**Supplementary Material**

1. **Supplemental Tables**

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1. **Supplemental Figures**

**Fig. S1** Taxonomic composition of the microbial community under different treatments as detected by GeoChip at phylum level at soil depths of 0-5cm (a) and 5-15cm (b).

**Fig. S2** Detrended correspondence analysis (DCA) of all detected *gyrB* genes under different treatments at soil depths of 0-5cm (a) and 5-15cm (b).

**Fig. S3** Effect of eCO2 and irrigation on functional genes involved in methane metabolism process at soil depths of 0 to 5 cm (left) and 5 to 15 cm (right).

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**Fig. S5** Effect of eCO2 and irrigation on functional genes involved in S cycle at soil depths of 0 to 5 cm (left) and 5 to 15 cm (right).

**Fig. S6** Standardized direct effects (including direct and indirect effects) of moisture, aboveground plant biomass, plant N, and chemical property based on the structural equation models under eCO2 (a) and irrigation (b) treatments.

**A. Supplemental Tables**

**Table S1** Average seasonal soil moisture at ambient, eCO2 and irrigation treatments in the summer of 2008.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| ct | |  | Ct | |  | ct-i | |
| Plots NO. | moisture | Plots NO. | moisture | Plots NO. | moisture |
| 8\_4 | 12.5±0.51 | 3\_5 | 12.14±0.42 | 5\_4 | 14.73±0.47 |
| 12\_3 | 12.8±0.53 |  | 7\_4 | 16.08±0.48 |  | 6\_5 | 13.21±0.42 |
| 14\_5 | 11.09±0.42 |  | 11\_3 | 14.89±0.41 |  | 17\_3 | 11±0.34 |
| 22\_1 | 10.75±0.38 |  | 26\_1 | 14.39±0.43 |  | 24\_1 | 15.01±0.5 |
| 25\_5 | 13.28±0.56 |  | 27\_2 | 13.98±0.45 |  | 29\_2 | 11.87±0.41 |
| Average | 12.08±0.44 |  | 14.3±0.58 | |  | 13.16±0.7 | |

ct, ambient CO2 and no irrigation; Ct, eCO2 and no irrigation; ct-i, ambient CO2 and irrigation

**Table S2** Overall microbial community functional diversity detected by GeoChip under ambient, eCO2, and irrigation treatments.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | Treatment | Total no. of detected genes | Simpson's (1/D) | Shannon-Weaver (H’) |
| 0-5cm | ct | 1268.6±78.3b | 1261.6±77.6b | 7.13±0.06b |
| Ct | 2217±269.3a | 2206.1±267.1a | 7.67±0.12a |
| ct-i | 1700.4±87.2ab | 1693.5±86.7ab | 7.43±0.05a |
| 5-15cm | ct | 1834±86c | 1825.6±85.4c | 7.51±0.05c |
| Ct | 3442.4±185.3a | 3424.1±184.3a | 8.14±0.05a |
| ct-i | 2669.4±223.4b | 2656.4±222.1b | 7.87±0.08b |

The significant difference of detected gene numbers, Shannon index (H') and the Simpson’s reciprocal index (1/D) at each depth were analyzed by least significant difference (LSD) test at the *P*<0.05 level. a, b, and c represent significant difference among treatments at each soil depth.

**Table S3** Number of functional gene probes derived from major phyla as detected by GeoChip under different treatments.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Phylum | Total | ct | Ct | ct-i |
| 0-5cm |  |  |  |  |
| *Proteobacteria* | 1985(66.3%) | 767.2±48.9b | 1341±161a | 1049.2±55.1ab |
| *Actinobacteria* | 475(15.9%) | 172.4±11.1b | 328.6±42.3a | 275.8±17.7a |
| *Firmicutes* | 127(4.2%) | 41.8±4b | 66±8a | 36.2±1.6b |
| *Ascomycota* | 121(4%) | 40.2±3.3b | 70±10.3a | 45.6±1.7b |
| *Euryarchaeota* | 40 (1.3%) | 10.4±1.5b | 22.8±3.1a | 14.6±1.5b |
| 5-15cm |  |  |  |  |
| *Proteobacteria* | 2670(66.5%) | 1125.8±52.8c | 2045.4±103.1a | 1603±127b |
| *Actinobacteria* | 677(16.9%) | 279.6±15.5c | 521.6±32a | 390.6±28.3b |
| *Firmicutes* | 154(3.8%) | 53±3.5c | 101.4±5.9a | 84.2±6.7b |
| *Ascomycota* | 148(3.7%) | 42.2±1.5c | 87.2±6.3a | 73.6±9.4b |
| *Euryarchaeota* | 56(1.4%) | 16±0.7b | 37.8±2.9a | 29.6±3.6a |

