Supplementary information

Depth-related variation in the activity and community structure of nitrite- and nitrate-coupled anaerobic methanotrophs in freshwater lake sediment

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Table S1 Details of primer sequences and thermal cycles of PCR and quantitative PCR (qPCR)

Process	Primer	Sequence (5'-3')	Thermal profile	References
PCR	202F	GACCAAAGGGGGCGAGCG	95 °C for 3 min; 35 cycles of 95 °C for 1 min, 57 °C for 1 min, and 72 °C for 1 min; a final extension at 72 °C for 10 min.	(Ettwig et al., 2009)
	1545R	CAKAAAGGAGGTGATCC		(Juretschko et al., 1998)
	qp1F	GGGCTTGACATCCCACGAACCTG	95 °C for 3 min; 30 cycles of 95 °C for 1 min, 63 °C for 1 min, and 72 °C for 1 min; a final extension at 72 °C for 10 min.	(Ettwig et al., 2009)
	qp2R	CTCAGCGACTTCGAGTACAG		(Ettwig et al., 2009)
PCR	McrA169F	GCAGCAATCACCAAGAAGAGAGG	95 °C for 3 min; 30 cycles of 95 °C for 1 min, 57 °C for 1 min, and 72 °C for 1 min; a final extension at 72 °C for 10 min.	(Xu et al., 2018)
	McrA1360R	TGCCTCTTTGTGGAGGTACATGGA		(Xu et al., 2018)
	McrA997F	ATCTGGCTCGGYGGCTACATGT	95 °C for 3 min; 30 cycles of 95 °C for 1 min, 62 °C for 1 min, and 72 °C for 1 min; a final extension at 72 °C for 10 min.	(Vaksmaa et al., 2017)
	McrA1360R	TGCCTCTTTGTGGAGGTACATGGA		(Xu et al., 2018)
qPCR	qp1F	GGGCTTGACATCCCACGAACCTG	95 °C for 3 min; 40 cycles of 95 °C for 30 s, 63 °C for 30 s, and 72 °C for 1 min; a final extension at 72 °C for 5 min.	(Ettwig et al., 2009)
	qp1R	CGCCTTCCTCCAGCTTGACGC		(Ettwig et al., 2009)
qPCR	McrA 159F	AAAGTGCGGAGCAGCAATCACC	95 °C for 3 min; 40 cycles of 95 °C for 30 s, 58 °C for 30 s, and 72 °C for 1 min; a final extension at 72 °C for 5 min.	(Vaksmaa et al., 2017)
	McrA 345R	TCGTCCCATTCCTGCTGCATTGC		(Vaksmaa et al., 2017)

Table S2 Vertical and horizontal variations in sediment physicochemical properties

Sampling sites	Depth (cm)	pH	SOC (mg/kg)	DOC (mg/kg)	NH_4^+-N (mg/kg)	NO_3^--N (mg/kg)	NO_2^--N (mg/kg)
LA	0-10	7.83±0.01a	12.45±0.06c	64.54±4.33b	31.25±0.37a	3.56±0.23a	1.64±0.11a
	10-20	7.71±0.01b	18.78±0.49b	65.32±4.22b	22.99±0.89b	3.68±0.08a	0.20±0.03b
	20-30	7.67±0.00c	20.75±0.11a	85.58±3.89a	33.39±0.56a	3.64±0.06a	0.17±0.01b
		B	A	AB	B	B	A
LB	0-10	7.82±0.00a	15.20±0.34a	69.48±2.71a	27.64±0.26c	5.23±0.73a	0.48±0.09a
	10-20	7.72±0.01b	15.38±0.05a	73.95±4.49a	60.89±1.13b	5.04±0.43a	0.35±0.06a
	20-30	7.73±0.00b	15.61±0.15a	74.39±3.41a	72.05±2.55a	5.14±0.31a	0.40±0.03a
		B	A	A	A	A	A
LC	0-10	7.84±0.00a	12.68±0.03c	61.84±3.28b	18.82±1.57c	4.85±0.25a	0.23±0.02a
	10-20	7.75±0.01b	16.84±0.05b	70.85±2.26a	35.80±0.13b	5.24±0.44a	0.15±0.00b
	20-30	7.74±0.00b	20.08±0.14a	57.29±1.65b	57.39±1.25a	5.86±1.01a	0.25±0.03a
		AB	A	B	AB	A	B
LD	0-10	7.83±0.01a	8.50±0.20a	55.91±2.88c	10.21±1.05c	2.74±0.39b	0.13±0.02a
	10-20	7.84±0.00a	7.22±0.15b	66.90±3.00b	20.08±0.54b	5.77±0.78a	0.38±0.09b
	20-30	7.83±0.01a	9.17±0.36a	78.56±2.40a	28.40±0.32a	4.24±0.33ab	0.23±0.06b
		A	B	AB	B	AB	AB

Note: Different lowercase letters indicate a significant difference among sediment layers, and different uppercase letters indicate a significant difference among sampling sites. Physicochemical properties of 0–10 cm sediment were obtained from a previous study (Bai et al., 2024).

