From Community Structure to Function: Metagenomics-Enabled Predictive Understanding of Microbial Communities to Climate Warming at the Temperate Grassland Ecosystems in Oklahoma

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Project Goals: Determining the response, adaptation and feedback mechanism of biological communities to climate change is critical to project future states of the earth and climate systems, but poorly understood in microbial communities. Thus, the overall goal of this study is to provide system-level, predictive mechanistic understanding of the temperature sensitivity of soil carbon decomposition to climate warming by using cutting-edge integrated metagenomic technologies. Towards this goal, the following four objectives will be pursued: (i) To determine phylogenetic composition and metabolic diversity of microbial communities in the temperate grassland and tundra ecosystems; (ii) To delineate the response of microbial community structure, function and activity to climate change in the temperate grassland and tundra ecosystems; (iii) To determine the temperature sensitivity of microbial respiration in soils with different mixtures of labile versus recalcitrant carbon, and the underlying microbiological basis for temperature sensitivity of these pools; and (iv) To synthesize all experimental data for revealing microbial control of ecosystem carbon processes in response to climate change. We have carried out our studies at two contrasting long-term experimental facilities, the temperate grassland ecosystems in OK (this poster) and tundra ecosystems in Alaska (Poster led by Schuur et al).

Feedback responses of microbial communities to climate warming. We have used integrated metagenomic technologies to analyze the responses of microbial communities in a long-term (10 years) experimental warming grassland ecosystem in Oklahoma. Our results showed that microorganisms play crucial roles in regulating soil carbon (C) dynamics through three primary feedback mechanisms: (i) shifting microbial community composition, which most likely led to the reduced temperature sensitivity of heterotrophic soil respiration, (ii) differentially stimulating genes for degrading labile but not recalcitrant C so as to maintain long term soil C stability and storage, and (iii) enhancing nutrient cycling processes to promote plant nutrient use efficiency and hence plant growth. Elucidating microbially mediated feedbacks is fundamental to understanding ecosystem responses to climate warming and provides a mechanistic basis for C-climate modeling.

Responses of microbial communities to clipping within the context of climate warming. We have also examined the responses of microbial communities to clipping within the context of climate warming using the GeoChip-based metagenomics technology. Various statistical analyses showed that clipping had significant impacts on microbial community structure, and altered the directions of the warming effects. The results clearly indicated that clipping substantially mediated warming effect on soil microbial community. The stimulated genes in relative more recalcitrant C decomposition under warming with

clipping may have important implications for the stability of soil C storage. The diminished warming effect by clipping for N genes may affect plant production and further influence soil C cycling.

Metagenomic sequencing analysis of microbial communities under long-term warming. We employed the Illumina Hi-Seq2000 platform (2 X 100 paired end) to obtain shotgun metagenomes from 12 replicate samples, with 6 each for warming and control. Each sample yielded 10-15 Gb of sequence data with >60 Gb per community. Using an assembly-free bioinformatic pipeline, our results showed that more than 90% of the genes and organisms did not differ in abundance between the two communities while a higher abundance of genes related to sporulation was observed under warming. Significant differences were observed among the top four most abundant phyla (*Proteobacteria, Acidobacteria, Planctomycetes,* and *Bacteriodetes*) between warming and control. Interestingly, the microbial populations of these phyla from the warmed samples showed significant increase in G+C% content. Furthermore, several metabolic pathways were significantly increased under warming, including pathways directly related to the emission of greenhouse gases (e.g., CH_4, NO, and CO_2), nitrogen cycling (e.g., fixation, nitrification, nitrate/nitrite reduction), and organic carbon utilization (e.g., mixed acid fermentation, mannitol utilization).

Belowground net primary productivity under warming and clipping. The dynamics of belowground net primary productivity (BNPP) is of fundamental importance in understanding carbon (C) allocation and storage in grasslands. Thus we have examined the changes of BNPP under warming and clipping. Warming increased BNPP by 42-67% with a significant increase observed in wet years. Clipping also had significant positive impacts on BNPP. Overall, f_{BNPP}, the fraction of BNPP to NPP, increased under both warming and clipping treatments, more in dry years. Water availability (either precipitation or soil moisture) was the most limiting factor for both BNPP and f_{BNPP}. It strongly dominated the interannual variability of NPP and f_{BNPP} and their responses to warming and clipping. Our results suggested that water availability might regulate tallgrass prairie's responses to warming and land use change, which may eventually influence the global C cycling.

The stability of organic carbon in deep soils. The majority of C in deep soils is recalcitrant and old with turnover times of hundreds to thousands of years, whose fate is critical to project future climate warming. Therefore, we determined the warming effect on old, recalcitrant organic C decomposition by combining long-term (9 yr) *in-situ* field and short-term (9 wk) laboratory incubations. Our results showed that warming of approximately 2°C significantly facilitated the loss of C with several thousand years old in the deep soil layer. Coupled stable isotope probing and meta-genomic analysis indicated that warming-induced old C decomposition was closely related to changes in the functional structure of microbial communities. Our findings suggest that warming may significantly reduce the size of the vast pool of old C in global soils and thus reinforce the positive feedback between the C cycle and climate.

Development of amplicon-sequencing approaches for uncovering functional gene diversity. Eco-functional genes involved in nitrogen and carbon cycling were targeted for pyrosequencing in order to resolve changes in microbial functional community structure underlying process changes. For nitrogen cycling, the functional genes *nifH* (N fixation), *nirK* (denitrification), *amoA* (nitrification), and *Archaeal-amoA* (nitrification) were initially targeted. Bacterial laccases, which catalyze the oxidation of various substituted phenolic groups were also sequenced. The FunGene Pipeline and Repository has been enhanced to provide the full line of capability of processing/analyzing sequences of these eco-functional genes. In addition, primers are currently being developed for the nitrogen cycle (*nosZ* and *nirS*) and the carbon cycle (*pmoA*, *ligE*, and fungal peroxidase).