

Supplementary materials

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Unique Phlotypes of Nitrite-dependent Anaerobic Methane Oxidation Bacteria in Coastal Sediments of the Mai Po Wetland by PCR Amplification of 16S rRNA and *pmoA* genes

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Table S1 Summary and analysis of n-damo bacteria from different habitats based on *pmoA* gene sequences*

Source/Environment	N-damo related clone	OTU	Shannon -Wiener	Chao1	Type	Accession number in GenBank	Reference**
CF5S	21	2	0.6365	1	M/R	KJ023443, KJ023450	Chen et al. (2014b)
CF5B	4	1	0	1	M/R	KJ023444	Chen et al. (2014b)
E201S	8	2	0.3768	1	M/R	KJ023445-KJ023446	Chen et al. (2014b)
E407S	6	2	0.4506	1	M/R	KJ023447-KJ023449	Chen et al. (2014b)
E407B	24	2	0.3768	1	M/R	KJ023451, KJ023455	Chen et al. (2014b)
E510S	16	2	0.2338	1	M/R	KJ023452, KJ023456	Chen et al. (2014b)
E525S	24	1	0	1	M/R	KJ023453	Chen et al. (2014b)
E525B	1	1	--	--	M/R	KJ023454	Chen et al. (2014b)
SCS E704S	24	3	1.0055	1	M	KF528962-KF528968 KF528973-KF528984 KF742451-KF742455 KF528961	Chen et al. (2014a)
SCS E401	13	3	0.9110	1	M	KF528969-KF528972 KF742444-KF742450	Chen et al. (2014a)
Lake Constance	15	6	1.4878	10.5	F	HQ906565-HQ906579	Deutzmann and Schink (2011)
Lake Biwa	21	1	0	1	F	AB661605-AB661625	Kojima et al. (2012)
Reed bed	12	1	0	1	F	JX898463-JX898474	Han and Gu (2013)
Reservoir	9	7	1.8892	13.2500	F	JX898486-JX898494	Han and Gu (2013)
WWTP^	10	7	1.8344	19.5	E	JX898495-JX898504	Han and Gu (2013)
Paddy soil	11	2	0.3046	1	F	JX898475-JX898485	Han and Gu (2013)
Paddy soil	14	1	0	1	F	JN704402-JN704415	Wang et al. (2012)
Paddy field	20	2	0.1985	1	F	KC341611-KC341630	Zhu et al. (2013a)
Jingshan paddy field	27	1	0	1	F	KC905857-KC905883	Hu et al. (2014)

Xixi wetland	21	3	0.3805	1	F	KC905884-KC905904	Hu et al. (2014)
XZ wetland	4	1	0	1	F	KC905905-KC905908	Hu et al. (2014)
FreshwaterWetlands (Jingshan Paddy; Xixi wetland; XZ wetland)	52	5	1.0575	1	F	KC905857-KC905908	Hu et al. (2014)
Baiyangdian Lake	24	1	0	1	F	KC341248-KC341271	Zhu et al. (2013a)
Bosten Lake	16	2	0.4826	1	F	KC341301-KC341316	Zhu et al. (2013a)
Chaohu Lake	19	6	1.1513	1	F	KC341317-KC341335	Zhu et al. (2013a)
Dongting Lake	21	2	0.6920	1	F	KC341336-KC341356	Zhu et al. (2013a)
Jiaxing Constructed wetland-winter	13	8	1.8393	26	F	KC341375-KC341387	Zhu et al. (2013a)
Jiaxing Constructed wetland-summer	11	8	2.0198	12.1667	F	KC341388-KC341398	Zhu et al. (2013a)
Subsurface North canal	18	2	0.6870	1	U	KC341272-KC341289	Zhu et al. (2013a)
North canal-12 m	3	1	0	1	U	KC341298-KC341300	Zhu et al. (2013a)
North canal-15 m	8	1	0	1	U	KC341290-KC341297	Zhu et al. (2013a)
Panjin swamp	21	1	0	1	F	KC341437-KC341457	Zhu et al. (2013a)
Peat land China	23	6	1.3944	8	F	KC341414-KC341436	Zhu et al. (2013a)
Poyang Lake	21	2	0.5983	1	F	KC341458-KC341478	Zhu et al. (2013a)
Shahe River	18	5	1.2094	1	F	KC341479-KC341496	Zhu et al. (2013a)
Songhuajiang River	18	7	1.6715	9.25	F	KC341497-KC341514	Zhu et al. (2013a)
Tarim River	19	2	0.6918	1	F	KC341515-KC341533	Zhu et al. (2013a)
Tiaoxi River	20	3	0.9973	1	F	KC341534-KC341553	Zhu et al. (2013a)
Tibetan Lake	10	1	0	1	S/R	JQ429431-JQ429432	Yang et al. (2012)
Tulufan River	18	3	0.8487	3	F	KC341357-KC341374	Zhu et al. (2013a)
Wuliangshuhai Lake	24	4	0.9366	4.5	F	KC341554-KC341577	Zhu et al. (2013a)

