# Alternations of Structure and Functional Activity of Below Ground Microbial Communities at Elevated Atmospheric Carbon Dioxide

**Q-2850**


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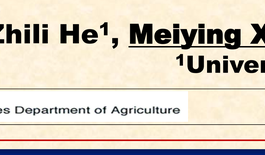
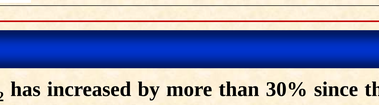
## Abstract


The global atmospheric concentration of $CO_2$ has increased by more than 30% since the industrial revolution. Although the stimulating effects of elevated $CO_2$ ($eCO_2$) on plant growth and primary productivity have been well studied, its influences on belowground microbial communities are poorly understood and controversial. In this study, we showed a significant change in the structure and functional potential of soil microbial communities at $eCO_2$ in a grassland ecosystem, the BioCON (Biodiversity, $CO_2$ and Nitrogen) experimental site (http://www.biocon.umn.edu/) using a comprehensive functional gene array, GeoChip 3.0, which contains about 28,0000 probes and covers approximately 57,000 gene variants from 292 functional gene families involved in carbon, nitrogen, phosphorus and sulfur cycles as well as other functional processes. GeoChip data indicated that the functional structure of microbial communities was markedly different between ambient $CO_2$ ($aCO_2$) and $eCO_2$ by detrended correspondence analysis (DCA) of all 5001 detected functional gene probes although no significant differences were detected in the overall microbial diversity. A further analysis of 1503 detected functional genes involved in C, N, P, and S cycles showed that a considerable portion (39%) of them were only detected under either $aCO_2$ (14%) or $eCO_2$ (25%), indicating that the functional characteristics of the microbial community were significantly altered by $eCO_2$. Also, for those shared genes (61%) detected, some significantly ($p<0.05$) changed their abundance at $eCO_2$. Especially, genes involved in labile C degradation, such as *amyA*, *egl*, and *ara* for starch, cellulose, and hemicelluloses, respectively, C fixation (e.g., *rbcL*, *pcc*/*acc*), N fixation (*nifH*), and phosphorus utilization (*ppx*) were significantly increased under $eCO_2$, while those involved in decomposing recalcitrant C, such as *glx*, *lip*, and *mnp* for lignin degradation remained unchanged. This study provides insights into our understanding of belowground microbial communities and their feedbacks to terrestrial ecosystems at $eCO_2$.


## BioCon (Biodiversity, $CO_2$ and Nitrogen) Study

The BioCON experiment site is located at the Cedar Creek Natural History Area in Minnesota, USA (http://www.swan.lter.umn.edu/biocon/).



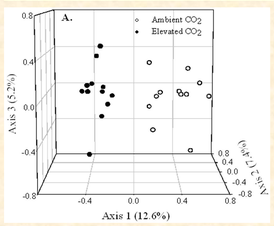
**Elevated $CO_2$: 560 µmol mol$^{-1}$** **Ambient $CO_2$: 368 µmol mol$^{-1}$**

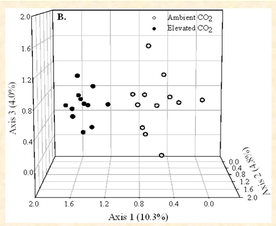
**24** soil samples were taken from 24 plots (2×2m) with 16 plant species under ambient $CO_2$ ($aCO_2$, green color) or elevated $CO_2$ ($eCO_2$, red color) without nitrogen addition.

## Methods

- **DNA extraction, amplification and labeling:** Soil DNA was extracted by freeze-grinding methods. 50ng purified DNA was amplified using a TempliPhi kit, and the amplification products were labeled with Cy-5 using random priming method.
- **GeoChip hybridization, scanning and image analysis**: A functional gene array (GeoChip 3.0) was used for soil DNA hybridization. All hybridizations were carried out in triplicate at 45°C for 10 hours with 50% formiade using a TECAN HS4800. The array was scanned by a ScanArray Express Microarray Scanner at 633 nm. ImaGene version 6.0 was then used for image quantification.
- **Statistical analysis of GeoChip data**: Functional gene diversity indices were calculated. Response ratios were used to examine the significance of $eCO_2$ on plant, soil variables and the abundance of functional genes with $aCO_2$ samples as the control. Detrended correspondence analysis (DCA) was used to determine the overall functional changes. Multivariate statistical analyses including the Mantel test, CCA and partial CCA analyses were performed to link microbial communities to soil and plant variables.

