

156. Expanding the toolkit for comparative metagenomics, implementing it in K-Base, and applying it to the study of the effects of experimental warming in Midwestern and Alaskan soils

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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 K-Base.

Abstract: Under this project, we have begun investigations on microbial communities from Alaskan tundra permafrost (AK) and Oklahoma temperate grassland (OK) soils, both of which have been experimentally warmed 2 to 4 °C for one and half year above ambient temperature *in-situ*. Our analyses of well-replicated 16S rRNA gene amplicon, meta-transcriptomic, and whole-community shotgun metagenomic datasets from these soils showed small but significant shifts in community composition, gene expression, and functional metabolic potential compared to control (un-warmed) adjacent communities. The specific microbial populations and genes/pathways enriched by warming were different between the two locations. Greater taxonomic composition differences were observed at the OK site relative to AK, presumably resulting from longer generation times due to the less optimal conditions for growth at permafrost soils. Analysis of fragments of rRNA genes recovered in the shotgun-metagenomic data revealed no significant shifts in fungal taxa at both sites, but that the ratio of fungi to bacteria decreased by warming, indicating that the warming treatment is more favorable for bacteria, at least in the short term. The most pronounced bacterial taxon shifts observed at OK site, which were somewhat also observed at the AK site, were increased in abundance of *Actinobacteria* and decreased in *Planctomycetes*, both representing major phyla in soils, particularly in regards to C-cycling. In terms of functions, the communities of AK warmed plots were enriched in metabolic pathways related to labile carbon mobilization and oxidation whereas fewer of these patterns were observed in the OK communities, indicating that soil C is more vulnerable to microbial respiration at AK. The OK microbial communities were instead enriched in genes involved in heat shock response and cellular surface structures, particularly, trans-membrane transporters for glucosides and ferrous iron. These results, which were consistent with independent

physicochemical measurements and process rates determined *in-situ*, were linked with higher primary productivity of the aboveground plant communities stimulated by warming. Collectively, our findings suggest that microbial communities of grassland soils play important roles in mediating feedback responses of the soil ecosystem to climate change and that even short periods of warming induce significant changes in microbial community function and composition.

To enable this research, we have also developed several bioinformatics tools that addressed practical limitations during the comparative analysis of the soil metagenomes such as how to assess the fraction of the community captured by a metagenomic dataset and estimate the sequencing effort required in study design (Nonpareil tool; Rodriguez-R and Konstantinidis, *Bioinformatics* 2013), how to determine the taxonomic affiliation of a metagenomic sequence (MyTaxa; Luo et al., in revision), how to bin assembled contigs into population genomes based on time-series metagenomes (BinGeR; Luo et al., in preparation), and how to determine differentially present genes between metagenomic datasets (Luo et al., *Methods Enzymol.* 2013). Furthermore, we have developed new tools for the analysis of high-throughput gene amplicon data, including a tool for frameshift correction and nearest-neighbor classification (FrameBot; Wang et al., *mBio*2013), and a 16S rRNA classifier for short read data (Cole et al., *Nucleic Acids Res.* 2014). Altogether, these make up our *Microbial Process Toolkit* for gene, metagenomic and metatranscriptomic data integration, modeling and visualization. We are in the process of implementing our toolkit in K-Base and we will report on these efforts as well.

References

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