Interactive comment on "Methane emissions from a sediment-deposited island in a Lancang-Mekong reservoir" by Wenging Shi et al.

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Referee # 2

The manuscript presents an interesting strategy to estimate the spatial contribution of CH₄ emissions from a sediment-deposited island in the Lancang-Mekong reservoir. While most of the studies in reservoirs are focused in estimate CH₄ emissions in water sites, this study calls the attention to consider the sediment-island CH₄ dynamics during reservoir operations (water level fluctuations). I think the study is relevant as strategy in such ecosystems (sediment-deposited island), but there are major clarifications that should be addressed before going a step forward in the acceptance.

Microbial analysis issues.

- 1. Due to the methodology of sampling (no with a core system) and different qPCR programs (I understand but you need explain it); methanotrophy and methanogenesis statements are overstated in the whole manuscript. I would recommend focusing more into the water level fluctuation to explain the CH₄ dynamics obtained. Additionally, Figure 5 contains an artifact in the axis to pronounce your statements in the manuscript, please use the same axis level to avoid confusion, even a statistical analysis comparing each site (methanogens vs methanotrophs) and whole samples may help to approve such patterns.
 - Many thanks for the valuable suggestions that have enabled us to improve the manuscript.
 - In this study, frequent reservoir operation induced lateral hyporheic exchange and created oxygen gradients along the hyporheic flow path across the island (Fig. 1), regulating microbes for CH₄ emissions. We analyzed the horizontal heterogeneity of microbial abundance here to clarify spatial patterns of CH₄ emission. For the

vertical heterogeneity of bacterial community structure, we can design and conduct further studies using a core system according to the referee's suggestions.

- The qPCR program mainly depends on the sequence and length of functional genes and primers used, and different qPCR programs for archaeal 16S rDNA and *pomA* were applied in this study. This information has been updated. Please see line 176-181 on page 9 in the revised manuscript.
- In this study, we found that microbial processes regulated by water level fluctuation were responsible for CH₄ emission patterns, had presented more statements on water level fluctuation than microbial processes (See the Discussion Section). Nevertheless, we have further simplified the statements about methanotrophy and methangenesis according to the referee's suggestions. Please see line 272-275 on page 14 in the revised manuscript.
- Methanogens and methanotrophs are two different microbial species, and their abundances often have different orders of magnitude in nature. For the comparison between methanogens and methanotrophs, it might be more meaningful to compare their relative values among sampling sites (Fig. 2 below). The Fig.2 below has been combined into Fig.5 and this information has been accordingly updated. Please see line 234-235 on page 12 in the revised manuscript.



Fig. 1 The horizontal heterogeneity in the interior of island under reservoir operation



Fig. 2 The ratio of methanogen to methanotroph abundance at different sampling sites

CH₄ measurements.

- 2. While, the title of the manuscript is about CH₄ emission. You have only one figure (Figure 4), and a small result description from the same figure (section 3.3) about CH₄ emission patterns. Moreover, Figure 4 is over interpreted by the extrapolation, which is valid, but you should explain the range of uncertain.
 - The objective of this study is to explore spatial patterns of CH₄ emissions from the sediment-deposited island and the underlying mechanisms. The data were presented in the manuscript as follows: Under reservoir operation, frequent water level fluctuations enhanced hyporheic exchange (Fig. 3) and created oxygen gradients (Fig. 2) along the hyporheic flow path, regulating microbial processes (Fig. 5) for CH₄ emission patterns (Fig. 4). We have revised the title to "Methane emissions from a sediment-deposited island in a Lancang-Mekong reservoir: Spatial heterogeneity and mechanisms" to make it more appropriate.
 - The range of uncertain was 0.05 mg/h m². This information has been updated, and please see line 160-161 on page 8 in the revised manuscript.
- 3. I have seen CH₄ consumption in flux measurements in soils, but in water determinations are very strange. If you are confident of your measurements, I encourage you to include the data obtained, and explore relations with other parameters measured in the study.

- Thanks for the comments. There might be some misunderstanding here. It is CH₄ production in flux measurements in water determinations. All the original data about CH₄ fluxes at each sampling site (Fig. 3) are presented in Table 1 below.
- In this study, the oxygen gradients induced by reservoir operation were responsible for the spatial patterns of oxygen-sensitive CH4 emissions. Some other parameters, such as temperature, EC and pH, are indeed irrelevant and have been removed, and please see line 195-201 on page 10 in the revised manuscript.



