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: i) results from field experiments in a temperate grassland located in central Oklahoma established in 2008 to reveal the influence of elevated temperature, altered precipitation and plant biomass clipping on long-term and seasonal succession of plant and microbial communities, and ii) a primer design tool for highly parallel qPCR analysis of microbial communities.

Long-term succession of plant and microbial communities. To understand the long-term successional dynamics of microbial communities in response to warming, clipping and alternated precipitation, the 264 annual soil samples were analyzed by sequencing of 16S rRNA gene and ITS, and a functional gene array (GeoChip 5.0). Dissimilarity and permutational analyses of variance indicated that the three treatments all significantly ($P<0.05$) affected the structure and functions of the microbial communities. Warming was the most influential factor on bacterial and fungal communities over time. Interestingly, a warming effect started to be significant ($P<0.05$) from the third year of operation on bacterial communities, while it was significant ($P<0.05$) from the very first year on fungal communities. Species richness values (Shannon index) of warmed microbial communities were similar in 2009 and 2010, but gradually decreased ($P<0.05$) in the last three years whereas the control plots remained unchanged. The community composition was altered over time with *Actinobacteria* and *Firmicutes* ($P<0.05$) becoming more abundant, and *Proteobacteria*, *Bacteroidete*s, and *Acidobacteria* showing reduced abundances in warmed samples in 2014, compared with those in 2009 and 2010. In the first two years, warming increased ($P<0.05$) the relative abundance of genes involved in C degradation, nitrogen (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 ($P<0.05$). Precipitation alternations significantly ($P<0.05$) affected the phylogenetic compositions of bacterial and fungal communities, but not their functional gene structures. Annual clipping significantly ($P<0.05$) changed some bacterial and fungal populations, such as *Actinobacteria*, *Bacteroidetes*, *Ascomycota*, *Zygomycota*. 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 were also increased ($P<0.05$) by annual clipping. Soil microbial communities under different treatments had different ($P<0.05$) temporal turnover rates, such as species-time relationships (STR) and time-decay relationships. The temporal turnover rates of bacterial communities were stimulated by warming, but decreased by double precipitation, and not significantly changed by clipping. The temporal turnover rates of fungal communities were accelerated by warming, half and double precipitation, but decreased by clipping. These results indicate that warming, altered precipitation, and clipping have differential effects on the diversity, composition, and structure of soil microbial communities over time.

Short-term microbial succession. Surface (0-15cm) soil samples were collected monthly during 2012 from both warmed and control plots, and were analyzed by sequencing of 16S rRNA genes and GeoChip 5.0. Soil respiration and geochemical properties were also measured to link the soil microbial community structures with environmental factors. Both warming and sampling month significantly ($P < 0.005$) affected the soil microbial taxonomic groups and functional genes. *Acidobacteria*, *Actinobacteria*, *Bacteroidetes*, *Firmicutes*, *Verrucomicrobia*, and α- and δ-*Proteobacteria* had significant monthly variations under warming, while only *Gemmatimonadetes*, δ-*Proteobacteria* and *Chloroflexi* showed monthly changes in the control. This higher temporal divergence of warmed communities was confirmed by a higher taxonomic β-diversity (Sørensen index, $P = 0.02$) of warmed samples compared with the control. Network analysis indicated the network for warmed bacterial communities exhibited more links, a higher average degree, and a higher average clustering coefficient than that of control communities. The functional gene intensities also showed a significant interactive effect of sampling month and warming, in which the C degradation genes tended to increase, but the C fixation and N cycling genes tended to decrease during peak plant biomass months (Apr. to May and Sept. to Oct.). Together, these results revealed a higher temporal variation of soil microbial communities related to seasonal succession in a warmer environment. The species-time relationship exponent (STR-w) was slightly higher in warmed than in control plots, suggesting a faster species accumulation over time in response to warming. These exponents were higher than most reported values from long-term studies on soil microbial communities, suggesting a quicker species accumulation under short term than long-term succession.

Efficient high-throughput primer design tool for highly parallel qPCR. To provide a new quantitative and high throughput microbial community analysis approach, over the past year, by utilizing our FunGene database and repository (http://fungene.cme.msu.edu), we have been developing and testing an efficient high-throughput primer design tool. Protein-coding genes are, in general, less conserved than structural RNA genes, meaning that often no single probe or primer pair is able to target a gene's full range of diversity. Our tool helps with the design of multiple primers from potentially large reference sets of 3,000 sequences or greater. We cast the problem as a variant of the well-known "maximum coverage problem" from computer science. Since this problem has no practical exact solution, we use a "greedy" algorithm to choose a set of primer pairs from the candidates that maximizes the diversity covered by the primer sets. During tool testing, we developed new primer sets for nitrogen cycling genes (amoA, nifH), recalcitrant carbon degradation genes (*cutC*, *cntN*), antibiotic resistance genes (tet_sul2, tetA-G), an integrase gene involved in mobile elements (intI1). We have experimentally validated a set of three non-degenerate primer pairs targeting *cntN* sequences. The sequencing results showed adequate sensitivity, satisfactory amplicon size, and 99% PCR efficiency with the three primers used. This tool is also being employed in the design of primers targeting ACC deaminase (*acdS*) genes, involved in reducing plant stress, for investigations into the role of disease suppressive soil microbial communities in promoting plant health. The current tool is already in use by several research groups. We have developed the tool to be "KBase ready" and intend to help integrate this functionality both into FunGene and into KBase.

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.*