Yellow River	15	1	0	1	F	KC341399-KC341413	Zhu et al. (2013a)
Yuanmingyuan Lake	12	3	0.7215	3.5	F	KC341698-KC341709	Zhu et al. (2013a)
Pearl River-winter	20	5	1.0098	6	F	KC341578-KC341597	Zhu et al. (2013a)
Pearl River-summer	13	2	0.6172	1	F	KC341598-KC341610	Zhu et al. (2013a)
Donghu Lake	15	3	0.8033	1	F	KC341631-KC341645	Zhu et al. (2013a)
Honghaitan Tidal Land	17	4	1.1151	4.5	C	KC341646-KC341662	Zhu et al. (2013a)
Shangqiu Reservoir	17	7	1.6100	8.5	F	KC341663-KC341679	Zhu et al. (2013a)
Tianchi Lake	18	9	1.8121	18	F	KC341680-KC341697	Zhu et al. (2013a)
WWTP [^]	128	3	0.8487	1	U	KC700842-KC700715	Gao et al. (2013)
Soils [^]	40	3	0.3813	1	U	KC700675-KC700714	Gao et al. (2013)
Qiantang River	50	16	2.1437	29.5	F	KC503613-KC503662	Li-Dong et al. (2014a)
West Lake	24	8	1.5359	12	F	JX531974-JX531997	Zhu et al. (2013b)
Jiaojiang estuary	80	20	2.3787	30.1250	C	KC512302-KC512381	Li-Dong et al. (2014b)
Freshwater habitats (Alpine peat bog WWTPs [^] Drainage ditch Contaminated aquifer [^])	12	9	NA	NA	F/R	HQ698926-HQ698937	Luesken et al. (2011c)
WWTPs [^]	19	10	NA	NA	F/R	JF706214-JF706196	Luesken et al. (2011b)
SBR co-enrichment with anammox [^]	7	3	NA	NA	E/R	JN006731-JN006737	Luesken et al. (2011a)
Sewage treatment plants [^]	12	8	NA	NA	E/R	KC112371-KC112382	Ho et al. (2013)
Minerotrophic peatland [^]	3	3	NA	NA	E/R	JX262153-JX262155	Zhu et al. (2012)
Lab scale reactor [^]	13	3	1.0579	1	E/?	AB767281-AB767293	Masashi et al. (2013)

*based on percentage sequence identity of 95% of *pmoA* gene-based PCR amplification and analysis using Fastgroup (Yu et al. 2006);

Abbreviations: NA, not applicable; M, marine; F, freshwater; S, saline lake; C, costal; U, unknown; E, enrichment; R, representative sequences available in the GenBank database only;

**Unpublished reference indicates that the related DNA sequences have already been released in the database without a published journal paper until 17-April-2014 when the information was accessed;

^Isolation sources/environments are not sediments.

Table S2 Statistical analyses of physicochemical parameters and n-damo bacteria diversity*

Parameter	OTU		Shannon-Wiener		Chao1		Abundance
	16S rRNA	<i>pmoA</i>	16S rRNA	<i>pmoA</i>	16S rRNA	<i>pmoA</i>	
Depth	0.6642	0.1111	0.8714	0.0401	0.4946	-0.0546	0.2918
pH	0.3893	-0.0906	0.4429	0.2817	0.4327	-0.3926	0.8428
Redox	-0.4577	-0.4001	-0.4725	-0.7031	-0.4115	-0.0002	-0.5632
Temperature	-0.6597	0.0648	-0.7509	0.4254	-0.5595	0.2391	0.3246
Salinity	-0.5406	0.1490	-0.3528	-0.1283	-0.7425	0.7672	-0.4087
NH ₄ ⁺	0.9299	0.1092	0.7962	0.0988	0.9974	-0.6086	0.0116
NO ₂ ⁻	0.8718	0.2132	0.6714	0.1132	0.9473	-0.5184	-0.2747
NO ₃ ⁻	-0.1376	0.0803	-0.4558	0.2308	0.0793	-0.2384	-0.2158
NH ₄ ⁺ /(NO ₃ ⁻ +NO ₂ ⁻)	0.8169	-0.2087	0.7809	-0.1245	0.9018	-0.7598	0.3587

