Supplementary Information for

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

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4. The supplementary materials include

Supplementary Figures S1-S4

Supplementary Tables S1-S4

Supplementary results

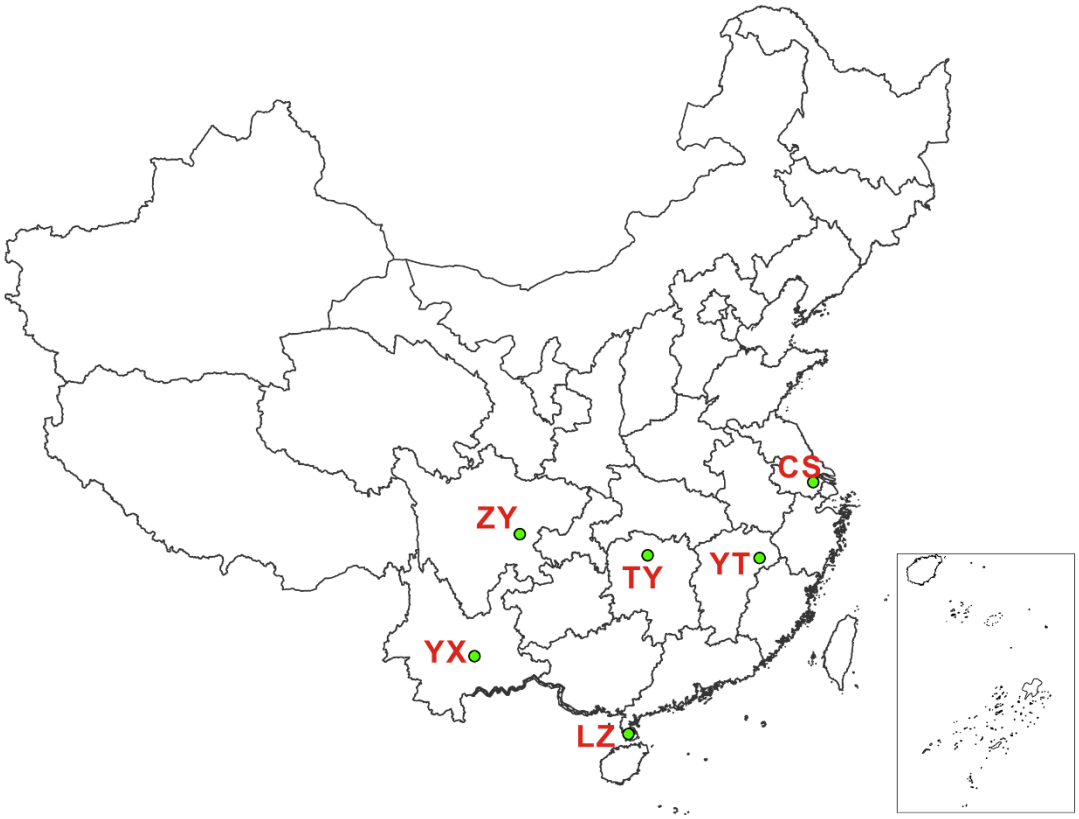

Figure S1 Geographic locations of six paddy soils tested in this study. YX, YT, TY, ZY, CS and LZ were short for Yu-Xi, Ying-Tan, Tao-Yuan, Zi-Yang, Chang-Shu and Lei-Zhou, respectively.

