

Supplemental Information

Elevated CO₂ shifts the functional structure and metabolic potentials of soil microbial communities in a C₄ agroecosystem

JinboXiong, Zhili He, Shengjing Shi, Angela Kent, Ye Deng, Liyou Wu, Joy D. Van Nostrand, and Jizhong Zhou

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33 **Table S1** Quantitatively evaluates the effects of eCO₂ and soil depths on the functional gene
 34 categories by parametric permutational multivariate analysis of variance (perMANOVA). R²
 35 value is the constrained proportion of the parameter.

Gene category	eCO ₂		Depth		eCO ₂ :Depth	
	R ²	<i>P</i>	R ²	<i>P</i>	R ²	<i>P</i>
C fixation	0.105	0.001	0.056	0.008	0.036	0.055
C degradation	0.116	0.001	0.054	0.002	0.031	0.068
N fixation	0.102	0.001	0.048	0.011	0.033	0.067
Nitrification	0.056	0.009	0.067	0.007	0.061	0.005
Denitrification	0.129	0.001	0.054	0.009	0.037	0.039
Assimilatory N reduction	0.104	0.001	0.046	0.018	0.063	0.002
Dissimilatory N reduction	0.131	0.001	0.052	0.016	0.037	0.059
Ammonification	0.093	0.001	0.056	0.001	0.033	0.043
P utilization	0.128	0.001	0.048	0.017	0.04	0.023
CH ₄ production	0.127	0.001	0.085	0.001	0.061	0.003
CH ₄ oxidation	0.094	0.001	0.048	0.007	0.031	0.097

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37 **Table S2** The correlation of functional process involved in soil C and N dynamics with soil
 38 variables by Mantel test.

Functional category	TN [§]		TC	
	<i>r</i>	<i>p</i>	<i>r</i>	<i>p</i>
C fixation	0.074	0.137	-0.017	0.481
C degradation	0.08	0.133	0.032	0.266
N fixation	0.146	0.024	0.141	0.051
Nitrification	0.183	0.012	0.201	0.031
Denitrification	0.057	0.192	-0.016	0.487
Ammonification	0.076	0.175	-0.03	0.534
Assimilatory N reduction	0.046	0.235	0.016	0.343
Dissimilatory N reduction	0.067	0.146	-0.043	0.687
CH ₄ production	0.105	0.111	-0.116	0.894
CH ₄ oxidation	0.166	0.049	0.037	0.294
P utilization	0.074	0.113	0.028	0.304
S cycling	0.067	0.151	0.016	0.336

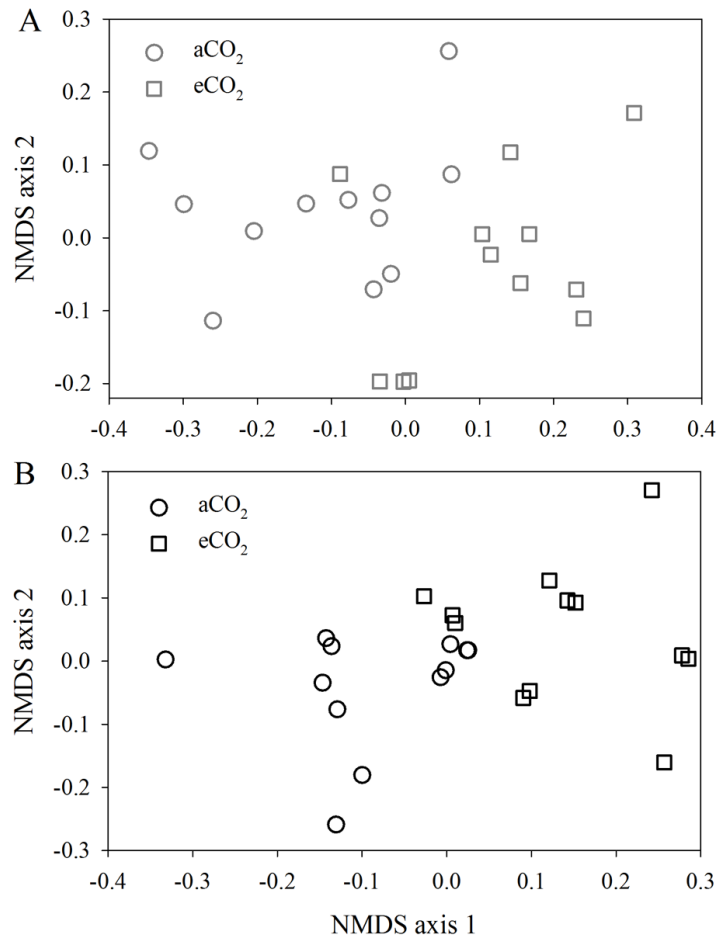
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 40 [§]:The other soil variables, including NO₃⁻, NH₄⁺, C:N ratio and corn yield that showed no
 41 significant trends to any functional processes are not represented in this table.

