

85. Successional Dynamics of Grassland Microbial Communities in Response to Warming, Precipitation Alternation, and Clipping

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Project goal: The overall goal of this project is to advance systems-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 specific objectives are to: (i) reveal the responses of soil microbial communities to climate warming and soil moisture alteration in both tundra and temperate grassland ecosystems; (ii) determine temperature sensitivity of recalcitrant C decomposition and the underlying microbiological basis; and (iv) develop integrated bioinformatics and modeling approaches to scale information across different organizational levels. As a part of the integrated project, this study focused on field experiments in a temperate grassland to reveal the influences of elevated temperature, altered precipitation and clipping on soil microbial communities and ecosystem processes. This study consists of three major parts: (i) development and evaluation of next generation functional gene arrays for discerning the impacts of climate change factors on microbial community structure; (ii) long-term succession of plant and microbial communities through sequencing analysis of 16S rRNA gene amplicons and plant communities; and (iii) short-term microbial succession through analyses of 16S rRNA amplicons and functional genes. Overall, this study provides valuable new insights into our understanding of the temporal dynamics of soil microbial communities in response to multiple climate change factors.

Development and evaluation of next generation functional gene arrays. To analyze the functional diversity, composition, structure, dynamics, and metabolic potential/activity of soil microbial communities, we have developed a new generation of functional gene array, GeoChip 5.0 with 167,044 probes targeting 395,894 coding sequences from 1,593 functional gene families involved in C, nitrogen (N), sulfur, and phosphorus cycling, electron transfer, metal homeostasis, organic remediation, stress response, secondary metabolism, and virus and virulence activity. As reported previously, GeoChip 5.0 is highly specific (>98% probes have 100-fold higher signal than the corresponding mismatch probes), sensitive (250ng DNA for detection of >50% targets), and quantitative (99.1% probes have good linearity between signal intensity and DNA concentration; $R^2 > 0.81$, $P < 0.05$). Also, the reproducibility of GeoChip hybridization (180K probes per array) was compared to 16S rRNA gene amplicon sequencing (very deep, >80,000 reads per sample) and shotgun metagenomic sequencing (average data size of 18 Gb per sample) using the same soil DNA, showing GeoChip had significantly higher reproducibility (>90% by both Sørensen and Bray-Curtis similarities) than 16S rRNA sequencing (62% by Sørensen, 80% by Bray-Curtis), which was in turn higher than shotgun metagenomic sequencing (5% by Sørensen, 55% by Bray-Curtis). These results provided direct experimental evidences for the presence of artifacts associated with random sampling processes, and indicate that closed-format technologies (e.g., microarrays) do lead to higher reproducibility than open-format technologies (e.g., sequencing). In addition, the sources of variation that could affect reproducibility in GeoChip-detected functional gene communities were

estimated using warmed and control soils. DNA extraction and hybridization each accounted for 1% of the community total variance. Differences among technical replicates were insignificant. Compared to the treatment effects and the biological blocking factor (13% and 27% total variance explained, respectively), variations caused by experimental processes for functional gene array hybridization are negligible.

Long-term succession of plant and microbial communities. The long-term experimental site was established on a temperate grassland of Central Oklahoma (OK) in 2008. Environmental factors simulated include air warming (two levels: control and warming), precipitation alteration (three levels: double, normal, and half) and clipping (two levels: unclipped and clipped). Surface layer (0-15cm) soil samples from all plots were collected annually at the summer peak plant biomass season (Sep. or Oct.) from 2009 to 2014. At the same time, plant biomass and community structure were surveyed. Air and soil temperature, soil moisture and the C flux rate were measured in field. Soil pH, and C and N content were determined in lab for each soil sample taken. Since the beginning of operation, soil temperature at 7.5cm depth was 3°C higher ($P<0.001$) in warmed compared to control plots and 0.8°C ($P<0.001$) lower in unclipped plots compared to clipped plots, due to the insulating effect of plant and litter cover. Soil moisture was significantly ($P<0.001$) lower in clipped and in warmed plots, and higher in the double precipitation plots compared with their controls. Plant biomass fluctuated greatly across all years largely due to differences in wet and dry years, with an observable ($P<0.05$) decrease with reduced precipitation. Pointedly, C3 plant biomass was reduced with warming and elevated precipitation, while C4 plants thrived with more precipitation as indicated by their greater biomass. To examine microbial community changes under treatments, the 264 annual soil samples taken from 2009 to 2014 were analyzed by sequencing 16S rRNA gene amplicons. Dissimilarity analysis showed that all treatments alone as well as year affected the microbial community structure. Permutational analysis of variance indicated that over time, warming became the most influential treatment. α -diversities of microbial communities under different treatments were similar in 2009 and 2010, but gradually decreased in warming ($P<0.05$) samples in later years. The community composition was also altered over time with significantly ($P<0.05$) more Actinobacteria and Firmicutes, and less Proteobacteria, Bacteroidetes and Acidobacteria in warmed samples in 2014, compared to 2009 and 2010 when the communities for control and warming plots were similar. Differences in precipitation level and clipping also impacted the community composition. By 2014, the half precipitation soil samples had less abundant proteobacteria and more abundant firmicutes than the double precipitation soils ($P<0.05$), while in clipped plots, Actinobacteria were less abundant. These results indicate that warming, altered precipitation and clipping have differential effects on the diversity, composition and structure of soil microbial communities over time. GeoChip analysis of these samples is in progress.

Short-term microbial succession. To understand the short-term dynamics of microbial communities in response to warming, 96 monthly soil samples were collected in 2012 from in both warmed and control plots and analyzed by sequencing 16S rRNA gene amplicons and GeoChip hybridization. Both warming and sampling month significantly ($P<0.005$) affected the soil microbial community structure for both taxonomic groups and functional genes, while the interaction between these variables was not significant. Also, in general, warming increased the relative abundance of Actinobacteria and Crenarchaeota, and N assimilation and denitrification genes, and decreased the abundance of δ -Proteobacteria, Planctomycetes, OD1 and Chlamydiae, and phosphorus and sulfur metabolism genes. C cycling genes were differentially influenced depending on the pathways involved. In addition, a significantly ($P=0.02$) higher β -diversity (Sørensen index) of warmed communities suggested that they were taxonomically more divergent than the control communities. Finally, more taxa/functional genes showed seasonal dynamics compared to the warming effect. Specifically, Euryarchaeota, Actinobacteria, Bacteroidetes, and Alphaproteobacteria reached their highest abundances concurrently with the highest plant biomass.

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