Microbial Food Web Mapping: Linking carbon cycling and community structure in soils through pyrosequencing 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 pyrosequencing enabled stable isotope probing (SIP) and to use this approach to dissect the microbial food web in soil. Pyrosequencing enabled 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

While strides have been made in understanding environmental controls on decomposition we still lack a coherent concept of the soil microbial food web. There is a general assumption of functional equivalence for different soil microbial communities with respect to the soil C cycle, but the validity of this hypothesis has been questioned. This deficiency in our knowledge results from the absence of in situ methods for identifying microorganisms involved in the soil C cycle and as a result we have a glaring lack of information about which organisms actually mediate critical soil processes. The pyrosequencing enabled stable isotope probing 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 (ie: polymers and sugars) are metabolized by different components of the community.

Initial experiments have explored 1) what are the dynamics of degradation for ^{13}C-cellulose plant simulant and how does the ^{13}C move through community nucleic acids over prolonged incubation, 2) how does the community respond to the addition of ^{13}C-cellulose plant simulant relative to the addition of only 13C-cellulose, and 3) how does the ^{13}C assimilation into microbial nucleic acids from ^{13}C-xylose plant simulant vary from that of ^{13}C-cellulose plant simulant when all aspects of the experiment are identical except for the isotopic label. $^{12}CO_2$ and $^{13}CO_2$ generated from microbial respiration is determined over a month long incubation and soils are sampled destructively over time. Samples from different times are subject to DNA and RNA stable isotope probing and 454 pyrosequencing of gradient fractions. These data are used to determine the buoyant density profile for individual OTUs in ^{13}C treatments relative to

unlabeled controls and to observe how the degree of 13C-label incorporation by individual OTUs changes over time.

Future experiments will explore how food web dynamics differ between edaphically similar soils that differ in land management practice. To prepare for these experiments we have identified a series of suitable sites that represent a gradient of management impact resulting in accumulation of soil organic matter. The sites include an intensively managed corn field, fields that were removed from intensive management for 10 or 20 years and subsequently managed for organic grain crops, and long term pasture. Initial characterization of the soil bacterial and fungal communities by 454 pyrosequencing is currently underway in order to determine the community composition in these sites and develop protocols for pyrosequencing enabled ^{13}C-SIP. Using these data, OTUs identified by ^{13}C-SIP can then me mapped back to the landscape in space and time to evaluate their distribution and importance in native soil systems.

The method and the results generated by this project will improve our ability to examine the impacts of management decisions, soil history, and environmental change on the behavior of microbial communities in terrestrial ecosystems, revealing the ecological mechanisms by which microbes regulate both C mineralization and C retention in soils, and improving our ability to predict changes in terrestrial ecosystem processes in the face of accelerating global change.