Applied and Environmental Microbiology

Supplemental material for:

Spatial-temporal pattern of sulfate-dependent anaerobic methane oxidation in an intertidal zone of the East China Sea

Jiaqi Wang[1], Miaolian Hua[1], Chaoyang Cai[1], Jiajie Hu[1], Junren Wang[1], Hongrui Yang[1], Fang Ma[2]*, Haifeng Qian[3], Ping Zheng[1], Baolan Hu[1,4]*

1. Department of Environmental Engineering, Zhejiang University, Hangzhou 310058, China

2. State Key Laboratory of Urban Water Resource and Environment, Harbin Institute of Technology, Harbin 150090, China

3. College of Environment, Zhejiang University of Technology, Hangzhou 310032, China

4. Zhejiang Province Key Laboratory for Water Pollution Control and Environmental Safety, Hangzhou 310058, China

***For correspondence**

Baolan Hu

Department of Environmental Engineering,

Zhejiang University

Hangzhou, 310058, China

Tel.: 0086 0571 88982340

Fax: 0086 0571 88982340

E-mail: blhu@zju.edu.cn

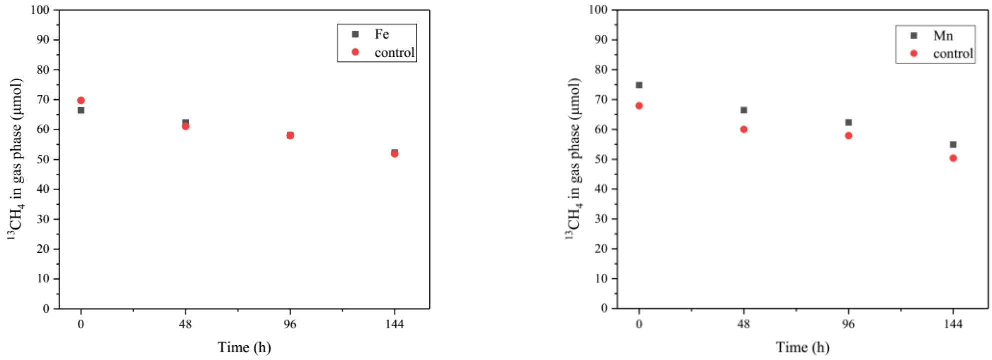

Fig. S1 $^{13}CH_4$ consumption in incubation of iron- and manganese-dependent anaerobic methane oxidation. Control represents the group in which only $^{13}CH_4$ was added.

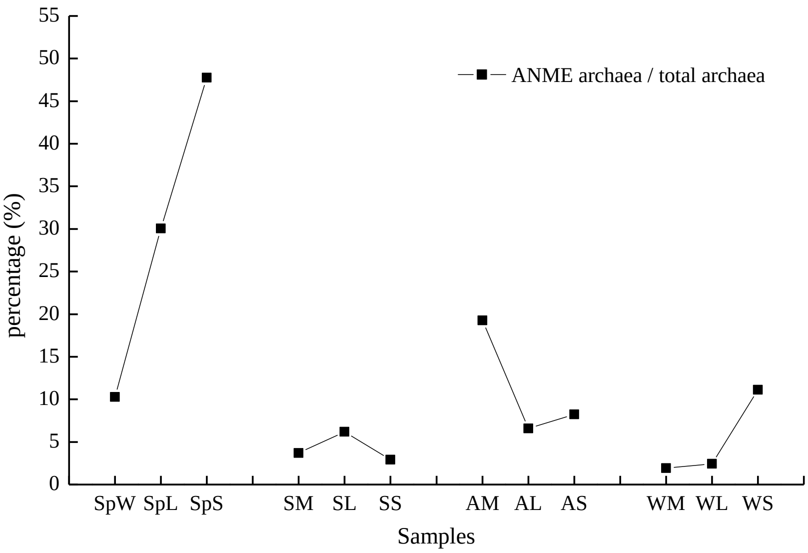

Fig. S2 The ratio of ANME archaea to total archaea measured by quantitative PCR in sediments collected from Zhoushan Archipelago intertidal zone