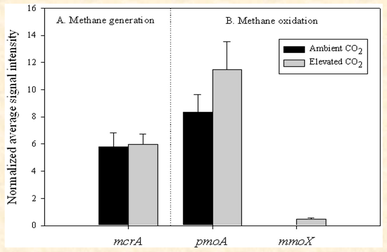
## Overall responses of microbial communities to $eCO_2$

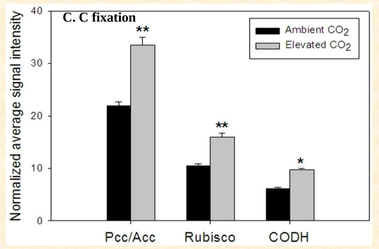



**Detrended correspondence analysis (DCA) of the functional gene array, GeoChip 3.0 (A) and 454 sequencing (B) data showed that $eCO_2$ significantly altered the soil microbial structure and composition.**

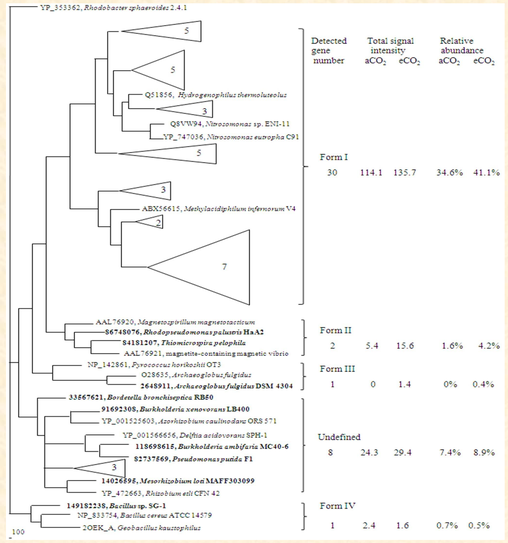
## Effects of $eCO_2$ on genes involved in carbon cycling

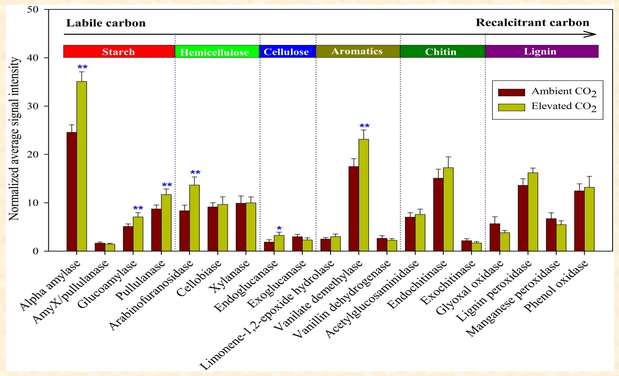



**The detected signal intensity increased for genes involved in carbon fixation (C), but no significant differences were observed for genes involved in methane generation (A) or methane oxidation (B) under $eCO_2$.**

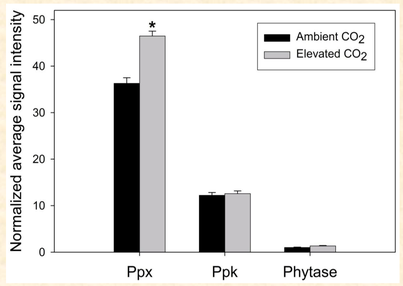
**Maximum-likelihood phylogenetic tree of the detected Rubisco sequences. 46 *rbcL* probes had positive signals with 27 shared by both $CO_2$ conditions and 8 and 11 only detected at $aCO_2$ or $eCO_2$, respectively. Four forms of Rubisco were identified with Form I as the dominant that also contained significantly changed gene sequences. Form I is a major form for $CO_2$ fixation. However, it is not known about the rates and extent of C fixation stimulated, or the impacts of the fixed C on overall soil C dynamics.**



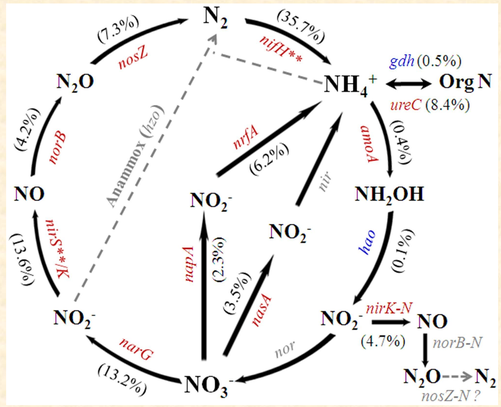

**The detected signal intensity increased for genes involved in labile C degradation, but remained unchanged for those involved in recalcitrant C degradation at $eCO_2$.**

## Effects of $eCO_2$ on genes for phosphorus cycling



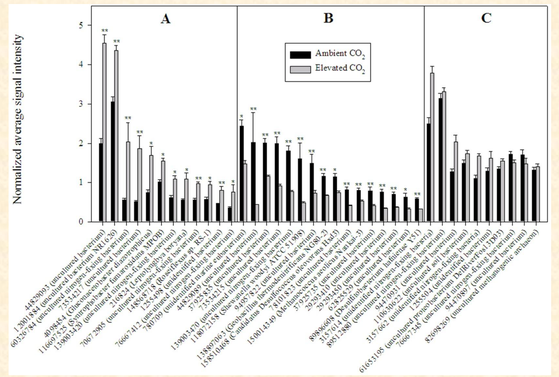

**GeoChip targets three enzymes involved in P utilization, exopolyphosphatase (PPX) for inorganic polyphosphate degradation, polyphosphate kinase (PPK) for polyphosphate biosynthesis, and phytase for phytate degradation. While no significant differences of signal intensity were observed for PPK and phytase, the total signal intensity of PPX was significantly increased at $eCO_2$.**

