Supplement of

The pH-based ecological coherence of active canonical methanotrophs in paddy soils

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Figure S1 Geographic locations of six paddy soils tested in this study. YX, YT, TY, ZY, CS and LZ were short for Yu-Xi, Ying-Tan, Tao-Yuan, Zi-Yang, Chang-Shu and Lei-Zhou, respectively.
Figure S2 Decreasing curves of headspace methane concentrations in the soil microcosms. The initial headspace methane concentration of all microcosms was approximate 50,000 ppmv and the incubation was ended when the concentration dropped below 5,000 ppmv or after 42 days.
Figure S3 Community compositions of $^{13}$C-labeled methanotrophs based on both 16S rRNA and pmoA gene analyses. All reads assigned to methanotroph genera were classified into type Ia, Ib, II or unclassified MOB groups, and the percentage of each group were expressed as the ratio of affiliated gene reads to the total methanotroph-affiliated gene reads.
Figure S4 Phylogenetic tree of the major $^{13}$C-labeled OTUs based on pmoA or 16S rRNA gene sequencing. Bootstrap values $\geq$60% are indicated at branch nodes. The reference strains with red color are used in the phylogeny of both pmoA and 16S rRNA genes.
### Table S1. Primers and conditions used in this study

| Primer Name | Primer sequence (5’-3’ | Target gene | Thermal Profile | Molecular analysis | Reference |
|-------------|-------------------------|--------------|-----------------|-------------------|-----------|
| 515F        | GTG CCAGCMGCGCG G       | universal 16S rRNA genes | 95°C, 4 min; 30×(95°C, 30 s; 55°C, 30 s; 72°C, 45 s); 72°C, 10 min | Illumina sequencing | (1)       |
| 907R        | CCG TCAATTCMTTTR AGT TT | bacterial pmoA gene | 95°C, 3 min; 40×(95°C, 10 s; 55°C, 30 s; 72°C, 30 s; 80°C, 5 s with plate read); Melt curve 65.0 to 95.0°C, increment 0.5°C, 0.05+ plate read | Real-Time PCR | (2)       |
| A189        | GNGACTGGGACCTT CTGG     | bacterial pmoA gene | 95°C, 4 min; 35×(95°C, 30 s; 55°C, 45 s; 72°C, 45 s); 72°C, 10 min | Illumina sequencing | (3)       |
| Mb661       | CCGGMGCAACGCTCY TTACC   | bacterial pmoA gene | 95°C, 4 min; 30×(95°C, 30 s; 55°C, 45 s; 72°C, 45 s); 72°C, 10 min | Illumina sequencing | (3)       |
Table S2. Assimilation rates of $^{13}$CH$_4$