42 **Table S3** *P* values of correlations between soil properties and signal intensities of functional
 43 genes by Mantel test. Bold face indicates significantly changed *P* values (*P* < 0.05).

Gene/enzyme	Function	NO ₃ ⁻	NH ₄ ⁺	TN	TC	C/N
0-5 cm soil						
<i>AceA</i>	C degradation	0.763	0.977	0.179	0.009	0.025
<i>amyA</i>	C degradation	0.551	0.694	0.038	0.1	0.22
<i>norB</i>	Denitrification	0.854	0.965	0.607	0.129	0.032
<i>alkB</i>	Aromatics	0.414	0.006	0.496	0.646	0.399
<i>alkK</i>	Aromatics	0.288	0.98	0.244	0.02	0.027
CDD	Aromatics	0.765	0.469	0.391	0.446	0.048
<i>nagL</i>	Aromatics	0.265	0.753	0.471	0.072	0.006
<i>nhh</i>	Aromatics	0.448	0.909	0.062	0.009	0.069
<i>oxdB</i>	Aromatics	0.552	0.926	0.279	0.039	0.032
<i>phdG</i>	Aromatics	0.634	0.008	0.268	0.463	0.572
<i>pheA</i>	Aromatics	0.922	0.824	0.032	0.02	0.074
<i>tmoABE</i>	Aromatics	0.211	0.83	0.067	0.033	0.099
<i>xylXY</i>	Aromatics	0.211	0.902	0.058	0.022	0.061
<i>nphA</i>	Aromatics	0.438	0.365	0.035	0.189	0.694
<i>dxnA</i>	Herbicides	0.345	0.364	0.027	0.497	0.662
<i>mhpC</i>	Herbicides	0.888	0.947	0.13	0.031	0.103
<i>pcpA</i>	Herbicides	0.7	0.694	0.056	0.017	0.046
<i>pcpE</i>	Herbicides	0.703	0.049	0.235	0.389	0.792
<i>cpnA</i>	Hydrocarbons	0.384	0.752	0.126	0.051	0.097
<i>nitA</i>	Hydrocarbons	0.639	0.854	0.165	0.043	0.078
<i>bco</i>	Pesticides	0.214	0.393	0.425	0.067	0.019
<i>dbdC</i>	Aromatics	0.882	0.618	0.009	0.186	0.408
<i>nphA</i>	Aromatics	0.438	0.365	0.035	0.189	0.694
5-15 cm soil						
Acetylglucosaminidase	C degradation	0.521	0.331	0.367	0.07	0.037
<i>ara</i>	C degradation	0.324	0.817	0.016	0.251	0.7
Pectinase	C degradation	0.02	0.625	0.443	0.062	0.041
Xylanase	C degradation	0.973	0.939	0.004	0.104	0.534
<i>mmoX</i>	CH ₄ consume	0.632	0.595	0.142	0.048	0.125
<i>dbdC</i>	Aromatics	0.882	0.618	0.009	0.186	0.408
<i>nphA</i>	Aromatics	0.438	0.365	0.035	0.189	0.694
BADH	Hydrocarbons	0.877	0.727	0.024	0.512	0.9
<i>cpnA</i>	Hydrocarbons	0.969	0.703	0.043	0.282	0.699
<i>rd</i>	Herbicides	0.18	0.587	0.045	0.381	0.666

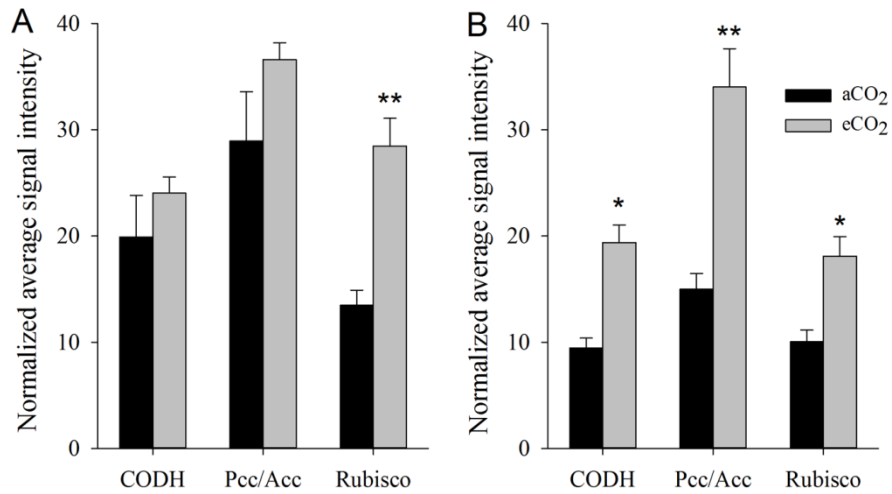
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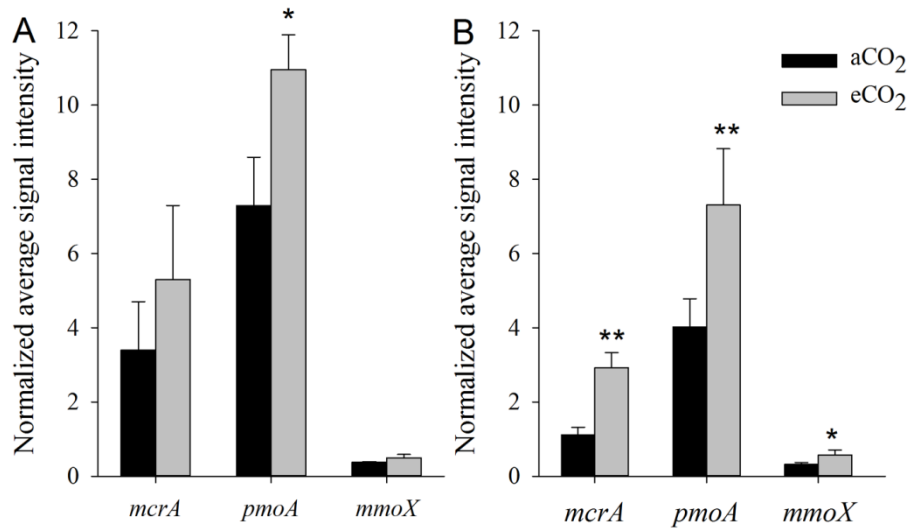
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48 **Figure S1** Non-metric multidimensional scaling (NMDS) analysis of elevated CO₂ (eCO₂)
49 and ambient CO₂ (aCO₂) samples at the depths of 0-5 cm (A) and the 5-15 cm (B) based on
50 the Bray-Curtis values of GeoChip detected *gyrB* gene.