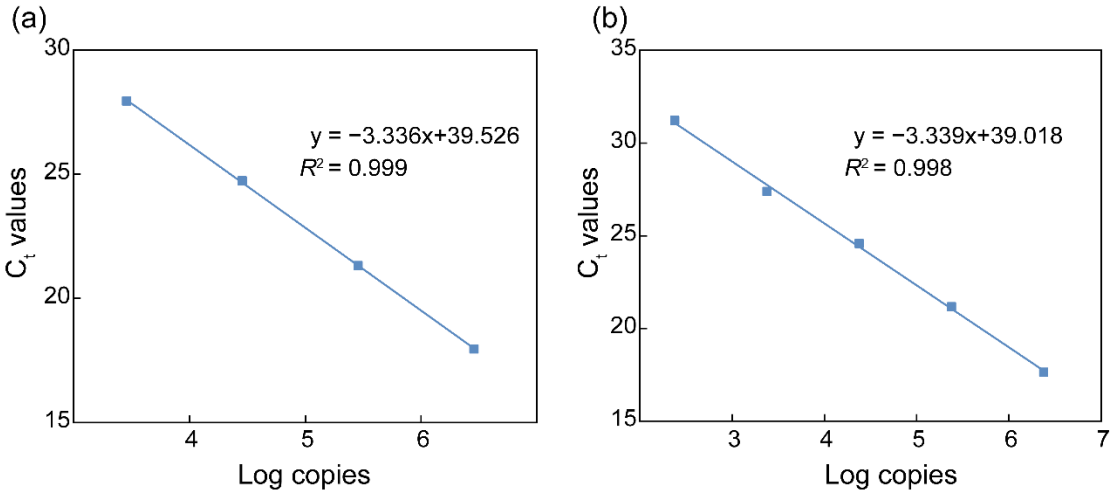

Fig. S1 Standard curves of quantitative PCR on *Methylomirabilis*-like 16S rRNA gene (a) and *Methanoperedens*-like *mcr*A gene (b).

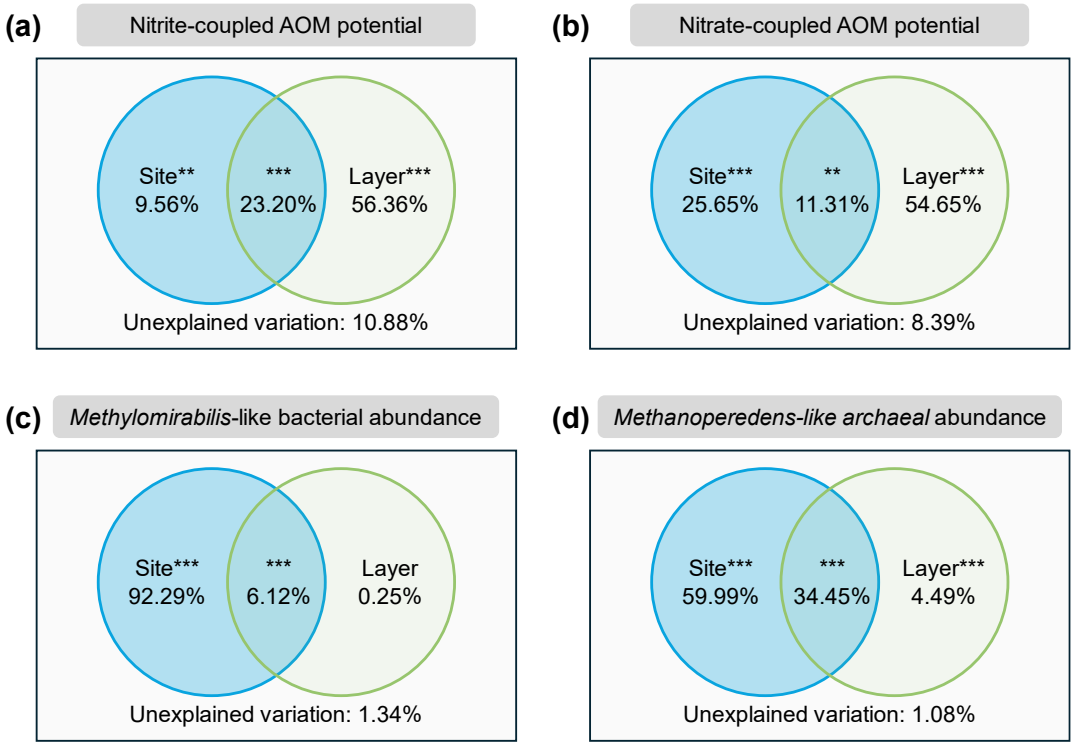

Fig. S2 Relative importance of sediment layer and sampling sites in influencing potential rates of nitrite- (a) and nitrate- coupled AOM (b), and abundance of *Methylomirabilis*-like bacteria (c) and *Methanoperedens*-like archaea (d).

