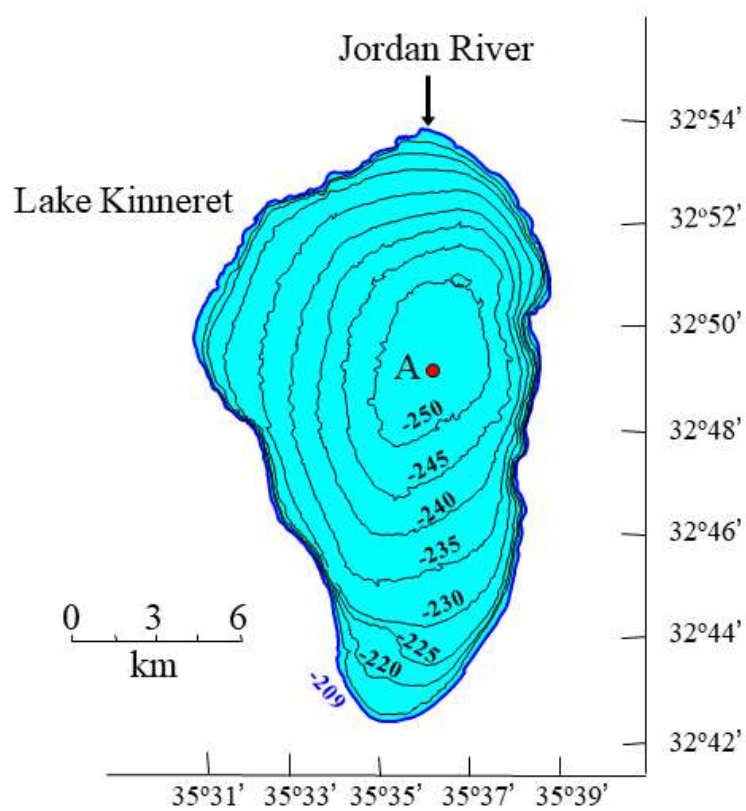


## Supplementary material

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



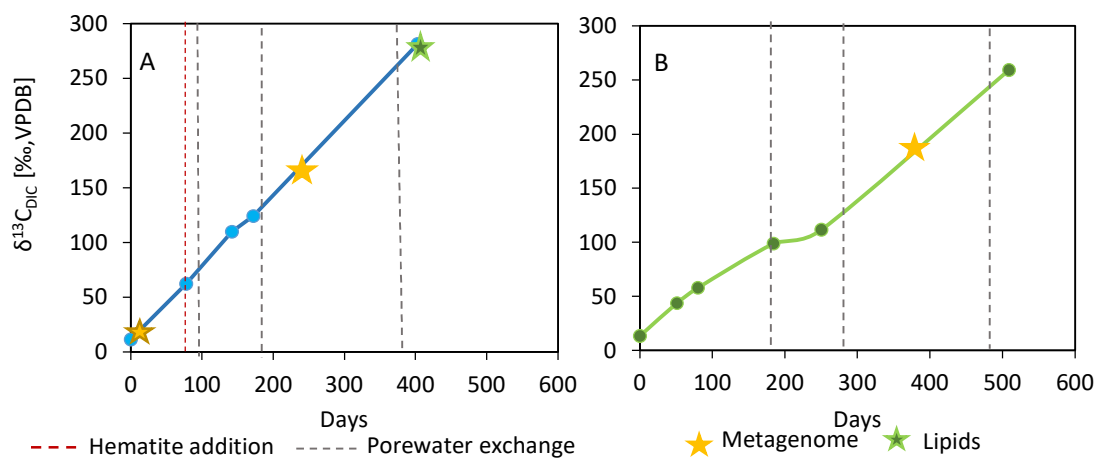


Figure S2: The change of the  $\delta^{13}\text{C}_{\text{DIC}}$  value of two pre- incubated slurries amended with  $^{13}\text{C}\text{-CH}_4$  (1.5-2 mL) with time: (A) set up in November 2017 with hematite addition, and (B) set up in August 2017 without an electron acceptor addition. Sampling time for different biological analyses (metagenome and lipids) is 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. Red dashed line represents the time hematite was added.

