

Supplementary Information

Rapid shifts in methanotrophic bacterial communities mitigate methane emissions from a tropical hydropower reservoir and its downstream river

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Table S1. Details of the samples for MOB community characterization analyzed in this study. Site codes and location as shown in Fig. 1.

Site	Sampling year	Location	Depth (m)	DNA and RNA	CARD-FISH
E	2017	Upstream	0	x	x
G	2017	Upstream	0	x	x
F	2017	Upstream	0	x	x
C	2017	Res. Mix. Layer	3	x	
B	2017	Res. Mix. Layer	0	x	
A	2017	Res. Mix. Layer	3	x	
A	2017	Res. Mix. Layer	7	x	
C	2017	Res. Oxic Hypo.	19	x	x
B	2017	Res. Oxic Hypo.	11	x	
B	2017	Res. Oxic Hypo.	22	x	x
A	2017	Res. Oxic Hypo.	14	x	
B	2017	Res. Hypoxic Hypo.	27	x	x
A	2017	Res. Hypoxic Hypo.	20	x	x
Dw-I	2017	Downstream	0	x	x
Dw-II	2017	Downstream	0	x	x
H	2018	Upstream	0	x	x
E	2018	Upstream	0	x	
G	2018	Upstream	0	x	
F	2018	Upstream	0	x	
C	2018	Res. Mix. Layer	0	x	
B	2018	Res. Mix. Layer	0	x	
A	2018	Res. Mix. Layer	0	x	
I	2018	Res. Mix. Layer	0	x	
C	2018	Res. Oxic Hypo.	6	x	
C	2018	Res. Oxic Hypo.	23	x	x
B	2018	Res. Oxic Hypo.	10	x	
B	2018	Res. Oxic Hypo.	24	x	x
A	2018	Res. Oxic Hypo.	10	x	
C	2018	Res. Hypoxic Hypo.	10	x	x
A	2018	Res. Hypoxic Hypo.	16	x	x
A	2018	Res. Hypoxic Hypo.	20	x	x
A	2018	Res. Hypoxic Hypo.	32	x	x
I	2018	Res. Hypoxic Hypo.	24	x	x
I	2018	Res. Hypoxic Hypo.	12	x	
I	2018	Res. Hypoxic Hypo.	13	x	x
Dw-I	2018	Downstream	0	x	x
Dw-II	2018	Downstream	0	x	x

Table S2. Group specific 16S rRNA targeted probes used for the detection of Proteobacterial methane-oxidizing bacteria. Probes M γ 84 and M γ 705 were used together as suggested in Eller et al. (2001).

Probe	Target group	Probe sequence
M α 450	Alphaproteobacteria MOB	5'-ATC CAG GTA CCG TCA TTA TC-3'
M γ 84	Gammaproteobacteria MOB	5'-CCA CTC GTC AGC GCC CGA-3'
M γ 705	Gammaproteobacteria MOB	5'-CTG GTG TTC CTT CAG ATC-3'

Reference:

Eller G, Stubner S, Frenzel P. 2001. Group-specific 16S rRNA targeted probes for the detection of type I and type II methanotrophs by fluorescence in situ hybridisation. *FEMS Microbiol Lett*; 198: 91–97.