Fig. 3 The map of sampling sites in this study

Table 1 The data about CH₄ fluxes at each sampling site

Sites		CH ₄ concentration in the chamber (×10 ⁻⁶ mol/L)						Flux	Flux	Average flux
Sites		0 min	10 min	20 min	30 min	40 min	(×10 ⁻⁶ mol/h)	(×10 ⁻⁶ mol/h m ²)	(mg/h m ²)	(mg/h m ²)
R1	Rep-1	0.452	0.773	1.174	1.375	1.656	1.806	57.5	0.92	
	Rep-2	0.641	0.921	1.280	1.680	2.160	2.279	72.6	1.16	1.07
	Rep-3	0.541	1.106	1.272	1.637	2.103	2.193	69.8	1.12	
R2	Rep-1	0.797	0.887	1.232	1.778	2.081	2.075	66.1	1.06	
	Rep-2	1.166	1.431	1.722	2.112	2.778	2.343	74.6	1.19	1.13
	Rep-3	0.823	0.869	1.197	3.825	4.273	NA	NA	NA	
R3	Rep-1	0.678	1.075	1.809	2.243	3.264	3.804	121.1	1.94	
	Rep-2	0.822	1.261	2.282	2.702	3.704	4.323	137.7	2.20	2.10
	Rep-3	1.243	1.543	2.246	2.969	4.043	4.216	134.3	2.15	

R4	Rep-1	0.573	0.660	0.759	1.018	1.189	0.954	30.4	0.49	
	Rep-2	0.390	0.553	0.665	0.797	0.829	0.673	21.4	0.34	0.39
	Rep-3	0.476	0.622	0.762	0.863	0.909	0.665	21.2	0.34	
R5	Rep-1	1.867	2.871	4.802	6.432	8.739	10.383	330.7	5.29	
	Rep-2	1.294	2.554	4.565	6.582	9.349	12.082	384.8	6.16	5.47
	Rep-3	1.188	2.165	4.289	4.913	7.935	9.745	310.4	4.97	
	Rep-1	0.493	1.175	1.608	2.142	2.678	3.203	102.0	1.63	
R6	Rep-2	0.881	1.411	1.881	2.751	3.561	4.021	128.1	2.05	1.84
	Rep-3	0.677	1.432	1.381	2.988	3.463	NA	NA	NA	
	Rep-1	0.388	0.802	0.923	1.224	1.482	1.566	49.9	0.80	
R7	Rep-2	0.653	0.876	1.875	1.896	2.165	NA	NA	NA	0.84
	Rep-3	0.794	0.930	1.420	1.609	1.902	1.737	55.3	0.89	
	Rep-1	0.971	1.626	2.515	3.401	4.015	4.718	150.3	2.40	
R8	Rep-2	0.879	0.997	1.377	1.995	2.469	2.508	79.9	1.28	1.84
	Rep-3	0.726	0.726	1.543	1.945	2.807	3.605	114.8	1.84	
	Rep-1	0.463	0.434	0.358	0.295	0.200	-0.399	-12.7	-0.20	-0.21
1	Rep-2	0.454	0.414	0.358	0.255	0.157	-0.452	-14.4	-0.23	
	Rep-3	0.386	0.314	0.258	0.214	0.139	-0.356	-11.3	-0.18	
2	Rep-1	0.878	1.213	1.556	2.009	2.810	2.796	89.0	1.42	
	Rep-2	0.784	0.970	1.432	1.945	2.442	2.575	82.0	1.31	1.26
	Rep-3	0.993	1.187	1.534	1.898	2.358	2.065	65.8	1.05	
3	Rep-1	1.068	1.483	2.425	3.567	4.738	5.654	180.1	2.88	
	Rep-2	1.282	1.919	2.799	3.304	4.482	4.671	148.8	2.38	2.78
	Rep-3	1.482	1.949	2.876	3.967	5.519	6.055	192.8	3.09	
4	Rep-1	2.893	6.897	8.251	12.608	16.811	20.13	641.1	10.26	
	Rep-2	3.892	7.996	10.451	15.906	17.211	20.73	660.2	10.56	10.41
	Rep-3	1.278	2.301	4.061	5.868	8.291	NA	NA	NA	
	Rep-1	0.786	NA	2.784	4.324	NA	NA	NA	NA	
5	Rep-2	0.876	1.805	3.180	4.625	5.769	7.564	240.9	3.85	4.27
	Rep-3	1.069	2.075	3.208	5.141	7.203	9.201	293.0	4.69	
	Rep-1	1.221	1.519	1.697	1.875	1.933	1.068	34.0	0.54	
6	Rep-2	0.782	0.819	0.999	1.153	1.338	0.868	27.6	0.44	0.49
	Rep-3	1.887	1.267	1.756	2.154	2.375	NA	NA	NA	
-	Rep-1	0.456	0.476	0.481	0.493	0.507	0.071	2.3	0.04	0.02
7	Rep-2	0.479	0.489	0.499	0.505	0.515	0.05	1.0	0.03	0.05
	Rep-3	0.378	0.387	0.002	0.011	0.02	0.003	2.1	0.03	
	Rep-1	0.399	0.408	0.417	0.422	0.428	0.045	1.4	0.02	0.02
0	Rep-2	0.430	0.471	0.482	0.505	0.512	0.080	2.7	0.04	0.05
	Rep-3	0.400	0.497	0.505	0.517	0.519	0.049	1.0	0.02	
0	Rep-1	0.078	0.0708	0.531	0.528	0.521	-0.003	-0.2	0.00	0.01
,	Rep-2	0.487	0.330	0.331	0.528	0.321	-0.032	-1.0	-0.02	-0.01
	Rep-1	0.407	0.703	0.40	0.583	0.472	0.023	-0.7	-0.01	
10	Rep-2	0.495	0.502	0.506	0.512	0.519	0.035	1.2	0.02	0.02
10	Rep-2	0.785	0.701	0.500	0.802	0.517	0.035	1.1	0.02	0.02
	Kep-5	0.765	0.791	0.795	0.802	0.009	0.0555	1.1	0.02	

