, indicating that decomposition is undertaken by a series of different organisms as the root grows. However, while many rhizosphere taxa appear to have the capacity for lignocellulose degradation, only a limited group is actively filling this niche at a given time. This suggests that rhizosphere priming is influenced by the stage of root growth, and that the organisms catalyzing this decomposition may be more limited than previously expected based on genomic or metagenomic surveys.

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