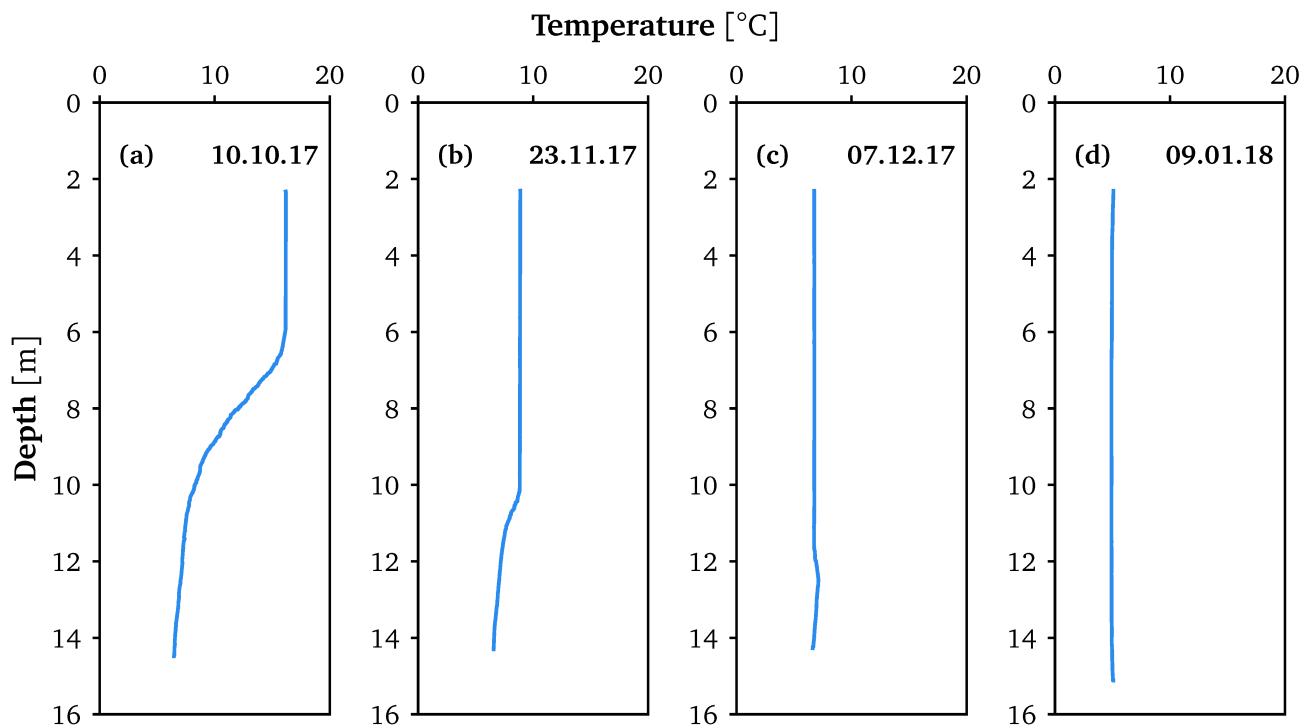
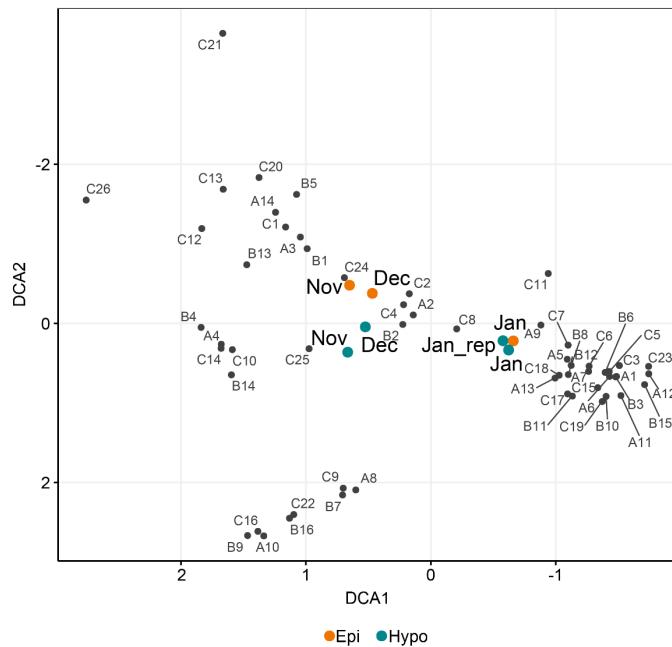


**Supplementary Figures**



**Supplementary Figure 1** Temperature profiles recorded at our sampling dates. A straight line from the top indicates the depth of the mixed layer, which has a homogeneous temperature profile. The inverse stratification in (c) can be explained by an increase in salinity towards the bottom.