	Rep-1	0.555	0.5545	0.5538	0.5535	0.5528	-0.003	-0.1	0.00	
11	Rep-2	0.453	0.454	0.454	0.455	0.456	0.004	0.1	0.00	0.00
	Rep-3	0.768	0.745	0.755	0.777	0.815	NA	NA	NA	
	Rep-1	0.787	1.199	1.443	2.108	2.588	2.707	86.2	1.38	
12	Rep-2	0.722	0.805	1.119	1.403	1.844	1.705	54.3	0.87	1.12
	Rep-3	0.477	0.674	0.846	0.876	1.532	NA	NA	NA	
	Rep-1	0.491	1.118	1.694	2.171	2.847	3.46	110.2	1.76	
13	Rep-2	0.493	1.015	1.290	1.965	2.390	2.85	90.8	1.45	1.61
	Rep-3	NA	0.564	NA	0.762	1.438	NA	NA	NA	
	Rep-1	0.393	0.444	0.477	0.521	0.532	0.021	0.7	0.01	
14	Rep-2	0.420	0.425	0.430	NA	0.439	0.029	0.9	0.01	0.01
	Rep-3	0.422	0.427	0.432	0.435	0.440	0.0265	0.8	0.01	
	Rep-1	0.754	0.733	0.695	0.677	0.644	-0.166	-5.3	-0.08	
15	Rep-2	0.472	0.463	0.455	0.447	0.435	-0.054	-1.7	-0.03	-0.05
	Rep-3	0.511	0.493	0.475	0.457	0.447	-0.098	-3.1	-0.05	
	Rep-1	0.567	0.540	0.525	0.511	0.503	-0.094	-3.0	-0.05	
16	Rep-2	0.853	0.840	0.835	0.821	0.809	-0.064	-2.0	-0.03	-0.04
	Rep-3	0.454	0.440	0.435	0.411	0.398	-0.085	-2.7	-0.04	
	Rep-1	0.263	0.564	0.672	0.675	0.785	NA	NA	NA	
17	Rep-2	0.567	0.570	0.572	0.574	0.575	0.012	0.4	0.01	0.01
	Rep-3	0.368	0.370	0.375	0.377	0.381	0.02	0.6	0.01	

- 4. Did you sample only once per time in each chamber? If you did it, you have large uncertain in your values, since there are a lot of risk to have leaks and lost sample before determining in the gas chromatograph. Which was the volume of gas pre-evacuated in each vial?
 - We are sorry for the unclear descriptions about gas flux analysis. Here, methane fluxes at each site were measured in triplicate by placing three individual chambers. This information has been updated, and please see line 157-158 on page 8 in the revised manuscript.
 - The volume of pre-evacuated vial is 12 ml. During gas collection, the vial was filled with 20 ml of gas sample for storage, and it is easy to draw 10 ml of gas sample from the vial using the syringe for analysis using gas chromatograph. The information about the vial size has been updated. Please see line 152 on page 8 in the revised manuscript.

General statements.

- 5. There are several statements that you are not able to support with your data, it would help you make a list of different parameters obtained and see how to relate them. Then, avoid such interpretation about redox changes, exudates, among others.
 - Thanks for the critical comments. We have deleted the statements that we are not able to support with our data, such as exudates and the strategy for the mitigation of methane emission hotspots in reservoir islands, and changed the description about redox changes to oxygen changes. Please see line 24 on page 2, line 67, 71 on page 4, line 76 on page 5, line 238 on page 12, line 260, 265 on page 13, line 285 on page 14, line 309 on page 15, and line 321, 322 on page 16 in the revised manuscript.
- 6. Sections 4.2 and 4.3 contain several discussions unnecessary (they are more introduction than discussion) about the relevance of the CH₄ emission from aquatic ecosystems, how methane is produced biologically, the environment relevance of reservoirs, shelf mitigation, limitations using Darcy fluxes.
 - Many thanks for the valuable comments that have enabled us to improve the manuscript. We have deleted or removed the introduction-like discussions to Introduction Section (eg., the descriptions about hyporheic zone, the relevance of the CH₄ emission from aquatic ecosystems, how methane is produced biologically), updated inappropriate discussions (e.g., shelf mitigation), and deleted unnecessary discussions (e.g., limitations using Darcy fluxes). Please see line 43-47 on page 3, line 81 on page 5, line 295-299 on page 15 and line 318, 323-325 on page 16 in the revised manuscript.