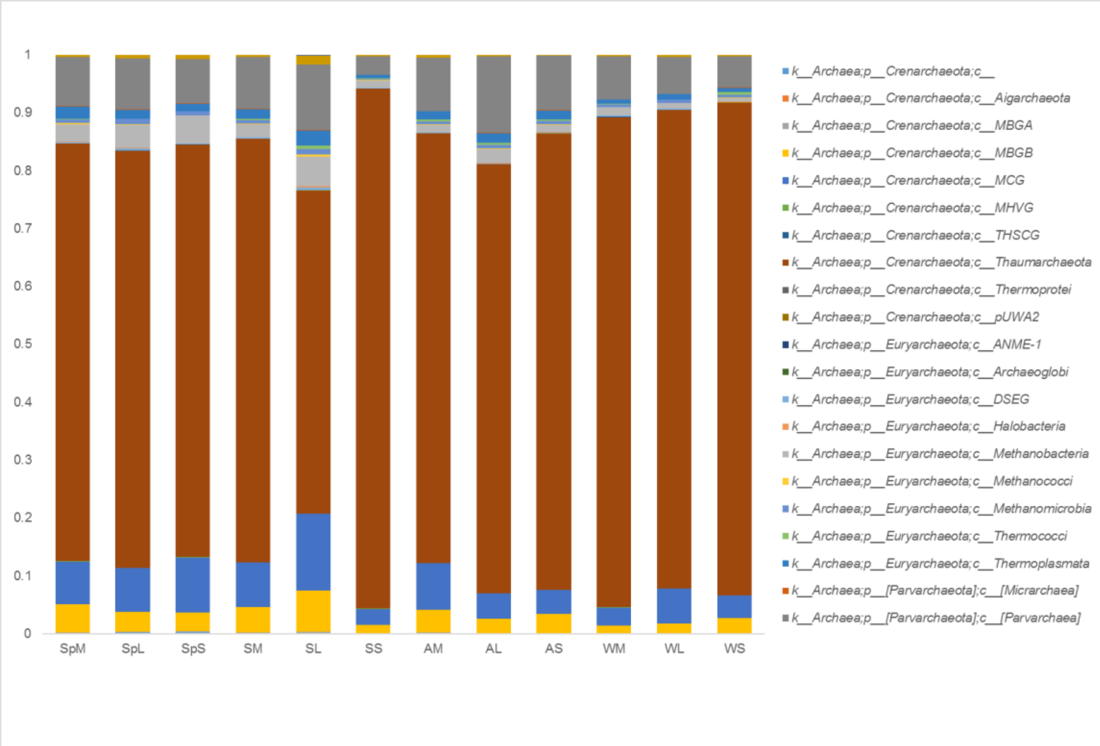

Fig. S3 The relative abundance of total archaea at class level in sediments collected from Zhoushan Archipelago intertidal zone

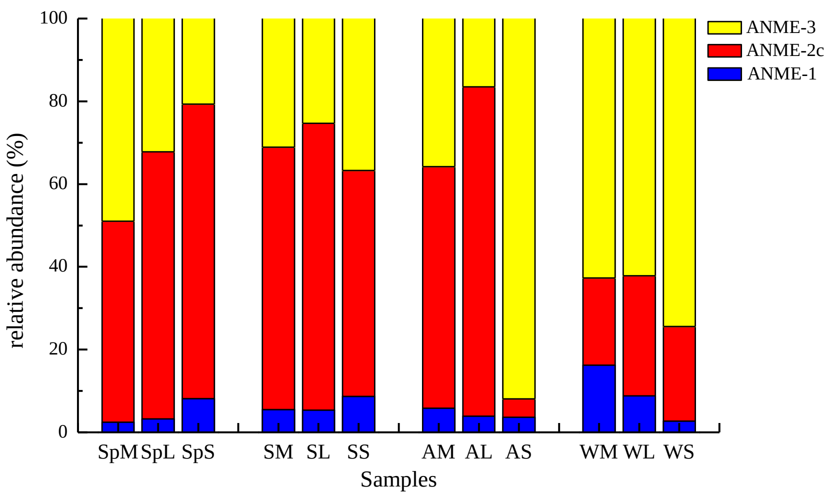

Fig. S4 The community structure of ANME archaea detected by quantitative PCR in sediments collected from the intertidal zone of Zhoushan Archipelago