**Supplementary Figure 2** Detrended correspondence analysis (DCA) of combined pmoABC TPM data (square root transformed) in November, December and January 2017/2018 during overturn in Rotsee. Sample scores are shown as big colored dots (orange = epilimnion and cyan = hypolimnion). Sequence variant scores are shown as smaller dots in grey. The DCA is based on transcripts per million mapped at 99% identity. pmoABC variants were assembled from metagenomes and metatranscriptomes sampled at same depths and dates as shown in Figure 1. Identified variants of each gene are numbered and abbreviated as *pmoA* (A), *pmoB* (B), *pmoC* (C).

## Supplementary Tables

**Supplementary Table 1 Summary of estimated parameters for Monod-kinetics of methane oxidation rates based on incubation experiments with natural water samples from above and below oxycline in Rotsee. Further, MOB cell numbers based on CARD-FISH data and in-situ measurements of methane oxidation rates are shown.**

	Oct Epilimnion	Oct Hypolimnion	Nov Epilimnion	Nov Hypolimnion	Dec Epilimnion	Dec Hypolimnion	Jan Epilimnion	Jan Hypolimnion
Sampling date	10.10.2017	10.10.2017	23.11.2017	23.11.2017	07.12.2017	07.12.2017	09.01.2018	09.01.2018
Sampling depth (m)	2	10	4	13	4	13.5	4	15
$K_m$ - Half-saturation constant ( $\mu\text{M}$ )	0.7	15.2	2.1	7.1	3.3	6.1	1.2	2.7
Standard deviation error for $K_m$	0.2	3.3	0.4	1.1	0.4	0.8	0.3	0.5
Distance of the 95% confidence interval around the optimal value for $K_m$	0.5	7.1	0.9	2.3	0.9	1.7	0.7	1.0
$V_{max}$ - Cell specific maximum methane oxidation rate ( $\text{fmol h}^{-1} \text{ cell}^{-1}$ )	1.8	0.8	8.4	0.6	4.7	1.6	0.8	0.9
Standard deviation error for $V_{max}$	0.2	0.1	0.5	0.0	0.2	0.1	0.0	0.0
Distance of the 95% confidence interval around the optimal value for $V_{max}$	0.4	0.2	1.2	0.1	0.4	0.2	0.1	0.1
$a_0$ - Specific affinity ( $V_{max}/K_m$ ) ( $10^{-12} \text{ L h}^{-1} \text{ cell}^{-1}$ )	2664	52	4170	83	1467	264	671	338
Standard deviation error for $a_0$	813	12	774	13	195	36	169	52
Distance of the 95% confidence interval around the optimal value for $a_0$	1875	26	1724	27	413	77	356	111
$V_{max}$ - Maximum methane oxidation rate ( $\mu\text{M h}^{-1}$ )	0.02	0.06	0.52	0.09	0.93	0.19	0.19	0.38
Standard deviation error for $V_{max}$	0.00	0.01	0.03	0.01	0.04	0.01	0.01	0.02
Distance of the 95% confidence interval around the optimal value for $V_{max}$	0.00	0.01	0.08	0.01	0.08	0.02	0.03	0.04
Degrees of freedom (number of samples minus one)	8	12	10	16	16	16	18	16
MOB cell numbers ( $10^5 \text{ cells mL}^{-1}$ )	0.1	0.8	0.6	1.5	2.0	1.2	2.5	4.3
<i>In-situ</i> methane oxidation rate ( $\mu\text{M h}^{-1}$ ) average of duplicates	0.01	0.11*	0.13	0.06	0.12	0.17	0.00	0.33

\*single measurement

**Supplementary Table 2** Transcripts per million (TPM) table for genes encoding pMMO (particulate methane monooxygenase) as generated from metagenomic and metatranscriptomics analysis. “MOB type” was determined by blastx against the NCBI protein\_refseq database. Type Ia and type Ib belong to *Methylococcales* (*Gammaproteobacteria*) and type II are alphaproteobacterial MOB as classified in Knief et al. (2015). Epi=Epilimnion and Hypo=Hypolimnion. Jan\_Hypo(r) is a replicate sample.