- 1 Methane emissions from a sediment-deposited island in a Lancang-Mekong
- 2 reservoir: Spatial heterogeneity and mechanisms
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12 Abstract

In dammed rivers, sediment accumulation creates potential methane emission hotspots, 13 which have been extensively studied in forebays. However, methane emissions from 14 sidebays remain poorly understood. We investigated methane emissions from a 15 sediment-deposited island situated in the sidebay of the Manwan Reservoir, Lancang-16 Mekong River. High methane emissions (maximum 10.4 mg $h^{-1}m^{-2}$) were observed at 17 the island center, while a ring-like zone of low-to-negative methane emission was 18 discovered at the drawdown area around the island edge, whose flux varied between -19 $0.2-1.6 \text{ mg h}^{-1}\text{m}^{-2}$. The ring-like zone accounted for 89.1 % of the island area, of which 20 9.1 % was a methane sink zone. Microbial processes in the drawdown area, regulated 21 by hydrological variations, were responsible for the low methane flux in this area. 22 23 Under reservoir operation, frequent water level fluctuations enhanced hyporheic exchange and created oxygen gradients along the hyporheic flow path. Dissolved 24 oxygen in hyporheic water decreased from 4.80 mg L^{-1} at the island bank edge to 0.43 25 mg L^{-1} at the center, which in turn decreased methanogen abundance for methane 26 production and increased methanotroph abundance for methane oxidation at the ring-27 like zone. This study quantified the methane emissions from sediment deposited islands 28 in the reservoir and helps to evaluate the global warming effects of hydropower systems. 29

30 **1 Introduction**

Natural rivers form continuous ecosystems, in which physical and chemical factors 31 32 drive biological processes from headwaters to river deltas (Butman and Raymond, 2011; Wilkinson et al., 2015). Along this continuum, rivers receive terrestrial organic carbon 33 (OC) and deliver it to the ocean at a global average rate of approximately 400–900 Tg 34 OC per year (Butturini et al., 2016; Seitzinger et al., 2005; Ran et al., 2013). In the past 35 two decades, many rivers have become intensively regulated by dams for a variety of 36 purposes, including improved navigation, water supply, flood control, and hydropower 37 38 production (Maavara et al., 2015). These engineering works decrease water velocity, converting rivers into a series of lentic reservoirs, where sediment accumulates in 39 forebays and sidebay islands (Maeck et al., 2013). Globally, the sediment accumulation 40 41 process has reduced the river-to-ocean flux of terrestrial OC by 26 % (Syvitski et al., 2005). 42

Methane is the second most important greenhouse gas, contributing approximately 43 44 18 % to total global warming effects (Smith et al., 2013; Wuebbles and Hayhoe, 2002). 45 Inland waters (lakes, rivers, and reservoirs) are significant sources of atmospheric methane, which is mainly released from anoxic sediment (Bastviken et al., 2011; Sobek 46 et al., 2012). After river damming, settling particles aggregate to form cohesive 47 sediment layers, which often become anoxic after oxygen is consumed but not 48 replenished through diffusive exchange (Rubol et al., 2013; Maeck et al., 2013). 49 Subsequently, large amounts of methane may be produced and released into the 50 atmosphere (Thornton et al., 1990; Maeck et al., 2013; Wilkinson et al., 2015), thereby 51

reducing the green credentials of hydropower. This issue has received considerable 52 attention in dammed rivers (Giles, 2006; Hu and Cheng, 2013). Maeck et al. (2013) 53 54 identified reservoirs as methane emission hotspots by comparing reservoir and riverine reaches, and estimated that global methane emissions have increased by 7 % due to 55 sedimentation in dammed rivers. In sidebays, the deposited sediments often created the 56 drawdown area under water level fluctuations, where water, heat, nutrients and 57 chemicals are exchanged and many biogeochemical reactions preferentially occur 58 (Tonina and Buffington, 2011; Cardenas and Markowski, 2010), potentially emitting 59 60 large amounts of greenhouse gases. Previous studies have mainly focused on methane emissions from dam forebays (Yang et al., 2013; DelSontro et al., 2010; DelSontro et 61 al., 2011), while the understandings of methane emissions from sediments deposited in 62 63 sidebays remain poor.

In reservoirs, frequent water level fluctuations often occur following hydropower 64 production demands, which enhances hyporheic exchange by driving water flow in and 65 66 out of the drawdown area (Tonina and Buffington, 2011; Hucks Sawyer et al., 2009). This may lead to changes of oxygen conditions in the interior of the drawdown area. 67 Zarnetske found a redox gradient along the hyporheic flow paths in a third-order stream 68 in the Willamette River basin, USA (Zarnetske et al., 2011a). Methane from sediments 69 is mainly produced by anaerobic methanogens, and is consumed by aerobic 70 methanotrophs (Borrel et al., 2011). We suppose the shift in oxygen conditions in 71 sediments may affect the microbial processes, thereby altering the methane emission 72 scheme. 73

In this study, methane emissions from a sediment-deposited island were investigated 74 in the sidebay of Manwan Reservoir, Lancang-Mekong River. Monitoring wells were 75 76 established to probe hyporheic exchange and oxygen gradients across the island. Methanogen and methanotroph abundances in the sediment were analyzed using 77 78 quantitative polymerase chain reaction (qPCR) to reveal the associated molecular mechanism. The objective of this study was to explore methane emissions from 79 sediment-deposited zones in a sidebay of a dammed river, with the goal to guide future 80 thorough assessments on the global warming effects of hydropower development. 81

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Methods 83 2

2.1 Study area 84

85 The Lancang-Mekong River, a trans-boundary river in Southeast Asia, originates from the Tibetan Plateau and discharges into the South China Sea. It has a length of 4909 km, 86 a watershed area of 760,000 km², and a mean annual runoff of 457 km³ at a discharge 87 of 14,500 m³ s⁻¹ (Li et al., 2013). The Lancang-Mekong Basin can be divided into two 88 parts: the "upper basin" in China, and the "lower basin" from Yunnan in China to the 89 90 Southeast Asia. Until 2016, seven dams have been built for hydropower production in the upper Lancang-Mekong River in China, including Miaowei, Gongguogiao, 91 Xiaowan, Manwan, Dachaoshan, Nuozhadu and Jinghong. The locations and main 92 features of these dams were shown in Fig. 1 and Table S1 in the Supplements, 93 94 respectively.



