Metagenomics recovers 100s of population genomes from Alaskan permafrost and Oklahoma prairie soils and provides insights into their roles in microbial community response to 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. Regarding this goal, we are pursuing the following objectives: **(i)** To improve our understanding of soil microbial communities indigenous to temperate and tundra ecosystems through whole-community analysis, and through the classification of novel taxa recovered directly from metagenomes and/or obtained in pure culture; **(ii)** To determine the microbiological basis underlying temperature sensitivity of soil organic matter decomposition; **(iii)** To determine the ubiquity of recovered bacterial populations and genes across large geographic regions spanning several hundred kilometers; 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 ℃ above ambient temperature *in-situ*. Well-replicated whole-community shotgun metagenomic sequencing of soils collected after 1 and 5 years of warming yielded near-complete representation of microbial community 'sequence richness' at AK and OK sites. A custom-made assembly and contig binning strategy has allowed for the recovery of many near-complete bacterial population genomes from both locations. In particular, populations recovered from AK soils collectively made up to ~15% of the total microbial community. These genomes represented diverse taxonomic groups and metabolic lifestyles tuned toward sulfur cycling, hydrogen metabolism, methanotrophy, and organic matter oxidation. While short-read analysis of soil metagenomes collected after 1-year of warming revealed small shifts in pathways related to SOM-decomposition (Xue et al., *in press*), recently-sequenced metagenomes collected after 5-years of experimentation revealed dominant bacterial populations shifting in abundance by as much as 80% in response to the warming treatment (Johnston et al., *in preparation*). Further, several bacterial populations

recovered from AK tundra soils were also present and/or dominant in geographically distant (~100-530 kilometers apart) tundra habitats (full genome representation and >98% genome-derived average nucleotide identity). Therefore, their relative contribution to various ecosystem functions is expected to be high and their individual responses to climate warming may be of significance to large geographic regions.

In addition to studying the *in-situ* response to warming, we have also incubated soil taken from both sites and two different depths under elevated temperatures in the laboratory for 3 years. By combining shotgun metagenomic sequencing with respiration data and soil indices, we hope to gain a more detailed view of the soil taxa, and the underlying mechanisms, modulating responses to warming and the activities responsible for greenhouse gas release. To this end, we have assembled >200 draft genomes, most of which represent previously uncharacterized (novel) taxa, and collectively making up ~50% of the incubation metagenomes. We will report on the significant differences in the abundance of these genomes over time in the laboratory incubations as well as the differences between soil ecosystems and depths. To further support this work and enable testing of the emerging hypotheses from comparative metagenomics, we have been implementing cultivation on dilute nutrient, minimal salt and soil extract media under reduced oxygen stress. A total of 660 culture plates under varying salt conditions (0-10% NaCl) and dilutions (10^{-6} – 10^{-8}) were cultivated for >8 weeks from Alaskan shallow (<55 cm) and deep (>55 cm) soils at 25 ℃. The majority of colonies appearing after 2 weeks remained small (<2 mm diameter), with varying colony morphologies. Our current workflow to identify target cultures for physiological characterization and sequencing involves amplification and identification of 16S rRNA genes in order to relate each culture to the metagenomic binning results.

References

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Funding statement: Our work is supported by the U.S. DOE Office of Science, Biological and Environmental Research Division (BER), Genomic Science Program, Award No. DE-SC0010715.