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

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

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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 changes of microbial communities to this phenomenon. In this study, functional gene changes at the mRNA level were analyzed by newly 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 Kouder Farm Field Laboratory, a 137.6-ha farm located in Central Redcliff Plains, in McClain County, Oklahoma. The experiment was a paired factorial design with warming as the primary factor and clipping as a secondary factor. An infrared heater was used to simulate global warming, and clipping was used to remove half of the biomass. Twelve 2m × 2m plots were divided into six pairs of warm and control plots. The heater generates a constant output of 10W/m², which increases soil temperature above the ambient plots, which is at the low range of the projected climate warming by IPCC. GeoChip whole microbial communities mRNA was extracted, amplified, labeled and hybridized with our GeoChip 3.0, a functional gene array covering genes 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 warm plots compared to control. Detrended correspondence analysis (DCA) for the 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 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 community increase functional gene expression under warming with or without clipping in order to adapt to the new environment. More detailed analysis is underway.

RESULTS: Overall Review

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

<table>
<thead>
<tr>
<th>Community</th>
<th>Shannon-Weaver index</th>
<th>Simpson’s index</th>
<th>Genes detected</th>
</tr>
</thead>
<tbody>
<tr>
<td>CW</td>
<td>7.185 ± 7.842</td>
<td>0.959 ± 0.046</td>
<td>5,534,306</td>
</tr>
<tr>
<td>UW</td>
<td>7.185 ± 7.842</td>
<td>0.959 ± 0.046</td>
<td>5,534,306</td>
</tr>
<tr>
<td>CC</td>
<td>7.185 ± 7.842</td>
<td>0.959 ± 0.046</td>
<td>5,534,306</td>
</tr>
<tr>
<td>UC</td>
<td>7.185 ± 7.842</td>
<td>0.959 ± 0.046</td>
<td>5,534,306</td>
</tr>
</tbody>
</table>

* Genes were used as ‘species’ and abundance was indicated by the normalized signal intensity, density of each gene from GeoChip 3.0. CW represents clipped warmed, UC represents unclipped control. UC represents unclipped control.

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

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 unclipped control (CW/UC). 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 or clipping plots; however, these changes in clipped plots were decreased with warming.

METHODS

• Sampling and RNA extraction: Soil samples (10 g) were taken from the warming site, and both bulk RNA and DNA was simultaneously extracted and sequenced with the method described by Zhou et al.
• Shewanella oneidensis Functional gene array: The third version of functional gene array GEOCHIP 3.0 was used to detect the microbial communities in the soils with different treatments.
• RNA purification, amplification, labeling, and hybridization: Raw RNA was purified by RNeasy MinElute kit (QIAGEN) and 18μg of purified RNA of each sample was amplified using whole-community RNA saturation amplification protocol described by Zhou et al. (2007). 18μg total RNA was divided into two parts, 4μg were used for the GeoChip 3.0 hybridization and the other 4μg were used for the GeoChip 3.0 hybridization.
• Microscopy scanning and data processing: Hybridized microarray slides were scanned using a Scanning Confocal Microarray Scanner (Affymetrix). The raw fluorescence data were analyzed using Bioconductor Lumi packages. Four spots were scanned for each slide, and the signal intensities were normalized by the positive controls with the output of 10000.
• Data Analysis: Functional gene diversity was calculated utilizing Shannon’s reciprocal index (SIR) and Shannon-Weaver index (SIW). Detrended correspondence analysis (DCA) were employed to analysis the microbial data.

RESULTS: Functional gene analyses

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

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 analysis and test statistics.
• Microbial community, diversity and total detected functional gene numbers were increased by warming and clipping treatment; however, the simultaneous treatment of warming and clipping caused the decrease of diversity and functional gene numbers.
• The abundances of functional genes involved in carbon degradation detected by GeoChip hybridization were altered to the expected changes with increased carbon abundance in unclipped plots; however, these changes in clipped plots were significant for most of the genes, implying that the increased availability of carbon degradation was limited by the increased consumption in the unclipped.
• Functional genes involved in different nitrogen processes, including nitrogen fixation, assimilation, denitrification, ammonification, and other processes were significantly up-regulated by warming or unclipping plots indicating accelerated nitrogen processes. These changes could be stimulated by warming or clipping, which increase carbon input, and in return, possibly increase nitrogen availability to support more biomass increase of plant under warming conditions.

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