64. Metagenomics-Enabled Predictive Understanding of Microbial Communities to Climate Warming: Results from Long Term Soil Incubations

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Project goal: 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 carbon (C) cycling processes. The main objectives of this integrative project are to (i) determine the responses of microbial community structure, functions and activities to climate warming, altered precipitation and soil moisture regime a in a tundra and temperate grassland ecosystem; (ii) determine temperature sensitivity on recalcitrant C decomposition; (iii) determine microbiological basis that is underlying temperature sensitivity of recalcitrant C decomposition; and (iv) develop integrated bioinformatics and modeling approaches to scale information across different organizational levels. This study focused on using laboratory incubations of soils as an isolated system to understand the influence of microbial processes on the release of C, and their response to changes in temperature.

Long-term aerobic laboratory incubations were performed at two temperatures (15 & 25°C) to determine the temperature sensitivity of microbial respiration (Q_{10}) in soils from a field warmed tundra (Alaska, AK) and a temperate grassland site (Oklahoma, OK). Three different layers were incubated from the AK site: two surface layers (0 – 15 cm & 15-25 cm with high carbon content) and a layer perennially frozen (45-55 cm) while 0-25 cm soils were incubated from the grassland site. The OK site included soils from control and field-warmed plots combined with a root exclusion treatment over 8 years. Carbon fluxes were measured periodically over the course of the incubation and total C respired at each time (C_T) was fitted with a two-pool C model to estimate pool sizes and decay rates of fast (C_F) and slow (C_S) decomposing C fractions. We estimated Q_{10} using three different methods: (1) a short-term method ($Q_{10\text{-}S}$), where soils were exposed to 6 different temperatures ranging from 5 to 30°C over the period of <1 week, while measuring C fluxes over this range at 14, 100 and 280 days during the long-term incubation; (2) the equal carbon method ($Q_{10\text{-}EC}$), which estimates the ratio of time that it takes for a soil to respire a given amount of carbon at each incubation temperature through the incubation experiment. $Q_{10\text{-}EC}$ was estimated for fast and slow decomposing C pools; (3) a separate modeling approach, which is an assimilation method of estimating Q_{10} for bulk soils and decomposing C pools from incubation data. The method includes a one pool (1P) model, a two pool (2P) model, a three pool (3P) model, and a three pool model with C transfer between the pools (3PX). The microbial community phylogenetic and functional composition, structure, and dynamics were analyzed by 16S rDNA sequencing and functional gene array GeoChip 5.0.

Results from the AK soils showed that after one year of incubation, C_T in the top 15cm could be as high as 25% and 15% of the initial soil C content at 25°C and 15°C incubations respectively. The fast decomposing C pool (C_F) accounted for up to 5% of the initial C content in the top 15 cm soils. Both, C_T and C_F decreased with depth but no field warming effect was detected. Overall average turnover time for C_F was ~ 60 days at these laboratory conditions.

Turnover time for C_S varied from 10 years in top soils to ~60 years in soils near the permafrost at 15°C incubation and decreased by half at the higher incubation temperature. Total C respired (C_T) in soils from OK accounted for 5% at 15°C and 10% of the initial soil C content at 25°C after one year of incubation. Fast decomposing C reached up to 4% of the initial soil C content at 25°C with an average turnover time of up to 100 days. The overall short term $Q_{10\text{-}S}$ for AK soils was 2.55 ± 0.03. Neither treatment nor depth nor day of incubation, nor incubation temperature had any effect on Q_{10}-s, however, interactions of field treatment*depth and treatment*day of incubation were significant. Estimated $Q_{10\text{-}EC}$ was 1.2 ± 0.4 and 2.2 ± 0.06 for C_F and C_S, respectively, with no significant differences with field treatment, varying depth or incubation temperature. For OK soils, the overall $Q_{10\text{-}S}$ was 3.2 ± 0.3. The AIC analysis indicated that the two-pool model was the best fit given the incubation data over one year.

The results of the dissimilarity analysis of the 16S rRNA amplicon sequencing data from the OK soils showed warming effect on microbial communities. After two weeks at 25°C, samples from the deep collar had more abundant populations of *Acidobacteria, Actinobacteria, Crenarchaeota, Firmicutes, Gemmatimonadetes, Nitrospira, Planctomycetes, Verrucomicrobia*, but *Armatimonadetes, Bacteroidetes, Chloroflexi, Proteobacteria* were less abundant. When incubated at 25°C for three months, more differences were observed; however after nine months incubation, the community differences had disappeared. At the bacterial phylum level, no significant warming effect was observed for the soils outside of the deep collar across all sampling time points. These results indicate that the warming effects were more significant when roots were excluded and for those populations responding to labile carbon. GeoChip results showed significant differences in microbial functional diversity in the OK soils. For the incubations at 25˚C, most of the carbon degradation genes had higher abundances in the warmed soils outside deep collars after two weeks, but lower abundance after nine months. With the soils inside deep collars, the carbon degradation genes had lower abundances across the three time points. Warming effects were much less for the incubation at15°C.

The dissimilarity analysis of 16S rRNA sequences was also done for the CiPEHR soils. A significant warming effect was observed in the soils from 0-15 cm when incubated at 25°C for two weeks. In those samples, *Proteobacteria, Actinobacteria, Acidobacteria, Chloroflexi, Firmicutes,* and *Gemmatimonadetes* had higher abundances in the warmed soils, while *Planctomycetes, Chlamydiae, Armatimonadetes, Verrucomicrobia, Bacteroidetes* had lower abundances. Significant differences in microbial population abundances between warmed and control soils from 0-15 cm were also observed when incubated at 15°C after three months, and the soils from >35 cm after 9 months. From GeoChip analyses, differences were observed among soil depths, between treatments, incubation temperatures, and incubation time. For the incubations at 25°C, consistent differences in carbon degradation genes between warmed and un- warmed soils from 15-25 cm were observed along the incubation time points.

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