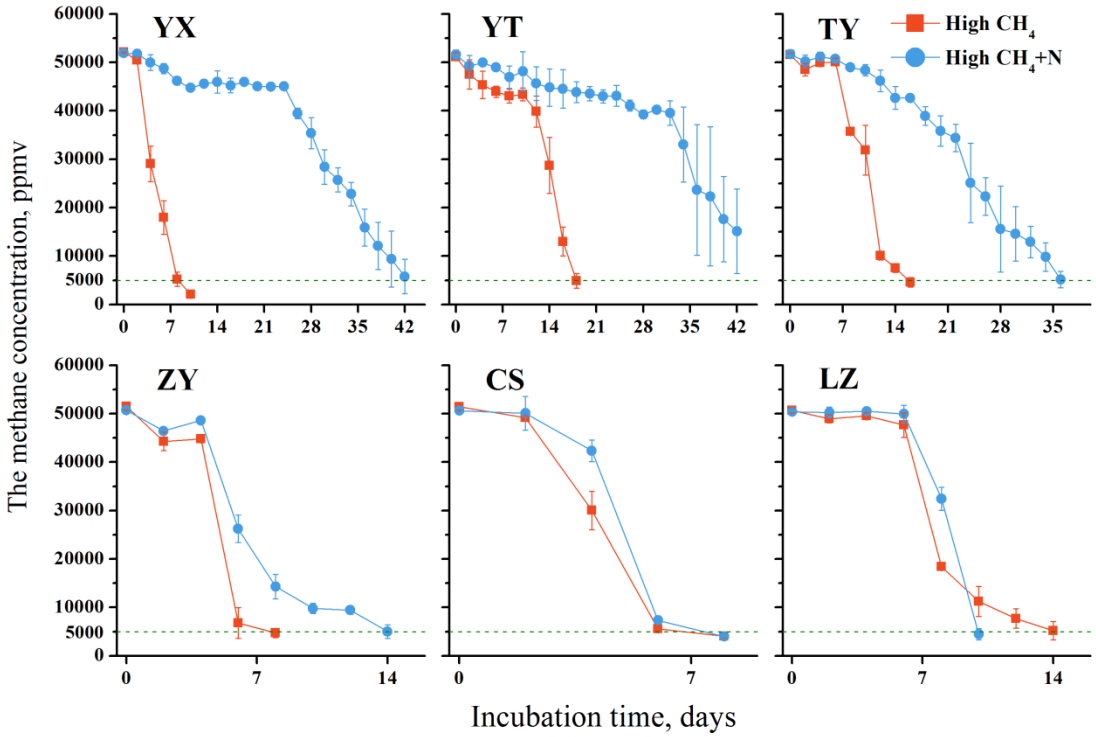

Figure S2 Decreasing curves of headspace methane concentrations in the soil microcosms. The initial headspace methane concentration of all microcosms was approximate 50,000 ppmv and the incubation was ended when the concentration dropped below 5,000 ppmv or after 42 days.

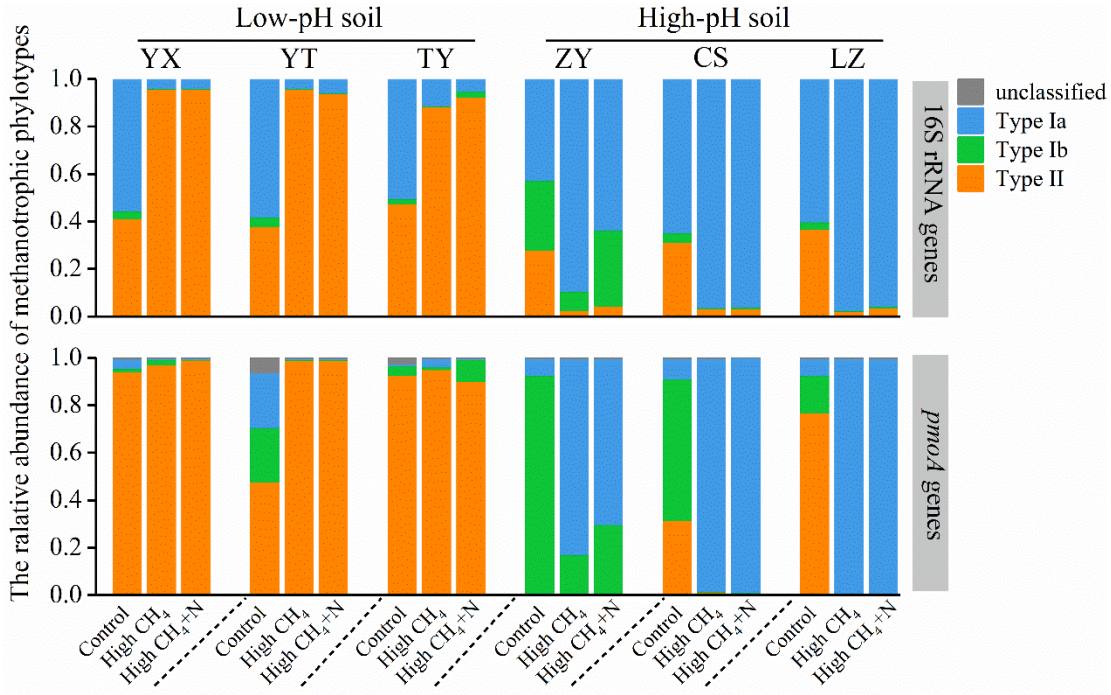

Figure S3 Community compositions of ^{13}C-labeled methanotrophs based on both 16S rRNA and *pmoA* gene analyses. All reads assigned to methanotroph genera were classified into type Ia, Ib, II or unclassified MOB groups, and the percentage of each group were expressed as the ratio of affiliated gene reads to the total methanotroph-affiliated gene reads.

