Supplementary material

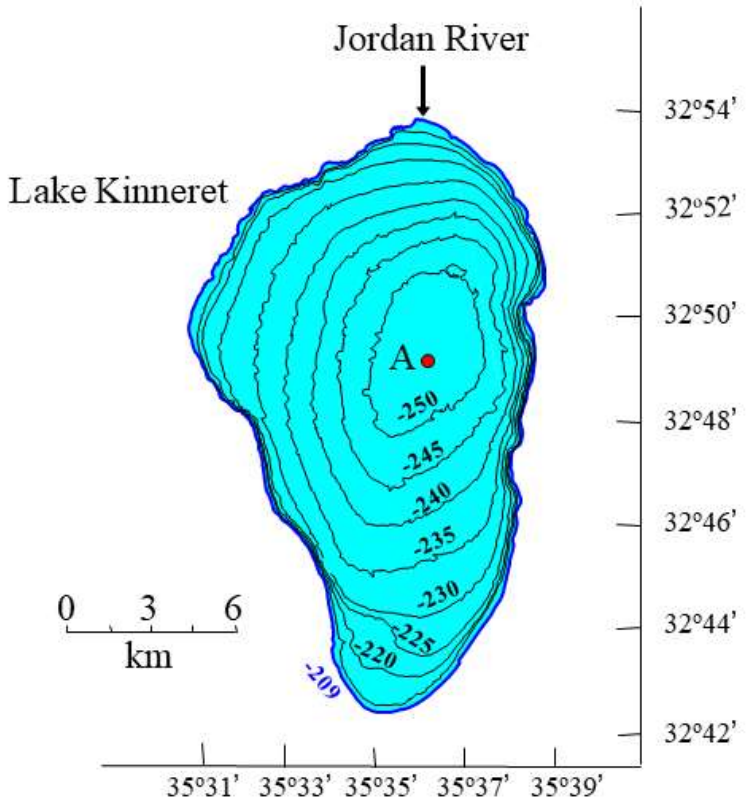

Figure S1: Map of Lake Kinneret showing the location of Station A in the center of the lake.

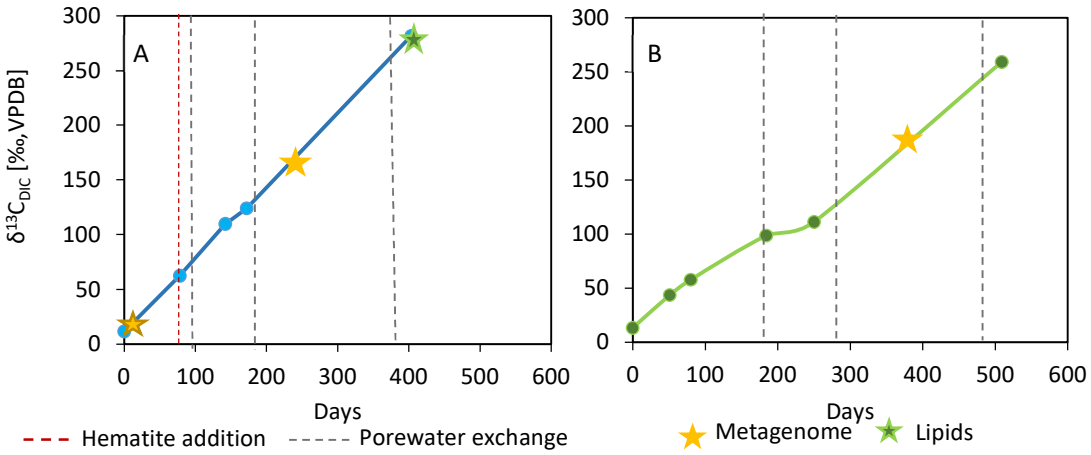

Figure S2: Change over time in the $\delta^{13}C_{DIC}$ values of two pre-incubated slurries amended with ^{13}C-CH_4 (1.5-2 mL): (A) setup used in November 2017 with hematite addition, and (B) setup used in August 2017 without an electron acceptor addition. Sampling times for the different biological 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.