*Pearson's r is given by

$$r = \frac{\sum_i (x_i - \bar{x})(y_i - \bar{y})}{\sqrt{\sum_i (x_i - \bar{x})^2} \sqrt{\sum_i (y_i - \bar{y})^2}};$$

Numbers in bold are regarded as significant ($P < 0.05$). The significance is computed using a two-tailed t test with $n-2$ degrees of freedom and

$$t = r \sqrt{\frac{n-2}{1-r^2}}.$$

16S rRNA gene abundance was positively correlated with depth, pH, temperature, NH₄⁺ and NH₄⁺/Σ(NO₃⁻+NO₂⁻). In addition, depth, NO₂⁻ and NH₄⁺ positively correlated with both 16S rRNA and *pmoA* OTUs and diversity, while redox negatively correlated with all OTU, diversity, richness and abundance indexes.



Fig. S1 Map of Mai Po Nature Reserve (Adapted from WWF_HongKong (2006))

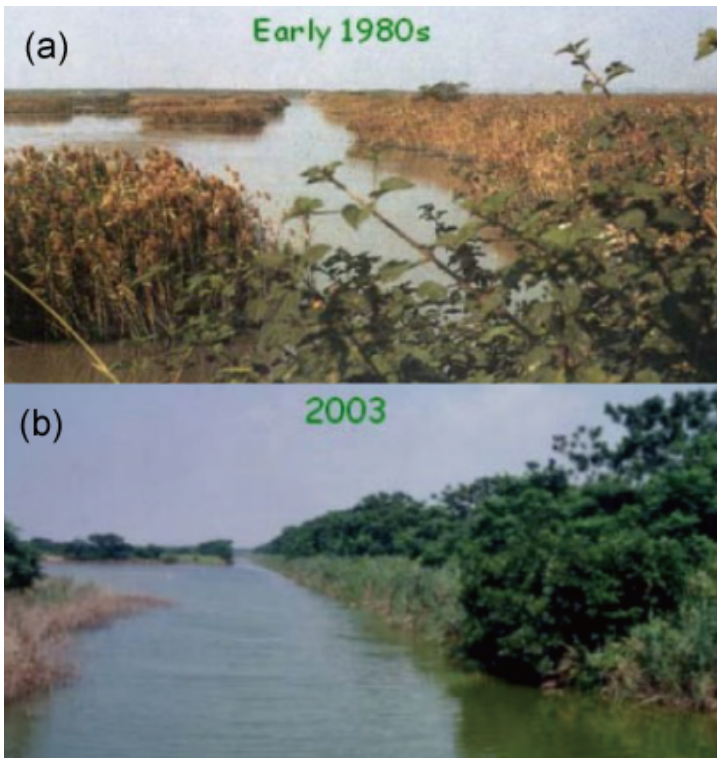
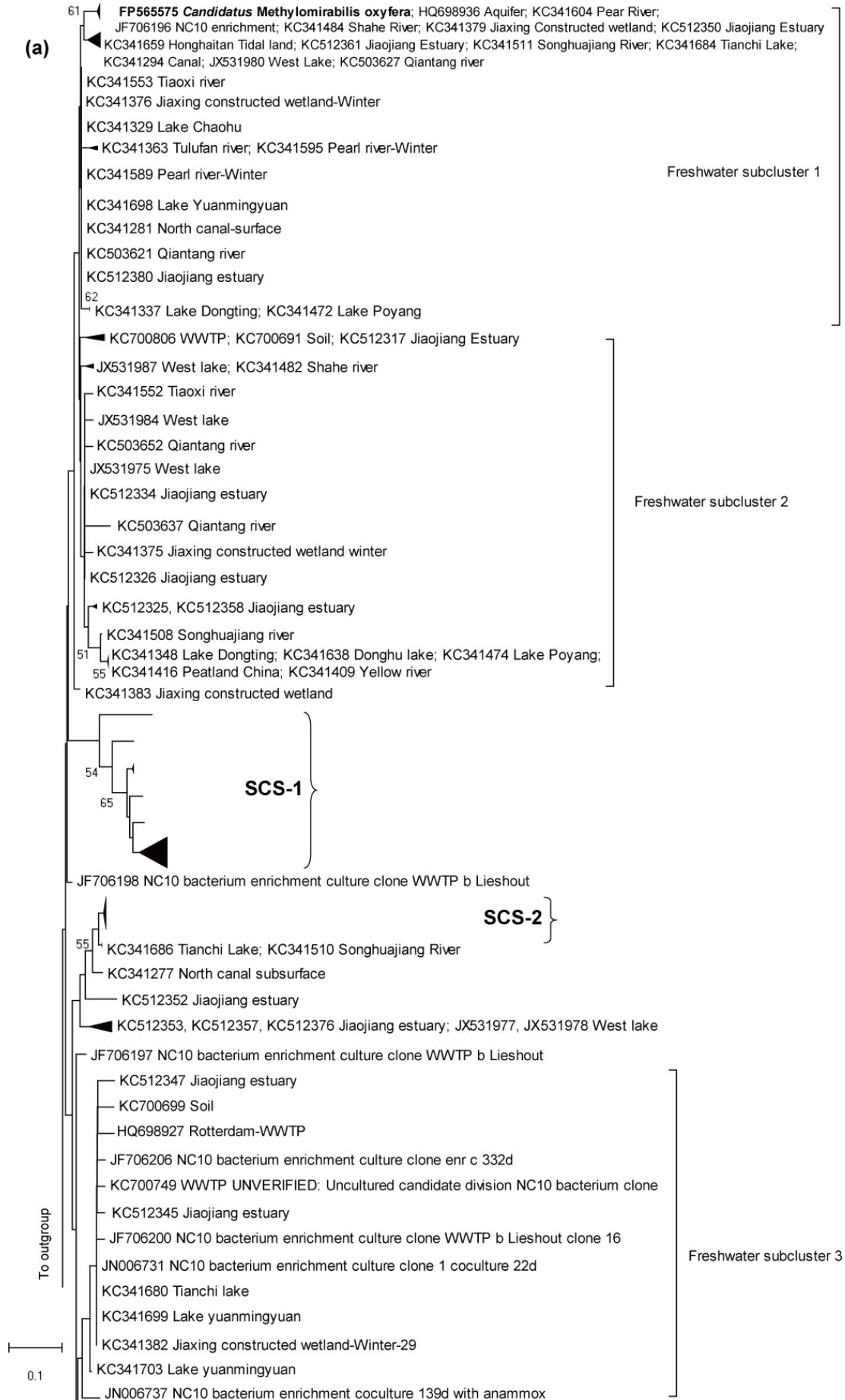