After impoundment, several different types of islands formed in the reservoir (Fig.

S1). This study selected a typical island for investigation (182 m in length, 90 m in
width), which is located at the convex bank (24°43′44″ N, 100°23′5″ E) in the sidebay
of Manwan reservoir, 30 km away from the dam (Fig. 1). Manwan has a subtropical
plateau monsoon climate, featuring no distinct seasons. Under reservoir operation, the
island bank is frequently flooded (Fig. S2).

101 **2.2 Monitoring wells**

Ten monitoring wells were installed in the island bank at 0.5 (W1), 1.5 (W2), 3.5 (W3), 102 6.5 (W4), 10.5 (W5), 15.5 (W6) 20.5 (W7), 25.5 (W8), 30.5 (W9), and 35.5 m (W10) 103 104 from the waterline, respectively (Fig. S2). The wells were 90-mm diameter perforated polyvinylchloride pipes, reaching a depth of 2.0 m below the ground surface. To prevent 105 flooding, the wells were extended 2.0 m aboveground. Due to hydropower production, 106 107 the reservoir runs in a pseudo-periodic hydrological regime with cyclic water level fluctuations. Here, we monitored a complete cycle of water level fluctuation within 115 108 h. Water levels were measured every 10 min from 11 to 16 September 2016 using 109 110 automated water level recorders (U2000101, OneSetHoBo, USA), which were mounted at the bottom of W5, W7, W10, and the reservoir (Fig. S3). 111

Instantaneous lateral fluid fluxes (q) across the island bank per unit length werecalculated following the Darcy Eq. (1) (Gerecht et al., 2011; Hucks Sawyer et al., 2009):

114
$$q(t) = -Kb \cdot \left[\frac{\vartheta h(x,t)}{\vartheta x}\right]$$
(1)

where *Kb* is sediment transmissivity, m d⁻¹; *h* is hydraulic head, m; *x* is distance, m; and *t* is time, d. A positive *q* value indicates flow from the reservoir to the island. The island *Kb* was 0.99 m d⁻¹, which was measured according to Philip (1993).

118 **2.3 Sampling and physicochemical analysis**

After water level receded at the monitoring time of 100 h, groundwater (100 ml) was 119 120 carefully sampled in triplicate from each monitoring well with a portable peristaltic pump (SC-1/253Yx, Chongqing Jieheng Peristaltic Pump Co., Ltd., China), and then 121 filtered *in situ* using portable syringe filters for water DOC analysis. Sediment (5 g) 122 was synchronously collected in triplicate from 10 cm below the surface adjacent to each 123 well using a hand shovel, and then homogenized before the storage for the analyses of 124 sediment OC and microbe. At a reservoir site adjacent to W1, water and surface 125 126 sediment samples were also collected in triplicate using a stainless-steel bucket and an Ekman grab sampler, respectively. The collected water and sediment samples were kept 127 frozen in an ice box (-5 \circ C--10 \circ C) and transported to the laboratory for analysis within 128 129 three days.

Dissolved oxygen (DO) at each well was measured in situ using a multi-sensor probe 130 (YSI 6600, Yellow Springs Instruments, USA). Analysis of dissolved organic carbon 131 132 (DOC) in the water was conducted on filtered samples (Whatman GF/F, UK) using a total organic carbon analyzer (Liqui TOC II, Elementar Inc., Germany). Sediment OC 133 was determined using a vario MACRO cube elementar (Elementar Inc., Germany). 134 Fresh sediment was freeze-dried and ground before analysis. Approximately 30 mg of 135 each sample was weighed in a tin cup and acidified with two drops of 8 % H₃PO₄ to 136 remove inorganic carbonates before OC analysis. 137

