35. Microbial Food Web Mapping: Linking carbon cycling and community structure in soils through next generation sequencing enabled stable isotope probing

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Project Goals: This research program explores fundamental aspects of carbon cycling in soil microbial communities. Major goals are to develop and validate methods for next generation sequencing enabled stable isotope probing (NGS-SIP) and to use this approach to dissect the microbial food web in soil. NGS-SIP offers a means to study the microorganisms that facilitate soil processes as they occur in soil, to characterize novel organisms that have escaped detection previously, and to make significant advances in our understanding of the biological principles that drive soil processes. With this approach we will examine connections between microbial community composition and soil carbon cycle dynamics. Specific objectives include 1) determine whether carbon input parameters (composition, quantity, timing of carbon additions) alter the route of carbon through the soil community, 2) determine whether these shifts interact with respect to microbial community structure, and 3) evaluate whether microbial community structure is functionally equivalent across edaphically similar soils that differ in management history.

The terrestrial biosphere contains a large fraction of global C and nearly 70% of the organic C in these systems is found in soils. Much of the organic C in soils is respired and on an annual basis soil respiration produces 10 times more CO_2 than anthropogenic emissions, but it remains difficult to predict the response of soil processes to anthropogenic changes in the environment. Our difficulty in predicting how soil processes will respond to environmental change suggests a need for a greater understanding of the biotic mechanisms that govern the soil C-cycle. It is important to examine the internal dynamics of soil microbial communities, and the manner in which they influence community function, in order to understand the how the terrestrial C-cycle responds to environmental change. The NGS-SIP approach that we are developing will allow for pulse chase style experiments that allow 13C-isotopes to be tracked through the soil community over time. The approach will involve the application of synthetic biomass containing a mix of carbon sources designed to approximate the plant biomass. The use of synthetic biomass allows substitution of 13C-labeled substrates into the mixture to track the manner in which different types of C are metabolized by different components of the community.

Experiments have explored the metabolism of cellulose (an insoluble polymer) and xylose (a soluble sugar monomer resulting from breakdown of hemicellulose) by soil communities over time. The NGS-SIP approach has provided data for more than 6,000 bacterial taxa. Soil C amendment caused change in community composition over time but only a subset of microorganisms in the community assimilated 13C from xylose or cellulose. Xylose incorporation into DNA occurred by day 1 but could no longer be detected by day 14, indicating biomass turnover. Cellulose degradation proceeded more slowly with 21% of cellulose C respired by day 7 and 60% respired by day 30. Incorporation of cellulose-C into DNA was observed only after 14 days. DNA incorporation of 13C from xylose suggest partial labeling which indicates assimilation of C from sources other than xylose. In contrast, taxa which incorporated 13C from cellulose were highly labeled indicating assimilation of C almost entirely from cellulose. Dominant taxa that assimilated C from xylose include *Arthrobacter*, *Agromyces*, *Rhizobium*, and *Paracoccus*, among others. Dominant taxa that assimilated C from cellulose include *Cellvibrio*, a novel non-cultivated lineage of *Chloroflexi*, and *Verrucomicrobia* among others. These data support the succession hypothesis of decomposition in that

sugars are degraded by fast growing opportunistic organisms and insoluble polymers are degraded more slowly by polymer degrading specialists. In addition, the results have identified novel taxa that mediate cellulose deconstruction in soil.

Several follow up experiments have been performed to explore the dynamics of C assimilation. In particular, we have performed parallel analysis of rRNA to determine if assimilation of 13C into rRNA provides additional insights not provided by DNA analysis. We have also performed parallel analysis of fungal taxa that incorporate 13C from cellulose. Finally, we have performed metagenomic analysis of NGS-SIP gradient fractions to identify genome fragments from bacteria that have assimilated 13C from 13C-cellulose. Analysis of rRNA indicates that rRNA is labeled more quickly than DNA in soil. In particular, 23% of community rRNA is labeled at day 7 while only 2% of community DNA has been similarly labeled. At day 14, 54% of community rRNA is labeled while only 6% of community DNA is labeled. The dramatic difference between the extent of rRNA and DNA labeling on a weekly timescale suggests a phenomenon other than metabolic shift up must be invoked to explain this result. We are currently evaluating the hypothesis that uncoupled growth dynamics results in 13C-cellulose degradation and incorporation of 13C into rRNA by cells that are not actively dividing and replicating DNA. This hypothesis makes the prediction that 'uncoupled taxa' will show labelling of rRNA but not DNA whereas replicating taxa will demonstrate labelling of both rRNA and DNA. If verified this hypothesis would reveal differences in ecological strategies between microbial taxa with implication on C use efficiency and C fate in soil.

In a second series of NGS-SIP experiments we have evaluated the effect of priming on cellulose decomposition in soils. The addition of labile C to soils has been shown to alter decomposition dynamics with the manner of application resulting in different responses. In particular, small additions of labile C added over time have been observed to have a larger impact on decomposition than the equivalent addition of labile C made in a single dose. This phenomenon has been hypothesized to relate to the ability of plant roots to prime organic matter mineralization by exuding labile C to stimulate microbial activity and thereby facilitate access to associated macro and micronutrients. We performed an NGS-SIP experiment with 13C-cellulose and priming doses of glucose to determine if the microbial community mediates the altered decomposition dynamics observed in response to different priming regimes. Analysis of these experiments is ongoing.

Finally, NGS-SIP results are being extended to evaluate the distribution of xylose and cellulose responsive taxa across a series of agricultural plots that vary in organic matter management history over a span of 50 years. Changes in management practice associated with crop rotation have resulted in a gradient of soil organic matter content. We have shown that soil organic matter content is the primary factor explaining differences in microbial community composition across these plots. We hypothesize that generalist taxa are favored in intensively managed low organic matter sites while cellulose specialists will be favored in sites where soil organic matter accumulates in response to plant biomass inputs.