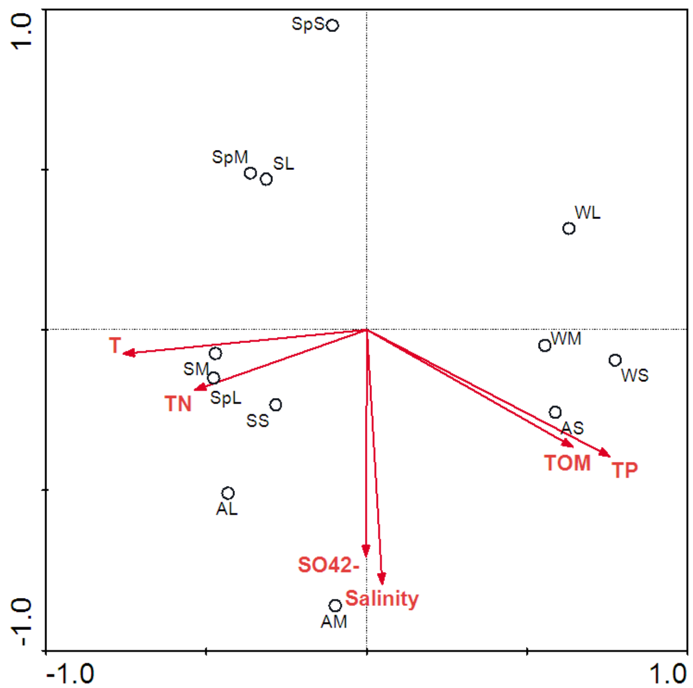

Fig. S5 RDA analysis of the correlations between ANME archaea community structures and environmental factors in sediments collected from Zhoushan Archipelago intertidal zone

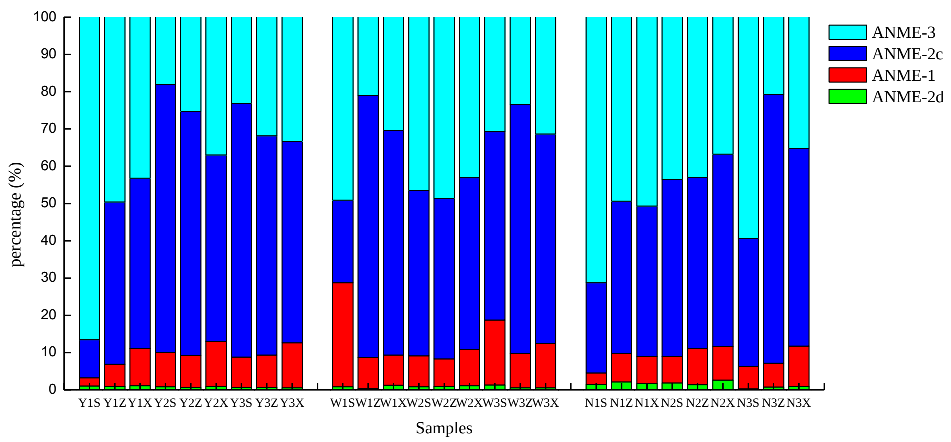

Fig. S6 The community structure of anaerobic methane oxidation archaea detected by quantitative PCR in the samples collected from three simulated columns

