Supplementary Material

**Differentiated mechanisms of biochar mitigating straw-induced greenhouse gas emissions in two contrasting paddy soils?**

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# Supplementary Figures and Tables

## Supplementary Figures

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**Supplementary Figure 1.** Dynamics of CH4 fluxes (mg CH4-C m-2 h-1) among five treatments in two soils over rice growing stages in 2016 (left side) and 2017 (right side). The same water regime of continuously flooding with a mid-drainage is applied, and indicated by light gray (flooded) and dark gray (drained), respectively. The rice growth stages are indicated by light green for seedling stage, pink for tillering stage and yellow for heading stage. The downwards arrow in black means topdressing date of nitrogen fertilizer. The bar represents the standard error of means (n=3). And the five treatments are differentiated from the diverse shapes (square in black for S0, scarlet circle for S1, blue circle for BS1, pink triangle for S2 and green triangle for BS2, respectively). The sub-graph magnified the CH4 fluxes where cannot be distinguished clearly.



**Supplementary Figure 2.** Dynamics of N2O fluxes (ug N2O-N m-2 h-1) among five treatments in two soils over rice growing stages in 2016 (a) and 2017 (b). The legend is same as Supplementary Figure 1.

-200

-100

0

100

TY\_S0

TY\_S1

TY\_S2

TY\_BS1

TY\_BS2

BH\_S0

BH\_S1

BH\_S2

BH\_BS1

BH\_BS2

*Eh* (mV)

**Supplementary Figure 3.** *Eh* in the subsurface determined at day 10 after straw addition.

**Supplementary Table 1.** The primer sets and thermal conditions used in the PCR amplifications.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Target genes** | **Primers** | **sequence (5' - 3')** | **length of fragments** | **Thermal profile** | **Reference** |
| Archaeal *amoA* | Arch-amoAF | STAATGGTCTGGCTTCTTC | 635 | 95℃ for 3min; 35 circles of (95℃ for10s, 55℃ for 30s,72℃ for 60s and plate read at 83℃ for 10s) | [[1](#_ENREF_1)] |
| Arch-amoAR | GCGGCATCCATCTGTATGT |  |  |
|  |  |  |  |
| Bacterial *amoA* | amoA-1F | GGGGTTTCTACTGGTGGT | 490 | 95℃ for 3min; 35 circles of (95℃ for10s, 55℃ for 30s,72℃ for 60s and plate read at 83℃ for 10s) | [[2](#_ENREF_2)] |
| amoA-2R | CCCCTCKGSAAAGCCTTCTTC |  |  |
|  |  |  |  |
| *nirK* | F1aCu | ATCATGGTSCTGCCGCG | 476 | 95℃ for 5min; 95℃ for 30s, 58℃ (a touchdown from 63℃ to 58℃, -1.0℃, 6 circles) for 30s, 72℃ for 1min and plate read at 83℃ for 10s (40 circles) | [[3](#_ENREF_3)] |
| R3Cu | GCCTCGATCAGRTTGTGGTT |  |  |
|  |  |  |  |
| *nirS* | cd3aF | GTSAACGTSAAGGARACSGG | 420 | 94℃ for 2min; 94℃ for 30s, 53℃ (a touchdown from 58℃ to 53℃, -1.0℃, 5 circles) for 1min, 72℃ for 30s and plate read at 83℃ for 10s (30 circles) | [[4](#_ENREF_4)] |
| R3cd | GASTTCGGRTGSGTCTTGA |  |  |
|  |  |  |  |
| *nosZ* clade I | nosZ\_2R | CGCRACGGCAASAAGGTSMSSGT | 453 | 95℃ for 10min; 95℃ for 30s, 60℃ (a touchdown from 65℃ to 60℃, -1.0℃, 5 circles) for 30s, 72℃ for 1min and plate read at 83℃ for 10s (40 circles) | [[5](#_ENREF_5)] |
| nosZ\_2F | CAKRTGCAKSGCRTGGCAGAA |  |  |
|  |  |  |  |
| *nosZ* clade II | nosZ-II-F | CTIGGICCIYTKCAYAC | 698 | 95°C for 2 min, 95°C for 30s, 54°C for 30s, 72°C for 40s and plate read at 83℃ for 10s (40 circles) | [6] |
| nosZ-II-R | GCIGARCARAAITCBGTRC |  |  |
|  |  |  |  |  |  |
| *mcrA* | ME1 | GCMATGCARATHGGWATGTC | 719 | 94℃ for 3min; 94℃ for 45s, 50℃ for 45s, 72℃ for 90s and plate read at 83℃ for 10s (35 circles) | [[7](#_ENREF_6)] |
| ME2 | TCATKGCRTAGTTDGGRTAGT |  |  |
|  |  |  |  |
| *pmoA* | A189f | GGNGACTGGGACTTCTGG | 491 | 94℃ for 2min; 94℃ for 45s, 53℃ (a touchdown from 58℃ to 52℃, -1.0℃, 6 circles) for 1min, 72℃ for 2min, and plate read at 83℃ for 10s (35 circles) a94℃ for 2min; 94℃ for 30s, 60℃ for 30s, 72℃ for 45s (35 circles); 72℃ for 5min b | [8] |
| mb661r | CCGGMGCAACGTCYTTACC |  |  |
|  |  |  |  |
|  |  |  |  | 94℃ for 2min; 94℃ for 30s, 60℃ for 30s, 72℃ for 45s (35 circles); 72℃ for 5min b |  |