Fig. S2 The reed bed in Mai Po Nature Reserve in the 1980s (a) and 2003 (b) (Adapted from WWF_HongKong (2006)).



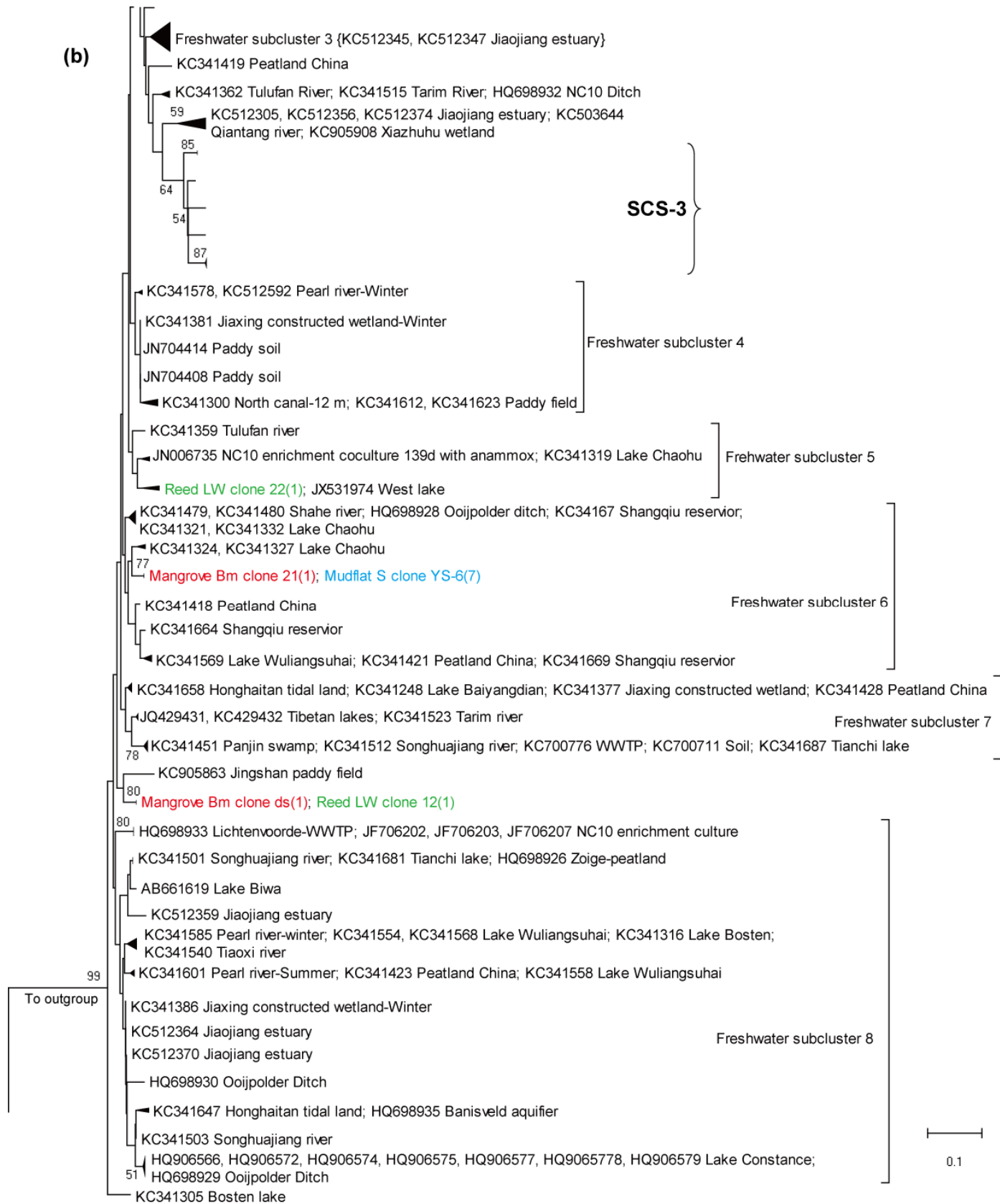


Fig. S3 Consensus phylogenetic tree subclusters 1-3(a) and 4-8(b) conducted after subjecting deduced *pmoA* amino acid sequences to neighbor joining analysis. The topology in this figure is strictly the same with that in Figure 2b. OTUs were defined with a Percent

Sequence Identity of 95% (*pmoA* gene) similarity by Fastgroup II. Sequences in the present work were marked in red, blue and green for those from mangrove, mudflat and reed sediments, respectively. Numbers in parenthesis refer to how many detected sequences fell in the branch or subcluster. Numbers at the nodes represent the levels of bootstrap support based on 1000 re-sampled data sets (only >50% values are shown).