<i>pmoA</i>				Length	Transcripts per million													
MOB type	variant	gene_id	contig		Nov	Epi	Nov	Hypo	Dec	Epi	Dec	Hypo	Jan	Epi	Jan	Hypo	Jan	Hypo
type_Ia	pmoA_1	CKCHICCI_895815	k121_412951_5	744	45.1	24.8	435.3	61.7	5102.7	5395.0	9085.7							
type_Ia	pmoA_2	CKCHICCI_686699	k121_49099_2	750	6500.3	612.2	5010.5	1092.7	1436.7	1820.3	2568.0							
type_Ia	pmoA_3	CKCHICCI_783929	k121_217620_32	513	2704.4	154.8	3609.5	713.8	150.9	158.1	521.6							
type_Ib	pmoA_4	CKCHICCI_21493	k141_66195_2	744	1026.6	161.3	1695.2	301.4	41.7	27.4	29.8							
type_Ia	pmoA_5	CKCHICCI_48608	k141_151227_2	750	19.8	1.6	186.2	24.5	284.1	386.1	651.8							
type_Ia	pmoA_6	CKCHICCI_125679	k141_392308_3	750	3.7	1.3	44.4	7.3	411.5	470.3	390.5							
type_Ia	pmoA_7	CKCHICCI_291010	k141_945231_2	744	6.2	1.1	76.3	12.1	320.8	362.2	288.6							
type_Ia	pmoA_8	CKCHICCI_63876	k141_198477_2	744	18.4	316.9	12.6	271.9	8.7	100.8	53.8							
type_Ia	pmoA_9	CKCHICCI_90158	k141_280678_2	744	43.6	4.0	79.9	7.1	123.3	108.1	291.1							
type_Ia	pmoA_10	CKCHICCI_432695	k141_1455349_1	744	1.2	187.3	1.2	86.3	1.5	12.3	6.2							
type_Ia	pmoA_11	CKCHICCI_59670	k141_185354_2	750	0.1	0.0	1.5	8.5	54.1	124.4	99.0							
type_Ia	pmoA_12	CKCHICCI_648838	k141_2173000_2	744	0.0	0.0	0.7	0.1	21.4	33.9	99.2							
type_Ia	pmoA_13	CKCHICCI_602520	k141_2016551_1	621	2.1	0.6	11.1	2.2	29.0	29.0	21.2							
type_Ia	pmoA_14	CKCHICCI_261103	k141_831066_2	744	35.4	2.5	39.1	5.4	1.7	1.9	2.8							
<i>pmoB</i>				Length	Transcripts per million													
MOB type		gene_id	contig		Nov	Epi	Nov	Hypo	Dec	Epi	Dec	Hypo	Jan	Epi	Jan	Hypo	Jan	Hypo
type_Ia	pmoB_1	CKCHICCI_783928	k121_217620_31	1245	15456.6	922.2	21421.0	4342.6	916.4	1013.0	3445.7							
type_Ia	pmoB_2	CKCHICCI_1400682	k121_1348134_1	666	12404.2	1261.0	7515.9	1881.2	2191.7	2932.0	4262.6							
type_Ia	pmoB_3	CKCHICCI_2164529	k121_2924228_1	1245	53.0	29.0	501.3	76.3	5736.5	6117.8	10476.6							
type_Ib	pmoB_4	CKCHICCI_21494	k141_66195_3	1233	847.6	122.1	1505.6	234.6	13.6	18.3	20.7							
type_Ia	pmoB_5	CKCHICCI_74829	k141_232614_1	759	830.3	42.1	1041.1	171.3	58.5	37.3	123.0							
type_Ia	pmoB_6	CKCHICCI_2811216	k121_4549403_2	1245	7.3	1.9	78.2	12.1	660.1	663.0	534.7							
type_Ia	pmoB_7	CKCHICCI_63875	k141_198477_1	1245	21.6	447.2	15.4	365.7	9.4	120.7	62.6							
type_Ia	pmoB_8	CKCHICCI_48609	k141_151227_3	1245	20.2	2.7	158.4	22.5	317.2	393.4	676.0							
type_Ia	pmoB_9	CKCHICCI_432696	k141_1455349_2	1245	1.8	212.0	1.8	114.5	0.9	12.8	6.7							
type_Ia	pmoB_10	CKCHICCI_28138	k141_86940_1	1245	2.6	0.7	2.8	2.4	16.2	182.6	131.4							
type_Ia	pmoB_11	CKCHICCI_103359	k141_321668_2	1245	1.8	0.4	8.1	20.4	49.5	60.8	123.8							
type_Ia	pmoB_12	CKCHICCI_129565	k141_404791_1	825	2.0	0.4	13.0	2.9	41.6	42.7	34.5							
type_Ia	pmoB_13	CKCHICCI_261102	k141_831066_1	1245	38.4	3.3	42.7	8.0	1.1	1.8	1.8							