**a. The thermal condition used in the qPCR for *pmoA* gene;** **b. The thermal condition used in the PCR for illumina sequence**

**Supplementary Table 2.** Spearman’s correlation among the soil properties, functional gene abundances, and the CH4 and N2O emissions during the rice growing season in 2016.

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Soil |  | pH | *Eh-surface* | *Eh-subsurface* | NH4+ | NO3- | DOC | TN | TC | C/N | **Bacterial** | **archaeal** | ***nirK*** | ***nirS*** | ***nosZ*** | ***mcrA*** | ***pmoA*** | ***mcrA/pmoA*** |
|  | ***amoA*** |  ***amoA*** |
| TY | *Eh-*surface | 0.354\* | 1 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|  | *Eh-*subsurface | 0.222 | 0.757\*\* | 1 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|  | NH4+ | -0.12 | -0.671\*\* | -0.658\*\* | 1 |  |  |  |  |  |  |  |  |  |  |  |  |  |
|  | NO3- | -0.294 | 0.218 | 0.176 | -0.268 | 1 |  |  |  |  |  |  |  |  |  |  |  |  |
|  | DOC | 0.722\*\* | 0.125 | -0.052 | 0.184 | -0.364\* | 1 |  |  |  |  |  |  |  |  |  |  |  |
|  | TN | 0.148 | -0.427\*\* | -0.206 | 0.16 | -0.32 | 0.073 | 1 |  |  |  |  |  |  |  |  |  |  |
|  | TC | 0.488\*\* | -0.088 | 0.137 | -0.095 | -0.291 | 0.26 | 0.720\*\* | 1 |  |  |  |  |  |  |  |  |  |
|  | C/N | 0.541\*\* | 0.021 | 0.226 | -0.156 | -0.259 | 0.288 | 0.567\*\* | 0.979\*\* | 1 |  |  |  |  |  |  |  |  |
|  | **bacterial *amoA*** | -0.061 | 0.499\*\* | 0.483\*\* | -0.621\*\* | 0.24 | -0.441\*\* | -0.034 | 0.051 | 0.067 | 1 |  |  |  |  |  |  |  |
|  | **archaeal *amoA*** | -0.069 | -0.218 | -0.067 | 0.258 | -0.098 | -0.082 | -0.034 | -0.103 | -0.118 | -0.028 | 1 |  |  |  |  |  |  |
|  | ***nirK*** | 0.133 | 0.067 | 0.078 | -0.2 | 0.144 | 0.157 | -0.016 | 0.006 | 0.007 | 0.005 | 0.168 | 1 |  |  |  |  |  |
|  | ***nirS*** | -0.427\*\* | -0.769\*\* | -0.655\*\* | 0.531\*\* | -0.045 | -0.282 | 0.301\* | -0.109 | -0.214 | -0.23 | 0.17 | 0.019 | 1 |  |  |  |  |
|  | ***nosZ*** | -0.06 | 0.501\*\* | 0.555\*\* | -0.550\*\* | 0.086 | -0.378\* | -0.023 | 0.075 | 0.107 | 0.587\*\* | -0.226 | -0.105 | -0.386\*\* | 1 |  |  |  |