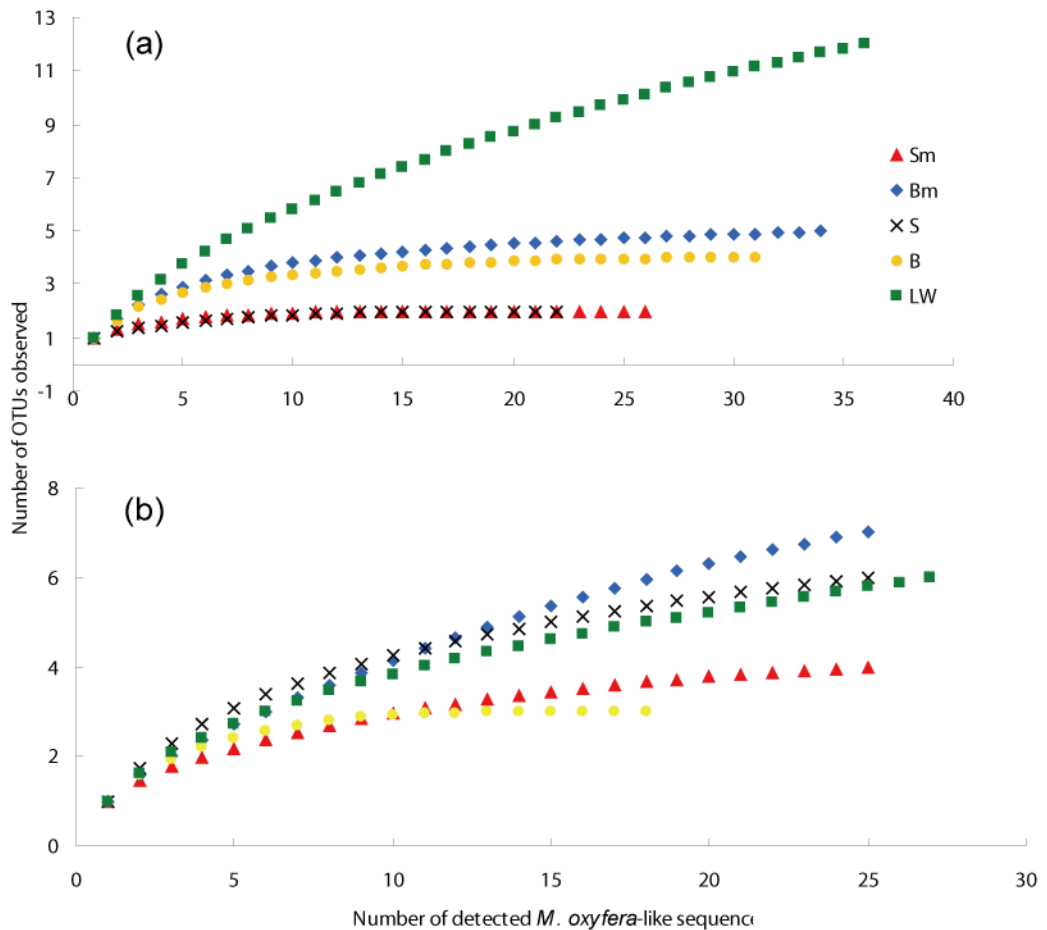


Fig. S4 Rarefaction analyses of n-damo bacteria communities based on amplified 16S rRNA (a) and *pmoA* (b) gene sequences in Mai Po sediments. Fastgroup II was used with 3% or 5% nucleotide sequence variation for OTU determination for 16S rRNA or *pmoA* gene, respectively.

References

- Chen J, Jiang XW, Gu J-D (2014a) Existence of Novel Phylotypes of Nitrite-dependent Anaerobic Methane-oxidizing Bacteria in Surface and Subsurface Sediments of the South China Sea. *Geomicrobiol J*. doi: 10.1080/01490451.01492014.01917742
- Chen J, Zhou ZC, Gu J-D (2014b) Occurrence and Diversity of Nitrite-dependent Anaerobic Methane Oxidation Bacteria in the Sediments of the South China Sea Revealed by Amplification of both 16S rRNA and *pmoA* Genes. *Appl Microbiol Biotechnol* 98: 5685-5696
- Deutzmann JS, Schink B (2011) Anaerobic Oxidation of Methane in Sediments of Lake Constance, an Oligotrophic Freshwater Lake. *Appl Environ Microbiol* 77: 4429-4436
- Gao J, Luo X, Wu G, Peng Y (2013) Occurrence of nitrite-dependent anaerobic methane oxidizing bacteria from different soil and wastewater sludge. NCBI Genbank web <http://www.ncbi.nlm.nih.gov/nuccore/> Accessed 17 April 2014
- Han P, Gu J-D (2013) A newly designed degenerate PCR primer based on *pmoA* gene for detection of nitrite-dependent anaerobic methane-oxidizing bacteria from different ecological niches. *Appl Microbiol Biotechnol* 97: 10155-10162
