

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 characteristics of the microbial degraders; and (iii) develop integrated bioinformatics and modeling approaches to scale information across different organizational levels.

As a part of the integrated project, here we present results from; i) field experiments established in 2008 in a temperate grassland of central Oklahoma to reveal the influence of elevated temperature, altered precipitation and plant biomass clipping on long-term succession of plant and microbial communities, and ii) soil respirations under long-term warming.

Long-term succession of microbial communities. To determine successional dynamics of microbial communities in response to warming, clipping, altered precipitation and their combinations, 264 annual soil samples from 2009 to 2014 were analyzed by sequencing of 16S rRNA genes for bacteria and archaea, ITS regions for fungi, and 18S rRNA genes for other micro-eukaryotes excluding fungi, and by functional gene arrays (GeoChip 5.0). Our analyses indicated that global change factors including warming, clipping, half precipitation, double precipitation and most of their combinations differently shifted the temporal successional patterns of the taxonomic composition and phylogenetic structure of different microbial populations. Importantly, among these global change factors, climate warming played a dominant role in accelerating divergent succession of all soil microbial communities as evidenced that experimental warming enhanced microbial temporal divergences under the context of various global changes. Secondly, our results also showed these global change factors and most of their interactions significantly ($P < 0.05$) changed species-time relationships (STRs) of different soil microbial populations including bacteria, fungi and micro-eukaryotes. And climate warming significantly ($P < 0.05$) promotes temporal scaling rates (STR exponent) of all microbial populations even under the context of various global changes. All of these results indicated that warming plays a dominant role in accelerating temporal succession rates of soil microbial communities.

In general, both deterministic (i.e. species traits, interspecies interactions and environmental conditions) and stochastic (i.e. birth, death, extinction, and speciation) processes simultaneously influence the assembly of local communities. To quantify the relative importance of deterministic vs. stochastic processes in

shaping soil microbial communities under climate change treatments, stochastic ratios were calculated based on taxonomic and phylogenetic metrics. Our results indicated that the stochastic processes contributed the majority of community variations across all treatments in taxonomic (48.2% ~ 68.1%) and phylogenetic (54.1% ~ 86.5%) levels across 6 years of manipulated experiment. Secondly, warming significantly ($P < 0.05$) decreased the relative importance of stochastic processes (4.6-17.6%) in controlling bacterial and fungal community composition, and the similar results were also obtained in many comparisons between combined warming treatments and their corresponding controls. Furthermore, the roles of stochastic processes in all microbial populations under single warming and most combined warming treatments decreased substantially along with time, indicating that deterministic processes play more important roles over time in controlling microbial communities in response to climate warming. These findings have important implications for predicting ecological consequences of climate changes. Because warming reduced stochasticity over time, the communities could converge quickly to less variable states, and hence the future community states could be more predictable under warmed climate if we have enough knowledge on the successional trajectories of the contemporary microbial communities.

Network analysis of microbial temporal successions. Soil microorganisms coexist in complex arrays in which interactions among members are essential for community assembly and ecosystem. However, most of studies in the last decades examined the responses of ecological communities to climate changes by quantifying diversity, but whether and how climate changes affect ecological community organization and the interactions among members of ecological communities, particularly microbial communities, remains elusive. Our network analysis revealed that warming predominantly led to larger and more complex bacterial and fungal networks along time under the context of various global change factors, as indicated that the warmed soil networks significantly increased in size ($r^2 = 0.836$, $P = 0.011$) and connectivity ($r^2 = 0.916$, $P = 0.003$) over time. Secondly, more and larger modules with more positive and negative links were found in the warmed soil networks, suggesting that more mutualistic and competitive interactions may occur under climate warming. Thirdly, we identified more putative keystone taxa including module hubs, connectors and network hubs in the warmed soil networks. Almost all of these keystone taxa had low relative abundances (0.002% ~ 2.593%), suggesting low-abundance taxa may significantly contribute to soil microbial function. Intriguingly, no network hubs were identified in any of non-warmed soil networks, but one network hub was detected only in the last year of warmed soil network, which were assigned to the typical oligotrophic phylum *Acidobacteria* and exhibited 91% identity to an isolate of *Acidobacteria* Gp16. These results indicated that oligotrophic taxa may play more important roles than those copiotrophic taxa in the warmed soil communities.

Soil respiration under long-term warming. The warming-induced stimulation of soil carbon release through respiration could amplify future increases in atmospheric CO₂ levels and associated climate warming. However, the magnitude and persistence of the warming-induced stimulation of soil carbon release under future climates, including more frequent climate extremes, are poorly understood. In the field experiment, ecosystem C fluxes and soil respirations were monitored monthly from 2009 to 2016, during which rainfall greatly changed in different years, containing one of the driest years and the wettest year on the historic record. Therefore, we obtained an opportunity to evaluate the magnitude and persistence of the warming-induced stimulation of soil carbon release under future climates, including more frequent climate extremes. Our results showed, after an eight-year field study of a native, tall-grass prairie ecosystem under warming and control, that although the warming response of autotrophic respiration is differentially shifted under drier (-36%) and wetter (+8%) conditions, the stimulation of heterotrophic respiration by warming is consistent and persistent under these extreme conditions (18%). Integrated metagenomic analyses indicated that warming-induced shifts in microbial community structure and function were consistent and did not significantly vary under drier or wetter conditions. More genes for soil carbon decomposition and nitrogen-cycling processes were consistently ($P < 0.05$) stimulated by warming under dry, normal and wet conditions. Furthermore, Mantel test revealed that very strong correlations were observed between community structure and ecosystem functional processes, such as plant GPP and total soil respiration, and the functional

gene groups involved in C degradation, N cycling, P utilization and S cycling ($P < 0.05$). Remarkably, soil heterotrophic microbial respiration specifically showed strong correlations with the genes involved in degrading recalcitrant C, including vanillin, lignin and cellulose, suggesting that the increased heterotrophic respiration may contribute to the degradation of recalcitrant C. All of these results indicated that the dynamic succession of microbial community greatly contributed to the consistent warming-induced stimulation of heterotrophic respiration under climate extremes. Our findings indicate that microbial dynamic succession may be important for understanding the magnitude and persistence of ecosystem-scale responses to global change.

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