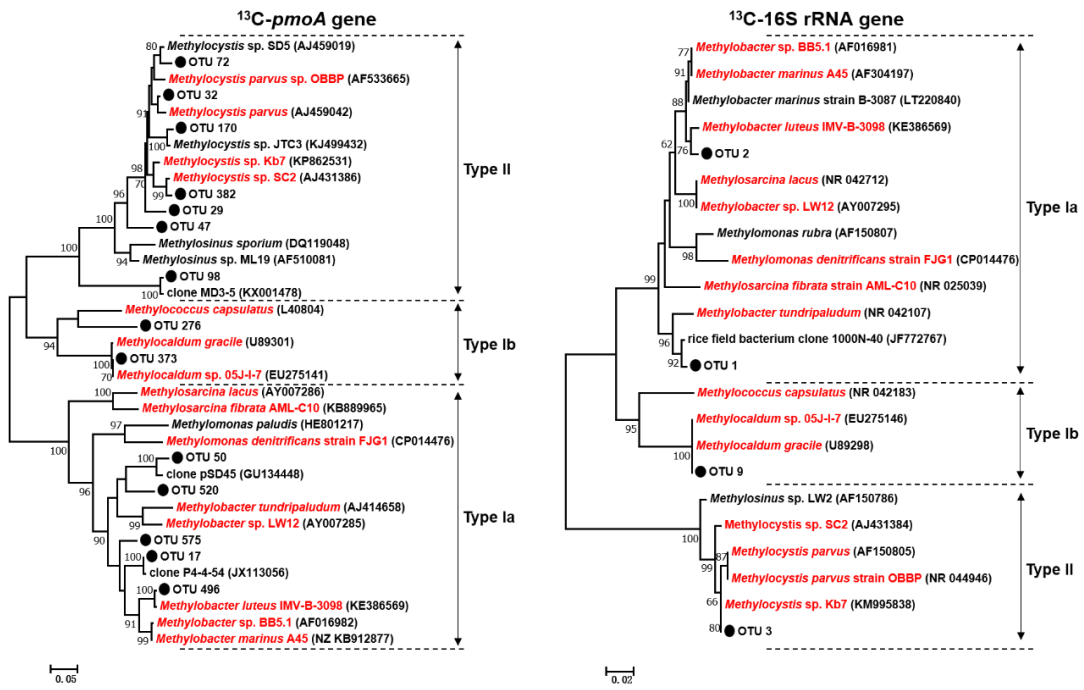

Figure S4 Phylogenetic tree of the major ^{13}C-labeled OTUs based on *pmoA* or 16S rRNA gene sequencing. Bootstrap values ≥60% are indicated at branch nodes. The reference strains with red color are used in the phylogeny of both *pmoA* and 16S rRNA genes.

Table S1. Primers and conditions used in this study

Primer Name	Primer sequence (5'-3')	Target gene	Thermal Profile	Molecular analysis	Reference
515F	GTG CCAGCMGCCGCG 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				
A189	GGNGACTGGGACTT CTGG	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)
Mb661	CCGGMGCAACGTCY TTACC		95°C, 4 min; 35×(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 CH4+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 CH_4 content	End-point CH_4 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‡
		μmol	μmol	hours	nmol CH_4 g^{-1} *d.w.s* h^{-1}	10^6 g^{-1} *d.w.s*	10^6 g^{-1} *d.w.s*		10^6 g^{-1} *d.w.s*	10^6 g^{-1} *d.w.s*	fmol CH_4 g^{-1} *d.w.s* h^{-1} $cell^{-1}$	fmol CH_4 g^{-1} *d.w.s* h^{-1} $cell^{-1}$
YX	$^{13}CH_4$	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
	$^{13}CH_4$+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	$^{13}CH_4$	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
	$^{13}CH_4$+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	$^{13}CH_4$	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
	$^{13}CH_4$+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	$^{13}CH_4$	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
	$^{13}CH_4$+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	$^{13}CH_4$	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
	$^{13}CH_4$+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	$^{13}CH_4$	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
	$^{13}CH_4$+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 ^{13}C-labed DNA fraction, which represented actively growing methanotrophs stimulated by methane addition.

‡ "Type II cell specific activity rate" and "Type II 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.