Supplement of

Long-term incubations provide insight into the mechanisms of anaerobic oxidation of methane in methanogenic lake sediments

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Figure S1: Map of Lake Kinneret showing the location of Station A in the center of the lake.
Figure S2: Change over time in the $\delta^{13}$C$_{\text{DIC}}$ values of two pre-incubated slurries amended with $^{13}$C-CH$_4$ (1.5-2 mL): (A) setup used in November 2017 with hematite addition, and (B) setup used in August 2017 without an electron acceptor addition. Sampling times for the different biological analyses (metagenome and lipids) are presented in the graphs. Black dashed lines represent the time at which 20 ml of the porewater in the bottles was exchanged with fresh porewater. The red dashed line represents the time at which hematite was added.
Figure S3: The change of dissolved Fe(II) in the two-stage experiment SN-6 with time. Error bars represent the average deviations of the data points from their means of duplicate/triplicate bottles.
Table S1: Lake Kinneret sediment composition from depths greater than 17 cm measured by X-ray diffraction analysis.

| Compound | Concentration (%) | Absolute error (%) |
|----------|-------------------|--------------------|
| Na$_2$O  | 0.219             | 0.01               |
| MgO      | 2.027             | 0.04               |
| Al$_2$O$_3$ | 10.299          | 0.09               |
| SiO$_2$  | 26.992            | 0.1                |
| P$_2$O$_5$ | 0.403            | 0.02               |
| SO$_3$   | 2.286             | 0.04               |
| Cl       | 0.178             | 0.01               |
| K$_2$O   | 0.69              | 0.02               |
| CaO      | 48.925            | 0.1                |
| TiO$_2$  | 1.201             | 0.03               |
| MnO      | 0.117             | 0.01               |
| Fe$_2$O$_3$ | 6.567          | 0.07               |
| ZnO      | 0.012             | 0.003              |
| SrO      | 0.074             | 0.008              |
| ZrO$_2$  | 0.009             | 0.003              |

*X-ray diffraction analysis

Sediment from depths of 17 to 40 cm was taken from a sediment core collected in July 2020. It was homogenized, dried at 60 °C in an oven, and ground into powder. The sediment was analyzed by an X-ray diffraction analyzer (Philips 1050/70) outfitted with a Philips ceramic sealed tube (2.2kW) and a scintillation point detector with a curved graphite monochromator. The semi-quantitative (± 5 wt %) interpretation was conducted by the system’s software (Crystal Logic tool).
Table S2: Experimental data from the representative two-stage slurry experiment and the calculated slopes (methanogenesis rates) over the duration of the experiment. Slope \textit{a} represents the rate between the first two time points (1-62 days), and slope \textit{b} represents the rate between the second and the third time points (62-138 days).

| Treatment | Time [day] | 1 nmol/g DW | 62 nmol/g DW | 138 nmol/g DW | slope a nmol/g DW*d | slope b nmol/g DW*d |
|-----------|-----------|-------------|--------------|---------------|--------------------|--------------------|
| No CH4    | 1         | 427         | 1334         | 3006          | 15                 | 22                 |
| No CH4    | 62        | 454         | 1511         | 3127          | 17                 | 21                 |
| No CH4    | 138       | 532         | 1658         | 3222          | 18                 | 21                 |
| 13CH4     | 1         | 9488        | 10923        | 11144         | 24                 | 3                  |
| 13CH4     | 62        | 9384        | 11116        | 12298         | 28                 | 16                 |
| 13CH4     | 138       | 8743        | 10198        | 11996         | 24                 | 24                 |
Table S3: Comparison of methanogenesis rates and mcrA copy numbers of sediment incubation experiments from the methanogenic zone in Lake Kinneret and from the methanogenic zone in the SE Mediterranean.

| Site                        | Methanogenesis rate [nmol/g DW* d] | mcrA copy no. | Sediment type                        | Dilution | Reference               |
|-----------------------------|-----------------------------------|---------------|--------------------------------------|----------|-------------------------|
| Lake Kinneret methanogenic zone | 17                                | 1x10^6        | Two-stage slurry                     | 1:3      | This work               |
|                             | 25                                | 1x10^6        | Two-stage slurries with ^13^CH_4     | 1:3      | This work               |
| SE Mediterranean methanic zone | 6                                 | 3x10^5        | Slurry incubation experiment with ^13^CH_4 | 1:4      | Yorshensky, 2019       |
Protocols for the two-stage experiments

Hematite experiment protocol (SN-1):

- Experiment was set up from pre-incubated sediments collected in December 2018
Subsamples of 18 gr pre-incubation slurry (1:1) were inserted into four 60-ml glass bottles.
The slurry was diluted with 18 gr natural anoxic porewater (> 20 cm sediment depth) to a final dilution of 1:3.
Hematite (64 mg) was added as a powder to two of the bottles.
The bottles were sealed with a rubber stopper and a crimp cap.
The bottles were flushed with N$_2$ gas for 5 min and shaken vigorously (3 times).
One ml of $^{13}$C-CH$_4$ was injected into the headspace of each bottle.
The experiment ran for 201 days at 20 ℃.

