55. Multi-‘Omic’ Analyses of the Dynamics, Mechanisms, and Pathways for Carbon Turnover in Grassland Soil

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Project Goals: Climate change will alter terrestrial ecosystems. However, the strength and the direction of change will be shaped by feedbacks, most of which will be difficult to predict. Of primary importance in this regard is how the distribution of carbon between the atmosphere and the subsurface will change in response to altered rainfall, temperature and vegetation patterns. Metagenomics, proteomics, transcriptomics, and metabolomics will be used to compare the membership and functioning of soil communities at three different depths below the grass root zone. Overall, this program will result in critical advances in our understanding of soil microbial community carbon cycling to address the question of how the grassland ecosystem will respond to future climate change.

Abstract:
In this project, we will investigate how different rainfall impacts the dynamics of carbon stored in grassland soil, and the potential consequences for release of carbon and other nutrients from soils to streams. However, little is known of the microorganisms that play vital roles in the processing of this largely vegetation-derived soil carbon and how the metabolic activities that occur at different soil depths ultimately impact DOC discharged into streams. We are studying the period around the first Fall rainfall event, when soil-associated carbon fixed during Spring growth is rapidly metabolized, focusing on climate manipulations that differ 1) in the amount of Spring rainfall (above-ground carbon stocks) and 2) on the period of time following the first Fall rain events (soil microbial communities) and 3) soil depth.

Genomic information was obtained for ten soil samples from two sites at 10- 20 cm and 30- 40 cm depth, two of which were collected before the first rainfall and the rest after. Metagenomic data were assembled and draft genomes, including dozens of partial to near complete genomes, were binned and reconstructed based on time series coverage analysis and tetranucleotide frequency using ggKbase and emergent self-organizing maps. While present in most samples, many microbes were more abundant (estimated by coverage) at certain depths. Chloroflexi, Actinobacteria, and Verrucomicrobia were more abundant in the 10- 20 cm sample than 30- 40 cm while the Archaea domain, Methylomirabilis, GAL15, and NC10 were more abundant at 30- 40 cm than the 10- 20 cm depth.

Phylogenetic analysis showed that the soil is dominated by Archaea and several phyla of Bacteria. Most of the organisms clustered with closely related species, creating a dandelion-like structure at the branches’ termini. In many cases there were no reference sequences of considerable similarity, representing new phyla and many novel classes and orders within the Gemmatimonadetes, Verrucomicrobia, Deltaproteobacteria, Acidobacteria (most novelty here), and Chloroflexi phyla.

Polar metabolites were extracted from selected pre- and post-rain soil samples using an aqueous extraction method and detected on an Agilent 6550 iFunnel Q-TOF LC/MS system following separation on a zwitterionic stationary phase column. Approximately 140 putative compounds were tracked across soil depth profiles and preliminary analysis of the metabolite composition indicated that carbon and
nitrogen sources (simple sugars and quaternary ammonium compounds) clustered with depth, decreasing from 10 cm to 40 cm.

Total protein samples were extracted from soils using a modified MoBio NoviPure protocol and cleaned up and digested using the Filter-Aided Sample Preparation (FASP) method. The proteome digest samples were analyzed using 2-dimensional liquid chromatography coupled with high-resolution tandem mass spectrometry on a LTQ-Orbitrap Elite mass spectrometer. The mass spectral data were searched using the Sipros algorithm on the Titan supercomputer against a combined soil metagenome database constructed above. On average approximately two thousand proteins or protein groups were identified per soil sample with a peptide-level false discovery rate at 1% and a protein-level false discovery rate at ~2%. Proteomics analysis revealed that corresponding sugar transporters are present and that there are abundant proteins in all samples are those involved in methanotrophy and ammonification.

The results from these “omics” methods are quickly coming together to form a narrative about the dynamic microbial community and its processes below the grass root zone with respect to carbon flow during the Fall rainfall event. We anticipate these below ground terrestrial system feedbacks will impact the grassland ecosystem and the global carbon cycle.

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