| Soil | Treatment | Initial $^{13}$CH$_4$ content μmol | End-point $^{13}$CH$_4$ content μmol | $^{13}$CH$_4$ consumed μmol g$^{-1}$ d.w.s | Soil total organic C content mg g$^{-1}$ d.w.s | Soil $^{13}$C-atom abundance % | Soil $^{13}$C content μmol g$^{-1}$ d.w.s | Soil $^{13}$C production μmol g$^{-1}$ d.w.s | Ratio of $^{13}$C production to total organic C % | $^{13}$C-assimilation ratio % |
|------|-----------|------------------------------------|-------------------------------------|-----------------------------------------------|-----------------------------------------------|----------------------------------|-----------------------------------------------|-----------------------------------------------|-----------------------------------------------|-----------------------------------------------|
| YX   | Control   | -                                  | -                                   | -                                             | 15.9±0.4                                      | 1.08±0.00                        | 13.20±0.34                                    | -                                             | -                                             | -                                             |
|      | High CH$_4$ | 255±1                             | 10±2                                | 40.83                                         | 16.2±0.1                                      | 2.16±0.15                        | 26.91±2.03                                    | 13.71                                         | 1.02                                         | 33.6                                         |
|      | High CH$_4$+N | 254±2                         | 28±18                               | 37.66                                         | 16.0±0.2                                      | 2.00±0.28                        | 24.55±3.22                                    | 11.35                                         | 0.85                                         | 30.1%                                        |
| YT   | Control   | -                                  | -                                   | -                                             | 13.1±0.4                                      | 1.08±0.00                        | 10.83±0.29                                    | -                                             | -                                             | -                                             |
|      | High CH$_4$ | 251±4                             | 24±7                                | 37.77                                         | 12.9±0.1                                      | 1.89±0.03                        | 18.79±0.34                                    | 7.97                                          | 0.74                                         | 21.1                                         |
|      | High CH$_4$+N | 253±5                         | 74±43                               | 29.78                                         | 13.1±0.2                                      | 1.59±0.38                        | 16.06±3.92                                    | 5.23                                          | 0.48                                         | 17.6                                         |
| TY   | Control   | -                                  | -                                   | -                                             | 23.1±0.1                                      | 1.08±0.00                        | 19.12±0.06                                    | -                                             | -                                             | -                                             |
|      | High CH$_4$ | 253±2                             | 22±5                                | 38.37                                         | 23.7±0.3                                      | 1.36±0.01                        | 24.89±0.33                                    | 5.76                                          | 0.29                                         | 15.0                                         |
|      | High CH$_4$+N | 253±2                         | 25±8                                | 37.97                                         | 23.4±0.1                                      | 1.28±0.07                        | 23.07±1.37                                    | 3.95                                          | 0.203                                        | 10.4                                         |
| ZY   | Control   | -                                  | -                                   | -                                             | 29.9±0.1                                      | 1.09±0.00                        | 25.10±0.07                                    | -                                             | -                                             | -                                             |
|      | High CH$_4$ | 252±4                             | 23±5                                | 38.19                                         | 30.2±0.2                                      | 1.38±0.06                        | 32.11±1.70                                    | 7.01                                          | 0.28                                         | 18.4                                         |
|      | High CH$_4$+N | 248±3                         | 25±7                                | 37.31                                         | 30.2±0.2                                      | 1.34±0.05                        | 31.19±1.14                                    | 6.09                                          | 0.24                                         | 16.3                                         |
| CS   | Control   | -                                  | -                                   | -                                             | 27.7±0.3                                      | 1.08±0.00                        | 22.98±0.25                                    | -                                             | -                                             | -                                             |
|      | High CH$_4$ | 252±4                             | 20±2                                | 38.66                                         | 27.8±0.6                                      | 1.61±0.05                        | 34.35±0.74                                    | 11.37                                         | 0.49                                         | 29.4                                         |
|      | High CH$_4$+N | 248±2                         | 20±3                                | 38.06                                         | 27.8±0.6                                      | 1.53±0.00                        | 32.69±0.78                                    | 9.71                                          | 0.42                                         | 25.5                                         |
| LZ   | Control   | -                                  | -                                   | -                                             | 13.4±0.0                                      | 1.08±0.00                        | 11.12±0.03                                    | -                                             | -                                             | -                                             |
|      | High CH$_4$ | 248±3                             | 25±9                                | 37.15                                         | 13.5±0.1                                      | 2.36±0.12                        | 24.52±1.39                                    | 13.40                                         | 1.19                                         | 36.2                                         |
|      | High CH$_4$+N | 247±3                         | 22±6                                | 37.51                                         | 13.7±0.1                                      | 2.42±0.12                        | 25.43±1.16                                    | 14.31                                         | 1.25                                         | 38.1                                         |
Table S3. The significance of correlation between soil parameters and the $^{13}$C-labelled active methanotrophic compositions based on Mantel tests. * indicate significant correlation ($p<0.05$).

| Tested soil parameter | Significance ($p$ value) |
|-----------------------|--------------------------|
| pH                    | 0.00278*                 |
| SOM                   | 0.67083                  |
| TOC                   | 0.11528                  |
| TN                    | 0.63889                  |
| CN                    | 0.30278                  |
| Cu content            | 0.20278                  |
| OXC                   | 0.25694                  |
| Exchangeable inorganic N | 0.80556                |
Table S4. Summary of methane oxidation rates, increased methanotrophic cell numbers and assumed cell specific activity rates