138 **2.4 Methane flux analysis**

139 Methane fluxes from the reservoir (eight sampling sites) and island (seventeen sampling

sites) were analyzed using bifunctional chambers according to the static chamber 140 method (Duchemin et al., 1999). The sampling sites are shown in Fig. S4. The 141 142 plexiglass bifunctional chamber consisted of a 6.28-L cylinder (20 cm in diameter, 20 cm in height) and a removable Styrofoam collar. During gas collection in the reservoir, 143 the chamber was fitted with the Styrofoam collar, which maintained the upper closed 144 portion of the chamber about 10 cm above the water surface (Fig. S5). The outlet of the 145 chamber was open during the chamber deployment, and was left to stand for 20 min to 146 equilibrate with ambient pressure outside before sample collection. During gas 147 148 collection on the island, the chamber was carefully inserted 5 cm deep into the sediment, leaving 15 cm above the sediment surface. The outlet of the chamber was also open 149 during the chamber deployment, and was left to stand for 90 min to equilibrate before 150 151 sample collection. Gas samples (20 ml) were collected every 10 min over a 40-min period using a 25-ml polypropylene syringe and injected into a 12-ml pre-evacuated 152 Exetainer® vial (839 W, Labco, UK) for storage until analysis using a gas 153 154 chromatograph (7890B, Agilent Technologies, USA). Gas fluxes were calculated using linear regression based on the concentration changes of five samples over time. Linear 155 regression correlation coefficients of less than 0.95 were not accepted for further 156 calculations (Duchemin et al., 1999). Methane fluxes at each site were measured in 157 triplicate by placing three individual chambers. Simple spline interpolation was used to 158 interpolate the methane emissions from the sampling sites into space in the reservoir 159 and island separately (Immerzeel et al., 2009), and the range of the uncertain was 0.05 160 $mg h^{-1} m^{-2}$. Methane emission areas at eight different categories were also calculated in 161

the island.

163 **2.5 Microbial abundance analysis**

After being transported to the laboratory, the frozen sediment samples were stored 164 immediately at -80 $\,^{\circ}$ C for further molecular analysis. The sediment methanogens and 165 methanotrophs adjacent to each monitoring well across the island (ten sediment samples) 166 were quantified using qPCR. DNA extraction was undertaken using a FastDNA Power-167 Max Soil DNA Isolation Kit (MP Biomedical, USA) according to the manufacturer's 168 instructions. The qPCR assay was performed using primers targeting methanogenic 169 170 archaeal 16S rDNA (primer set, 1106F/1378R) and methanotrophic pmoA genes (primer set, A189F/M661R) (Watanabe et al., 2007; Ma and Lu, 2011). Gene copies 171 were amplified and quantified in a Bio-Rad cycler equipped with the iQ5 real-time 172 173 fluorescence detection system and software (version 2.0, Bio-Rad, USA). All reactions were completed in a total volume of 20 µL containing 10 µL SYBR[®] Premix Ex TagTM 174 (Toyobo, Japan), 0.5 mM of each primer, 0.8 μL of BSA (3 mg mL⁻¹, Sigma, USA), 175 176 ddH₂O, and template DNA. The qPCR program mainly depends on the sequence and length of functional genes and primers used, and different qPCR programs for archaeal 177 16S rDNA and *pomA* were applied in this study. The qPCR program for archaeal 16S 178 rDNA was as follows: 95 $^{\circ}$ C for 60 s, followed by 40 cycles of 95 $^{\circ}$ C for 25 s, 57 $^{\circ}$ C 179 for 30 s, and 72 °C for 60 s, and the qPCR program for *pomA* referred to: 95 °C for 60 180 s, followed by 40 cycles of 95 °C for 25 s, 53 °C for 30 s, and 72 °C for 60 s. A standard 181 curve was established by serial dilution $(10^{-2}-10^{-8})$ of known concentration plasmid 182 DNA with the target fragment. All PCRs were run in triplicate on 96-well plates (Bio-183

184 Rad, USA) sealed with optical-quality sealing tape (Bio-Rad, USA). Three negative185 controls without the DNA template were included for each PCR run.

186 **2.6 Data analysis**

One-way analysis of variance (ANOVA) was employed to test the statistical significance of differences between sampling sites. Post-hoc multiple comparisons of treatment means were performed using the Tukey's least significant difference procedure. All statistical calculations were performed using the SPSS (v22.0) statistical package for personal computers. The level of significance was P < 0.05 for all tests.

192

193 **3 Results**

3.1 Physicochemical characteristics

As shown in Fig. 2, the island groundwater had lower DO but higher DOC, compared with that of the bulk reservoir water. Lateral gradients of groundwater DO and DOC were observed in the island. From the island edge to the center, DO and DOC decreased significantly from 4.80 ± 0.19 to 0.43 ± 0.09 mg L⁻¹ and 7.30 ± 0.54 to 1.70 ± 0.39 mg L⁻¹, respectively (P < 0.05) (Fig. 2a,b). In general, sediment OC was higher near the island edge, decreasing from 6.37 ± 0.69 mg g⁻¹ at the edge to 2.42 ± 0.60 mg g⁻¹ at the center of the island. Sediment OC in the reservoir was 6.63 ± 0.09 mg g⁻¹ (Fig. 2c).