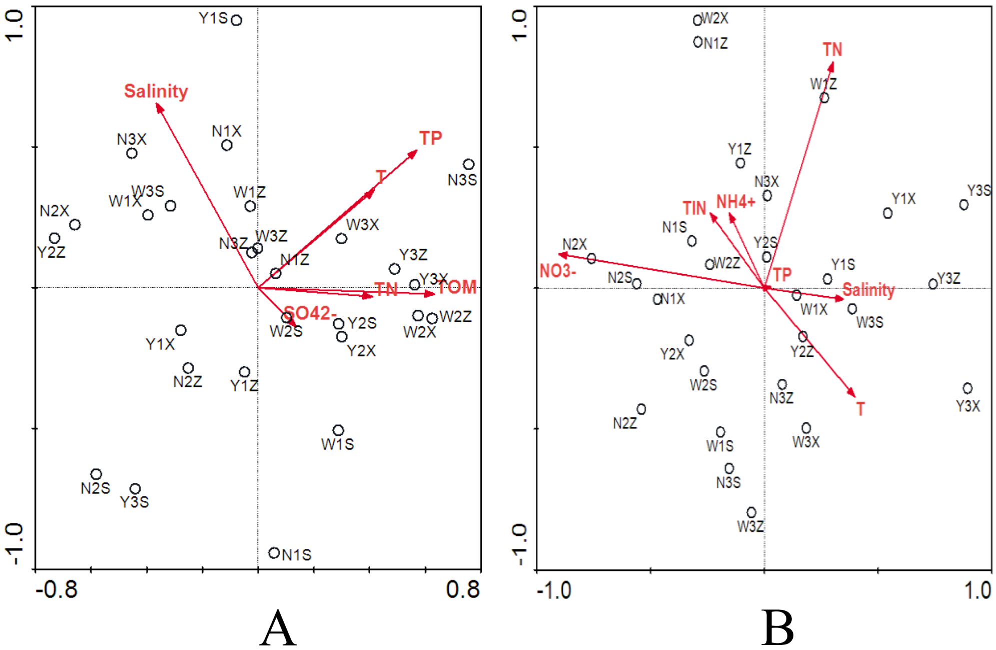

Fig. S7 RDA analysis to show the correlations between ANME archaea or ANME-2d archaea community structures and environmental factors in simulated columns