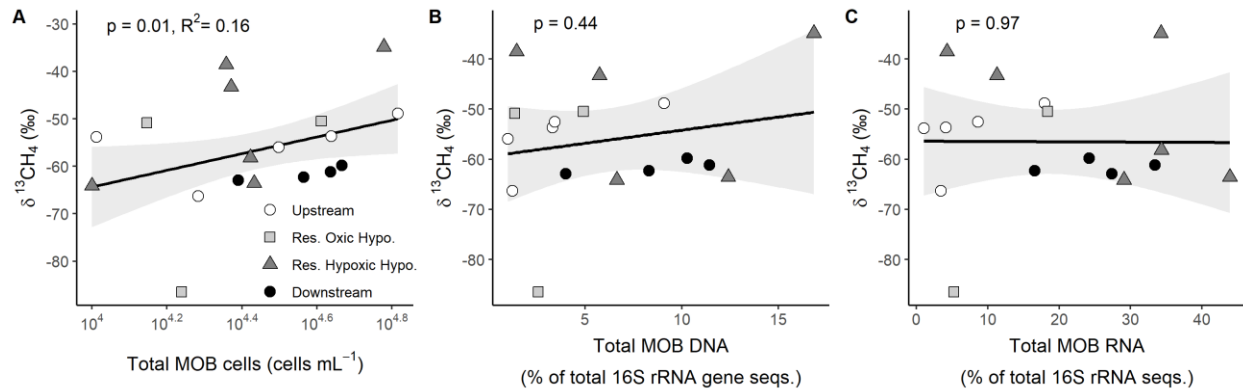


Figure S1. Correlation between methane-oxidizing bacteria (MOB) and $\delta^{13}\text{CH}_4$ in Batang Ai river-reservoir system. A) Total MOB cells (determined by CARD-FISH). B) Total MOB DNA (% of total 16S rRNA gene sequences). C) Total MOB RNA (% of total 16S rRNA sequences). Plotted data does not include the reservoir mixed layer, since MOB cells count was not determined in this layer due to the very low MOB sequences abundance (<0.1%). Note log scale in the x axis of A.