|  | ***mcrA*** | -0.466\*\* | -0.767\*\* | -0.575\*\* | 0.475\*\* | -0.202 | -0.314\* | 0.503\*\* | 0.146 | 0.027 | -0.128 | 0.018 | -0.046 | 0.710\*\* | -0.099 | 1 |  |  |
|  | ***pmoA*** | -0.492\*\* | -0.665\*\* | -0.418\*\* | 0.308\* | -0.096 | -0.440\*\* | 0.366\* | 0.12 | 0.032 | 0.039 | 0.211 | 0.023 | 0.732\*\* | -0.075 | 0.754\*\* | 1 |  |
|  | ***mcrA/pmoA*** | -0.126 | -0.355\* | -0.371\* | 0.442\*\* | -0.076 | 0.079 | 0.172 | -0.098 | -0.167 | -0.371\* | -0.152 | -0.048 | 0.147 | -0.107 | 0.503\*\* | -0.127 | 1 |
|  | CE (CH4) | 0.08 | 0.118 | 0.164 | 0.058 | -0.185 | 0.157 | -0.104 | -0.041 | -0.025 | -0.083 | -0.053 | 0.028 | -0.356\* | 0.029 | 0.034 | -0.223 | 0.476\*\* |
|  | CE(N2O) | 0.410\*\* | 0.063 | 0.244 | -0.104 | -0.337\* | 0.302\* | 0.470\*\* | 0.697\*\* | 0.682\*\* | 0.119 | -0.141 | 0.08 | -0.234 | 0.059 | 0.136 | 0.005 | 0.149 |
|  | CH4 flux | -0.171 | -0.408\*\* | -0.251 | 0.449\*\* | 0.101 | 0.014 | 0.296\* | 0.074 | -0.002 | -0.197 | -0.011 | -0.094 | 0.147 | -0.03 | 0.514\*\* | 0.209 | 0.587\*\* |
|  | N2O flux | -0.141 | 0.25 | 0.359\* | -0.314\* | 0.207 | -0.341\* | -0.116 | -0.07 | -0.052 | 0.341\* | 0.142 | 0.061 | -0.131 | 0.297\* | -0.065 | 0.032 | -0.161 |
|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| BH | *Eh-*surface | 0.694\*\* | 1 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|  | *Eh-*subsurface | 0.797\*\* | 0.796\*\* | 1 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|  | NH4+ | 0.772\*\* | 0.690\*\* | 0.639\*\* | 1 |  |  |  |  |  |  |  |  |  |  |  |  |  |
|  | NO3- | 0.436\*\* | 0.401\*\* | 0.558\*\* | 0.111 | 1 |  |  |  |  |  |  |  |  |  |  |  |  |
|  | DOC | -0.522\*\* | -0.554\*\* | -0.632\*\* | -0.202 | -0.65\*\* | 1 |  |  |  |  |  |  |  |  |  |  |  |
|  | TN | 0.019 | -0.171 | -0.12 | -0.072 | -0.136 | 0.094 | 1 |  |  |  |  |  |  |  |  |  |  |
|  | TC | -0.002 | -0.24 | -0.084 | -0.1 | -0.076 | 0.145 | 0.877\*\* | 1 |  |  |  |  |  |  |  |  |  |