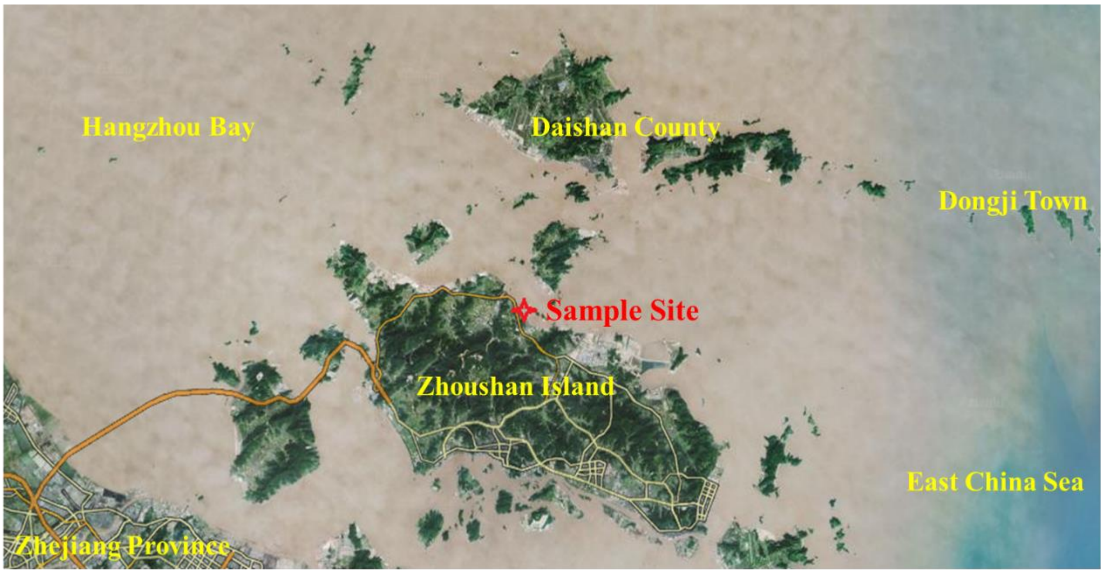

Fig. S8 Map of the sampling sites within Zhoushan Archipelago

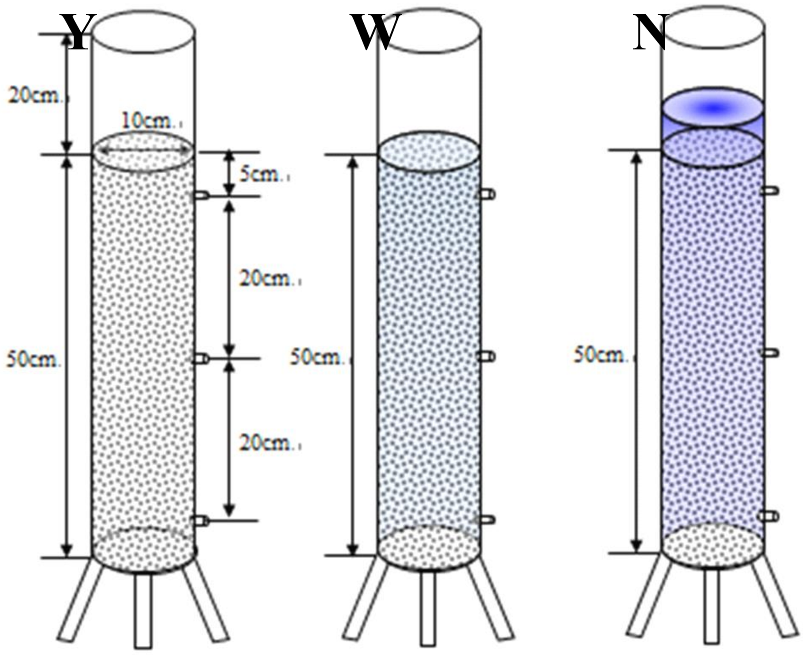

Fig. S9 Three simulated columns used in this study

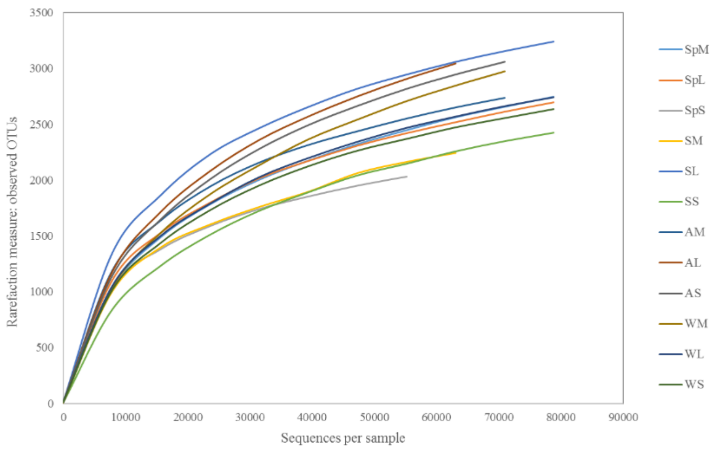

Fig. S10 Rarefaction curves of the number of observed OTUs from 16S rRNA sequencing

Table S1 Correlation analysis of environmental factors and the abundance of ANME archaea and potential S-AOM activity in sediments collected from Zhoushan Archipelago intertidal zone

Environment Factors	Pearson correlation coefficients						
	ANME-1 abundance	ANME-2c abundance	ANME-3 abundance	*M. oxyfera* abundance	ANME-2d abundance	S-AOM activity	D-AOM activity
T	**0.555****	**0.504****	-0.132	-0.124	**0.308****	**0.405***	**0.309***
ORP	0.389	0.456	-0.189	-0.123	0.006	0.456	0.398
SO_4^{2-}	**0.433***	0.359	0.113	-0.045	-0.098	0.589	0.667
NH_4^+	0.017	-0.060	-0.039	-0.045	-0.098	-0.009	-0.129
NO_3^-	**0.462***	0.336	0.332	**0.428***	**0.629****	-0.359	**0.785****
TIN	0.067	-0.022	-0.005	0.003	0.279	-0.012	-0.223
TP	**0.412***	0.160	-0.114	-0.036	-0.199	0.122	-0.111
Salinity	0.062	0.111	0.137	0.036	-0.105	0.109	0.291
TN	**-0.459****	-0.296	-0.178	-0.047	-0.048	0.349	0.219
TOM	**0.660****	**0.524****	0.004	0.048	0.049	**0.578****	**0.346****

*denotes $p < 0.05$, **denotes $p < 0.01$

Table S2 The diversity of ANME archaea in sediments collected from Zhoushan Archipelago intertidal zone

Sample	Sequence number	Coverage (%)	OTUs number	Shannon index	Chao 1 index
SpM	1430	96.7	46	3.09	104.20
SpL	1413	95.2	52	4.09	130.88
SpS	1546	98.3	39	3.15	106.78
SM	1620	92.1	42	2.89	95.37
SL	895	91.5	59	4.12	148.32
SS	1170	92.6	53	3.14	106.76
AM	886	95.0	44	2.77	96.95
AL	1592	96.3	70	3.98	143.28
AS	1500	97.1	61	4.00	134.80
WM	1670	93.2	50	4.23	143.397
WL	1283	96.3	63	4.35	160.95
WS	1633	97.5	49	2.98	101.32

The coverage was calculated as $C = [1 - (n_1/N)] \times 100$, where n_1 is the number of unique OTUs and N is the total number of sequences in the sample.