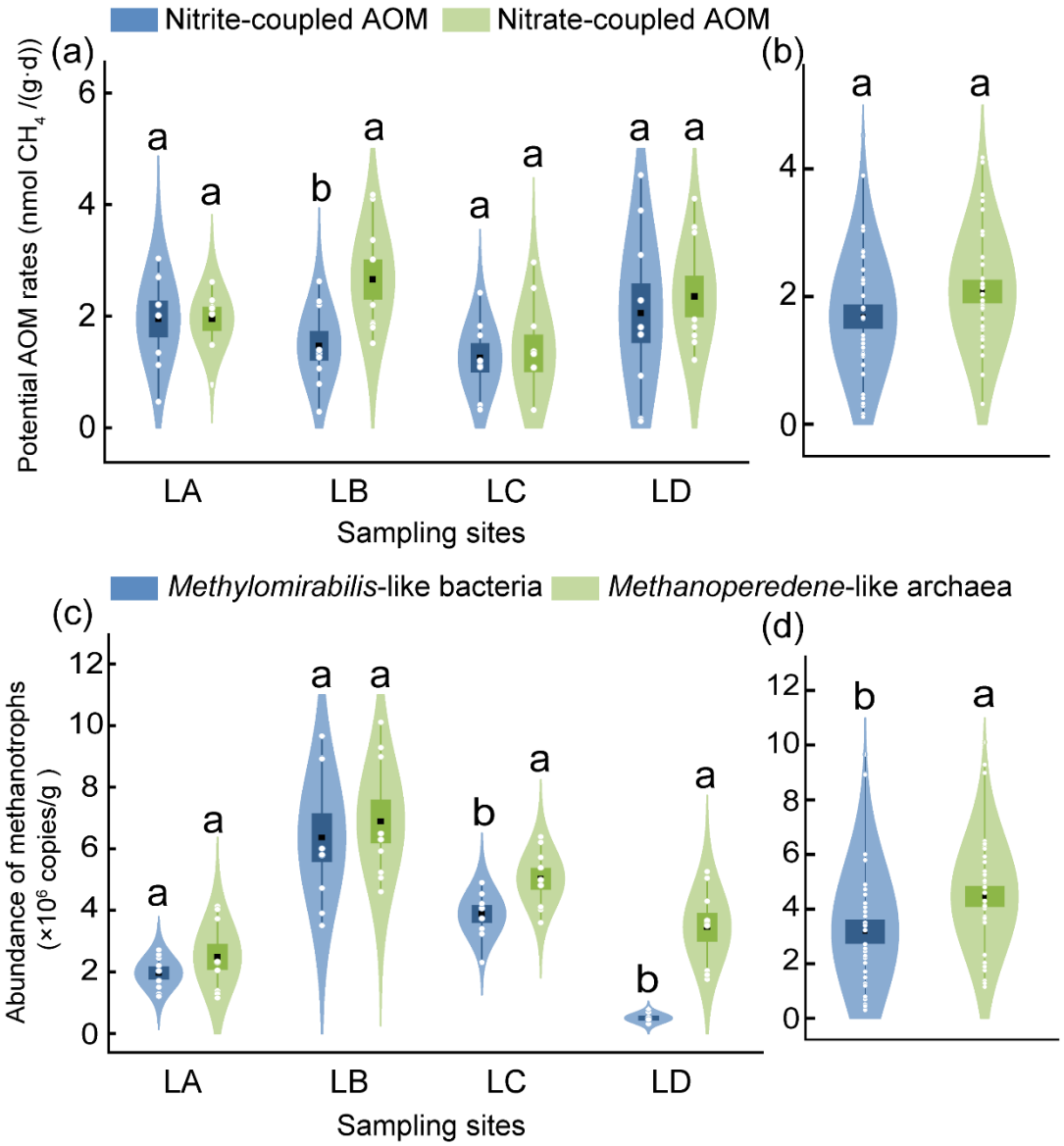

Fig. S3 Comparison between potential nitrite- and nitrate-coupled AOM rates at each sampling site (a) or across all sampling sites (b), and comparison between abundance of *Methylomirabilis*-like bacteria and *Methanoperedens*-like archaea at each sampling site (c) or across all sampling sites (d).

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