|  | C/N | -0.01 | -0.211 | 0.006 | -0.086 | 0.023 | 0.152 | 0.430\*\* | 0.808\*\* | 1 |  |  |  |  |  |  |  |  |
|  | **bacterial *amoA*** | -0.075 | -0.193 | -0.118 | -0.313\* | 0.237 | -0.007 | 0.262 | 0.266 | 0.179 | 1 |  |  |  |  |  |  |  |
|  | **archaeal *amoA*** | -0.204 | -0.226 | -0.046 | -0.29 | 0.021 | 0.069 | 0.367\* | 0.437\*\* | 0.366\* | 0.119 | 1 |  |  |  |  |  |  |
|  | ***nirK*** | -0.12 | -0.167 | -0.189 | 0.013 | -0.091 | 0.311\* | 0.381\*\* | 0.366\* | 0.205 | 0.276 | 0.221 | 1 |  |  |  |  |  |
|  | ***nirS*** | -0.330\* | -0.533\*\* | -0.445\*\* | -0.334\* | -0.138 | 0.418\*\* | 0.288 | 0.349\* | 0.287 | 0.579\*\* | 0.163 | 0.535\*\* | 1 |  |  |  |  |
|  | ***nosZ*** | 0.581\*\* | 0.367\* | 0.428\*\* | 0.466\*\* | 0.359\* | -0.198 | 0.343\* | 0.302\* | 0.163 | 0.370\* | 0.026 | 0.437\*\* | 0.323\* | 1 |  |  |  |
|  | ***mcrA*** | -0.325\* | -0.395\*\* | -0.329\* | -0.411\*\* | -0.101 | 0.269 | 0.174 | 0.089 | -0.076 | 0.09 | 0.533\*\* | 0.305\* | 0.237 | -0.035 | 1 |  |  |
|  | ***pmoA*** | 0.152 | -0.17 | 0.012 | -0.109 | 0.368\* | -0.219 | 0.370\* | 0.312\* | 0.112 | 0.543\*\* | 0.288 | 0.359\* | 0.222 | 0.428\*\* | 0.368\* | 1 |  |
|  | ***ratio*** | 0.075 | -0.049 | -0.11 | 0.239 | -0.360\* | 0.175 | -0.09 | -0.18 | -0.235 | -0.374\* | -0.166 | -0.065 | 0.011 | -0.132 | 0.117 | -0.314\* | 1 |
|  | CE (CH4) | 0.051 | -0.216 | -0.05 | 0.053 | -0.118 | 0.041 | 0.077 | -0.037 | -0.182 | -0.149 | 0.286 | 0.22 | 0.043 | 0.006 | 0.466\*\* | 0.172 | 0.299\* |
|  | CE(N2O) | 0.536\*\* | 0.288 | 0.347\* | 0.580\*\* | 0.15 | -0.232 | -0.033 | -0.155 | -0.249 | -0.195 | -0.156 | -0.141 | -0.129 | 0.288 | -0.046 | 0.098 | 0.424\*\* |
|  | CH4 flux | -0.536\*\* | -0.494\*\* | -0.449\*\* | -0.525\*\* | -0.265 | 0.448\*\* | 0 | -0.019 | -0.048 | 0.095 | 0.355\* | 0.222 | 0.24 | -0.242 | 0.730\*\* | 0.087 | 0.084 |
|  | N2O flux | -0.005 | -0.137 | 0.117 | -0.189 | 0.300\* | -0.192 | 0.187 | 0.234 | 0.165 | 0.22 | 0.236 | 0.17 | 0.134 | 0.04 | 0.267 | 0.537\*\* | -0.316\* |