3.2 Water level fluctuation and hyporheic exchange

203 The reservoir stage fluctuated frequently during the field survey, showing three distinct

peaks, with a maximum of 3.80 m in the first 37 h and gradual decline to below 1.30 m

in the next 60 h, yielding a maximum oscillation of 2.54 m. Similar oscillations were

206	observed in the island water table, but were damped and lagged relatively to the
207	reservoir stage fluctuations (Fig. 3a). In W5, W7, and W10, the water levels reached
208	3.27, 3.41, and 3.33 m, then fell to 1.74, 2.09, and 2.01 m, for a maximum oscillation
209	of 1.53, 1.33, and 1.32 m, respectively. Data from the automated water level recorders
210	indicated that the water level responses in W5, W7, and W10 lagged the reservoir stage
211	by 20, 25, and 30 min, respectively. Lateral hyporheic exchanges across the island bank
212	were calculated according to the Darcy Law, showing that the flux was largest at the
213	island edge and decreased from the edge to the center. The water exchange across the
214	0-10.5 m island edge zone was 1.2 and 4.7 times higher than those across the $10.5-20.5$
215	m and 20.5–35.5 m zones, respectively. The flow rates at the reservoir-W5, W5-W7,
216	and W7-W10 zones were relatively consistent at -0.55-1.35, -0.89-0.28, and -0.39-
217	0.17 m ² d ⁻¹ (Fig. 3b), resulting in a water exchange volume of 2.61, 2.26, and 0.56 m ³ ,
218	respectively, over the 115-h observation period.

219 **3.3 Methane emissions**

High methane emission rates were observed at the island sites, with a maximum of 10.4 220 $mg h^{-1}m^{-2}$ at the center. However, a large ring-like low methane emission zone appeared 221 at the drawdown area around the island edge, where the methane flux was maintained 222 at -0.2–1.6 mg $h^{-1}m^{-2}$ (Fig. 4a). The negative flux values also suggest the occurrence of 223 a methane sink at the island edge. The ring-like zone accounted for 89.1 % of the island 224 area, of which 9.1 % accounted for the methane sink zone (Fig. 4b). Compared with the 225 island, the methane flux from the adjacent reservoir was moderate at 0.4–5.5 mg h⁻¹m⁻ 226 ² (Fig. 4a). 227

228 **3.4 Methanogen and methanotroph abundances**

Methanogens and methanotrophs were distributed non-uniformly across the island. In 229 230 general, methanogen counts were low at the island edge but high at the center, whereas methanotrophs were abundant at the island edge but scarce in the center. From the island 231 232 edge to the center, the methanogenic archaeal 16S rDNA gene increased from 0.12 \times 10^5 to 5.34 $\times 10^5$ copies g⁻¹, and the methanotrophic *pmoA* gene decreased from 1.57 \times 233 10^6 to 0.64×10^6 copies g⁻¹ (Fig. 5a). The ratio of methanogen to methanotroph 234 abundance increased from 0.01 at the island edge to 0.83 at the center (Fig. 5b). 235 236 **4** Discussion 237 4.1 Hyporheic exchange and oxygen gradients 238

In hydropower reservoirs, the release of water pulses is often employed to increase 239 power production and meet daily electricity peak demand (Bonalumi et al., 2012; 240 Toffolon et al., 2010). Such hydropeaking creates daily water level fluctuations in the 241 242 reservoir. In this study, frequent water level fluctuations were observed within the 115h observation period, with a maximum of 3.80 m (Fig. 3a). A hysteretic response 243 occurred in the island bank water table (Fig. 3a), driving water exchange between the 244 reservoir and island (Fig. 3b). The water exchange flux was largest close to the island 245 edge and decreased from the edge to the center, as water table fluctuations were 246 attenuated (Fig. 3a). 247

During a storage-release cycle, the island switched from water gaining to losing at daily or hourly scales, creating a ring-like drawdown area of enhanced hyporheic

exchange around the island. The drawdown area extended tens of meters into the island 250 bank (Fig. 3b). If the river system was unregulated, however, hydrodynamics within the 251 252 drawdown area would likely exhibit seasonal or annual patterns, or keep pace with snowmelt and rainstorm events, under a natural base flow-fed regime. In this case, the 253 254 drawdown area may be limited or altogether absent (Boano et al., 2008; Cardenas and Wilson, 2007). 255

Exchange across the sediment-water interface involves mixing of surface water and 256 groundwater through hyporheic flow (Hester et al., 2013; Naranjo et al., 2015). In this 257 258 study, when the reservoir water entered the hyporheic flow path, it was typically rich in oxygen (Fig. 2d). As oxygen was consumed through aerobic respiration, other terminal 259 electron acceptors were utilized (Klupfel et al., 2014), creating an oxygen gradient 260 261 along the hyporheic flow path (Fig. 2d). Changes in sediment moisture can speed up the mineralization of organic matter (Wang et al., 2010; Rubol et al., 2014). 262 Groundwater DOC showed a general decrease from the island edge to center (Fig. 2e). 263 264 This hyporheic exchange clearly affected biogeochemical processes, and had important effects on hyporheic microbial communities, especially oxygen-sensitive species. For 265 example, we detected poor methanogen abundance at the island edge, but rich 266 abundance at the center, with methanotrophs showing the opposite pattern (Fig. 5). 267

268

4.2 Spatial heterogeneity of methane emissions

In dammed rivers, riverbed sediment accumulation creates potential methane emission 269 270 hotspots. In this study, however, high methane emissions were only observed at the island center, with a ring-like low methane emission zone or even methane sink 271

appearing around the island edge (Fig. 4a). This was attributed to the spatial 272 heterogeneity of methanogens and methanotrophs across the island (Fig. 5), leading to 273 274 an increase in methane production and a decrease in methane consumption from the island edge to the center. The methane sink at the island edge (Fig. 4) was mainly 275 276 attributed to the strong oxidation by methanotrophs, which may consume methane to below equilibrium with the atmosphere. Methane emissions may not only rely on 277 bacterial abundance but also bacterial activity (Schwarz et al., 2008). This deserves 278 further studies using other molecular biology techniques, such as DNA/RNA-based 279 280 stable isotope probing (Dumont and Murrell, 2005).

