Effects of experimental warming and clipping on metabolic change of microbial community in a US Great Plains tallgrass prairie

Jianping Xie[1,2,3], Xinxing Liu[1], Xueduan Liu[1], Joy D. Van Nostrand[1,2], Ye Deng[1,2], Liyou Wu[1,2], Zhili He[1,2], Guanzhou Qiu[3], Jizhong Zhou[1,2]

[1]Institute for Environmental Genomics & Department of Botany/Microbiology, University of Oklahoma, Norman, OK, [2]Department of Botany and Microbiology, University of Oklahoma, Norman, OK,
[3]School of mineral processing and bioengineering, Central south university, Changsha, Hunan, 410083, China

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ABSTRACT

While more and more studies are being conducted on the effects of global warming, little is known regarding the response of metabolic change of whole soil microbial communities to this phenomenon. In this study, functional gene changes at the mRNA level were analyzed by our new developed GeoChip 3.0. Soil samples were taken from a long-term climate warming experiment site, which has been conducted for ~8 years at the Kessler Farm Field Laboratory, a 137.6-ha farm located in the Central Redbed Plains, in McClain County, Oklahoma. The experiment uses a paired factorial design with warming as the primary factor nested with clipping as a secondary factor. An infrared heater was used to simulate global warming, and clipping was used to mimic mowing hay. Twelve 2m × 2m plots were divided into six pairs of warmed and control plots. The heater generates a constant output of ~100 Watts m^{-2} to approximately 2 °C increase in soil temperature above the ambient plots, which is at the low range of the projected climate warming by IPCC. Soil whole microbial communities' mRNA was extracted, amplified, labeled and hybridized with our GeoChip 3.0, a functional gene array covering genes involved in N, C, P, and S cycling, metal resistance and contaminant degradation, to examine expressed genes. The results showed that a greater number and higher diversity of genes were expressed under warmed plots compared to control. Detrended correspondence analysis (DCA) of all detected genes showed that the soil microbial communities were clearly altered by warming, with or without clipping. The dissimilarity of the communities based on functional genes was tested and results showed that warming and control communities were significantly different (P<0.05), with or without clipping. Most genes involved in C, N, P and S cycling were expressed at higher levels in warming samples compared to control samples. All of the results demonstrated that the whole microbial communities increase functional gene expression under warming with or without clipping in order to adapt the changed out environment. More detail analysis is underway.

EXPERIMENTS SITE DESCRIPTION

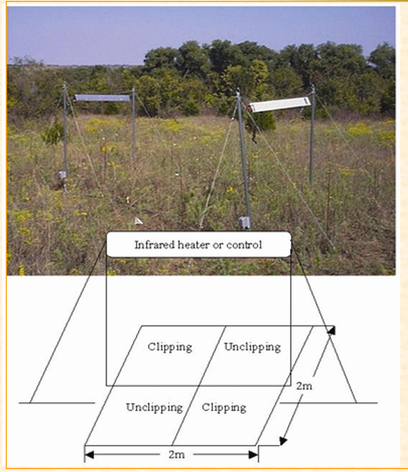

Figure 1. Picture of the experimental site and the experimental design. The experiment uses a paired nested design with warming as the main factor and clipping as a secondary factor. Twelve 2m × 2m plots are divided into six pairs of control (i.e. un-warmed) and warmed plots. The warming treatment was implemented by installing a twin-tube quartz radiator above ground by 1.5m. Each plot is divided into four 1m × 1m subplots. Two diagonal subplots are clipped at 10cm above the ground yearly. The other subplots were the unclipped controls.

METHODS

- **Sampling and RNA extraction.** Soil samples (15 g) were taken from the warming site, and soil bulk RNA and DNA was simultaneous extracted and separated using the method describe by Zhou et al. (2001).
- **50mer Oligonucleotide Functional gene array.** The third version of functional gene array (GeoChip 3.0) was used to study the structures and compositions of the microbial communities in the soils with different treatments.
- **RNA purification, amplification, labeling, and hybridization.** Raw RNA was purified by RNeasy® Mini kit (QIAGEN) and 100ng of purified RNA of each sample was amplified using whole-community RNA amplification approach describe by Zhou et al (2007). 10 µg aRNA was labeled with cy5 and hybridized to FGA III slides with MAUI hybridization system.
- **Microarray scanning and data processing.** Hybridized microarray slides were scanned using a ScanArray® 5000 and the image displays were analyzed using ImaGene™ version 6.1. Empty and poor spots were removed before the signal intensities were normalized by the signals of universal standard.
- **Data Analysis.** Functional gene diversity was calculated using Simpson's reciprocal index (1/D) and Shannon-Weaver index (H). Detrended correspondence analysis (DCA) were employed to analyze the microarray data.