- Ho A, Vlaeminck SE, Ettwig KF, Schneider B, Frenzel P, Boon N (2013) Revisiting methanotrophic communities in sewage treatment plants. *Appl Environ Microbiol* 79: 2841-2846
- Hu BL, Shen LD, Lian X, Zhu Q, Liu S, Huang Q, He ZF, Geng S, Cheng DQ, Lou LP, Xu XY, Zheng P, He YF (2014) Evidence for nitrite-dependent anaerobic methane oxidation as a previously overlooked microbial methane sink in wetlands. *Proc Natl Acad Sci U S A* 111: 4495-4500
- Kojima H, Tsutsumi M, Ishikawa K, Iwata T, Musmann M, Fukui M (2012) Distribution of putative denitrifying methane oxidizing bacteria in sediment of a freshwater lake, Lake Biwa. *Syst Appl Microbiol* 35: 233-238
- Li-Dong S, Qun Z, Shuai L, Ping D, Jiang-Ning Z, Dong-Qing C, Xiang-Yang X, Ping Z, Bao-Lan H (2014a) Molecular evidence for nitrite-dependent anaerobic methane-oxidising bacteria in the Jiaojiang Estuary of the East Sea (China). *Appl Microbiol Biotechnol*. doi: 10.1007/s00253-00014-05556-00253
- Li-Dong S, Qun Z, Shuai L, Ping D, Jiang-Ning Z, Dong-Qing C, Xiang-Yang X, Ping Z, Bao-Lan H (2014b) Molecular evidence for nitrite-dependent anaerobic methane-oxidising bacteria in the Jiaojiang Estuary of the East Sea (China). *Appl Microbiol Biotechnol*
- Luesken FA, Sanchez J, van Alen TA, Sanabria J, Op den Camp HJM, Jetten MSM, Kartal B (2011a) Simultaneous Nitrite-Dependent Anaerobic Methane and Ammonium Oxidation Processes. *Appl Environ Microbiol* 77: 6802-6807

