Time-series metagenomics of experimentally warmed Alaskan tundra and Oklahoma temperate soils enables fine-resolution assessment of belowground C cycling feedbacks to climate change

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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 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 K-Base.

Abstract: Soils contain more carbon (C) in the form of soil organic matter (SOM) than both aboveground plant and atmospheric pools combined. Higher land temperatures are expected to cause the release of considerable amounts of CO_2 and CH_4 to the atmosphere, primarily through the stimulation of microbial-mediated turnover of SOM. However, the direction, magnitude, and underlying basis of soil ecosystem feedbacks to climate warming remain poorly understood. To this end, we have investigated microbial communities from Alaskan tundra permafrost (AK) and Oklahoma temperate grassland (OK) soils, both of which have been experimentally warmed *in-situ* (2 to 4°C above ambient temperature in the field) and under laboratory conditions (15 and 25℃). By combining well-replicated soil metagenomes with continuous environmental monitoring, respiration data, and soil measurements, we hope to gain an improved understanding of microbial responses to climate warming, particularly those involved in the turnover of (SOM) and release/sequestration of greenhouse gases.

Metagenomic datasets representing soils sampled after 1.5 and 5 years of field warming yielded near-complete representation of microbial community 'sequence richness' at AK and OK sites, and revealed that OK soil communities are an order of magnitude more diverse than their tundra counterparts. Sequence assembly and binning techniques allowed for the recovery of several near-complete bacterial population genomes from both ecosystems, most of which represent previously uncharacterized taxa, allowing for prediction of their metabolic lifestyles,

regional prevalence (based also on publically-available datasets from nearby locations), and response to elevated temperatures. Several of the recovered AK populations were regionally ubiquitous, e.g., found at several locations ~100-530 kilometers apart (Johnston et al., 2016). Warming favored bacterial populations encoding diverse metabolisms for recalcitrant and labile SOM degradation, including abundant members of the community (0.25-2% of total), which increased by 30-100% of their original abundance after just 5 years of field warming. Whole-community assessment of 5-year AK field samples also revealed a uniform increase in many SOM catabolism pathways coincident with warming, including those for both the labile and recalcitrant fractions of SOM (Johnson et al., in preparation). These results were also consistent with GeoChip functional gene analysis and observations of increased ecosystem respiration reported at an earlier experimental phase (Xue et al., 2016). 5 years of experimental warming at the OK field site altered the functional composition of microbial communities (β-diversity distances) and increased microbial community sequence complexity/diversity.

Metagenomic sequencing and assembly of lab-incubated soils resulted in the recovery of several hundred-population genomes, which collectively represented 25-75% of the total soil community. This data allowed for more resolved associations between SOM-turnover and the community composition (i.e., members responsible for these activities) to be identified. For instance, a correlation coefficient of 0.6 was obtained by relating the presence and abundance of population genomes to soil C and respiration measurements. Also, a correlation coefficient of 0.7 was obtained by relating specific bacterial populations involved in major N-cycling processes (N-fixation, denitrification, etc.) to measured soil N. To further verify these results and obtain model organism for future studies, >600 bacterial cultures have been recovered from AK long-term laboratory incubation soils using dilute nutrient, minimal salt and soil extract media under reduced oxygen stress. The 16S rRNA genes from these isolates have been matched against assembled bins for identification, and Biolog plates are beings used to reveal their associated metabolisms. These efforts have recovered, for example, an *Acidobacteria* (*Terriglobus sp*.) organism capable of N-fixation that likely plays a N-cycling role in tundra soils, which are more severely N-limited relative to temperate grassland, constraining the rate of SOM-turnover. The recovery of several N-fixing isolates also complements recent efforts to describe nifH-harboring community members indigenous to the Alaska tundra ecosystem (Penton et al., 2016).

References

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