Supplemental Information with “Methane oxidation in anoxic lake water stimulated by nitrate and sulfate addition” (van Grinsven et al.)
**Fig. S1.** Methane concentration over time in the incubation experiment with 12 m depth summer samples, with oxygen (grey triangles) or humic substance (orange circles) added. No significant change in methane concentration over time was observed, the $R^2$ of the linear regression analysis was 0.04 for the oxygen addition experiment and 0.03 for the experiment with the addition of humic substances.
Fig. S2. PmoA distribution of incubation experiments in summer (A) and winter (B). Numbers are provided in Table S3.
Fig. S3. The abundance of methanotrophic community members (as detected by 16S rRNA gene amplicon sequencing) in the winter incubation experiments performed with water from 17 m depth.
**Fig. S4.** Genome-inferred metabolic pathways of MAG bin-63. Pathways indicated in green were detected, sequences of grey pathways were lacking. Genes were indicated where possible; numbers refer to EC database numbers. For details, see Supplementary File S1.

**Mixed acid fermentation**

\[ \text{Pyruvate} \rightarrow \text{Oxalacetate} \rightarrow \text{Malate} \rightarrow \text{Fumarate} \rightarrow \text{Succinate} \]

\[ \text{Lactate} \rightarrow \text{Acetyl-CoA} \rightarrow \text{Acetyl-P} \rightarrow \text{Acetate} \]

\[ \text{H}^+ \leftrightarrow \text{H}_2 \]

**Aerobic respiratory chain**

\[ \text{Cyt. bc1} \rightarrow \text{Cyt. bd} \rightarrow \text{Na}^+ \rightarrow \text{NQR} \rightarrow \text{type I NADH dehydr.} \rightarrow \text{type II NADH dehydr.} \]

\[ \text{O}_2 \rightarrow \text{Na}^+ \rightarrow \text{NAD}^+ \rightarrow \text{NADH} \rightarrow \text{H}^+ \]
**Fig. S5.** Maximum likelihood phylogenetic tree based on 34 concatenated single-copy, protein-coding genes (following the method of Dombrowski et al., 2018) of the three highest average abundance MAGs (i.e. bin-63, bin-37, and bin-19) detected in the winter incubation experiment under anoxic conditions and amended with methane.
Fig. S6. GC coverage plots including the contigs of the 10 most abundant bins obtained in the sequenced sample with (A) the taxonomic classification of GTDB-Tk at the level of family, and (B) with the taxonomic classification of CheckM, indicating that the MAG bin-63, indicated in dark blue in panel B, was affiliated to the family Methylomonadaceae, indicated in teal in panel A.
Table S1. Methane oxidation rates (MOR) and additional information regarding the rate measurements. The R² given is the R² of the linear regression analysis used to determine the methane oxidation rate. The oxidizing equivalents surplus/deficiency indicates the µM of methane that could have been oxidized by the electron acceptor, after the amount that was oxidized within the 24 h incubation experiment was deducted.

| Depth (m) | MOR (µM day⁻¹) | t₀ [CH₄] | oxidized in 24 h (%) | R² | [NO₃⁻] or [SO₄²⁻] (µM) | Potential CH₄ oxidation by [NO₃⁻] or [SO₄²⁻] (µM) | Oxidizing equivalents surplus/deficiency (µM) |
|----------|----------------|----------|---------------------|----|-------------------------|-----------------------------------------------|---------------------------------------------|
| 3        | -              | 2.7      | -                   | 0.01 | 116                     | 73                                           | 72                                          |
| 5        | -              | 2.2      | -                   | 0    | 146                     | 92                                           | 62                                          |
| 7        | 18             | 28       | 63                  | 0.96 | 124                     | 78                                           | 20                                          |
| 9        | 7.3            | 102      | 7                   | 0.2  | 74                      | 47                                           | -26                                         |
| 12       | 9.2            | 107      | 9                   | 0.25 | 146                     | 92                                           | 28                                          |
| 15       | 46             | 286      | 16                  | 0.42⁵ | 146                     | 92                                           | 28                                          |
| 17       | 36             | 398      | 9                   | 0.21⁵ | 146                     | 92                                           | 28                                          |
| 5        | 0.4            | 0.7      | 57                  | 0.8  | 2230                    | 2230                                         | 2229                                        |
| 7        | 30             | 9.6      | 100                 | 0.65 | 2159                    | 2159                                         | 2150                                        |
| 9        | 58             | 113      | 51                  | 0.98 | 2272                    | 2272                                         | 2155                                        |
| 12       | 72             | 158      | 46                  | 0.71 | 2267                    | 2267                                         | 2176                                        |
| 15       | 64             | 156      | 41                  | 0.9  | 2194                    | 2194                                         | 2037                                        |
| 5        | 0.5            | 0.7      | 70                  | 0.08 | 2230                    | 2230                                         | 2229                                        |
| 7        | 13             | 9        | 100                 | 0.84 | 2159                    | 2159                                         | 2150                                        |
| 9        | 52             | 117      | 44                  | 0.96 | 2272                    | 2272                                         | 2155                                        |
| 12       | 40             | 91       | 44                  | 0.76 | 2267                    | 2267                                         | 2176                                        |
| 15       | 74             | 158      | 47                  | 0.92 | 2194                    | 2194                                         | 2037                                        |
| Winter, methane addition | 3 | - | 176 | - | 0.01 | | |
| Winter, anoxic + methane addition | 7 | - | 134 | - | 0.01 | | |
| Winter, natural | 17 | 7.7⁴ | 151 | 5 | 0.06 | | |