- Luesken FA, van Alen TA, van der Biezen E, Frijters C, Toonen G, Kampman C, Hendrickx TL, Zeeman G, Temmink H, Strous M, Op den Camp HJ, Jetten MS (2011b) Diversity and enrichment of nitrite-dependent anaerobic methane oxidizing bacteria from wastewater sludge. *Appl Microbiol Biotechnol* 92: 845-854
- Luesken FA, Zhu BL, van Alen TA, Butler MK, Diaz MR, Song B, den Camp HJMO, Jetten MSM, Ettwig KF (2011c) pmoA Primers for Detection of Anaerobic Methanotrophs. *Appl Environ Microbiol* 77: 3877-3880
- Masashi H, Masafumi K, Masato K, Takashi Y (2013) Enrichment of denitrifying anaerobic methane oxidizing microorganisms using nitrate or nitrite as electron acceptor. NCBI Genbank web <http://www.ncbi.nlm.nih.gov/nuccore/> Accessed 17 April 2014
- Wang Y, Zhu G, Harhangi HR, Zhu B, Jetten MS, Yin C, Op den Camp HJ (2012) Co-occurrence and distribution of nitrite-dependent anaerobic ammonium and methane oxidizing bacteria in a paddy soil. *FEMS Microbiol Lett* 336: 79-88
- WWF_HongKong (2006) Management Plan for the Mai Po Nature Reserve 2006-2010. WWF Hong Kong, Hong Kong
- Yang J, Jiang HC, Wu G, Hou WG, Sun YJ, Lai ZP, Dong HL (2012) Co-occurrence of nitrite-dependent anaerobic methane oxidizing and anaerobic ammonia oxidizing bacteria in two Qinghai-Tibetan saline lakes. *Front Earth Sci* 6: 383-391
- Yu YN, Breitbart M, McNairnie P, Rohwer F (2006) FastGroupII: A web-based bioinformatics platform for analyses of large 16S rDNA libraries. *Bmc Bioinformatics* 7: 57
- Zhu BL, van Dijk G, Fritz C, Smolders AJP, Pol A, Jetten MSM, Ettwig KF (2012) Anaerobic Oxidation of Methane in a Minerotrophic Peatland: Enrichment of Nitrite-Dependent Methane-Oxidizing Bacteria. *Appl Environ Microbiol* 78: 8657-8665
- Zhu GB, Zhou LL, Jiang B, Wang Y, Wang SY, Jetten MSM, Yin CQ (2013a) Biogeographical distribution of nitrite dependent anaerobic methanotrophic bacteria in wetland ecosystems: from regional scale to global scale. NCBI Genbank web <http://www.ncbi.nlm.nih.gov/nuccore/> Accessed 17 April 2014
- Zhu Q, Shen LD, Hu BL, Lou LP, Cheng DQ (2013b) Molecular detection of denitrifying anaerobic oxidizing bacteria in the sediment of West Lake, Hangzhou. *Acta Scientiae Circumstantiae* 33: 1321-1325