Feedback Responses of Grassland Microbial Communities to Experimental 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 2009 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 and ecosystem C fluxes under long-term warming.

Warming reduces soil microbial biodiversity. To determine successional dynamics of microbial communities in response to warming, clipping, altered precipitation and their combinations, 264 annual soil samples from 2009 to 2016 were analyzed by sequencing of 16S rRNA genes for bacteria and archaea, ITS regions for fungi, and by functional gene arrays (GeoChip 5.0). Our analyses indicated that, experimental warming decreased bacterial and fungal species diversity as well as their phylogenetic diversity, irrespective of the types of clipping and precipitation treatments. Decreases were primarily linked to the decrease of plant functional diversity. By examining the functional traits of plants and microbes, warming also enhanced the dominance of fast-growing resource-acquisitive species in both plant and microbial communities, which could together contribute to the reduced temporal stability of community structure and ecosystem functioning.

Long-term succession of microbial communities. 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 bacteria and fungi. More 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, which is published in *Nature Climate Change*. 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, which is presented in our manuscript recently accepted by *Nature Ecology & Evolution*. All of these results indicated that warming plays a dominant role in accelerating temporal succession rates of soil 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 functions. However, most of the studies in the last decades examined the responses of ecological communities to climate changes by just focusing on 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% ~ 2.59%), 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.

Incorporating functional genes data into ecosystem modeling. To examine the temperature sensitivity of microbial respiration (Q_{10}), the measured field soil respiration data from individual plots were fitted with the Q_{10}-based Arrhenius equation, which fitted relatively well with the Rh data (r^2 = 0.19-0.99, $p < 0.05$). The Q_{10} estimates were significantly or marginally significantly higher under control than warming in various years. By fitting the respiration data from all 7 years, the overall Q_{10} of heterotrophic soil respiation was also significantly ($p < 0.001$) lower under warming (1.692 ± 0.041) than control (1.947 ± 0.055), suggesting that temperature sensitivity of heterotrophic soil respiation was possibly reduced under warming.

Due to the importance of microbes and their activities in controlling heterotrophic respiration, we further improved the Microbial-ENzyme Decomposition (MEND) model by incorporating GeoChip-detected functional genes information into the ecosystem models. We pooled the functional genes into two categories (i.e., oxidative and hydrolytic enzymes) corresponding to the MEND model. We constrained the model by achieving the highest correlation between MEND-modeled enzyme concentrations and GeoChip-detected enzyme densities in addition to a best fit between modeled and simulated Rh. The MEND mnodel simulated heterotrophic soil respiation agreed well with the observed heterotrophic soil respiation under warming and control. In addition, Also, our model simulation analysis revealed that some key model parameters, such as Q_{10}, intrinsic carbon use efficiency (CUE) and its temperature sensitivity, were better constrained with microbial information than without them. In addition, the MEND simulated oxidative and hydrolytic enzyme concentrations were significantly correlated with the observed gene abundances. The MEND modeling also produced satisfactory results on the enzyme concentrations under warming, as well as the responses of enzymes to warming.

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