

Title: Soil microbial food web mapping with high resolution stable isotope probing

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Project Goals: Short statement of goals. (Limit to 1000 characters)

Project Goals: This research program will reveal fundamental aspects of soil C-cycling and provide ecological and metabolic insights on diverse non-cultivated soil microorganisms that play major roles in the global C-cycle. Specific goals include: 1) Map the C assimilation dynamics for thousands of non-cultivated microorganisms in soil by harnessing a full cycle microbial food web mapping approach that employs an array of ^{13}C -labeled molecules; 2) Map the C assimilation dynamics of soil microorganisms across soil systems as a function of soil characteristics; and 3) Evaluate ecological and seasonal patterns of activity and abundance for discrete microbial taxa across gradients of soil characteristics and as a function of their C-assimilation dynamics. These goals will be achieved by employing a newly developed microbial food web mapping approach, enabled by advances in ^{13}C -stable isotope probing of nucleic acids and next generation sequencing.

Soils make up the largest active carbon pool on the planet. Although carbon cycling in soil is largely mediated by microbial life, the specific taxonomic groups that perform each role in the soil microbial food web have not been well resolved. High-resolution stable isotope probing (HR-SIP) leverages highly multiplexed high-throughput 16S rRNA sequencing to simultaneously map *in situ* substrate assimilation dynamics to potentially thousands of finely resolved microbial taxa. In this study, HR-SIP was performed with nine ^{13}C isotopes (cellulose, xylose, glucose, glycerol, vanillin, palmitic acid, amino acids, lactate, and oxalate) in order to identify carbon assimilators at multiple stages in the breakdown of plant biomass in soil.

During the 48 day incubation, we observed a coinciding succession of ^{13}C -substrate respiration and also incorporation into bacterial biomass. Relatively labile substrates (e.g., glucose) were utilized first (days ~1-6), followed by oxalate (days ~6-14), and finally by cellulose and palmitic acid (days ~14-30). The total amount of ^{13}C respired varied substantially between substrates, from ~35% for vanillin to nearly 100% for lactate. The number ^{13}C -incorporating taxa (“incorporators”) also varied among treatments, with ~4-6% of taxa incorporating cellulose or palmitic acid, compared to <2% of taxa incorporating any other substrate. Phylogenetic similarity was very high between cellulose and palmitic acid incorporators and also between lactate and oxalate incorporators. Interestingly, nearly all of the lactate incorporators identified on day 6 were also identified as oxalate incorporators, but not until day 14, suggesting diauxic growth is common for soil bacteria consuming these fermentation products. While certain *Gammaproteobacteria* and *Firmicutes* taxa consumed almost all substrates, many other taxa specialized on one or two substrates. For instance, certain *Firmicutes* taxa solely

incorporated glucose although all other substrates were present. These results suggest pervasive niche partitioning among bacterial taxa in the soil carbon cycle, with partitions for both the relatively transient dissolved organic matter pool and the more persistent particulate organic matter pool. More generally, these findings will help define ecologically relevant taxonomic groups of microbes with coherent functional roles in the soil microbial food web.

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