a the methane oxidation rate is based on the linear regression analysis, whilst the actual methane oxidation consumption in the incubation vials was lower due to methane limitation.

b a subset of the data points was used (t₈, t₁₂ and t₂₄)

c Using a 8:3 ratio of NO₃⁻:CH₄ and a 1:1 ratio of SO₄²⁻:CH₄ (Segarra et al. 2013)

d Low R² of regression analysis
Table S2. Relative abundances (%) of known methanotrophs detected in the Lacamas Lake water column and incubation experiments (C denotes control) as determined by 16S rRNA gene amplicon sequencing. Any group with a percentage <0.1% is considered not significant and displayed as zero. Total reads obtained are indicated. Total 16S rRNA gene copies per liter were determined using quantitative PCR of the total prokaryotic community, with SD being the standard deviation of 3 experimental replicates. The taxonomic assignment of each of the mentioned methanotrophs is shown in Fig. 3.

|               | SUMMER |               | WINTER |               |               |
|---------------|--------|---------------|--------|---------------|---------------|
|               | Natural conditions (depth in m) | Incubations (12 m) | Natural conditions (depth in m) | Incubations (12 m) | Incubations (17 m) |
|               | 3 5 7 9 12 15 17 | C NO$_3^-$ SO$_4^-$ | 3 5 12 15 17 | C CH$_4$/oxic CH$_4$/anoxic | C CH$_4$/oxic CH$_4$/anoxic |
| CABC2E06      | 0 0.2 0.5 0.3 0.1 0.1 0.1 | 0.9 1 0.4 | 0 0 0 0 0 | 0 0.3 1 | 0 0.4 0.9 |
| Crenotrix spp. | 0 0 0.1 0.1 0 0 0 | 0.3 0.4 0.6 | 0 0.1 0 0 0 | 0.1 0.3 | 0 0.1 0.4 |
| Methylobacter spp. | 0 0.1 0.3 0.4 0.6 0.7 0.8 | 12 11 17 | 0.4 0.5 0.8 0.6 0.9 | 0.7 7 35 | 0.2 6.9 13 |
| Methylophilus spp. | 0 0.1 0.5 0.4 0.2 0.1 0.1 | 1.7 3.1 2.3 | 0 0 0 0 0 | 0 0 0 | 0 0 0 |
| pLW-20       | 0 0.4 3.3 2.6 0.5 0.1 0 | 1.7 4 1.3 | 0 0 0 0 0 | 0 0.1 0.2 | 0 0 0.1 |
| Unidentified Methylococcaceae | 0 0.1 0.3 0.2 0.1 0 0 | 0.8 1.4 1.3 | 0 0 0 0 0 | 0 0 0 | 0 0.2 0.1 |
| Reads per sample (x10$^6$) | 1.7 1.4 1.9 1.8 0.9 1.5 1.7 | 1.5 1.8 1.5 | 8.8 1.7 1.4 2 1.6 | 1.7 1.6 1.3 | 1.4 1.3 1.5 |
| Total 16S rRNA gene copies L$^{-1}$ (x10$^9$) | 4.1 5 4.6 5.4 5.5 6.8 3.1 | 10 3.2 3.3 | 2 3.4 2.8 3.1 3.6 | 7.1 4.8 11 | 1.6 2.2 2.4 |
| SD            | 0.3 0.2 0.6 0.8 0.7 0.9 0.7 | 2.3 0.4 0.3 | 0.3 0.2 0.2 0.3 0.7 | 1.1 0.4 0.8 | 0.3 0.2 0.1 |
Table S3. *pmoA* relative abundance (%) in samples of the summer and winter water column and incubations (C denotes control). The tentative taxonomic assignment of the denovo sequences can be observed in Figure 5.