RESULTS: Overall Review

Table 1 The diversity indices of whole microbial communities and total gene numbers detected by GeoChip 3.0

	CW	UW	CC	UC
Shannon-Weaver index (H)	6.896±0.089	6.488±0.180	6.487±0.145	6.343±0.084
Simpson's index(1/D)	778.215±74.792	578.441±109.606	558.1266±87.367	454.13775±40.538
Gene number	1306±84	875±130	860±111	745±72

* Genes were used as 'species' and abundance was indicated by the normalized signal intensity data of each gene from GeoChip 3.0. CW represent clipped warmed; UW represent unclipped warmed; CC represent clipped control; UC represent unclipped control.

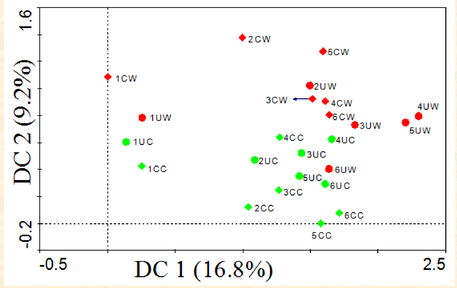

Figure 1 Detrended correspondence analysis (DCA) of whole microbial communities. Functional genes detected using the GeoChip 3.0 were used for DCA. Detected functional gene signal intensity was used as species and square root transform was used to transform gene's signal intensity. Red color represent warmed, the green color represent control. Cycle represent unclipped plots, diamond represent clipped plots.

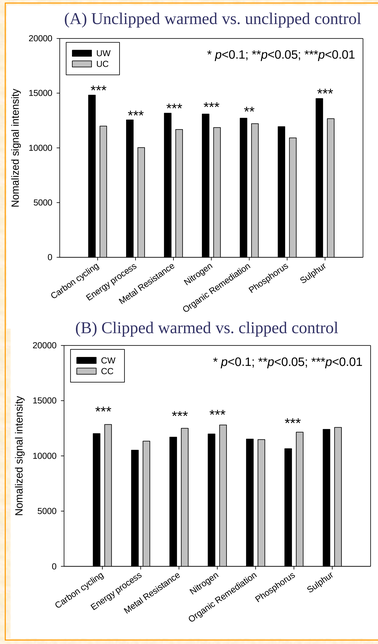

Figure 2 Relative abundance of functional gene categories detected. The normalized signal intensity was shown for different functional groups under different treatment. (A) Unclipped warmed versus unclipped control (UW/UC); (B) Clipped warmed versus clipped control (CW/CC). The results showed that the abundances of most functional genes group detected by GeoChip 3.0 hybridization were changed significantly, and mostly were increased by warming in unclipped plots; however , these changes in clipped plots were decreased with warming.

RESULTS: Functional gene analyses

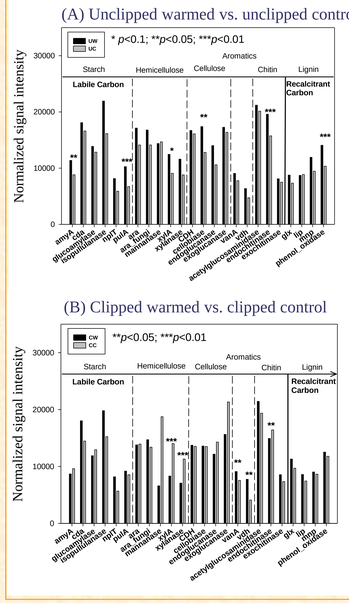

Figure 3 Changes of relative abundance for functional genes involved in carbon degradation. (A) Unclipped warmed versus unclipped control (UW/UC); (B) Clipped warmed versus clipped control (CW/CC). Signal intensities were the sum of detected individual sequences for each functional gene, and then averaged across 6 replicates at warmed and un-warmed. The complexity of carbon is presented in order from labile to recalcitrant. All data are presented as the mean with three stars (***) for significance at p<0.01, two stars (**) for significance at p < 0.05, and one star (*) for significance at p < 0.10 based on pair wise t test.

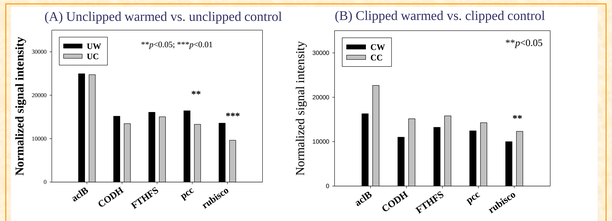

Figure 4 Changes of Functional genes involved in carbon fixation. (A) Unclipped warmed versus unclipped control (UW/UC); (B) Clipped warmed versus clipped control (CW/CC).