| Soil | Treatment | Initial CH4 content | End-point CH4 content | Incubation time | Methane oxidation rate | Increased pmoA copy number* | Increased MOB cell number* | type II /type I ratio† | Increased type II cell number | Increased type I cell number | Type II cell specific activity rate‡ | Type I cell specific activity rate‡ |
|------|-----------|---------------------|-----------------------|-----------------|------------------------|-----------------------------|-----------------------------|--------------------------|-----------------------------|-------------------------------|----------------------------------|----------------------------------|
| YX   | 13CH4     | 255±1               | 10±2                  | 240             | 170±1                  | 202±26                      | 101±13                      | 33.9                     | 98.1±12.6                   | 2.9±0.4                        | 1.8±0.2                          | 59.4±7.5                         |
|      | 13CH4+N   | 254±2               | 28±18                 | 1008            | 37±3                   | 65±28                       | 33±14                       | 130                      | 32.4±13.7                   | 0.3±0.1                        | 1.3±0.5                          | 168.3±65.6                      |
| YT   | 13CH4     | 251±4               | 24±7                  | 432             | 87±4                   | 319±112                     | 159±56                      | 101                      | 157.9±55.6                  | 1.6±0.6                         | 0.6±0.2                          | 61.4±24.5                       |
|      | 13CH4+N   | 253±5               | 74±43                 | 1008            | 30±8                   | 8±4                         | 4±2                         | 108                      | 3.88±1.91                   | 0.04±0.02                       | 9.6±6.8                         | 1034.6±735.2                    |
| TY   | 13CH4     | 253±2               | 22±5                  | 384             | 100±3                  | 1395±564                    | 698±282                     | 20.0                     | 664.3±268.6                 | 33.3±13.5                       | 0.2±0.1                          | 3.4±1.4                         |
|      | 13CH4+N   | 253±2               | 25±8                  | 864             | 44±1                   | 263±195                     | 131±98                      | 9.25                     | 118.7±88.2                  | 12.8±9.5                        | 0.6±0.4                          | 5.3±4.1                         |
| ZY   | 13CH4     | 252±4               | 23±5                  | 192             | 199±7                  | 809±213                     | 405±106                     | 0.003                    | 1.2±0.3                     | 403.3±106.0                    | 174.1±50.9                      | 0.5±0.2                         |
|      | 13CH4+N   | 248±3               | 25±7                  | 336             | 111±3                  | 290±65                      | 145±33                      | 0.004                    | 0.6±0.1                     | 144.6±32.5                     | 208.7±41.0                      | 0.8±0.2                         |
| CS   | 13CH4     | 252±4               | 20±2                  | 192             | 201±4                  | 1125±92                     | 563±46                      | 0.014                    | 7.9±0.7                     | 554.7±45.2                     | 25.5±2.7                        | 0.4±0.0                         |
|      | 13CH4+N   | 248±2               | 20±3                  | 192             | 198±1                  | 952±171                     | 476±85                      | 0.010                    | 4.7±0.9                     | 471.3±85.6                     | 43.0±8.2                        | 0.4±0.1                         |
| LZ   | 13CH4     | 248±3               | 25±9                  | 336             | 111±3                  | 316±87                      | 158±44                      | 0.005                    | 0.7±0.2                     | 157.2±43.4                     | 161.2±40.9                      | 0.7±0.2                         |
|      | 13CH4+N   | 247±3               | 22±6                  | 240             | 156±4                  | 446±126                     | 223±63                      | 0.002                    | 0.5±0.1                     | 222.6±62.7                     | 341.3±119.4                     | 0.8±0.3                         |

* “Increased pmoA copy number” indicated the increased pmoA gene copy number after methane amended microcosms compared to the controls, according to the qPCR results. “Increased MOB cell number” was then calculated by assuming each methanotrophic cell contained 2 copies of pmoA genes.

† “type II/type I ratio” was calculated based on the taxonomic classification of pmoA genes in the 13C-labeled DNA fraction, which represented actively growing methanotrophs stimulated by methane addition.

‡ “Type II cell specific activity rate” and “Type I cell specific activity rate” was calculated assuming the methane was oxidized exclusively by type II or type I cells, respectively.
Reference

1. Stubner, S., 2002. Enumeration of 16S rDNA of Desulfotomaculum lineage 1 in rice field soil by real-time PCR with SybrGreen™ detection. Journal of Microbiological Methods 50, 155-164.

2. Holmes, A., Costello, A., Lidstrom, M., Murrell, J., 1995. Evidence that particulate methane monooxygenase and ammonia monooxygenase may be evolutionarily related. FEMS Microbiology Letters 132, 203-208.

3. Costello, A., Lidstrom, M., 1999. Molecular characterization of functional and phylogenetic genes from natural populations of methanotrophs in lake sediments. Applied and Environmental Microbiology 65, 5066-5074.