 Abundance of each gene is in black bold.

CE means the cumulative emission of GHGs.

**Supplementary Table 3.** Alpha diversity of methanotrophs and *nosZ*-containing denitrifiers in two paddy soils.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Stage | Soil | Treatment | *pmoA* |  | *nosZ* |
| ace | simpson | shannon | chao |  | ace | simpson | shannon | chao |
| Seedling | TY | S0 | 130.1 | 0.048 | 3.565 | 131.6 |  | 393.2 | 0.028 | 4.674 | 421.2 |
| (18d) |  | S1 | 140.3 | 0.051 | 3.634 | 140.1 |  | 420.5 | 0.024 | 4.800 | 428.3 |
|  |  | S2 | 130.2 | 0.052 | 3.598 | 130.4 |  | 400.9 | 0.022 | 4.823 | 411.0 |
|  |  | BS1 | 142.9 | 0.049 | 3.611 | 153.8 |  | 463.4 | 0.025 | 4.785 | 537.3 |
|  |  | BS2 | 165.0 | 0.054 | 3.650 | 164.3 |  | 491.8 | 0.023 | 4.819 | 530.3 |
|  | BH | S0 | 139.4 | 0.077 | 3.271 | 138.6 |  | 511.9 | 0.013 | 5.082 | 550.1 |
|  |  | S1 | 143.6 | 0.070 | 3.312 | 145.4 |  | 590.0 | 0.010 | 5.243 | 603.2 |
|  |  | S2 | 144.3 | 0.061 | 3.485 | 144.7 |  | 552.3 | 0.026 | 5.010 | 584.4 |
|  |  | BS1 | 102.4 | 0.079 | 3.183 | 103.3 |  | 542.6 | 0.008 | 5.360 | 530.2 |
|  |  | BS2 | 135.4 | 0.076 | 3.338 | 136.3 |  | 635.3 | 0.014 | 5.167 | 655.4 |
| Tillering | TY | S0 |  |  |  |  |  | 422.2 | 0.026 | 4.667 | 442.9 |
| (58d) |  | S2 |  |  |  |  |  | 442.1 | 0.024 | 4.758 | 473.2 |
|  |  | BS2 |  |  |  |  |  | 494.2 | 0.024 | 4.795 | 514.5 |
|  | BH | S0 |  |  |  |  |  | 540.3 | 0.020 | 4.851 | 589.0 |
|  |  | S2 |  |  |  |  |  | 624.4 | 0.010 | 5.230 | 639.6 |
|  |  | BS2 |  |  |  |  |  | 863.9 | 0.014 | 5.274 | 976.0 |
| Heading | TY | S0 |  |  |  |  |  | 512.6 | 0.028 | 4.680 | 522.2 |
| (120d) |  | S2 |  |  |  |  |  | 415.4 | 0.026 | 4.689 | 423.8 |
|  |  | BS2 |  |  |  |  |  | 544.1 | 0.024 | 4.799 | 592.1 |
|  | BH | S0 |  |  |  |  |  | 457.5 | 0.014 | 5.061 | 451.5 |
|  |  | S2 |  |  |  |  |  | 443.5 | 0.026 | 4.652 | 474.2 |
|  |  | BS2 |  |  |  |  |  | 468.4 | 0.019 | 4.933 | 576.9 |
| Origin | TY |  | 129.6 | 0.083 | 3.207 | 134.0 |  | 374.2 | 0.018 | 4.734 | 374.3 |
| (0d) | BH |  | 123.8 | 0.058 | 3.420 | 126.2 |  | 441.8 | 0.018 | 4.641 | 441.7 |

**Supplementary Table 4.** The correlation analyses between soil parameters and community composition of *nosZ* gene containing denitrifiers via the envfit function.

|  |  |  |
| --- | --- | --- |
| Soil | TY | BH |
| Parameters | r2 | *Pr* | r2 | *Pr* |
| Moisture | 0.412 | 0.001 | 0.219 | 0.030 |
| pH | 0.167 | 0.053 | 0.400 | 0.001 |
| *Eh*-surface | 0.657 | 0.001 | 0.664 | 0.001 |
| *Eh*-sublayer | 0.487 | 0.001 | 0.455 | 0.001 |
| NH4+ | 0.158 | 0.060 | 0.675 | 0.001 |
| NO3- | 0.042 | 0.432 | 0.009 | 0.900 |
| DOC | 0.060 | 0.379 | 0.154 | 0.074 |
| TN | 0.344 | 0.001 | 0.296 | 0.002 |
| TC | 0.153 | 0.085 | 0.333 | 0.004 |
| C/N | 0.100 | 0.202 | 0.220 | 0.016 |

r2 represents the decisive coefficient of environmental factors on species distribution; *Pr* indicates the significant test of correlation, significant difference is accepted when *Pr* <0.050.

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