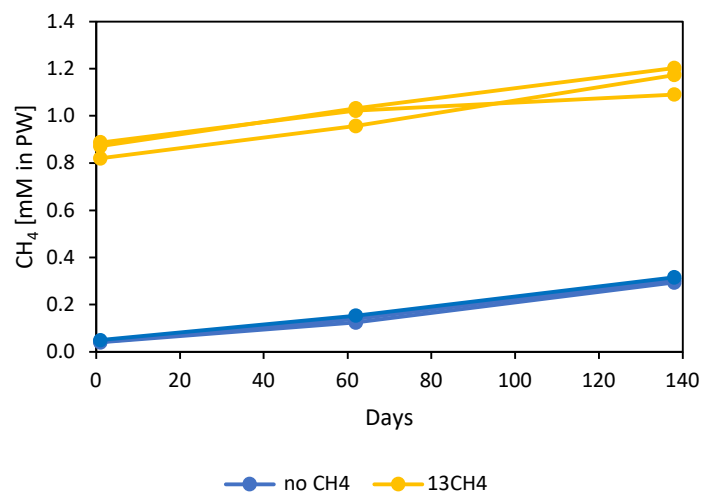


Figure S3: The change of CH<sub>4</sub> concentrations with time of a representative pre-incubated slurry experiment, showing apparent net methanogenesis with rate of 2  $\mu\text{M day}^{-1}$ .

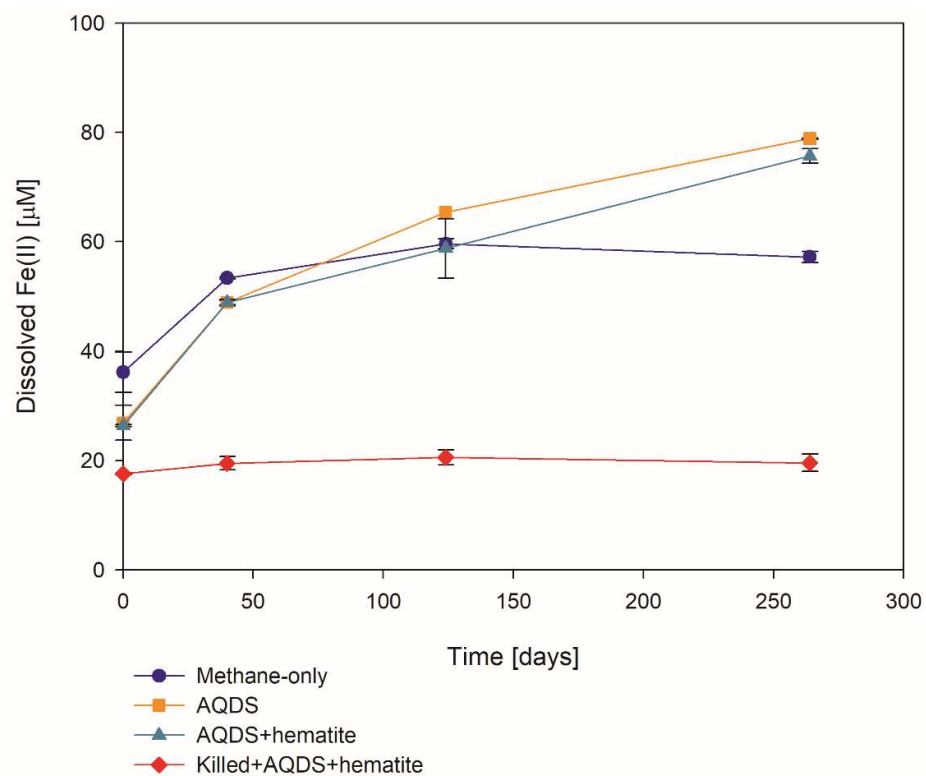


Figure S4: The change of dissolved Fe(II) in the two-stage experiment SN-6 with time, with the addition of AQDS. Error bars represent the average of the absolute deviations of data points from their average.