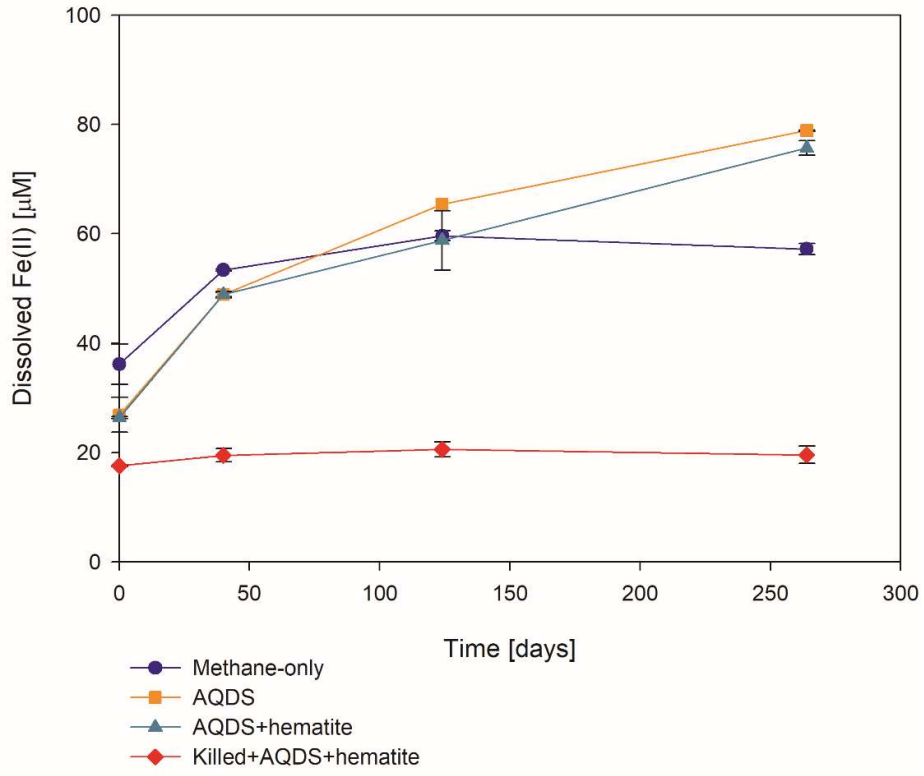

Figure S3: The change of dissolved Fe(II) in the two-stage experiment SN-6 with time. Error bars represent the average deviations of the data points from their means of duplicate/triplicate bottles.

Table S1: Lake Kinneret sediment composition from depths greater than 17 cm measured by X-ray diffraction analysis*.

Compound	Concentration (%)	Absolute error (%)
Na_2O	0.219	0.01
MgO	2.027	0.04
Al_2O_3	10.299	0.09
SiO_2	26.992	0.1
P_2O_5	0.403	0.02
SO_3	2.286	0.04
Cl	0.178	0.01
K_2O	0.69	0.02
CaO	48.925	0.1
TiO_2	1.201	0.03
MnO	0.117	0.01
Fe_2O_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 *a* represents the rate between the first two time points (1-62 days), and slope *b* represents the rate between the second and the third time points (62-138 days).

Time [day]	**1**	**62**	**138**	**slope a**	**slope b**
Treatment	nmol/g DW	nmol/g DW	nmol/g DW	nmol/g DW*d	nmol/g DW*d
No CH4	427	1334	3006	15	22
No CH4	454	1511	3127	17	21
No CH4	532	1658	3222	18	21
13CH4	9488	10923	11144	24	3
13CH4	9384	11116	12298	28	16
13CH4	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_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_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_3 solution was also injected into two bottles with hematite to obtain a final concentration of 0.2 mM.

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 experiment ran for 306 days at 20 ℃.

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_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.

$NaNO_2$ 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 ^{12}C-CH_4 and 0.5 ml of ^{13}C-CH_4 was injected into the headspace of each bottle.

The experiment ran for 493 days at 20 ℃.

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_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 ^{12}C-CH_4 and 0.5 ml of ^{13}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 N_2 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 ^{12}C-CH_4 and 0.5 ml of ^{13}C-CH_4 was injected into the headspace of each bottle.

Acetylene gas was injected into two bottles at different time points after ^{13}C-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 °C.

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 N_2 gas for 5 min and shaken vigorously (3 times).

One ml of ^{13}C-CH_4 was injected into the headspace of three bottles.

The experiment ran for 147 days at 20 °C.