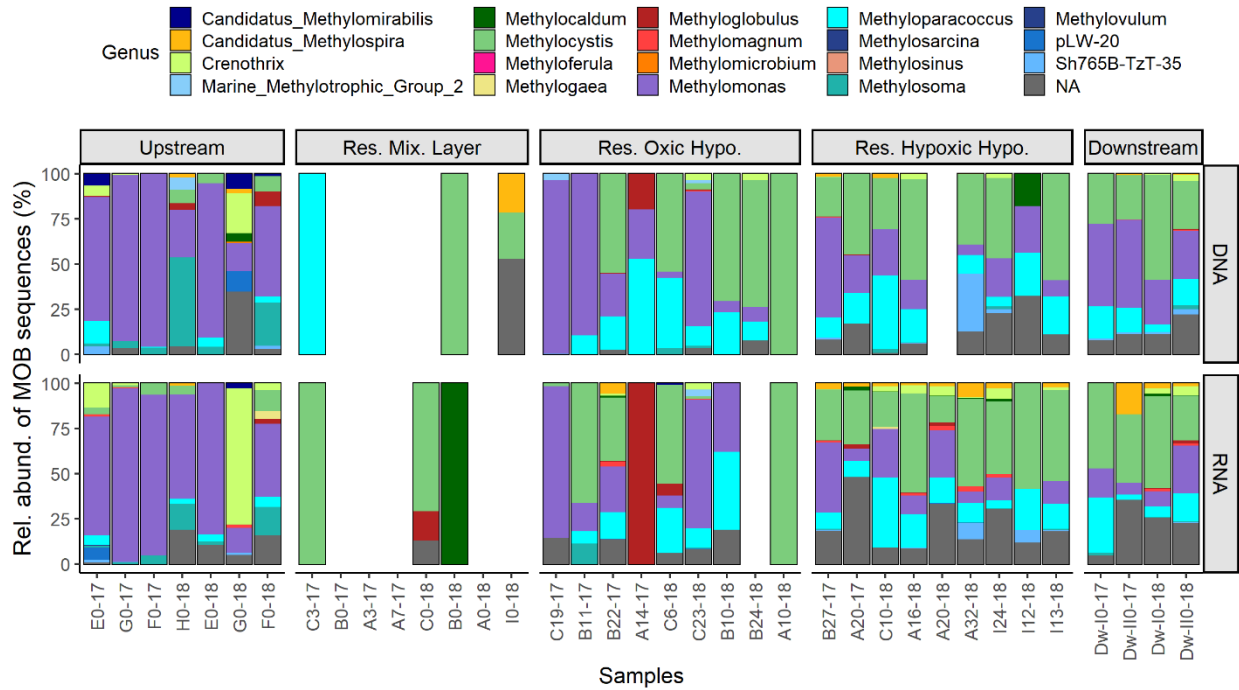


Figure S2. Relative abundance (%) of sequences belonging to all methane-oxidizing bacteria (MOB) genera detected across the dataset (sum of all MOB sequences in a sample = 1). Each bar represents a sample taken at a different campaign, site, and depth. In the x axis, the letters indicate the site code as in Fig. 1, followed by two numbers indicating the sampling depth and the sampling campaign, respectively (2017 or 2018). Empty slots mean that no MOB was detected in the sample (Res. Mixed Layer) or that sample was lost during rarefaction (Res. Oxidic Hypo. and Res. Anoxic Hypo.). NA genus includes unclassified Methylomonaceae and Methylococcaceae (Gamma-MOB) ASVs.

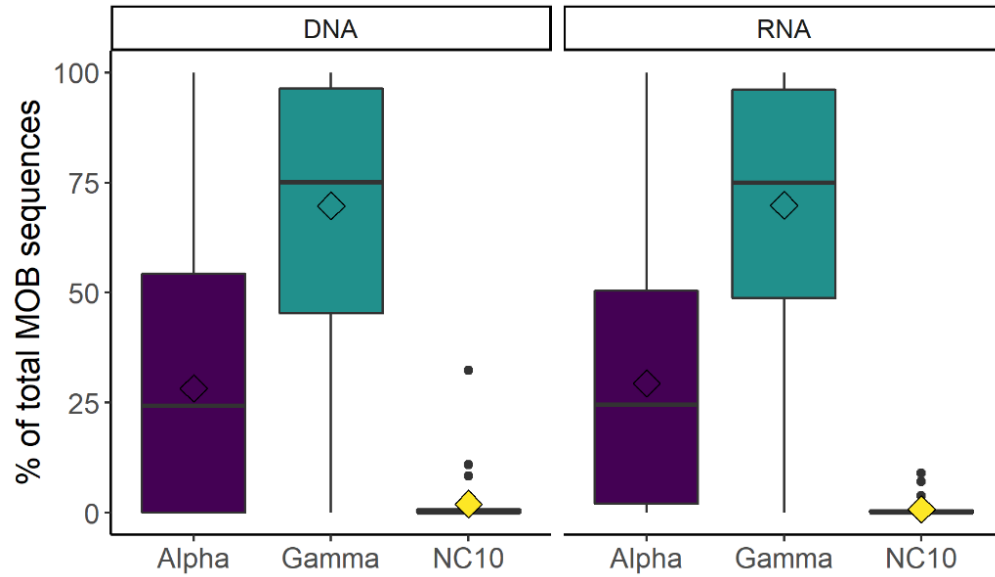


Figure S3. Contribution of each methane-oxidizing bacteria (MOB) group detected in this study to the total MOB 16S rRNA sequences across samples. Alpha: Alphaproteobacteria MOB; Gamma: Gammaproteobacteria MOB; NC10: candidate phyla NC10 MOB. Boxplots represent median, first and third quartiles (hinges), and 1.5 x interquartile range (whiskers). Diamonds denote means.

