

63. Developing mass spectrometry approaches enabling multi-'omic' analyses of the dynamics, mechanisms, and pathways for carbon turnover in grassland soil under two climate regimes

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Project goals: The goal of this project is to investigate how altered rainfall impacts carbon stored in grassland soil by characterizing effects on microbially mediated carbon decomposition and nitrogen cycle processes. The project will use genome targeted metagenomics, stable isotope resolved metaproteomics, and community metabolomic analyses. The study will leverage a long term ecological research site in California that features an ongoing experimental manipulation of water inputs to mimic shifting precipitation regimes that are relevant to climate change scenarios. New data visualization tools will be developed for the DOE Systems Biology Knowledgebase to facilitate integration of the information generated in the study and scaling of data to higher order process understanding.

Our program is focused on a decadal-scale climate manipulation experiment on a meadow in the Angelo Coastal Range Reserve in northern California. The reserve is a relatively pristine coastal environment with a Mediterranean climate, characterized by wet winters and long summer droughts. In environments such as this, changes in water abundance and the timing of rainfall may profoundly impact soil conditions, vegetation type, and ecological characteristics such as soil carbon storage and carbon turnover. For this reason, 13 years ago, UC Berkeley researchers initiated a series of experiments in which the intensity and seasonality of rainfall was manipulated (in 24 of the 36 experimental plots) to replicate two scenarios predicted by the current climate models. Prior research showed that extension of the spring rainy season significantly increased plant primary productivity (Suttle *et al.* 2007) and that the period immediately following the first autumn rainfall is critical for carbon breakdown.

Microorganisms are the primary agents responsible for breakdown and turnover of soil carbon compounds. After six years of rainfall manipulation, our group conducted preliminary 16S rRNA soil microbial community surveys that revealed seasonal responses that displayed the potential impact of extreme weather events (Cruz *et al.* 2009). The longer-term responses and soil system behavior are completely unknown. We will study 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.

We have developed powerful approaches to investigate community metabolism using metaproteomics and metabolomics in model Acid Mine Drainage Biofilms that we are actively working to extend to study the Angelo soils. Soil presents tremendous challenges to protein and metabolite analysis and so our current focus has been on developing extraction protocols using the Angelo soil samples. In 2013, eight total sets of samples were collected before and after the first fall rainfall. For each set, samples and 10-cm-deep soil cores were collected from four depths (0-40 cm) within two 0.25-m² quadrants and flash-frozen in the field in a dry ice/ethanol bath. Samples were aliquotted for metagenomic sequencing, community proteomics measurements, and metabolomics analysis.

A set of metagenomic samples from before the first fall rain and three different days afterward were extracted with a modified PowerMax Soil DNA Isolation Kit protocol. The 10-minute vortexing step was replaced with a non-shaking water bath at 65 °C and only gentle inversions every 10 minutes. This produced approximately 1 microgram per gram of soil, of high-quality DNA (260/280 ratios of 1.6-1.8) of fragment sizes larger than 23 Kb. These samples are currently in the JGI sequencing queue.

To optimize protein extraction for Angelo soils, we compared two methods: the SDS-boiling method (Chourey *et al.*, 2010) and the commercial MoBio Novipure kit. SDS-boiling co-extracted contaminants that complicated protein concentration estimation and sample clean-up. It was critical to minimize the presence of soil contaminants in proteome samples, such as humic acids, because they can create many challenges for liquid chromatography and mass spectrometry analysis. On the other hand, the Novipure kit produced colorless clean samples that are compatible with downstream preparation. The yield of protein extraction was estimated to be approximately 50 microgram of protein per gram of soil. The obtained samples were analyzed by shotgun proteomics using LTQ Orbitrap mass spectrometer. The quality of ion chromatograms was comparable to those from well-characterized systems, such as the Acid Mine Drainage microbial communities. Many peptides were detected with good chromatographic peaks and informative tandem mass spectra. This indicated effective protein extraction from Angelo soils, which will enable in-depth characterization of soil community proteomes.

For metabolomics analysis, total metabolites (extracellular and intracellular) were measured by fumigating soil samples with chloroform vapors for 24 hours. Unfumigated soil was used to analyze extracellular metabolites. Extractions were performed comparing buffers and the addition of organic solvents for soil metabolite analysis. Liquid chromatography/mass spectrometry and gas chromatography/mass spectrometry were used to evaluate the various extraction conditions. Fumigation is found to have the strongest effect on the range and abundance of metabolites detected, producing approximately two times more molecular features than unfumigated soil. Using this approach we detected a wide range of metabolites including amino acids, mono- and disaccharides, nucleosides, lipids and other metabolites consistent with our program goals.

The developed technologies provided the foundation for integrated -omics analyses of a representative grassland soil microbial community. The results will characterize dynamic metabolic processes within this community during a key period of carbon turnover.

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