Table S1: Lake Kinneret sediment composition from below 17 cm depth measured in X-ray diffraction analysis\*.

Compound	Concentration (%)	Absolute error (%)
<b>Na<sub>2</sub>O</b>	0.219	0.01
<b>MgO</b>	2.027	0.04
<b>Al<sub>2</sub>O<sub>3</sub></b>	10.299	0.09
<b>SiO<sub>2</sub></b>	26.992	0.1
<b>P<sub>2</sub>O<sub>5</sub></b>	0.403	0.02
<b>SO<sub>3</sub></b>	2.286	0.04
<b>Cl</b>	0.178	0.01
<b>K<sub>2</sub>O</b>	0.69	0.02
<b>CaO</b>	48.925	0.1
<b>TiO<sub>2</sub></b>	1.201	0.03
<b>MnO</b>	0.117	0.01
<b>Fe<sub>2</sub>O<sub>3</sub></b>	6.567	0.07
<b>ZnO</b>	0.012	0.003
<b>SrO</b>	0.074	0.008
<b>ZrO<sub>2</sub></b>	0.009	0.003

\*X-ray diffraction analysis

A sediment bulk between 17-40 cm depth was taken from a sediment core collected on July 2020. It was homogenized, dried in 60°C in the oven, and grounded to powder. The sediment was analyzed by X-ray diffraction analyzer (Philips 1050/70), containing a Philips ceramic sealed tube (2.2kW) and a Scintillation point detector with curved graphite monochomator. The semi-quantitative ( $\pm 5$  wt %) interpretation was conducted by the system's software (Crystal Logic tool).

Table S2: Read abundance of key denitrification genes in two different long-term 1:1 slurry incubation with/without hematite (normalized as counts per million).

KEGG ID	Gene name	Incubation+hematite T0	Incubation+hematite Tmiddle	Incubation T250	Incubation T450
<b>K15864</b>	<i>nirS</i>	54	32	28	26
<b>K00370</b>	<i>narG/narZ/nxrA</i>	139	82	75	43
<b>K00371</b>	<i>narH/narY/nxrB</i>	82	40	34	16

Table S3: Experimental data of 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 [days]	1	62	138	slope a	slope b
Treatment	mM in PW	mM in PW	mM in PW	μM/day	μM/day
no CH <sub>4</sub>	0.04	0.12	0.29	1.38	2.24
no CH <sub>4</sub>	0.04	0.14	0.31	1.60	2.19
no CH <sub>4</sub>	0.05	0.15	0.31	1.71	2.13
<sup>13</sup> CH <sub>4</sub>	0.89	1.02	1.09	2.20	0.90
<sup>13</sup> CH <sub>4</sub>	0.87	1.03	1.20	2.64	2.24
<sup>13</sup> CH <sub>4</sub>	0.82	0.96	1.17	2.24	2.86

Table S4: Comparison of methanogenesis rates and mcrA copy numbers of incubation experiments from the methanogenic zone in Lake Kinneret and the SE Mediterranean sediments.

Site	Methanogenesis rate [ $\mu\text{mol/gr}$ dry sediment * day]	mcrA copy no.	Sediment type	Dilution	Reference
Lake Kinneret methanogenic zone	0.017	$1 \times 10^6$	Two-stage slurry	1:3	This work
	0.025	$1 \times 10^6$	Two-stage slurries with $^{13}\text{CH}_4$	1:3	This work
SE Mediterranean methanic zone	0.006	$3 \times 10^5$	Slurry incubation experiment with $^{13}\text{CH}_4$	1:4	Yorshensky, 2019



## **Protocols for the two-stage experiments**

### **Hematite experiment protocol (SN-1):**

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 sed 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<sub>2</sub> gas for 5 minutes and shaken vigorously (3 times).

One ml of <sup>13</sup>C-CH<sub>4</sub> was injected to the headspace of each bottle.

