

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

Xue Guo¹, Qun Gao¹, Mengting Yuan¹, Zhou Shi¹, Xishu Zhou¹, **Liyu Wu^{1*}(lwu@rccc.ou.edu)**, **Zhili He¹**, Joy D. Van Nostrand¹, Lauren Hale¹, Daliang Ning¹, Aifen Zhou¹, Jiajie Feng¹, Ying Fu¹, Chang-Gyo Jung¹, Tong Yuan¹, Xuanyu Tao¹, **Jim Cole²**, **James M. Tiedje²**, **Edward A.G. Schuur³**, **Konstantinos Konstantinidis⁴**, **Yiqi Luo¹**, and **Jizhong Zhou¹**

¹Institute for Environmental Genomics and Department of Microbiology and Plant Biology, University of Oklahoma, Norman, OK; ²Center for Microbial Ecology, Michigan State University, East Lansing, MI; ³Center for Ecosystem Sciences and Society, Department of Biological Sciences, Northern Arizona University, Flagstaff, AZ; ⁴Center for Bioinformatics and Computational Genomics and School of Biology, Georgia Institute of Technology, Atlanta, GA

<http://www.ou.edu/ieg.html>

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) modeling ecosystem C pools and C flux under climate warming.

Long-term succession of microbial communities. To understand successional dynamics of microbial communities in response to warming, clipping, and altered precipitation, 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 OTU richness for all three marker genes significantly decreased in warmed plots compared to ambient plots, which remained true when clipping and reduced precipitation were coupled with warming. Among all twelve treatments, OTU richness under clipping with double precipitation was the highest for all three marker genes. To understand temporal patterns and their underlying mechanisms within the context of climate change factors, species-time relationships (STRs) were estimated. By examining different soil microbial populations, bacteria, fungi and micro-eukaryotes in this multifactor global change experiment, we demonstrated that all soil microbial populations exhibit significant ($P < 0.05$) STRs under various global changes. Secondly, our results showed warming significantly ($P < 0.05$) stimulated microbial temporal scaling rates (STR exponent) across different organization scales. This is the first demonstration that climate warming has significant impacts on temporal scaling of microbial communities and significantly ($P < 0.05$) promotes temporal scaling rates 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 scaling rates of soil microbial communities.

Dissimilarity and permutational analyses of variance indicated that global change factors including warming, clipping, half precipitation and double precipitation significantly ($P < 0.05$) altered the taxonomic composition and phylogenetic structure of different microbial populations. Among these global change factors, climate warming played a dominant role in altering the taxonomic composition and

phylogenetic structure of soil microbial communities in the long-term experiment. Second, since previous “snapshot” studies have well-documented the sensitivities of soil microbial communities to various global change factors, it is anticipated that warming and related climate factors have important effects on the temporal successional patterns of soil microbial communities. However, little is known about how microbial communities develop over time under multiple global change conditions. Our results showed that global change factors and their interactions differently shifted the successional patterns of soil microbial communities. To the best of our knowledge, this is the first demonstration that various global change factors have significant ($P < 0.05$) impacts on temporal succession patterns of soil microbial communities. Importantly, among these 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. These results are also consistent with some recent studies showing that temperature plays a primary role in shaping microbial communities. Third, global changes induced divergent succession of soil microbial communities significantly ($P < 0.05$) affected ecosystem function processes. Aboveground net plant productivity (ANPP), gross primary productivity (GPP) and soil respirations exhibited significant ($P < 0.05$) correlation with the changes of microbial community structures across different microbial populations. This could suggest that the global changes induced divergent successions of soil microbial communities have important effects on the terrestrial C and nitrogen (N) cycling. **Microbial functional traits under climate changes.** As revealed by GeoChip hybridization, a number of microbial functional groups important for C decomposition and N cycling were differentially impacted by warming across 6 years. In the first two years, warming increased ($P < 0.05$) the relative abundance of genes involved in C degradation, N cycling and phosphorus (P) utilization, while in the third year, warming had no significant effects on these genes. Then, in the most recent two years, these genes decreased in response to the warming treatment ($P < 0.05$). Mantel test revealed that very strong correlations were observed between community structure and ecosystem functional processes, such as plant GPP and soil total respiration, and the functional gene groups involved in C degradation, N cycling, P utilization and S cycling ($P < 0.05$). Remarkably, soil heterotrophic respiration (from soil microbes) specifically showed strong correlations with the genes involved in degrading recalcitrant C, including vanillin, lignin and cellulose, which could suggest that the increased heterotrophic respiration may be contributed to the degradation of recalcitrant C. Secondly, precipitation alternations significantly ($P < 0.05$) affected the phylogenetic compositions of bacterial and fungal communities, but not their functional gene structures. In addition, annual clipping significantly ($P < 0.05$) changed the abundance of genes involved in C decomposition and N cycling. Cumulative annual clipping effects on functional genes were observed over time. From the second year of operation, significant ($P < 0.05$) increases of the relative abundances of genes involved in degradation of both labile and recalcitrant C were observed. However, in the last two years, only the genes involved in the degradation of recalcitrant C increased ($P < 0.05$). Other genes involved in nutrient-cycling processes including N cycling and P utilization also increased ($P < 0.05$) by annual clipping.

Modeling of soil C pools and flux. To explore the effects of warming on the temperature sensitivity of soil respiration (Q_{10}), we conducted regression analysis using $R_s = ae^{bT}$, where R_s is soil respiration, T is soil temperature, coefficient a is the intercept of soil respiration when temperature is zero, and coefficient b represents the temperature sensitivity of soil respiration. Warming significantly ($P < 0.05$) reduced Q_{10} in every experimental year (from 2009 to 2014), indicating that respiratory acclimation has occurred after 6 years of warming. The variation of Q_{10} among different years was also observed with lower Q_{10} in 2012 and 2014. This was consistent with the year variation of precipitation, which showed lower precipitation in the year 2012 and 2014. Such results indicate that precipitation can influence the warming effect on respiratory acclimation. To understand the whole ecosystem C dynamic feedback to warming, and the microbial traits affecting this feedback, terrestrial ecosystem (TECO) modeling was performed to simulate different C pools as well as soil respiration under control and warming treatment. Microbial functional within-group similarity (n=4) of each year was then incorporated into model simulation.

Warming significantly ($P < 0.001$) decreased soil C storage compared with control treatments. This result was consistent with our experimental data that warming significantly ($P < 0.05$) increased heterotrophic respiration, suggesting warming increased microbial decomposition of soil C, thus could potentially decrease soil C. After incorporating microbial function gene information into the model, the simulation of heterotrophic respiration improved by 18.9%, suggesting that it is important to include microbes global change models.

Funding statement: *Our work is supported by the U.S. DOE Office of Science, Biological and Environmental Research Division (BER), Genomic Science Program, Award No. DE - SC0010715.*