Table S3 Correlation analysis of environmental factors and the diversity of ANME archaea in sediments collected from Zhoushan Archipelago intertidal zone

Environmental Factors	Pearson correlation coefficient		
	OUTs number	Shannon index	Chao1index
T	-0.095	-0.321	-0.313
SO_4^{2-}	**0.130****	**0.193***	0.0054
TP	0.286	0.226	0.269
Salt	0.313	-0.185	-0.108
TN	-0.287	-0.299	-0.261
TOM	**0.355****	0.515	**0.576***

*denotes $p < 0.05$, **denotes $p < 0.01$

Table S4 The potential methane oxidation activity and the contribution rate to potential total methane oxidation in the sediments collected from the simulated columns

Sample	Potential AMO rate (nmol g^{-1} (dry sediment) day^{-1})	AMO contribution rate (%)	Potential D-AOM rate(nmol g^{-1} (dry sediment) day^{-1})	D-AOM contribution rate (%)	Potential S-AOM rate(nmol g^{-1} (dry sediment) day^{-1})	S-AOM contribution rate (%)
Y1S	18.53	98.22%	0.34	1.78%	0.00	0.00%
Y1Z	8.17	93.52%	0.37	4.19%	0.20	2.29%
Y1X	5.10	76.69%	0.91	13.69%	0.64	9.62%
Y2S	24.09	98.83%	0.29	1.17%	0.00	0.00%
Y2Z	20.00	95.83%	0.52	2.49%	0.35	1.68%
Y2X	16.01	87.01%	1.61	8.75%	0.78	4.24%
Y3S	30.19	98.75%	0.38	1.25%	0.00	0.00%
Y3Z	26.00	96.61%	0.51	1.90%	0.40	1.49%
Y3X	20.25	88.47%	1.80	7.86%	0.84	3.67%
W1S	14.83	92.03%	1.03	6.38%	0.26	1.59%
W1Z	7.32	82.66%	1.11	12.55%	0.42	4.80%
W1X	2.55	54.24%	1.41	29.90%	0.75	15.86%
W2S	19.85	92.49%	1.19	5.54%	0.42	1.97%
W2Z	14.84	86.64%	1.53	8.95%	0.75	4.41%
W2X	12.52	80.92%	1.99	12.84%	0.97	6.24%
W3S	25.36	92.96%	1.47	5.39%	0.45	1.65%
W3Z	18.75	87.16%	1.91	8.89%	0.85	3.95%
W3X	15.78	82.16%	2.34	12.17%	1.09	5.67%
N1S	11.63	86.80%	0.97	7.23%	0.80	5.97%
N1Z	7.04	69.55%	1.48	14.65%	1.60	15.80%
N1X	0.36	7.56%	2.09	43.34%	2.37	49.10%
N2S	17.91	89.91%	1.25	6.27%	0.76	3.82%
N2Z	11.21	75.26%	2.01	13.53%	1.67	11.21%
N2X	3.54	43.00%	2.57	31.25%	2.12	25.75%
N3S	22.27	89.58%	1.70	6.84%	0.89	3.58%
N3Z	15.36	78.53%	2.51	12.83%	1.69	8.64%
N3X	5.63	49.13%	3.05	26.61%	2.78	24.26%

AMO, aerobic methane oxidation.

Table S5 Correlation analyses of environmental factors and the abundance of ANME archaea and D-AOM microorganism in the sediments collected from the simulated columns

Environmental Factors	Pearson correlation coefficient						
	ANME-1 abundance	ANME-2c abundance	ANME-3 abundance	*M. oxyfera* abundance	ANME-2d abundance	S-AOM activity	Nitrate-AOM activity
T	**0.555****	**0.504****	-0.132	-0.124	**0.308****	**0.405***	**0.309***
ORP	**-0.389***	**-0.456***	-0.189	-0.123	**-0.486***	**-0.456***	-0.398
SO_4^{2-}	**0.433***	0.359	0.113	-0.045	-0.098	**0.589****	0.667
NH_4^+	0.017	-0.060	-0.039	-0.045	-0.098	-0.009	-0.129
NO_3^-	0.462	0.336	0.332	**0.428***	**0.629****	-0.359	**0.785****
TIN	0.067	-0.022	-0.005	0.003	0.279	-0.012	-0.223
TOM	**0.660****	**0.524****	0.004	0.048	0.049	**0.578****	**0.346****

*denotes $p < 0.05$, **denotes $p < 0.01$

Table S6 The diversity indices for anaerobic methane oxidation archaea in the samples collected from the simulated columns