type_II	pmoB_14	CKCHICCI_531536	k141_1780115_1	786	40.9	8.0	17.1	5.5	1.3	0.7	1.9							
type_Ia	pmoB_15	CKCHICCI_648837	k141_2173000_1	1245	0.0	0.1	0.6	0.1	24.7	38.0	109.8							
type_Ia	pmoB_16	CKCHICCI_291739	k141_947998_2	303	3.4	146.5	1.6	73.6	1.7	11.8	11.1							
<i>pmoC</i>				Length	Transcripts per million													
MOB type		gene_id	contig		Nov	Epi	Nov	Hypo	Dec	Epi	Dec	Hypo	Jan	Epi	Jan	Hypo	Jan	Hypo
type_Ia	pmoC_1	CKCHICCI_834089	k121_303165_22	753	24801.3	1378.1	31740.6	6391.7	1273.7	1061.4	3716.8							
type_Ia	pmoC_2	CKCHICCI_686698	k121_49099_1	420	14163.3	1272.5	9145.5	2181.7	4563.7	2644.1	4059.9							
type_Ia	pmoC_3	CKCHICCI_895814	k121_412951_4	753	101.5	29.9	795.0	118.7	14303.3	9027.8	15648.9							
type_Ia	pmoC_4	CKCHICCI_142444	k141_445340_1	486	3616.0	382.7	1306.3	344.5	909.0	552.1	839.3							
type_Ia	pmoC_5	CKCHICCI_125678	k141_392308_2	753	11.9	4.8	100.3	21.4	1537.0	955.1	846.0							
type_Ia	pmoC_6	CKCHICCI_291009	k141_945231_1	753	19.2	3.9	186.1	35.7	1236.5	788.8	653.4							
type_Ia	pmoC_7	CKCHICCI_48607	k141_151227_1	753	51.9	5.2	323.2	47.8	1100.4	558.8	969.8							
type_Ia	pmoC_8	CKCHICCI_131046	k141_409481_1	753	254.8	24.1	351.5	88.9	226.9	107.4	174.1							
type_Ia	pmoC_9	CKCHICCI_63877	k141_198477_3	753	32.3	426.6	19.4	351.3	12.0	110.7	70.0							
type_Ib	pmoC_10	CKCHICCI_21492	k141_66195_1	456	316.5	51.2	429.8	83.8	19.8	9.8	7.5							
type_Ia	pmoC_11	CKCHICCI_90159	k141_280678_3	753	86.5	4.6	131.1	11.0	398.6	165.0	454.1							
type_Ia	pmoC_12	CKCHICCI_364319	k141_1229866_1	420	620.8	68.6	67.7	16.7	12.7	5.1	7.7							
type_II	pmoC_13	CKCHICCI_167310	k141_524698_3	702	351.9	30.5	73.8	14.7	8.9	6.5	5.0							
type_Ia	pmoC_14	CKCHICCI_266298	k141_850242_2	570	72.1	8.7	183.3	45.4	4.3	1.7	2.3							
type_Ia	pmoC_15	CKCHICCI_240594	k141_758815_3	753	5.9	0.4	6.3	2.9	20.2	210.9	162.7							
type_Ia	pmoC_16	CKCHICCI_482883	k141_1618043_2	753	1.5	141.1	1.6	71.8	0.8	10.3	4.7							
type_Ia	pmoC_17	CKCHICCI_82392	k141_255945_1	753	3.3	0.6	12.6	25.7	72.8	71.8	148.7							
type_Ia	pmoC_18	CKCHICCI_600687	k141_2010355_1	753	3.7	1.5	21.6	3.8	80.4	49.6	37.5							
type_Ia	pmoC_19	CKCHICCI_59669	k141_185354_1	753	0.1	0.3	2.8	8.1	36.8	78.9	81.8							
type_Ia	pmoC_20	CKCHICCI_261104	k141_831066_3	753	49.8	3.2	49.5	6.3	2.1	1.8	2.8							
type_II	pmoC_21	CKCHICCI_169023	k141_530397_1	375	65.8	2.6	23.9	2.9	1.8	1.3	0.6							
type_Ia	pmoC_22	CKCHICCI_394865	k141_1330390_2	753	1.7	45.8	0.5	23.5	0.0	6.7	4.9							
type_Ia	pmoC_23	CKCHICCI_648839	k141_2173000_3	753	0.0	0.0	1.0	0.1	34.5	32.7	114.7							
type_Ia	pmoC_24	CKCHICCI_921527	k121_458352_96	423	41.9	5.1	9.5	1.5	4.7	3.2	4.4							
type_II	pmoC_25	CKCHICCI_2982492	k121_4952763_1	375	28.1	3.5	15.7	5.4	3.0	3.0	1.7							
type_Ia	pmoC_26	CKCHICCI_237408	k141_748766_3	765	30.4	2.9	15.4	2.7	0.0	0.0	0.0							