The significant difference among three treatments at each depth were analyzed by least significant difference (LSD) test at the *P*<0.05 level, and marked with a and b.

**Table S4** Number of probes from detected functional genes involved in carbon, nitrogen, sulfur, and phosphorus cycling.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Gene category | 0-5cm | | | 5-15cm | | |
| ct | Ct | ct-i | ct | Ct | ct-i |
| Acetogenesis | 2 | 4 | 2 | 1 | 6 | 2 |
| Carbon degradation | 131 | 236 | 142 | 168 | 335 | 276 |
| Carbon fixation | 48 | 93 | 71 | 73 | 145 | 112 |
| Methane production | 3 | 8 | 2 | 4 | 6 | 7 |
| Methane oxidation | 9 | 13 | 8 | 9 | 19 | 14 |
| Carbon cycling | 193 | 354 | 225 | 255 | 513 | 412 |
| Ammonification | 23 | 39 | 24 | 25 | 48 | 43 |
| Assimilatory N reduction | 2 | 11 | 3 | 4 | 18 | 13 |
| Denitrification | 84 | 157 | 117 | 119 | 262 | 192 |
| Dissimilatory N reduction | 8 | 17 | 10 | 13 | 21 | 21 |
| Nitrification | 2 | 4 | 0 | 2 | 5 | 2 |
| Nitrogen fixation | 52 | 71 | 48 | 59 | 117 | 98 |
| Nitrogen cycling | 171 | 299 | 202 | 222 | 471 | 369 |
| Phosphorus utilization | 51 | 58 | 43 | 50 | 88 | 71 |
| Sulphur oxidation | 27 | 35 | 28 | 28 | 48 | 42 |
| sulfite reductase | 51 | 69 | 48 | 54 | 124 | 89 |
| Sulphur | 78 | 104 | 76 | 82 | 172 | 131 |
| Total | 493 | 815 | 546 | 609 | 1244 | 983 |

**Table S5** Description of significantly changed genes involved in carbon, nitrogen, sulfur, and phosphorus cycling among ct, Ct, and ct-i.

|  |  |  |
| --- | --- | --- |
| Gene category | Functional processes | Gene/enzyme |
| C cycling | C degradation | *amyA* |
| C degradation | glucoamylase |
| C degradation | *pulA* |
| C degradation | *ara* |
| C degradation | *xylA* |
| C degradation | xylanase |
| C degradation | *CDH* |
| C degradation | cellobiase |
| C degradation | endoglucanase |
| C degradation | exoglucanase |
| C degradation | acetylglucosaminidase |
| C degradation | endochitinase |
| C degradation | exochitinase |
| C degradation | pectinase |
| C degradation | *limEH* |
| C degradation | *vanA* |
| C degradation | *vdh* |
| C degradation | *glx* |
| C degradation | *lip* |
| C degradation | *mnp* |
| C degradation | phenol\_oxidase |
| Methane production | *mcrA* |
| Methane oxidation | *pmoA* |
| N cycling | Nitrogen fixation | *nifH* |
| Nitrification | *amoA* |
| Denitrification | *narG* |
| Denitrification | *nirK* |
| Denitrification | *nirS* |
| Denitrification | *norB* |
| Denitrification | *nosZ* |
| Dissimilatory N reduction to ammonium | *napA* |
| Dissimilatory N reduction to ammonium | *nrfA* |
| Ammonification | *gdh* |
| Ammonification | *ureC* |
| Assimilatory N reduction | *nasA* |
| P cycling | Phosphorus utilization | *ppk* |
| Phosphorus utilization | *ppx* |
| S cycling | Sulfite reductase | *dsrA* |
| Sulfite reductase | *dsrB* |
| Sulphur oxidation | *sox* |

**Table S6** Correlation between functional genes involved in C, N, P, and S cycle and environmental variables as revealed by Mantel analysis.