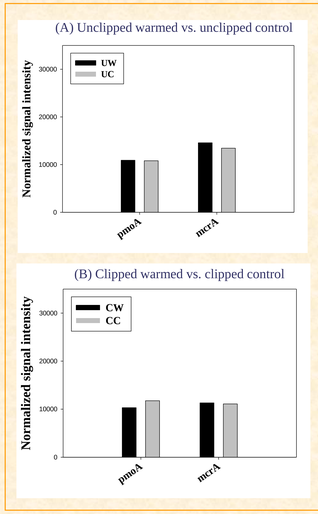

Figure 4 Changes of Functional genes involved in methane cycling. (A) Unclipped warmed versus unclipped control (UW/UC); (B) Clipped warmed versus clipped control (CW/CC). Signal intensities were the sum of detected individual sequences for each functional gene, and then averaged across 6 replicates at warmed and un-warmed.

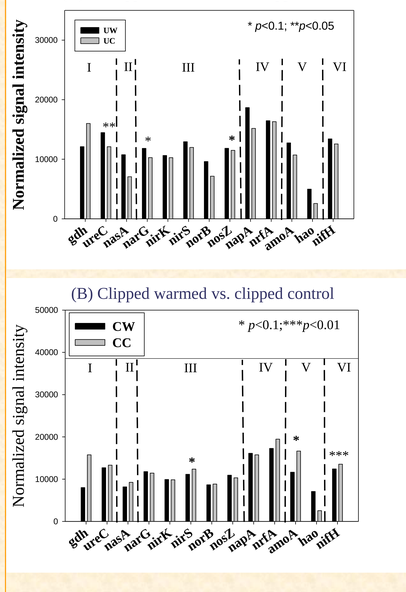

Figure 5 Changes of Functional genes involved in nitrogen cycling. (A) Unclipped warmed versus unclipped control (UW/UC); (B) Clipped warmed versus clipped control (CW/CC).

I-VI in the figures indicate different nitrogen processes:

I. Ammonification, including *gdh* for glutamate dehydrogenase and *ureC* encoding urease;

II. Assimilatory N reduction, including *nasA* encoding nitrate reductase;

III. Denitrification, including *narG* for nitrate reductase, *nirS* and *nirK* for nitrite reductase, *norB* for nitric oxide reductase, and *nosZ* for nitrous oxide reductase;

IV. Dissimilatory N reduction, including *napA* for nitrate reductase, and *nrfA* for c-type cytochrome nitrite reductase;

V. Nitrification, including genes: *amoA* encoding ammonia monooxygenase, *hao* for hydroxylamine oxidoreductase;

VI. N_2 fixation, including *nifH* encoding nitrogenase reductase.

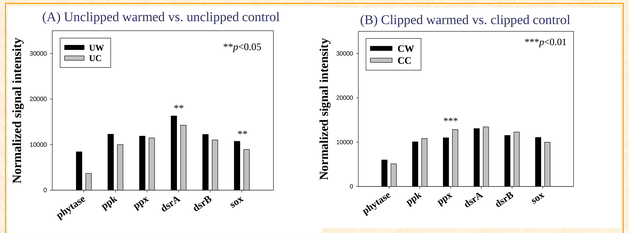

Figure 6 Changes of Functional genes involved in P & S cycling. (A) Unclipped warmed versus unclipped control (UW/UC); (B) Clipped warmed versus clipped control (CW/CC).

CONCLUSIONS

- Long term warming altered the microbial communities in both clipping and unclipping plots as indicated by detrended correspondence analyses and statistic tests;
- Microbial community diversity and total detected functional gene numbers were increased by warming and clipping treatment; however, the simultaneous treatment of warming and clipping cause the decrease of diversity and detected functional gene numbers;
- The abundances of functional genes involved in carbon degradation detected by GeoChip hybridization were changed significantly, and mostly were increased by warming with decreased abundances in unclipped plots; however , these changes in clipped plots were not significant for most of the genes, implying that the increased activity of carbon degradation is related to increase carbon input in the unclipped plots.
- Functional genes involved in different nitrogen processes, including nitrogen fixation, nitrification, denitrification, ammonification, and other processes were significantly upgraded by warming in unclipped plots indicating accelerated nitrogen processing, these changes could be stimulated by increase carbon input, and in return, probably increased nitrogen availability to support more biomass increase of plant under warming condition.

ACKNOWLEDGEMENT

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