|                | SUMMER |                | WINTER |                |                |
|----------------|--------|----------------|--------|----------------|----------------|
|                | Natural conditions (depth in m) | Incubations (12 m) | Natural conditions (depth in m) | Incubations (12 m) | Incubations (17 m) |
|                | 3 5 7 9 12 15 17 | C NO₃²⁻ SO₄²⁻ | 3 5 12 15 17 | C CH₄/oxic CH₄/anoxic | C CH₄/oxic CH₄/anoxic |
| LL-pmoA-1      | 12 0.6 0.2 0.6 16 85 93 | 60 39 78 | 94 96 96 97 96 | - 97 97 | 94 96 96 |
| LL-pmoA-2      | 0.3 0 0 0 0.3 1.5 1.5 | 1.3 1.7 1.7 | 1.7 1.9 1.7 1.8 1.8 | - 1.7 2 | 2.3 2.3 1.8 |
| LL-pmoA-3      | 78 43 9.9 4.9 3.8 0.6 0.8 | 9.9 6.4 0.6 | 3.4 1.7 1.7 1.5 1.9 | - 1.6 0.7 | 3.7 2.1 1.7 |
| LL-pmoA-4      | 9.9 56 90 95 80 13 4.2 | 29 53 19 | 0.8 0.4 0.5 0.2 0.1 | - 0.1 0.1 | 0.1 0 0.1 |
**Table S4.** Characteristics of the MAGs affiliated to methanotrophs of the Methylococcales which are discussed in the text. Classification was inferred by GTDB-Tk. An unassigned species (i.e., s__) indicates that the query genome is either i) placed outside a named genus or ii) the ANI to the closest intra-genus reference genome with an AF >=0.65 is not within the species-specific ANI circumscription radius. Classification was performed by placement of the genome in the reference tree and by using the relative evolutionary divergence (RED). Red value indicates the relative evolutionary divergence for a query genome. aa_percent: indicates the percentage of the multiple sequence alignment spanned by the genome (i.e. percentage of columns with an amino acid).

| bin id | Completeness (%) | Contamination (%) | Strain heterogeneity (%) | contigs | size (bp) | Average abundance | aa_percent | red_value | classification |
|--------|------------------|-------------------|--------------------------|---------|-----------|-------------------|------------|-----------|----------------|
| bin.63 | 96               | 0.74              | 0                        | 136     | 2225455   | 419               | 97         | 0.95023   | d__Bacteria;p__Proteobacteria; c__Gammaproteobacteria;o__Methylococcales; f__Methylomonadaceae;g__KS41 |
| bin.37 | 92               | 5.3               | 14                       | 282     | 2214630   | 77                | 87         | 0.951832  | d__Bacteria;p__Proteobacteria; c__Gammaproteobacteria;o__Methylococcales; f__Methylomonadaceae;g__KS41 |
| bin.19 | 88               | 0.46              | 50                       | 453     | 4678414   | 30                | 74         | 0.951132  | d__Bacteria;p__Proteobacteria; c__Gammaproteobacteria;o__Methylococcales; f__Methylomonadaceae;g__KS41 |
Table S5. Relative abundance (% of 16S rRNA gene reads) of *Sulfuritalea*, *Burkholderiales* and *Methylophilaceae* detected in Lacamas Lake by 16S rRNA gene amplicon sequencing (C denotes control).