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Gene | Category | TN | TC | NH4+-N | NO3--N | Moisture | pH | Plant | Plant N |
| All detected genes | | 0.265 | 0.119 | 0.15 | **0.009** | **0.001** | 0.724 | **0.027** | 0.067 |
| *amyA* | C degradation | 0.734 | 0.532 | 0.758 | 0.609 | **0.001** | 0.494 | 0.138 | 0.403 |
| glucoamylase | C degradation | 0.552 | 0.333 | 0.134 | **0.013** | **0.001** | 0.636 | **0.002** | **0.001** |
| *pulA* | C degradation | 0.705 | 0.443 | 0.867 | 0.372 | **0.001** | 0.463 | 0.051 | 0.188 |
| *ara* | C degradation | 0.44 | 0.217 | **0.01** | **0.001** | **0.001** | 0.849 | **0.003** | **0.002** |
| *xylA* | C degradation | 0.543 | 0.367 | 0.572 | 0.36 | **0.001** | 0.591 | **0.028** | 0.159 |
| xylanase | C degradation | 0.064 | **0.01** | **0.001** | **0.001** | **0.001** | 0.806 | **0.006** | **0.003** |
| *CDH* | C degradation | **0.043** | **0.031** | **0.002** | **0.004** | **0.001** | 0.777 | **0.016** | **0.03** |
| cellobiase | C degradation | 0.74 | 0.545 | 0.866 | 0.57 | **0.001** | 0.595 | 0.079 | 0.377 |
| endoglucanase | C degradation | 0.141 | 0.061 | 0.282 | 0.112 | **0.001** | 0.505 | **0.019** | **0.041** |
| exoglucanase | C degradation | 0.084 | **0.032** | **0.009** | **0.001** | **0.001** | 0.488 | **0.008** | **0.005** |
| endochitinase | C degradation | 0.366 | 0.126 | **0.015** | **0.007** | **0.001** | 0.582 | **0.009** | **0.004** |
| exochitinase | C degradation | 0.709 | 0.369 | 0.71 | 0.547 | 0.08 | 0.487 | **0.045** | **0.028** |
| pectinase | C degradation | 0.629 | 0.551 | 0.49 | 0.649 | **0.001** | 0.701 | **0.035** | 0.154 |
| *limEH* | C degradation | 0.21 | 0.133 | 0.666 | 0.077 | **0.001** | 0.774 | 0.184 | 0.479 |
| *vanA* | C degradation | 0.48 | 0.309 | 0.444 | 0.143 | **0.001** | 0.75 | 0.13 | 0.396 |
| *lip* | C degradation | 0.159 | 0.177 | 0.186 | 0.413 | **0.007** | 0.524 | 0.119 | 0.328 |
| phenol\_oxidase | C degradation | 0.224 | 0.124 | 0.435 | 0.099 | **0.001** | 0.801 | 0.186 | 0.274 |
| *mcrA* | Methane metabolism | 0.891 | 0.684 | 0.874 | 0.806 | 0.116 | 0.495 | **0.037** | 0.129 |
| *pmoA* | Methane metabolism | 0.211 | 0.069 | 0.363 | 0.114 | **0.001** | 0.807 | 0.067 | 0.077 |
| *nifH* | Nitrogen fixation | 0.494 | 0.277 | 0.569 | 0.117 | **0.001** | 0.815 | 0.057 | 0.128 |
| *amoA* | Nitrification | 0.178 | 0.093 | 0.165 | **0.042** | **0.002** | 0.616 | 0.654 | 0.865 |
| *napA* | Dissimilatory N reduction | 0.217 | 0.105 | 0.444 | 0.089 | **0.003** | 0.687 | 0.161 | 0.22 |
| *nrfA* | Dissimilatory N reduction | 0.59 | 0.49 | 0.774 | 0.543 | **0.001** | 0.635 | **0.044** | 0.315 |
| *gdh* | Ammonification | 0.63 | 0.34 | 0.505 | 0.574 | **0.01** | 0.265 | **0.012** | **0.013** |
| *ureC* | Ammonification | 0.313 | 0.222 | 0.062 | **0.007** | **0.001** | 0.827 | 0.146 | 0.238 |
| *nasA* | Assimilatory N reduction | 0.452 | 0.241 | 0.387 | 0.052 | **0.001** | 0.561 | 0.069 | 0.114 |
| *narG* | Denitrification | 0.341 | 0.234 | 0.394 | 0.143 | **0.001** | 0.554 | 0.094 | 0.279 |
| *nirK* | Denitrification | 0.199 | 0.102 | **0.047** | **0.005** | **0.001** | 0.558 | 0.142 | 0.199 |
| *nirS* | Denitrification | 0.158 | 0.069 | 0.077 | **0.008** | **0.001** | 0.693 | **0.023** | **0.033** |
| *norB* | Denitrification | 0.142 | 0.059 | **0.001** | **0.001** | **0.001** | 0.781 | **0.004** | **0.001** |
| *nosZ* | Denitrification | 0.231 | 0.094 | 0.094 | **0.012** | **0.001** | 0.828 | **0.045** | 0.077 |
| *ppk* | Phosphorus utilization | 0.562 | 0.277 | 0.118 | **0.037** | **0.001** | 0.702 | 0.066 | 0.192 |
| *ppx* | Phosphorus utilization | **0.011** | **0.005** | **0.005** | **0.001** | **0.001** | 0.796 | **0.008** | **0.007** |
| *dsrA* | sulfite reductase | 0.428 | 0.267 | 0.402 | 0.184 | **0.001** | 0.77 | 0.135 | 0.426 |
| *dsrB* | sulfite reductase | 0.795 | 0.671 | 0.467 | 0.18 | **0.001** | 0.813 | **0.036** | 0.096 |
| *sox* | Sulphur oxidation | 0.09 | **0.025** | **0.012** | **0.001** | **0.001** | 0.75 | **0.013** | **0.034** |

