Meta-omics Analysis of Microbial Carbon Cycling Responses to Altered Rainfall Inputs in Native Prairie Soils

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Project Goals: Our overarching goal is to harness the power of multiple meta-omics tools to gain greater understanding of the functioning of whole-soil microbial communities and their role in C cycling. This goal will be met through the following three objectives: (1) to further develop and optimize and use a combination of meta-omics approaches to understand how climate shifts (precipitation timing/amount) impact microbially-mediated C cycling functions at different levels of expression and regulation; (2) to determine the impacts of long-term climatic changes (precipitation) on soil C cycling using an existing long-term field manipulation; and (3) to conduct laboratory experiments of specific environmental variables (moisture, C inputs) to confirm field observations of the linkages between microbial communities and C cycling processes.

Soils process and store large amounts of C; however, there is considerable uncertainty about the details that influence microbial partitioning of C into soil C pools and what influential forces control the fraction of the C input that is stabilized. It is not clear how the microbial community will respond to climate-induced modification of precipitation and inter-precipitation intervals, and if this response will affect the fate of C deposited into soil by the local plant community. Part of this uncertainty lies with our ignorance of how the microbial community adapts physiologically or in composition to changes in soil moisture brought about by shifts in precipitation.

To explore the response of soil microbial communities and C cycling to altered precipitation, we are using the rainfall manipulation plots (RaMPs) established more than a decade ago at the Konza Prairie in Kansas. The RaMPs experiment consists of two treatments: (1) natural precipitation, and (2) extended precipitation interval. The extended precipitation interval consists of storing rainfall and reapplying it at an interval 50% longer than that between natural rainfall events. We employ a systems biology approach, considering the complex soil microbial community as a functioning system and using state-of-the-art metatranscriptomic, metaproteomic, and metabolomic approaches. Our experiments are designed to specifically identify microbial community members and processes that are instrumental players in processing of C in the prairie soils and how these processes are impacted by wetting and drying events. At this time, we have made progress on our first two objectives: optimizing omics protocols and sampling the RaMPs experiment.

Omics optimization. Although our ultimate goal is to use omics data to understand the mechanistic responses of soil microbial communities to water content fluctuations, it was important to first optimize our methods of metatranscriptome and metaproteome analysis. To do this, we set up a short-term laboratory incubation using soil collected from the Konza field site. Several treatments were set up, (1) an unamended control, (2) soil inoculated with an *Arthrobacter* strain whose genome has been sequenced containing that contains the *gfp* gene, (3) soil plus *Arthrobacter* and amended

with acetate (a generic source of readily available C), (4) soil plus *Arthrobacter* and amended with 4-chlorophenol (a xenobiotic C compound that is metabolized by the added *Arthrobacter* strain), (5) soil amended with acetate, and (6) soil amended with 4-chlorophenol. Respiration was followed for 72 hours and periodically samples were taken for RNA extraction to measure the expression of specific genes. At the end of the incubation, we extracted for RNA for metatranscriptome and protein for metaproteome analysis. We found no change in the abundance of the added *Arthrobacter* strain (based on 16S rRNA gene or *gfp* gene copy numbers) at 72 hours, although transcript activity shifted by orders of magnitude. The shift in transcriptional activity was accompanied by a shift in the proteome signature. For example, in the presence of acetate, there was an increase in ATP synthase abundance. More complete results from this experiment are shown in the poster by David et al.

RaMPs experiment. We sampled the RaMPs experiment twice during the summer of 2011, in June during active plant growth when soils are relatively moist and in September after plant growth had peaked and soils were relatively dry. At each sampling period, we collected soils just before a 2.5-cm rainfall event ("pre"), 1 day latter ("pulse"), and 5 days after wet up ("post"), when soils had dried to water contents similar to those before the rainfall event. At each of these times, we generated samples to analyze microbial community properties (pyrotagged analysis of ribosomal DNA and RNA, metatranscriptome, and metaproteome) and soil C cycling processes (soil respiration, extracellular enzyme activities, microbial biomass, etc.). Soil C cycling processes responded to the wetting and drying events at both sampling times, but showed only small differences between RaMPs treatments. Soil respiration responded most strongly, followed by changes in microbial biomass C, with relatively small changes in the activities of most extracellular enzymes. Samples are in process for omics analyses. The most current results can be found in the poster by Zeglin et al.

Additional laboratory experiments are planned to explore in greater detail the omics response of soil microbial taxa to wet/dry cycles, both in terms of temporal patterns of response and physiological adaptations that control utilization of C and allocation of C within and outside the cell.

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