Groundwater DOC and sediment OC at the island edge, which are carbon sources for 281 methane emission, were higher than that at the island center (Fig. 2b,c), suggesting that 282 283 both sediment heterogeneity and dilution effects of hyporheic exchange had limited contribution to the spatial pattern of methane emissions in the island. Hyporheic 284 exchange effectively shifted oxygen gradients across the island, resulting in substantial 285 mitigation of potential methane emissions. In this study, only 0.2 % of the island area 286 maintained a high methane flux $(9.6-11.2 \text{ mg h}^{-1}\text{m}^{-2})$ (Fig. 4b), suggesting that methane 287 emissions across the small island were attenuated, but only in the drawdown area where 288 hyporheic exchange occurred. 289

290 **4.3 Implications**

Greenhouse gas emissions significantly detract from the green credentials of hydropower, and have thus received considerable research attention (Giles, 2006; Hu and Cheng, 2013). Previous studies have revealed that damming causes significant

retention of carbon and creates deep, anoxic sediment strata, fueling methanogenesis 294 and net water-air methane flux (Maeck et al., 2013). This study demonstrated that 295 296 methane emissions at the most area of the sediment-deposited island were generally lower than the adjacent reservoir under reservoir operation (Fig. 4a), but higher than the 297 drawdown area at other reservoir bank, such as Three Gorge Reservoir (Chen et al., 298 2011). This was mainly due to the deep sediment strata (about 60 m in depth) in the 299 island. Given the widely distributed sediment-deposited islands in reservoirs, it should 300 be of concern in future estimations of greenhouse gas emissions from dammed rivers. 301 302 Prospective studies should assess the quantitative relationship between methane emissions from the drawdown area and hydropower operation scenarios. 303

Until now, few studies have concentrated on organic carbon mineralization in the 304 305 drawdown area in reservoirs, with most focusing on the process of denitrification (Zarnetske et al., 2011b). Carbon emissions in the drawdown area are poorly understood, 306 especially in regulated and dammed rivers. This study fills the knowledge gap and adds 307 to our understanding of the ecological impacts of hydropower exploitation. Under 308 reservoir operation, variable oxygen conditions and methane production may also affect 309 the mercury cycle in the drawdown area and thereby the release of methylmercury (a 310 bioaccumulative environmental toxicant) to the river (Marvin-DiPasquale et al., 2009), 311 a subject deserving of further study. 312

313

314 **5** Conclusions

In dammed rivers, sediment deposited islands are widely distributed in sidebays and are

potential hotspots of methane emission to the atmosphere. In this study, high methane 316 fluxes were only observed at the island center, while a ring-like zone of low methane 317 318 emission or even sink was found in the drawdown area around the island edge. We attribute this spatial heterogeneity of methane emissions to hyporheic exchange 319 between the reservoir and island. Under reservoir operation, frequent water level 320 fluctuations drove hyporheic exchange, creating oxygen gradients along the hyporheic 321 flowpath. These oxygen gradients affected the microbial communities associated with 322 methane production and consumption, producing the spatial heterogeneity in methane 323 324 emissions across sediment-deposited islands. This study will help us to evaluate the global warming effects of hydropower systems. 325

326

327 Data availability

328 The data presented here can be obtained upon request to Wenqing Shi (wqshi@nhri.cn).

329

330 Author contribution

Qiuwen Chen designed the research; Wenqing Shi, Yuyu Ji and Yuchen Chen performed
the research; Cheng Chen and Juhua Yu contributed new reagents/analytic tools;
Qiuwen Chen and Wenqing Shi analyzed the data; Jianyun Zhang and Bryce R. Van
Dam contributed significant discussions and inputs; Wenqing Shi and Qiuwen Chen
wrote the paper with input from all authors.

336 Competing interests

337 The authors declare that they have no conflict of interest.

338

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Fig. 1 Map of the studied island in Manwan reservoir, Lancang-Mekong River.





Fig. 3 Vertical water level fluctuation (a) and horizontal hyporheic flow rate (b). R =
reservoir, W = monitoring wells. Positive fluxes indicate net flow from the reservoir to
island, whereas negative values indicate net flow from the island to reservoir.



493

494 Fig. 4 Methane emissions from the island and reservoir. (a) Spatial pattern of methane

emissions; (b) Percentage of the island area emitting methane at a certain flux. Methane

496 fluxes were interpolated separately for the island and reservoir.



Fig. 5 Abundances of sediment methanogens and methanotrophs in the island. (a)
Spatial patterns of methanogen and methanotroph abundances across the island; (b) The
ratio of methanogen to methanotroph abundance at each site. W= monitoring wells.
Error bars indicate standard deviations.