Abbreviations: TN, total nitrogen content; TC, total carbon content; Plant, aboveground biomass; Plant N, aboveground plant nitrogen

Bold P values indicate statistically significant (*P* <0.05) correlations.

**B. Supplemental Figures**

**a**

**b**

**a**

**b**



**Fig. S1** Taxonomic composition of the microbial community under different treatments as detected by GeoChip at phylum level at soil depths of 0-5cm (a) and 5-15cm (b).

**a b**



**Fig. S2** Detrended correspondence analysis (DCA) of all detected *gyrB* genes under different treatments at soil depths of 0-5cm (a) and 5-15cm (b).



**Fig. S3** Effect of eCO2 and irrigation on functional genes involved in methane metabolism process at soil depths of 0 to 5 cm (left) and 5 to 15 cm (right). Significant differences were calculated by meta-analysis of response ratio. Error bars indicate 95% confidence interval. Asterisks indicate the significance level: \* at 95% confidence interval, \*\* at 99% confidence interval.



**Fig. S4** Effect of eCO2 and irrigation on functional genes involved in P cycle at soil depths of 0 to 5 cm (left) and 5 to 15 cm (right). Significant differences were calculated by meta-analysis of response ratio. Error bars indicate 95% confidence interval. Asterisks indicate the significance level: \* at 95% confidence interval, \*\* at 99% confidence interval.



**Fig. S5** Effect of eCO2 and irrigation on functional genes involved in S cycle at soil depths of 0 to 5 cm (left) and 5 to 15 cm (right). Significant differences were calculated by meta-analysis of response ratio. Error bars indicate 95% confidence interval. Asterisks indicate the significance level: \* at 95% confidence interval, \*\* at 99% confidence interval.

**a b**



**Fig. S6** Standardized direct effects (including direct and indirect effects) of moisture, aboveground plant biomass, plant N, and chemical property based on the structural equation models under eCO2 (a) and irrigation (b) treatments. Plant represents aboveground plant biomass, and plant N represents aboveground plant N.