The experiment ran for 201 days at 20 °C.

### **Magnetite experiment protocol (SN-2):**

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 sed 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<sub>2</sub> gas for 5 minutes 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 to two experiment bottles.

One ml of <sup>12</sup>C-CH<sub>4</sub> and 0.5 ml of <sup>13</sup>C-CH<sub>4</sub> was injected to the headspace of each bottle. The <sup>13</sup>C-CH<sub>4</sub> injected turned out to be not labeled. <sup>13</sup>C-CH<sub>4</sub> from a new stock was injected to all the experiment bottles again after 105 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 °C.

### **Manganese experiment protocol (SN-3):**

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 sed depth) to a final dilution of 1:3.

MnO<sub>2</sub> (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<sub>2</sub> gas for 5 minutes and shaken vigorously (3 times).

One ml of <sup>13</sup>C-CH<sub>4</sub> was injected to the headspace of each bottle. About 200 µl of <sup>13</sup>C-CH<sub>4</sub> was injected, then on the 24<sup>th</sup> day another 1 ml was added.

The experiment ran for 201 days at 20 °C.

### **Nitrate experiment protocol (SN-4):**

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 sed 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<sub>2</sub> gas for 5 minutes 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<sub>3</sub> solution was injected into two experiment bottles without hematite, two with hematite and to the killed control bottle to reach 1 mM final concentration. Then, it was injected into two bottles with hematite to reach a final concentration of 0.2 mM.

One ml of <sup>12</sup>C-CH<sub>4</sub> and 0.5 ml of <sup>13</sup>C-CH<sub>4</sub> was injected into the headspace of each bottle.

The experiment ran for 306 days at 20 °C.

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

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 sed 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<sub>2</sub> gas for 5 minutes 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<sub>2</sub> solution was injected into two experiment bottles with hematite and to the killed controls to reach a final concentration of 0.5 mM. Then, it was injected into two bottles with hematite to reach a final concentration of 0.1 mM.

One ml of <sup>12</sup>C-CH<sub>4</sub> and 0.5 ml of <sup>13</sup>C-CH<sub>4</sub> was injected into the headspace of each bottle.

The experiment ran for 493 days at 20 °C.

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

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 sed 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<sub>2</sub> gas for 5 minutes 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.

AQDS solution was injected to two experiment bottles with hematite, two without hematite and to the killed controls to reach a final concentration of 5 mM.

One ml of <sup>13</sup>C-CH<sub>4</sub> was injected into the headspace of each bottle.

The experiment ran for 264 days at 20 °C.

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

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 sed 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<sub>2</sub> gas for 5 minutes 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.

<sup>13</sup>C-CH<sub>4</sub> was injected to 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<sub>2</sub> gas and shaken vigorously (3 times).

One ml of <sup>13</sup>C-CH<sub>4</sub> was injected again to the headspace of each bottle on day 51.

The experiment ran for 169 days at 20 °C.

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

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 sed 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<sub>2</sub> gas for 5 minutes and shaken vigorously (3 times).

BES solution was injected to two bottles to reach final concentration of 20 mM.

One ml of <sup>12</sup>C-CH<sub>4</sub> and 0.5 ml of <sup>13</sup>C-CH<sub>4</sub> was injected to the headspace of each bottle.

The experiment ran for 493 days at 20 °C.

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

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 sed 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<sub>2</sub> gas for 5 minutes 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 <sup>12</sup>C-CH<sub>4</sub> and 0.5 ml of <sup>13</sup>C-CH<sub>4</sub> was injected to the headspace of each bottle.

Acetylene gas was injected to two bottles at different time points after <sup>13</sup>C-DIC enrichment was observed: after 120 days to the first bottle, and after 208 days to the second bottle.

The experiment ran for 321 days at 20 °C.

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

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 sed 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<sub>2</sub> gas for 5 minutes and shaken vigorously (3 times).

One ml of  $^{13}\text{C-CH}_4$  was injected to the headspace of three bottles.

The experiment ran for 147 days at 20 °C.