Multi-'Omic' Analyses of the Dynamics, Mechanisms, and Pathways for Carbon Turnover in Grassland Soil

Cristina N. Butterfield[1]* (butterfc@berkeley.edu), Zhou Li[2], Peter Andeer[3], Susan Spaulding[1], David Burstein[1], Susannah G. Tringe[3,4], Brian C. Thomas[1], Robert Hettich[2], Trent Northen[3,4], Chongle Pan[2], and **Jillian F. Banfield**[1]

[1]University of California, Berkeley; [2]Oak Ridge National Laboratory, Oak Ridge, Tennessee; [3]Lawrence Berkeley National Laboratory, Berkeley, CA; [4]DOE Joint Genome Institute, Walnut Creak, CA

http://ggkbase.berkeley.edu

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 are used to compare the membership and functioning of soil communities at three different depths below the root zone in a grassland that experiences a Mediterranean climate. We track microbial community composition and activity during the period of major carbon turnover in this ecosystem under two rainfall scenarios, identify key carbon currencies released from the soil zone and provide a basis for prediction of how grassland ecosystems will respond to future climate change.

Abstract (2 page limit):
We are investigating how varied rainfall events impact the dynamics of carbon storage in grassland soil, and the potential consequences for the transport of carbon and other nutrients from these soils to the underlying vadose zone and 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 flanking environments. 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), 2) on the period of time following the first Fall rain events (soil microbial communities), and 3) soil depth.

We couple genome-resolved metagenomics with proteomics and metabolomics to determine the metabolic capabilities of soil microbial communities and to map carbon flow through the sub-root soil. Our research is conducted in a well-monitored grassland in the Angelo Coast Range Reserve in Northern California that has been subjected to a long term rainfall manipulation to simulate climate change. This grassland experiences a

Mediterranean climate, which means that mild rainy winters drive spring plant growth, and hot dry summers drive the senescence of these plants. Thus, the period immediately following plant death, when the first rainfall events occur, leads to the rapid transformation and mobilization of plant-derived organic carbon and its microbial degradation products within this system as well as to lower soil depths. In our pilot year (2013), we observed a disturbance in the overall architecture of the bacterial and archaeal communities and a subsequent rapid return to pre-rain, baseline levels. We reconstructed 198 genomes, including 46 near-complete genomes from the relatively abundant organisms from every bacterial and archaeal phylum, and binned the remaining DNA sequence based on taxonomy of predicted genes. This information was used to create a protein sequence database for proteomics. We identified and quantified 2880~4700 proteins and their microbial origins from 10 soil communities. We found that much of the community was actively degrading plant biomass following the first rain event of the season. Some of the most abundant functions were rarely reported in the literature, and included the aromatic carbon degradation by Thermoplasmatales archaea and methylotrophy by Gemmatimonadetes and Rokubacteria bacteria. Metabolomics analyses also illuminated that most organic and nitrogenous compounds are efficiently degraded by the 30 cm depth level.

Currently, we are automating methods that were successfully applied to the 10 samples collected in the 2013 Fall rainy season to analyze 60 soil metagenomes and 20 soil proteomes collected in Fall 2014. This increase in bioinformatics capacity will make processing of the 148 samples from 2015 possible. We have both automated and optimized the application of the metagenomic assembly program IDBA-UD and achieved a significant improvement in both assembly throughput and recovered scaffold lengths for the 2014 samples over the 2013 samples. We have explored how both read sequencing and physical sample depth relate to the difficulty of assembly of sequence information from a highly complex soil environment. Finally, we are exploring the application of differential coverage binning from very high numbers of samples and the integration of multiple automated binning programs to improve both the throughput and quality of genome binning significantly. In particular, differential coverage binning allows us to leverage sequence information from 2013 samples to improve genome bins of 2014 data, and will subsequently improve the binning of all samples in future years.

The 2014 dataset includes samples from plots amended with water over the last 15 years to simulate the longer spring rainfall predictions indicated in some climate change models. Comparison of microbial communities present in the control vs. manipulations should indicate to us a shift in lifestyle associated with environmental change. Preliminary metagenomics analyses indicate that the 2013 and 2014 pre-rain samples are similar in terms of community composition. A priority objective for analysis of these and post rain samples is to improve functional predictions to facilitate metabolic substrate specificity assignment and link this information to metabolomics measurements.

To aid in the identification of specific transformation pathways, we have developed a strategy using isotopically-labeled metabolites to follow transformations of particular carbon substrates by the soil community. The identification of these transformations

should facilitate the putative identifications of relevant proteins and microbes within our larger in situ field data sets. We can then validate these assignments using targeted-, multi-omic analyses of stable isotope probing experiments, which will enable the tracking of nutrients through community members and thus a model of the system.

Overall, "omics" methods are being integrated to capture the dynamics of microbial communities in soil below the grass root zone. Below ground terrestrial system processes will impact grassland ecosystem function and the global carbon cycle.

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