Sample	Sequence number	Coverage (%)	OTUs number	Shannon index	Chao 1 index
Y1S	1622	98.5	72	4.03	136.21
Y1Z	1401	97.3	59	3.95	133.51
Y1X	1279	98.3	47	3.23	109.17
Y2S	1513	98.5	78	5.11	172.72
Y2Z	1513	99.1	74	5.01	169.34
Y2X	1298	95.8	50	3.76	127.09
Y3S	1279	94.5	52	3.45	116.61
Y3Z	1365	95.9	58	3.77	127.43
Y3X	1779	96.8	82	5.39	182.18
W1S	1194	99.1	49	3.02	102.08
W1Z	1808	95.9	89	5.59	188.94
W1X	1354	96.8	51	3.09	104.44
W2S	1304	99.3	57	3.98	134.52
W2Z	1663	100	73	4.98	168.32
W2X	1736	99.4	79	4.79	161.90
W3S	1814	97.8	84	5.34	180.49
W3Z	1595	98.5	69	4.93	166.63
W3X	1467	94.5	58	4.12	139.26
N1S	770	93.5	39	3.15	106.47
N1Z	1171	95.9	48	4.03	136.21
N1X	1371	98.9	54	4.47	151.09
N2S	1266	95.8	47	4.01	135.54
N2Z	1103	96.9	44	4	135.20
N2X	1132	99.1	38	3.98	134.52
N3S	1321	93.5	49	4.07	137.57
N3Z	1463	96.9	56	4.24	143.31
N3X	1273	99.5	55	4.22	142.64

Table S7 Correlation analyses of environmental factors and the diversity indices for anaerobic methane oxidation archaea in the samples collected from the simulated columns

Environment Factor	OTUs number	Shannon index	Chao 1 index
T	0.200	0.305	0.305
ORP	**-0.347***	-0.239	-0.129
SO_4^{2-}	**0.047****	**0.192****	**0.192****
NH_4^+	-0.159	-0.145	-0.145
NO_3^-	-0.311	0.032	0.032
TIN	-.0184	-0.131	-0.131
TOM	0.182	**0.449***	**0.449***

*denotes $p < 0.05$, **denotes $p < 0.01$

Table S8 The physiochemical parameters of different intertidal zone sediment samples collected from Zhoushan Archipelago

Season	Samples	pH	ORP (mv)	T (℃)	SO_4^{2-} (mg Kg^{-1})	TP (mg Kg^{-1})	TN (mg Kg^{-1})	TOM (g Kg^{-1})	Salinity (g Kg^{-1})	Total iron (g Kg^{-1})	Total Mn (g Kg^{-1})
Spring (May)	SpM	8.31	49.6 ±6.4	20.0	119.7	411.8	708.3	9.1 ±1.1	12.9 ±1.2	36.35	0.77
	SpL	8.36	48.9 ±1.5	19.9	159.6	416.8	637.4	9.3 ±0.6	12.6 ±0.2	30.66	0.61
	SpS	8.37	36.4 ±0.5	18.9	104.5	427.0	530.2	7.2 ±1.8	10.2 ±1.4	39.02	0.79
Summer (August)	SM	8.31	87.8 ±10.9	32.0	145.2	486.0	769.5	14.7 ±2.7	14.6 ±0.1	37.40	0.74
	SL	8.43	34.9 ±2.2	32.0	105.2	485.8	614.3	9.4 ±2.4	15.2 ±0.4	30.75	0.67
	SS	8.36	26.9 ±1.4	32.0	160.6	516.5	579.6	8.5 ±0.6	15.8 ±0.2	30.61	0.74
Autumn (November)	AM	8.30	70.7 ±2.2	22.4	165.1	520.4	755.8	14.1 ±1.4	21.0 ±4.0	40.42	0.90
	AL	8.30	40.8 ±5.2	20.7	122.1	441.9	554.6	13.2 ±0.1	17.5 ±1.5	33.90	0.74
	AS	8.30	61.7 ±2.2	20.0	125.9	591.2	453.3	13.7 ±2.9	19.9 ±0.2	38.78	1.00
Winter (February)	WM	8.12	72.4 ±3.8	9.0	129.9	527.3	697.5	24.6 ±0.6	14.3 ±1.5	37.09	0.81
	WL	8.24	62.8 ±7.1	9.0	132.8	517.6	521.6	13.8 ±1.0	13.5 ±1.2	40.08	0.79
	WS	8.12	53.5 ±8.0	9.0	150.9	558.8	461.3	18.8 ±2.9	13.2 ±1.5	34.34	0.69

ORP: Oxidation-Reduction Potential; *T*: Temperature;

TP: Total Phosphorus; *TN*: Total Nitrogen; *TOM*: Total Organic Matter.