|               | SUMMER |                     |                     | WINTER |                     |                     |
|---------------|--------|---------------------|---------------------|--------|---------------------|---------------------|
|               |        | Natural conditions (depth in m) | Incubations (12 m) |        | Natural conditions (depth in m) | Incubations (12 m) |
|               |        | 3 5 7 9 12 15 17 | C NO$_3$$^-$ SO$_4$$^{2-}$ | 3 5 12 15 17 | C CH$_4$/CH$_4$/ CH$_4$/ | C CH$_4$/CH$_4$/ CH$_4$/ |
| *Sulfuritalea* (genus) |        | 0.03 0.11 1.7 4.5 13 12 10 | 7.2 7.3 8.9 | 0.13 0.13 0.15 4.68 0.1 | 0.09 0.08 0.08 | 0.07 0.07 0.07 |
| *Burkholderiales* (order) |        | 4.5 7.1 14 15 17 11 8.6 | 12 8.7 11 | 28 23 35 22 28 | 19 17 8.5 | 19 15 10 |
| *Methylophilaceae* (family) |        | 0.85 1.4 2 1.3 1 0.64 0.69 | 3.7 5.2 3.7 | 0.72 0.7 1.2 1.1 1.5 | 0.94 2.6 6.8 | 1.4 3.1 3.1 |
Table S6. Experimental and sampling details. Samples for nutrient analysis were taken in duplicate (indicated with #), samples for methane oxidation rate linear regression analysis in quadruplicate (natural rate incubations indicated with +, amended incubations indicated with ^). For DNA analysis one filter per sampling moment and depth was taken (indicated with x).

| Depth (m) | Nutrients | Microbial community | Incubations |
|----------|-----------|---------------------|-------------|
|          | Nitrate   | Nitrite             | Sulfate     | DNA samples | Methane oxidation rate | Methane oxidizing community |
|          | 0.2 µm filtered | 0.2 µm filtered | Zn-Acetate (0.4 mg l) | 0.3 µm filtered | 24-hour incubations | 72-hour incubations, then 0.2 µm filtered |
| Summer  | Winter    | Summer  | Winter    | Summer | Winter | Summer | Winter | Summer | Winter |
| 0       |           |         |           | x     | +     | x     | +     | x^    | x^    |
| 1       |           |         |           |       | +     | x     | +     | x^    | x^    |
| 3       | #         | #       | #         | #     | x     | x     | +     | x^    | x^    |
| 5       | #         | #       |           | #     | x     | x     | +     |       |       |
| 7       | #         | #       | #         | #     | x     |       | +     | x^    |       |
| 9       | #         | #       |           | #     | x     |       |       | x^    | x^    |
| 12      | #         | #       | #         | #     | x     |       | +     | x^    |       |
| 15      | #         | #       | #         | #     | x     |       |       | x^    | x^    |
| 17      | #         | #       | #         | #     | x     |       | +     | x^    | x^    |
| Inlet   | #         | #       | #         | #     | x     |       | +     | x^    | x^    |

X. single sampling (for DNA purposes)
# . sampling in biological duplicate
+ . sampling in biological quadruplicate
**Table S7.** List of 34 single-copy marker genes used for phylogenetic analysis of MAGs.

| Gene ID          |
|------------------|
| DNGNGWU00001     |
| DNGNGWU00002     |
| DNGNGWU00003     |
| DNGNGWU00005     |
| DNGNGWU00006     |
| DNGNGWU00007     |
| DNGNGWU00009     |
| DNGNGWU00010     |
| DNGNGWU00011     |
| DNGNGWU00012     |
| DNGNGWU00014     |
| DNGNGWU00015     |
| DNGNGWU00016     |
| DNGNGWU00017     |
| DNGNGWU00018     |
| DNGNGWU00019     |
| DNGNGWU00021     |
| DNGNGWU00022     |
| DNGNGWU00023     |
| DNGNGWU00024     |
| DNGNGWU00025     |
| DNGNGWU00026     |
| DNGNGWU00027     |
| DNGNGWU00028     |
| DNGNGWU00029     |
| DNGNGWU00030     |
| DNGNGWU00031     |
| DNGNGWU00032     |
| DNGNGWU00033     |
| DNGNGWU00034     |
| DNGNGWU00036     |
| DNGNGWU00037     |
| DNGNGWU00039     |
| DNGNGWU00040     |
Table S8. Relative abundance of methanotroph OTUs as percentage of all reads in the sample (C denotes control). The taxonomic affiliation of the OTUs is shown in Figure 3.

