Title: Mapping microbial food web dynamics in soil 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. We performed an HR-SIP experiment that employed nine ^{13}C-labeled substrates chosen to represent organic matter present during plant biomass degradation (cellulose, xylose, glucose, glycerol, vanillin, palmitic acid, amino acids, lactate, and oxalate).

We observed a succession of ^{13}C-substrate respiration and incorporation into bacterial biomass. Relatively labile dissolved substrates (e.g., glucose) were utilized rapidly (days ~1-6), followed by oxalate (days ~6-14), and finally by insoluble substrates such as 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 a greater diversity of taxa incorporating insoluble substrates (cellulose or palmitic acid), than dissolved substrates. We find evidence for both generalist and specialist taxa. For example, certain *Gammaproteobacteria* and *Firmicutes* responded rapidly and consumed almost all substrates, though many other taxa in these clades specialized on one or two substrates. In contrast, many *Verrucomicrobia* grew slowly and specialized on insoluble substrates such as ^{13}C-cellulose and ^{13}C-palmitic acid. Clustering OTUs by their signal of incorporation in each treatment produced an assortment of groups with differing functional roles in the

carbon cycle (e.g., vanillin or cellulose specialists). Most functionally coherent groups contained multiple phyla. 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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