Mapping the pathways of root carbon flow into and through soil microbial food webs

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Project goals: The flow of carbon (C) from roots into soil is controlled by a complex array of interactions. Our project addresses how multi-trophic interactions mediate the flow and fate of root C into soil and how changing precipitation regimes alter these interactions. This newly initiated research project uses stable isotope probing (SIP) and genome-resolved metagenomics to identify and characterize the participants in root-C-based food webs and to understand the ecological interactions in the rhizosphere that ultimately control the fate of C entering soil. We are tracking ^{13}C-labeled C moving from roots into root-exudate and debris consumers and through the members of the soil food web supported by these primary consumers. Genome-resolved metagenomic analyses of the SIP-isolated DNA then allows us to better understand the functional characteristics of rhizosphere C-transformers and illuminate the carbon basis of these interdomain interactions in soil.

The zone of soil influenced by roots, the rhizosphere, is of great importance to plant and soil health and carbon cycling. While there have been numerous explorations of root-bacterial and root-fungal interactions, there has been little work on how complex biotic, multi-domain interactions mediate and control C flow in the rhizosphere. We hypothesize that the complex interactions among bacteria, archaea, fungi, bacteriophages, and fauna are primary controllers of the flow and fate of root C. We propose to identify the players and ultimately assess the quantitative importance of the multiple pathways of C-flow in the rhizosphere using stable isotopes (^{13}C) coupled with metagenomic analyses. We expect that metagenomic data will allow us to better understand interdomain interactions as well as bacteriophage-host interactions in soil. For the work reported here, we grew *Avena fatua*, common wild oat, in $^{13}CO_2$ (99 atom%) and collected starting soil, rhizosphere soil, and bulk soil (soil not associated with roots) at weeks 0, 6, and 9. The DNA extracted from these samples was then prepared using density-gradient centrifugation, yielding DNA samples with a range of ^{13}C label: unlabeled, partially labeled, and heavily labeled DNA. Samples for each time point were prepared using this procedure and the "purified" DNA was then sequenced (HiSeq 3000); the reads were assembled, and binned to yield genomes.

Using relevant marker genes and whole genomes, we investigated microbes living in the rhizosphere: those that grew and incorporated the ^{13}C into their DNA and those that did not. The microbes living in rhizosphere that do not incorporate root carbon into their DNA are more like the bulk soil community, suggesting that investigations of rhizosphere ecology that do not employ stable isotope probing may underestimate the degree of difference between the rhizosphere community and the bulk community. We were able to retrieve many genome bins from our soil samples that were at least 70% complete. Using whole genome information, we were able to functionally characterize the rhizosphere microbial community and better

understand the selective pressure that the root exerts. Using hidden Markov models and homology based searches we investigated genes involved in energy metabolism and carbon and nitrogen cycling in our recovered microbial genomes.

In addition to microbial functions, assembled metagenomic data from soil allow us to explore other members of the microbial world. This is especially true for the study of bacteriophages, for which there are no universal primers. Through genome resolved metagenomics, we could assemble and identify bacteriophage genomes; some bacteriophages were identified in the heavily labelled fraction indicating they must have parasitized microbes growing on root derived carbon. Using bioinformatic analyses of CRISPRs as well as tRNAs, and homologous matches, we were able to link a number of bacteriophages to their host bacteria with confidence. This suggests that not only are bacteriophages shaping bacterial communities in the rhizosphere but that bacteriophages may be critical in determining the flow and fate of C entering soil as root exudates and debris. In addition to metagenomic identification of bacteriophages, we were also able to identify labelled bacterivore nematode 18S rRNA genes indicating they are consuming labeled bacterial cells. The quantitative importance of these top-down control processes is a major focus of this ongoing project.

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