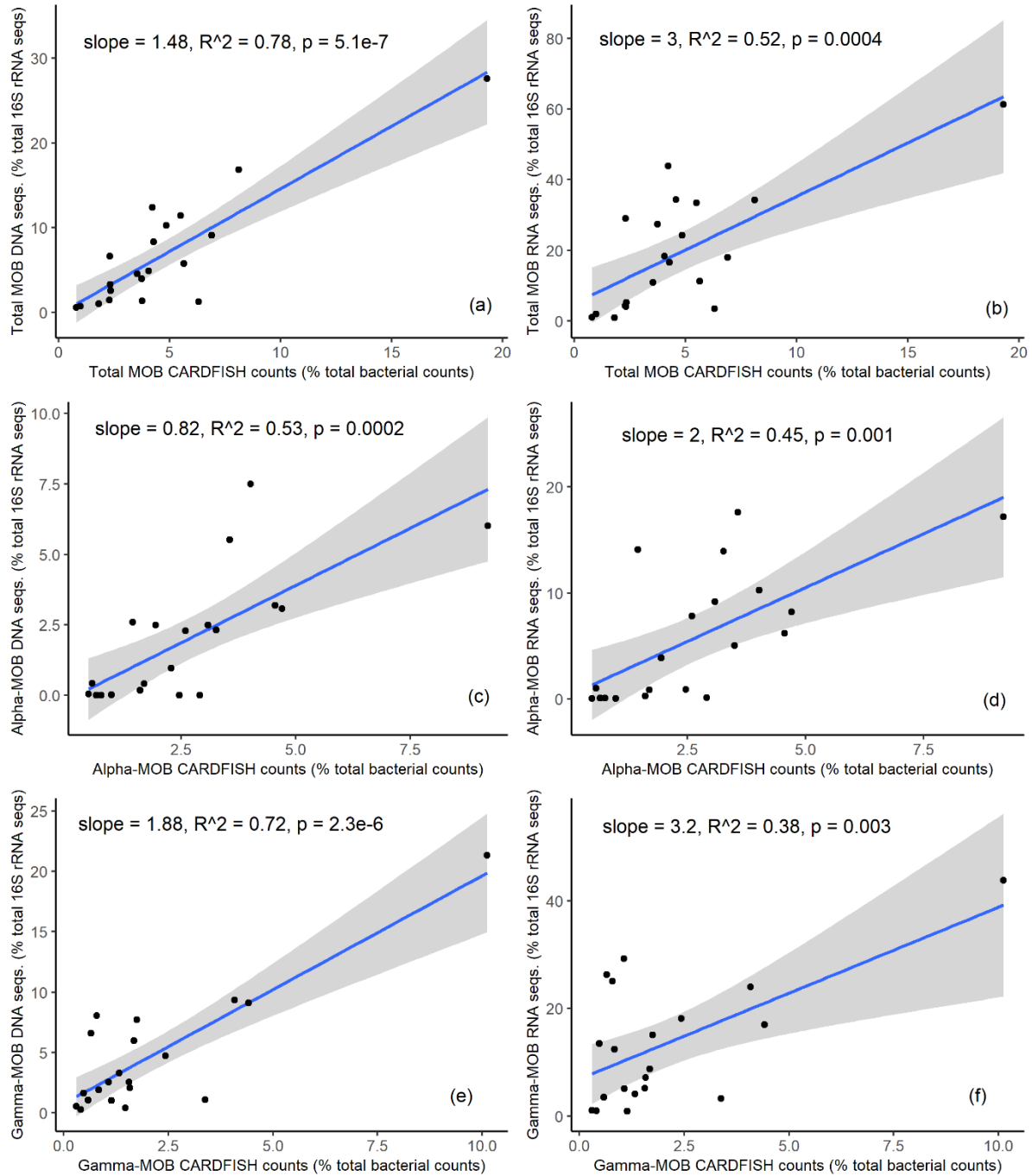


Figure S4. Correlation between CARD-FISH MOB cell counts (% of total DAPI counts) and MOB 16S rRNA sequences (DNA and RNA) (% of total bacterial 16S rRNA sequences) across samples in Batang Ai reservoir. a-b) total Proteobacteria MOB (Alpha- and Gamma-MOB). c-d) Alphaproteobacteria MOB. e-f) Gammaproteobacteria MOB.

