

## 69. From Structure to Function: Metagenomics-Enabled Predictive Understanding of Soil Microbial Feedbacks to Climate Warming

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**Project Goals:** The overall goal of this project is to advance system-level predictive understanding of the feedbacks of belowground microbial communities to multiple climate change factors and their impacts on soil C cycling processes. Towards this goal, we are pursuing the following objectives: (i) To determine the responses of microbial community structure, functions and activities to climate warming, altered precipitation, soil moisture regime and/or clipping in the tundra and temperate grassland ecosystems; (ii) To determine the temperature sensitivity and substrate priming on recalcitrant C decomposition; (iii) To determine microbiological basis underlying temperature sensitivity of recalcitrant C decomposition; and (iv) To develop integrated bioinformatics and modeling approaches to scale information across different organizational levels towards predictive understanding of ecosystem responses to multiple climate change factors, which will be collaborated and integrated with the Knowledge Base (KBase).

**Ecosystem responses to climate warming in tundra.** We have examined the tundra ecosystem responses to climate warming after one and half year in an experiment site of Carbon in Permafrost Experimental Heating Research (CiPEHR), which was established in September, 2008 at a moist acidic tundra site in Interior Alaska (AK). In this site, snow fences were used to increase soil temperature, coupled with early spring snow removal to control the snow-water equivalents in both warming and control plots. Our results showed that soil warming treatment increased both soil temperature and moisture significantly. In addition, the proportion of labile C pool 2 (mainly cellulose) was higher under warming than control. Furthermore, the gross primary productivity (GPP) was significantly increased by warming, mainly driven by the enhanced growth of graminoid. Warming also extended the length of growing season through earlier bud break (first appearance of bud) and delayed senescence. The ecosystem respiration (ER) was significantly higher under warming, but the net ecosystem exchange (NEE) did not differ between warming and control. Together, these results indicated that warming substantially altered plant and soil properties at the permafrost ecosystem.

We have used integrated metagenomic technologies to analyze the responses of microbial communities in experimental warming site of CiPEHR in tundra ecosystem. Although  $\alpha$ -diversity of GeoChip-based microbial functional community did not differ significantly between warming and control, the Inverse Simpson index based on both 16S and 28S rRNA sequences decreased marginally significantly by warming. Also, three complimentary non-parametric multivariate statistical tests (ANOSIM, Adonis, and MRPP) based on various distance indexes (Euclidean, Horn and Bray) and

detrended correspondence analysis consistently revealed that functional community structure differed substantially between warming and control, although significant differences were not detected with sequence data. Specifically, stimulations of broad groups of pathways/functional genes/populations involved in degrading both labile and recalcitrant C in permafrost were observed, which could potentially result in accelerated C decomposition. Moreover, warming stimulated not only functional genes involved in aerobic but also anaerobic microbial processes, which could lead to greater positive climate feedbacks by releasing more CO<sub>2</sub>, CH<sub>4</sub> and N<sub>2</sub>O. Furthermore, warming significantly enhanced functional genes involved in nutrient cycling processes and hence may result in higher nutrient availability. Overall, our results imply that soil microbial community was sensitive to short-term warming in tundra ecosystem we tested and highlighted the importance of microbial community mediated ecosystem feedbacks to climate warming in permafrost.

**Ecosystem responses to climate warming in temperate grassland.** We have investigated the ecosystem responses to climate warming in an experimental warming facility in a temperate grassland (tall grass prairie) ecosystem in the US Great Plains in Central Oklahoma (OK) after one and half years. Results showed that warming significantly increased the soil temperature, but decreased the soil moisture significantly. In addition, the proportion of labile C pool 2 (mainly cellulose) in total soil organic C was increased by warming at 39%. Moreover, though the increase of GPP and the decrease of ER were not significant, the NEE was significantly decreased by warming, representing more C were sequestered in the ecosystem. Together, these results indicated that warming changed some measured soil attributes, but not plant community.

We have also used metagenomic technologies, e.g. GeoChip and 16S rRNA amplicon sequencing, to analyze the responses of microbial communities at the experimental warming site in the temperate grassland ecosystem. Results showed that both functional and phylogenetic structures of soil microbial communities did not alter significantly by warming. Only a small portion of functional genes involved in carbon, nitrogen, phosphorus and sulfur cycling showed significant differences between warming and control. Interestingly, the magnitudes of changes in these functional genes induced by short-term warming (one and half year) in the temperate grassland ecosystem were much lower than in the tundra ecosystem as mentioned above. Overall, these results reveal that soil microbial communities in the temperate grassland we tested were less sensitive to short-term warming.

**Development of amplicon-sequencing approaches for uncovering functional gene diversity.**

Specific functional genes involved in nitrogen cycling and fungal community composition have been targeted for 454 amplicon pyrosequencing. The targeted genes included the fungal 28S LSU region, Archaeal *amoA* for ammonia oxidation, *nirK* and *nirS* for nitrite reduction, *nosZ* for nitrous oxide reduction and *nifH* for nitrogen fixation. Our findings showed that warming in temperate grassland significantly affects the overall community structure for *nifH*, *nirK* and *nosZ*. In addition, overall richness increased significantly for all genes except Archaeal *amoA*. Only *nirK* evenness, richness and overall structure were found to change in tundra.

*This work is supported by the US Department of Energy, Biological Systems Research on the Role of Microbial Communities in Carbon Cycling Program (DE-SC0004601).*