Magnetite experiment protocol (SN-2):

- Experiment was set up from pre-incubated sediments collected in August 2018
Subsamples of 15 gr pre-incubation slurry were inserted into seven 60-ml glass bottles.
The slurry was diluted with 15 gr natural anoxic porewater (> 20 cm sediment depth) to a final dilution of 1:3.
Magnetite (70 mg) was added as a powder to two of the bottles.
The bottles were sealed with a rubber stopper and a crimp cap.
The bottles were flushed with N$_2$ gas for 5 min and shaken vigorously (3 times).
The killed control bottle was autoclaved twice.
Magnetite was added to the control bottle after cooling under a laminar hood.
Liquid amorphous iron was injected into two experiment bottles. One ml of $^{12}$C-CH$_4$ and 0.5 ml of $^{13}$C-CH$_4$ were injected into the headspace of each bottle. The injected $^{13}$C-CH$_4$ turned out to be not labeled. $^{13}$C-CH$_4$ from a new stock was injected into all of the experiment bottles again after 105 days.
Na-molybdate solution was injected into the bottles with amorphous iron after 50 days.
Na-molybdate solution was injected into one of the “methane-only” and one of the magnetite treatments after 365 days.
The experiment ran for 447 days at 16 ℃.

Manganese experiment protocol (SN-3):

- Experiment was set up from pre-incubated sediments collected in December 2018
Subsamples of 18 gr pre-incubation slurry were inserted into four 60-ml glass bottles.
The slurry was diluted with 18 gr natural anoxic porewater (> 20 cm sediment depth) to a final dilution of 1:3.
MnO$_2$ (87 mg) was added as a powder to two of the bottles.
The bottles were sealed with a rubber stopper and a crimp cap.
The bottles were flushed with N$_2$ gas for 5 min and shaken vigorously (3 times).
One ml of $^{13}$C-CH$_4$ was injected into the headspace of each bottle. About 200 µl of $^{13}$C-CH$_4$ was injected, then on the 24$^{th}$ day, another 1 ml was added.
The experiment ran for 201 days at 20 ℃.

Nitrate experiment protocol (SN-4):
Experiment was set up from pre-incubated sediments collected in August 2017. Subsamples of 15 gr pre-incubation slurry were inserted into nine 60-ml glass bottles. The slurry was diluted with 15 gr natural anoxic porewater (> 20 cm sediment depth) to a final dilution of 1:3. Hematite (62 mg) was added as a powder to six of the bottles. The bottles were sealed with a rubber stopper and a crimp cap. The bottles were flushed with N\textsubscript{2} gas for 5 min and shaken vigorously (3 times). The killed control bottle was autoclaved twice. Hematite was added to the control bottle after cooling under a laminar hood. KNO\textsubscript{3} solution was injected into two experiment bottles without hematite, two with hematite and into one bottle with the killed control to obtain a final concentration of 1 mM. KNO\textsubscript{3} solution was also injected into two bottles with hematite to obtain a final concentration of 0.2 mM. One ml of \textsuperscript{12}C-CH\textsubscript{4} and 0.5 ml of \textsuperscript{13}C-CH\textsubscript{4} were injected into the headspace of each bottle. The experiment ran for 306 days at 20 °C.

**Nitrite experiment protocol (SN-5):**

- Experiment was set up from pre-incubated sediments collected in August 2018. Subsamples of 20 gr pre-incubation slurry were inserted into eleven 60-ml glass bottles. The slurry was diluted with 20 gr natural anoxic porewater (> 20 cm sediment depth) to a final dilution of 1:3. Hematite (64 mg) was added as a powder to four of the bottles. The bottles were sealed with a rubber stopper and a crimp cap. The bottles were flushed with N\textsubscript{2} gas for 5 min and shaken vigorously (3 times). Two killed control bottles were autoclaved twice. Hematite was added to the control bottles after cooling under a laminar hood. NaNO\textsubscript{2} solution was injected into two experiment bottles with hematite and into the killed controls to obtain a final concentration of 0.5 mM. Then, it was injected into two bottles with hematite to obtain a final concentration of 0.1 mM. One ml of \textsuperscript{12}C-CH\textsubscript{4} and 0.5 ml of \textsuperscript{13}C-CH\textsubscript{4} was injected into the headspace of each bottle. The experiment ran for 493 days at 20 °C.