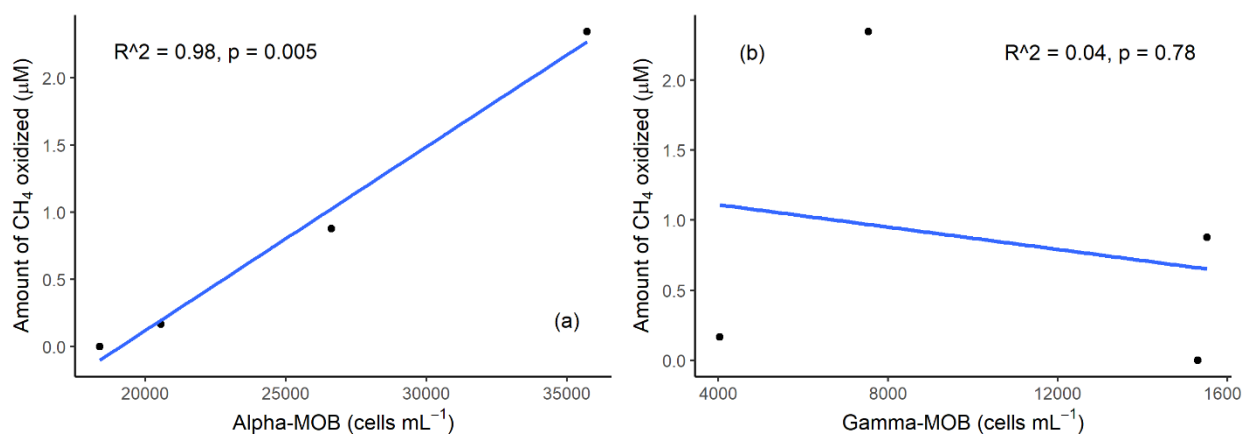


Figure S5. Correlation between MOB cells and amount of CH₄ oxidized from the intake of the turbines in the hypolimnion of the reservoir to the power station (0 Km) and from 0 Km to 2.7 Km further downstream at both sampling campaigns. a) Alphaproteobacteria MOB cells. b) Gammaproteobacteria MOB cells.