**Supplementary Table 3** Transcripts per million (TPM) table for *mmoX*, *mmoY*, *mmoZ*, which encode part of the soluble methane monooxygenase as generated from metagenomic and metatranscriptomics analysis. “MOB type” was determined by blasting (blastx) the gene against the NCBI protein\_refseq database. Type Ia belong to *Methylococcales* (*Gammaproteobacteria*) as classified in Knief et al. (2015). Epi=Epilimnion and Hypo=Hypolimnion. TPM of *mmoXYZ* showed a very low abundance throughout our sampling campaign.

<i>mmoX</i>					Transcripts per million														
MOB type	variant	gene_id	contig	Length	Nov	Epi	Nov	Hypo	Dec	Epi	Dec	Hypo	Jan	Epi	Jan	Hypo	Jan	Hypo	rep
type Ia	mmoX_1	CKCHICCI_119928	k141_374180_2	1584	0.9	0.1	0.9	0.3	0.1	0.0	0.0	0.3							
type Ia	mmoX_2	CKCHICCI_614426	k141_2057240_1	1584	0.0	3.3	0.0	1.1	0.0	0.0	0.0	0.0							
type Ia	mmoX_3	CKCHICCI_1871328	k121_2298038_2	1584	0.2	0.0	0.4	0.1	0.0	0.0	0.0	0.0							
type Ia	mmoX_4	CKCHICCI_3043207	k121_5099499_1	1218	0.0	0.2	0.0	0.1	0.0	0.0	0.0	0.0							
<i>mmoY</i>					Transcripts per million														
MOB type	variant	gene_id	contig	Length	Nov	Epi	Nov	Hypo	Dec	Epi	Dec	Hypo	Jan	Epi	Jan	Hypo	Jan	Hypo	rep
type Ia	mmoY_1	CKCHICCI_614427	k141_2057240_2	1179	0.0	2.2	0.0	1.0	0.0	0.0	0.0	0.0							
type Ia	mmoY_2	CKCHICCI_1160907	k121_893959_4	1179	0.9	0.0	0.8	0.3	0.1	0.0	0.0	0.1							
type Ia	mmoY_3	CKCHICCI_1871329	k121_2298038_3	1179	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0							
type Ia	mmoY_4	CKCHICCI_2602160	k121_4062417_1	570	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0							
<i>mmoZ</i>					Transcripts per million														
MOB type	variant	gene_id	contig	Length	Nov	Epi	Nov	Hypo	Dec	Epi	Dec	Hypo	Jan	Epi	Jan	Hypo	Jan	Hypo	rep
type Ia	mmoZ_1	CKCHICCI_207359	k141_653022_3	498	1.3	0.0	1.8	0.3	0.1	0.0	0.0	0.3							
type Ia	mmoZ_2	CKCHICCI_1871331	k121_2298038_5	501	0.1	0.0	0.2	0.1	0.0	0.0	0.0	0.0							
type Ia	mmoZ_3	CKCHICCI_2602162	k121_4062417_3	498	0.0	0.1	0.0	0.1	0.0	0.0	0.0	0.0							