**AQDS experiment protocol (SN-6):**

- Experiment was set up from pre-incubated sediments collected in July 2019. Subsamples of 18 gr pre-incubation slurry were inserted into eight 60-ml glass bottles. The slurry was diluted with 18 gr natural anoxic porewater (> 20 cm sediment depth) to a final dilution of 1:3. Hematite (58 mg) was added as a powder to two of the bottles. The bottles were sealed with a rubber stopper and a crimp cap. The bottles were flushed with N\textsubscript{2} gas for 5 min and shaken vigorously (3 times). Two killed control bottles were autoclaved twice. AQDS stock solution was prepared by adding 0.76 gr of AQDS to 10 ml of anaerobic DDW (flushed with N2 prior to the addition of AQDS).
Hematite was added to the control bottles after cooling under a laminar hood. The AQDS solution was injected into two experiment bottles with hematite, two without hematite and into the killed controls to obtain final concentrations of 5 mM.

One ml of $^{13}$C-CH$_4$ was injected into the headspace of each bottle.

The experiment ran for 264 days at 20 ℃.

**Natural humic acids and clay experiment (SN-7):**

- Experiment was set up from pre-incubated sediments collected in July 2019

Subsamples of 20 gr pre-incubation slurry were inserted into nine 60-ml glass bottles. The slurry was diluted with 20 gr natural anoxic porewater (> 20 cm sediment depth) to a final dilution of 1:3. Hematite (64 mg) was added as a powder to four of the bottles. Natural humic acids (22 mg) were added as a powder to two experiment bottles. The bottles were sealed with a rubber stopper and a crimp cap.

The bottles were flushed with N$_2$ gas for 5 min and shaken vigorously (3 times). The killed control bottle was autoclaved twice.

Hematite was added to the control bottle after cooling under a laminar hood.

$^{13}$C-CH$_4$ was injected into the headspace of each bottle.

Nontronite (Fe-bearing clay) was added under a laminar hood to two of the experiment bottles after 43 days. All the experiment bottles were flushed again with N$_2$ gas and shaken vigorously (3 times).

One ml of $^{13}$C-CH$_4$ was injected again into the headspace of each bottle on day 51.

The experiment ran for 169 days at 20 ℃.

**Bromoethanesulfonate (BES) experiment (SN-8):**

- Experiment was set up from pre-incubated sediments collected in August 2018

Subsamples of 20 gr pre-incubation slurry were inserted into four 60-ml glass bottles. The slurry was diluted with 20 gr natural anoxic porewater (> 20 cm sediment depth) to a final dilution of 1:3. Hematite was added as a powder to all bottles.

The bottles were sealed with a rubber stopper and a crimp cap.

The bottles were flushed with N$_2$ gas for 5 min and shaken vigorously (3 times).

BES solution was injected into two bottles to obtain a final concentration of 20 mM.

One ml of $^{13}$C-CH$_4$ and 0.5 ml of $^{12}$C-CH$_4$ was injected into the headspace of each bottle.

The experiment ran for 493 days at 20 ℃.

**Acetylene experiment (SN-9):**

- Experiment was set up from pre-incubated sediments collected in August 2017

Subsamples of 15 gr pre-incubation slurry were inserted into eight 60-ml glass bottles. The slurry was diluted with 15 gr natural anoxic porewater (> 20 cm sediment depth) to a final dilution of 1:3.

Hematite was added as a powder to six bottles.

The bottles were sealed with a rubber stopper and a crimp cap.
The bottles were flushed with N₂ gas for 5 min and shaken vigorously (3 times).
Two killed control bottles were autoclaved twice.
Hematite was added to the control bottle after cooling under a laminar hood.
One ml of ¹²C-CH₄ and 0.5 ml of ¹³C-CH₄ was injected into the headspace of each bottle.
Acetylene gas was injected into two bottles at different time points after ¹³C-DIC enrichment was observed: into the first bottle after 120 days, and into the second bottle after 208 days.
The experiment ran for 321 days at 20 ℃.

**No electron acceptor experiment (SN-10):**

- Experiment was set up from pre-incubated sediments collected in October 2019
Subsamples of 18 gr pre-incubation slurry were inserted into six 60-ml glass bottles.
The slurry was diluted with 18 gr natural anoxic porewater (> 20 cm sediment depth) to a final dilution of 1:3.
The bottles were sealed with a rubber stopper and a crimp cap.
The bottles were flushed with N₂ gas for 5 min and shaken vigorously (3 times).
One ml of ¹³C-CH₄ was injected into the headspace of three bottles.
The experiment ran for 147 days at 20 ℃.