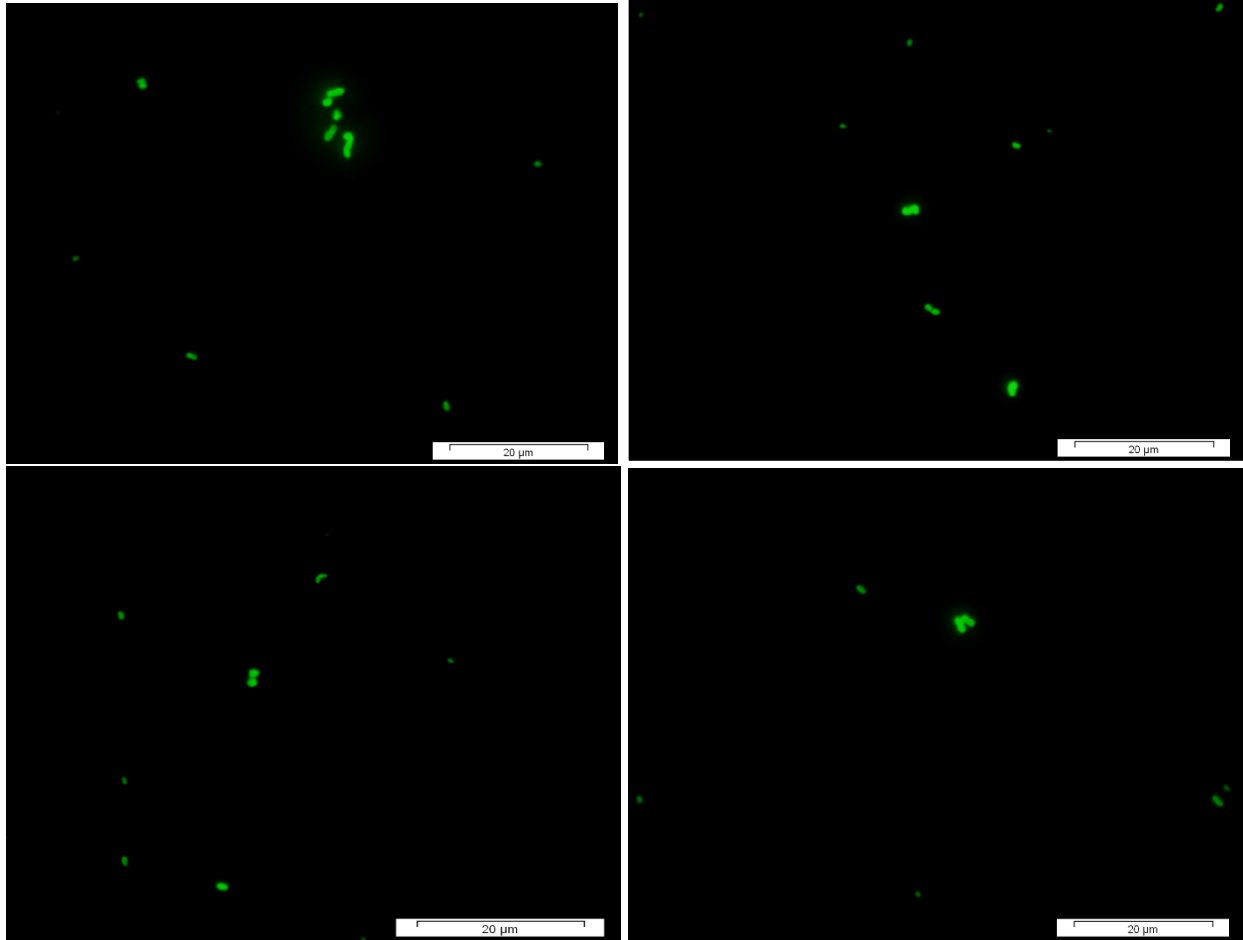


Figure S6. Potentially dividing Alphaproteobacteria MOB cells in the downstream river section between 0 and 2.7 Km downstream of the dam. Cells under the microscope visualized by CARD-FISH (Catalyzed Reporter Fluorescent In Situ Hybridization) using specific probes. Whole scale bars represent 20 µm.

Calculating MOB cell growth in the downstream segment

We estimated the potential increase in MOB cell abundance over the downstream segment (from 0 to 2.7 Km downstream of the dam) using the equation:

$$\Delta Cells_{MOB} = \left(\frac{Coxid \times MBGE}{BactC \times MOBVol} \right) \div 1000$$

where $\Delta Cells_{MOB}$ and $Coxid$ are the MOB cells produced ($cells\ mL^{-1}$) and the measured amount of CH_4 oxidized ($\mu g\ C\ L^{-1}$; see model developed in Soued and Prairie 2020 for details) within the river segment, respectively. $MBGE$ is the assumed methanotrophic bacterial growth efficiency, $BactC$ is bacterial carbon content in $\mu g\ C\ \mu m^{-3}$ ($63 \times 10^{-9}\ \mu g\ C\ \mu m^{-3}$; Fagerbakke et al. 1996), and $MOBVol$ is the average MOB cell volume in μm^3 calculated from the measured area of the DAPI subset of FISH positive cells in this dataset assuming a round shape of cells.

References:

- Fagerbakke, K. M., M. Heldal, and S. Norland. 1996. Content of carbon, nitrogen, oxygen, sulfur and phosphorus in native aquatic and cultured bacteria. *Aquat. Microb. Ecol.* 10: 15–27. doi:10.3354/ame010015
- Soued, C., and Y. T. Prairie. 2020. The carbon footprint of a Malaysian tropical reservoir : measured versus modeled estimates highlight the underestimated key role of downstream processes. *Biogeosciences* 17: 515–227. doi:10.5194/bg-2019-385