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

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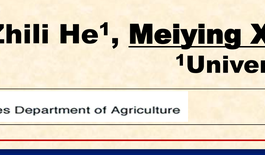
Abstract

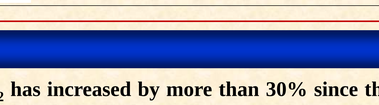
The global atmospheric concentration of CO_2 has increased by more than 30% since the industrial revolution. Although the stimulating effects of elevated CO_2 (eCO_2) on plant growth and primary productivity have been well studied, its influences on belowground microbial communities are poorly understood 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}



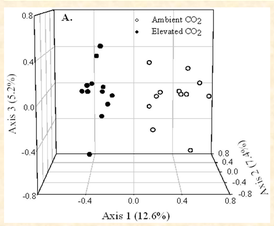
Ambient CO_2: 368 µmol mol^{-1}

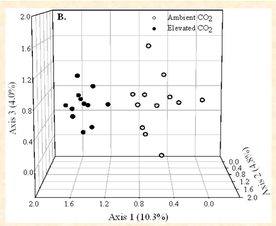
24 soil samples were taken from 24 plots (2×2m) with 16 plant species under ambient CO_2 (aCO_2, green color) or elevated CO_2 (eCO_2, red color) without nitrogen addition.

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.

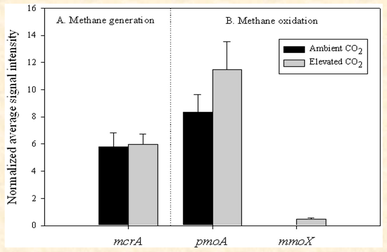
Overall responses of microbial communities to eCO_2

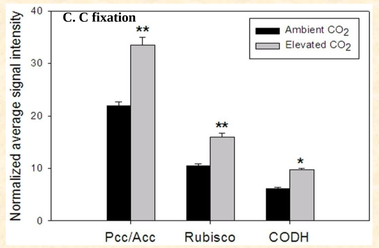




Detrended correspondence analysis (DCA) of the functional gene array, GeoChip 3.0 (A) and 454 sequencing (B) data showed that eCO_2 significantly altered the soil microbial structure and composition.

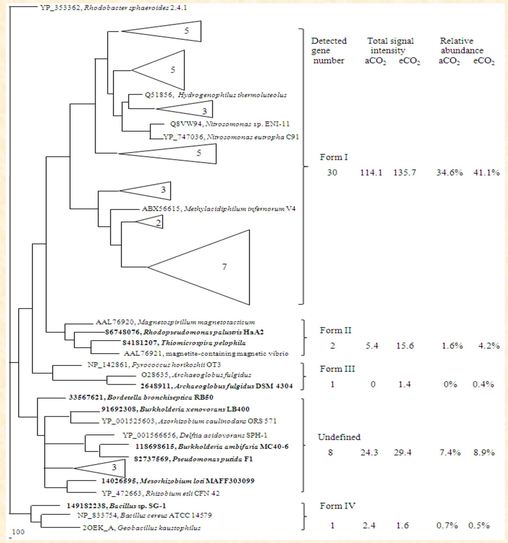
Effects of eCO_2 on genes involved in carbon cycling

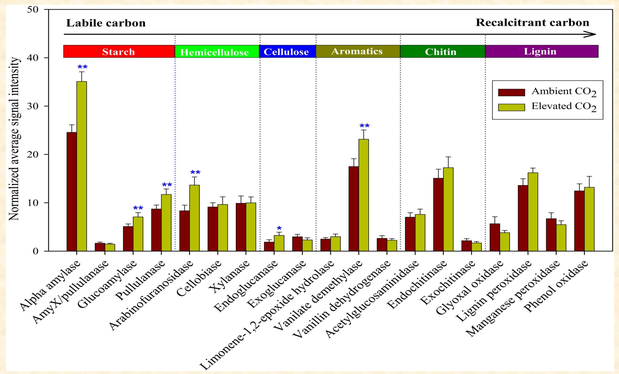




The detected signal intensity increased for genes involved in carbon fixation (C), but no significant differences were observed for genes involved in methane generation (A) or methane oxidation (B) under eCO_2.

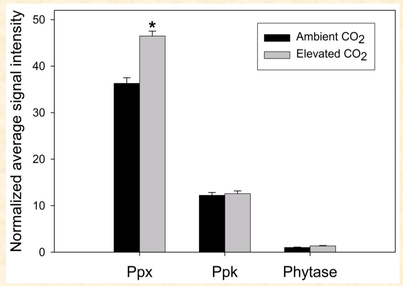
Maximum-likelihood phylogenetic tree of the detected Rubisco sequences. 46 *rbcL* probes had positive signals with 27 shared by both CO_2 conditions and 8 and 11 only detected at aCO_2 or eCO_2, respectively. Four forms of Rubisco were identified with Form I as the dominant that also contained significantly 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.





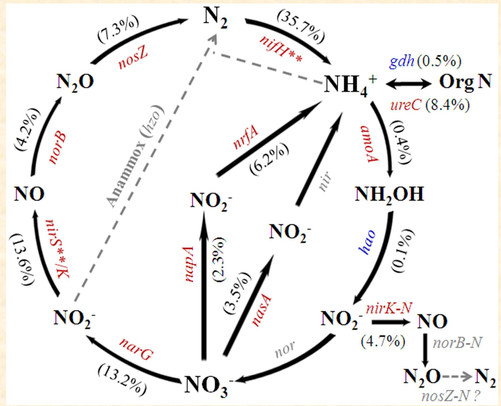
The detected signal intensity increased for genes involved in labile C degradation, but remained unchanged for those involved in recalcitrant C degradation at eCO_2.

Effects of eCO_2 on genes for phosphorus cycling



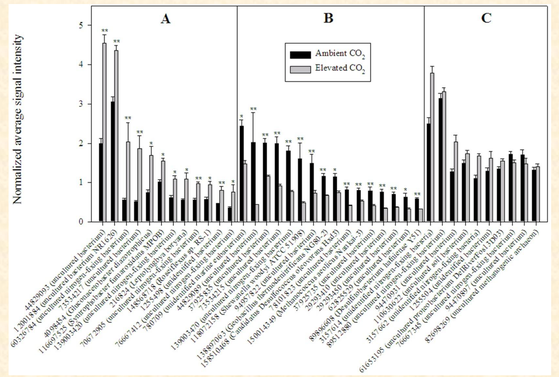
GeoChip targets three enzymes involved in P utilization, exopolyphosphatase (PPX) for inorganic polyphosphate degradation, polyphosphate kinase (PPK) for polyphosphate biosynthesis, and phytase for phytate degradation. While no significant differences of signal intensity were observed for PPK and phytase, 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).

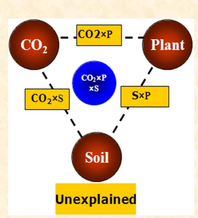


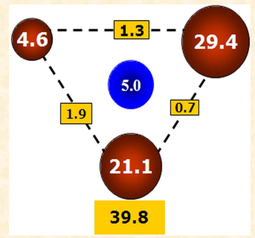
Linking microbial communities to soil, and plants

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

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