|        | SUMMER |        | WINTER |        |        |
|--------|--------|--------|--------|--------|--------|
|        | Natural conditions (depth in m) | Incubations (12 m) | Natural conditions (depth in m) | Incubations (12 m) | Incubations (17 m) |
|        | 3 5  7 9 12 15 17 | C NO$_3^-$ SO$_4^{2-}$ | 3 5 12 15 17 | C CH$_4$/oxic CH$_4$/anoxic | C CH$_4$/oxic CH$_4$/anoxic |
| LL-16S-01 | 0 0 0.33 0.29 0 0 0 | 0.11 0.04 0.01 | 0 0 0 0 0 | 0 0 0 | 0 0 0 |
| LL-16S-02 | 0 0.01 0 0.06 0 0 0 | 0.02 0.06 0 | 0 0 0 0 0 | 0 0 0 | 0 0 0 |
| LL-16S-03 | 0 0 0.03 0.06 0 0 0 | 0 0.01 0.04 | 0 0 0 0 0 | 0 0 0 | 0 0 0 |
| LL-16S-04 | 0 0 0.51 0.08 0 0 0 | 0 0 0.02 | 0 0 0 0 0 | 0 0 0 | 0 0 0 |
| LL-16S-05 | 0 0.02 0.02 0.08 0.02 0 0 | 0.1 1 0.04 | 0 0 0 0 0 | 0 0 0 | 0 0 0 |
| LL-16S-06 | 0 0.01 0.08 0.25 0 0 0 | 0.02 0.01 0.08 | 0 0 0 0 0 | 0 0 0 | 0 0 0 |
| LL-16S-07 | 0 0 0 0.07 0 0 0 | 0.01 0.01 0.01 | 0 0 0 0 0 | 0 0 0 | 0.01 0.86 0.05 |
| LL-16S-08 | 0 0 0 0 0 0 0 | 0.01 0.02 0.01 | 0 0 0 0 0 | 0 0 0 | 0 0 0 |
| LL-16S-09 | 0 0 0 0 0 0 0 | 0.01 0.01 0.01 | 0 0 0 0 0 | 0 0 0 | 0 0 0 |
| LL-16S-10 | 0 0 0 0 0 0 0 | 0 0 0 | 0 0 0 0 0 | 0 0 0 | 0 0 0 |
| LL-16S-11 | 0 0.01 0.01 0.02 0 0 0 | 0.14 0.2 0.01 | 0 0 0 0 0 | 0 0 0 | 0 0 0 |
| LL-16S-12 | 0 0.01 0 0 0 0 0 | 0.01 0.02 0 | 0 0 0 0 0 | 0 0 0 | 0 0 0 |
| LL-16S-13 | 0 0 0 0 0 0 0.01 | 0.04 0.12 0.03 | 0 0 0 0 0 | 0 0.01 0.03 | 0 0 0 |
| LL-16S-14 | 0 0 0 0 0 0 0 | 0 0 0 | 0 0 0 0 0 | 0 0 0 | 0 1.3 5 |
| LL-16S-15 | 0 0 0 0 0 0 0 | 0 0 0 | 0 0 0 0 0 | 0 0 0 | 0 0 0 |
| LL-16S-16 | 0.01 0 0.01 0.02 0.02 0.09 0.01 | 1.8 1.1 4.7 0 0 0 0 | 0.03 0.01 0.08 0.04 0.02 | 0.09 0.03 17 | 0 0 0 |
| LL-16S-17 | 0 0 0 0 0.01 0.01 0.07 | 0.01 0.51 0.01 | 0 0 0 0 0 | 0 1.8 0.04 | 0 0 0 |
| LL-16S-18 | 0 0 0 0 0 0 0 | 0.05 0.25 0.03 | 0 0 0 0 0 | 0 0 0 | 0 0 0 |
| LL-16S-19 | 0 0 0 0 0 0 0 | 0 1.1 0.01 | 0 0 0 0 0 | 0 0.06 0.02 | 0 0 0 |
| LL-16S-20 | 0 0 0 0 0 0 0 | 0.12 0.29 0.04 | 0 0 0.01 0 0 | 0 0 0.03 | 0 0 0 |
| LL-16S-21 | 0 0 0 0 0 0 0.03 | 1.8 0.88 0.08 | 0.01 0.01 0 0 | 0 0 0.81 | 0 0 0 |
| LL-16S-22 | 0 0 0 0 0 0 0 | 0.12 0.29 0.04 | 0 0 0.01 0 0 | 0 0 0.03 | 0 0 0 |
| LL-16S-23 | 0 0 0 0 0.02 0 0.03 | 0.12 0.01 0.04 | 0 0 0.03 0.03 0.13 | 0.01 0.65 0.47 | 0 0.1 0.06 |
| LL-16S-24 | 0 0 0 0 0 0 0.01 | 0.24 0.33 0.13 | 0 0 0 0 0 | 0 0.01 0.04 | 0.01 0.08 0.01 |
| LL-16S-25 | 0 0 0 0 0 0 0 | 0 0 0 | 0 0 0 0 0 | 0 0 0 | 0.02 0 0.01 |