Table S9 Physicochemical properties of the samples collected from the simulated columns

Samples	pH	ORP (mv)	T (°C)	NH_4^+-N (mg/Kg)	NO_3^--N (mg/Kg)	TIN (mg/Kg)	SO_4^{2-} (mg/Kg)	TOM (g/Kg)
Y1S	7.93	85.1	20.6	4.14	4.50	8.79	314.96	17.83
Y1Z	8.00	95.6	21.2	3.75	5.03	8.90	321.86	18.85
Y1X	7.89	76.4	20.7	23.10	3.91	27.09	294.51	22.85
Y2S	8.02	123.1	25.6	4.85	4.93	10.07	326.39	32.22
Y2Z	7.97	105.7	24.9	4.13	4.48	8.68	345.90	36.22
Y2X	8.00	88.7	25	5.43	5.42	11.00	310.55	30.76
Y3S	7.83	133.1	32.5	4.42	3.42	8.14	365.72	25.67
Y3Z	8.00	107.5	33.6	7.60	4.32	12.23	387.20	28.00
Y3X	7.99	78.7	31.7	12.04	3.69	16.04	358.47	27.66
W1S	8.08	75.6	20.9	12.31	4.61	17.03	282.95	16.17
W1Z	8.15	96.4	21.5	26.51	4.84	31.46	222.67	19.99
W1X	7.79	71.7	19.9	34.79	4.79	39.67	205.22	11.80
W2S	8.09	83.7	25.3	3.65	5.38	9.14	284.58	29.30
W2Z	8.04	109.6	26.8	7.53	5.71	13.38	295.18	28.61
W2X	7.89	100.8	25.1	22.37	6.39	28.85	287.92	34.28
W3S	8.03	93.7	32.9	4.40	4.33	9.13	481.00	29.09
W3Z	8.12	87.6	33.4	9.99	5.39	15.74	391.94	30.70
W3X	7.99	78.5	31.8	16.76	5.18	22.25	316.72	34.97
N1S	8.02	69.5	19.7	10.91	5.20	16.16	238.67	21.96
N1Z	8.00	83.5	20.6	19.15	5.87	25.08	238.85	23.84
N1X	7.89	65.3	19.6	19.03	5.69	26.10	153.13	23.71
N2S	8.01	67.4	25.9	11.10	6.74	17.89	385.43	25.50
N2Z	8.09	70.7	24.8	21.71	6.12	27.89	335.30	27.83
N2X	8.12	59.2	23.9	19.71	6.72	26.48	441.72	29.39
N3S	7.79	57.4	33	7.39	5.21	12.96	423.50	31.27
N3Z	8.00	60.5	34	8.12	5.33	13.82	426.94	33.15
N3X	8.04	55.3	35	8.32	5.51	14.28	424.30	36.46

ORP: Oxidation-Reduction Potential; *T*: Temperature;

TIN: Total Inorganic Nitrogen; *TOM*: Total Organic Material

Table S10 The different treatments in experiment groups to detect potential methane oxidation rates

Groups	Treatments						
	$^{13}CH_4$ 10% v/v	NO_3^- 0.1 mM	SO_4^{2-} 10 mM	O_2 10% v/v	Ferrihydrite 1 g L^{-1}	Birnessite 1 g L^{-1}	$BaCl_2$
A	+						+
B	+	+					+
C	+		+				+
D	+			+			+
E	+				+		+
F	+					+	+

+denotes that add different substrates in responding group

Group A was used to determine whether the preculture process had depleted the residual NO_x^-, O_2 and other electron accepters, and it was also set as control. Group B was supplemented with deoxygenated NO_3^- solution and the final NO_3^- concentration was controlled at 0.1 mmol L^{-1}. Group C was replenished with deoxygenated SO_4^{2-} solution and the final concentration was set at 10 mmol L^{-1} (approximate to the sulfate concentration in sediments collected from Zhoushan Archipelago intertidal zone). Group D was added with 10% volume O_2 in the gas phase. Ferrihydrite and birnessite were added to Group E and F respectively with the final concentration controlled at 0.1 g L^{-1}. 3 mL of $^{13}CH_4$ was used to replace the equivoluminal gas in all serum bottles and accounted for about 10% of the gas volume. The methane oxidation rate could be calculated by the following formulae: r(D-AOM) = Group B - Group A, r(S-AOM) = Group C - Group A, r(aerobic methane oxidation) = Group D - Group A, r(iron-AOM)= Group E - Group A, r(manganese-AOM)= Group F - Group A.