## Effects of $eCO_2$ on genes involved in nitrogen cycling




- **147 genes involved in $N_2$ fixation (*nifH*) were detected, and their signal intensity was significantly higher ($p<0.05$) under $eCO_2$ than $aCO_2$.**
- **The abundance of denitrification genes (*nirS*/*nirK*) also significantly increased at $eCO_2$.**
- **Most *nifH* genes were from uncultured microorganisms, suggesting that our understanding of $N_2$-fixing microorganisms and microbial $N_2$ fixation mechanisms are very limited.**

**The significantly changed *nifH* genes and other top 10 abundant *nifH* gene s detected by GeoChip 3.0. Among 147 detected genes, 92 were shared by both $aCO_2$ and $eCO_2$, and 15 and 40 only detected at $aCO_2$ or $eCO_2$, respectively. Among 92 shared gene sequences, 12 were significantly increased at $eCO_2$ (A) while 16 were significantly decreased (B). Other top 10 abundant *nifH* gene s are also shown (C).**

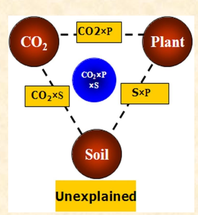


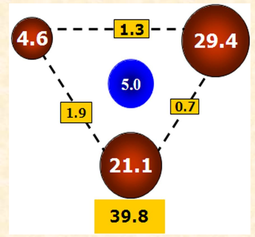

## Linking microbial communities to soil, and plants

The relationships of microbial community functional structure to soil C and N dynamics and aboveground plant characteristics revealed by partial Mantel test. Soil and plant variables were selected by the BIO-ENV procedure.

| In association with: | | Soil[a] | | Plant[b] | |
|---|---|---|---|---|---|
| Controlling for: | | Plant[b] | | Soil[a] | |
| Functional category | Gene No. | $r_M$ | $p$ | $r_M$ | $p$ |
| All detected | 5001 | 0.312 | **0.027** | 0.146 | 0.109 |
| C cycle | 576 | 0.351 | **0.014** | 0.134 | 0.144 |
| C fixation | 147 | 0.480 | **<0.001** | 0.184 | 0.072 |
| Labile C degradation | 259 | 0.296 | **0.005** | 0.126 | 0.150 |
| Recalcitrant C degradation | 127 | 0.193 | 0.068 | 0.020 | 0.443 |
| N cycle | 548 | 0.239 | 0.063 | 0.149 | 0.097 |
| $N_2$ fixation | 147 | 0.320 | **0.005** | 0.166 | 0.070 |
| Nitrification | 7 | 0.036 | 0.343 | 0.104 | 0.125 |
| Denitrification | 277 | 0.173 | 0.119 | 0.148 | 0.139 |
| N reduction to $NH_4^+$ | 55 | 0.202 | 0.064 | 0.068 | 0.256 |
| N mineralization | 62 | 0.095 | 0.176 | 0.069 | 0.234 |
| Phosphorous utilization | 74 | 0.197 | 0.069 | 0.008 | 0.448 |

**Partial CCA analysis of the effects of $CO_2$, soil and plant parameters on microbial communities**




## Summary

- **Elevated $CO_2$ ($eCO_2$) dramatically altered the composition and functional structure of belowground microbial communities.**
- **While genes involved in recalcitrant C degradation and methane metabolism remained unchanged, those involved in labile C degradation, and C and N fixation, and phosphorus release were increased at $eCO_2$.**
- **Some of such changes in microbial communities were significantly correlated with soil C and N and plants, mitigating the global climate change.**

## Acknowledgements


This work is supported by the United States Department of Agriculture (Project 2007-35319-18305) through NSF-USDA Microbial Observatories Program, and the National Science Foundation under Grant Numbers DEB-0716587 and DEB-0620652. The GeoChips and associated technologies were supported by the Virtual Institute for Microbial Stress and Survival (http://VIMSS.lbl.gov) supported by the U. S. Department of Energy, Office of Science, Office of Biological and Environmental Research, Genomics:GTL program through contract DE-AC02-05CH11231 between Lawrence Berkeley National Laboratory and the U. S. Department of Energy), and by the Oklahoma Bioengery Center (OBC) and the Oklahoma Applied Research Support (OARS), Oklahoma Center for the Advancement of Science and Technology (OCAST), the State of Oklahoma.

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