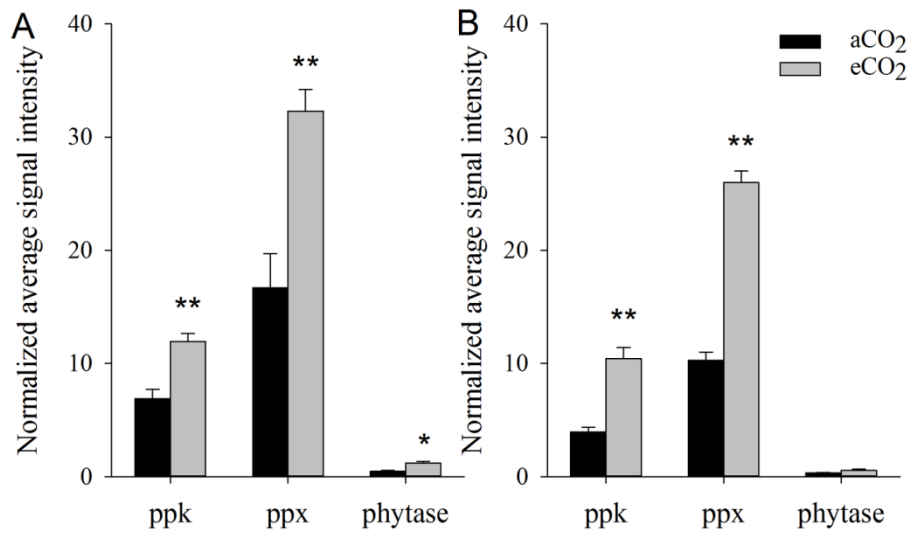
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53 **Figure S2** The normalized signal intensity of the detected key gene families involved in CO₂
 54 fixation. Rubisco: ribulose, 5-bisphosphate carboxylase/oxygenase; CODH: carbon
 55 monoxide dehydrogenase; Pcc/Acc: propionyl-CoA/acetyl-CoA carboxylase. Data are
 56 presented as mean ± SE (standard error, $n = 12$). *: $P < 0.05$; **: $P < 0.01$ based on t-test
 57 between elevated CO₂ (eCO₂) and ambient CO₂ (aCO₂) samples in the soil depths of 0-5 cm
 58 (A) and 5-15 cm (B).



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Figure S3 The normalized signal intensity of the detected key gene families involved in CH₄ cycling. *mcrA*: the alpha-subunit of methyl coenzyme M reductase for methane production; *pmoA*: particulate methane monooxygenase, and *mmoX*: methane monooxygenase for methane consumption. Data are presented as mean ± SE (standard error, $n = 12$). *: $P < 0.05$; **: $P < 0.01$ based on t-test between elevated CO₂ (eCO₂) and ambient CO₂ (aCO₂) samples in the soil depth of 0-5 cm (A) and 5-15 cm (B).



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69 **Figure S4** The abundance of detected key genes involved in P utilization in the soil depth of
70 0-5 cm (A) and 5-15 cm (B). Ppx, exopolyphosphatase for inorganic polyphosphate
71 degradation; Ppk, polyphosphate kinase for polyphosphate biosynthesis in prokaryotes; and
72 phytase for phytate degradation. Data are presented as mean \pm SE. *: $P < 0.05$; **: $P < 0.01$
73 based on the Student t-test.