Supplementary material

Table S1: Lake Kinneret sediment composition.

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

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-rey 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 (± 5 wt %) interpretation was conducted by the system's software (Crystal Logic tool).

Table S2: Details of the additions in each long-term 1:3 slurry experiment.

Experiment	Trearment	# of bottles	CH_4 [mL]	$^{13}CH_4$ [mL]	Fe_2O_3 [mM]	Fe_3O_4 [mM]	$Fe(OH)_3$ [mM]	MnO_2 [mM]	NO_2^- [mM]	NO_3^- [mM]	AQDS [mM]	Humic substances [mM]	Black coffee [mg]	PCA [mM]	Fe-bearing nontronite (clay) [gr]	Na_2-molybdate [mM]	BES [mM]	Acetylene [µL]	Temp [c°]	Comments
Hematite	$^{13}CH_4$	2		1															20	
	13CH4+hematite	2		1	10														20	
Magnetite	$^{13}CH_4$	2														1			16	The methane that was added at the beginning of the experiment was not labelled, so ^{13}C-labeled methane was added after 90 days. Na_2-molybdate was added to one of the bottles on day 365
	$^{13}CH_4$+magnetite	2				10										1			16	Na_2-molybdate was added to one of the bottles on day 365
	$^{13}CH_4+Fe(OH)_3$	2					10									1			16	
	Killed+$^{13}CH_4$+magnetite	1				10													16	
MnO_2	$^{13}CH_4$	2		1.2															20	200 µL $^{13}CH_4$ was added on day 1, then another 1 mL was added on day 24.
	$^{13}CH_4$+MnO2	2		1.2				10											20	200 µL $^{13}CH_4$ was added on day 1, then another 1 mL was added on day 24.
Nitrate	$^{13}CH_4+NO_3^-$(high conc.)	2	1	0.5	12					1									20	
	$^{13}CH_4+NO_3^-$(high conc.)+hematite	2	1	0.5	12					1									20	
	$^{13}CH_4+NO_3^-$(low conc.)+hematite	2	1	0.5	12					0.2									20	
	Killed+$^{13}CH_4+NO_3^-$(high conc.)+hematite	1	1	0.5	12					1									20	
Nitrite	$^{13}CH_4$	3	1	0.5															20	
	$^{13}CH_4+NO_2^-$(high conc.)+hematite	2	1	0.5	10				0.5										20	
	$^{13}CH_4+NO_2^-$(low conc.)+hematite	2	1	0.5	10				0.1										20	
	$^{13}CH_4$+hematite+PTIO	2	1	0.5	10														20	
	Killed+$^{13}CH_4+NO_2^-$(high conc.)+hematite	2	1	0.5	10				0.5										20	
AQDS	$^{13}CH_4$	3		1															20	
	$^{13}CH_4$+AQDS	2		1							5								20	
	$^{13}CH_4$+AQDS+hematite	2		1	10						5								20	
	Killed+$^{13}CH_4$+AQDS	1		1															20	
Natural humic acids, methanophenezines (PCA) and clay	$^{13}CH_4$	2		1															20	The head space of the experiment bottles was flushed with N_2 on day 51 and $^{13}CH_4$ was added. This was done in order to match the the clay bottles.
	$^{13}CH_4$+humic acid	2		1								0.5							20	
	$^{13}CH_4$+black coffee	1		1									23						20	
	$^{13}CH_4$+PCA	2		1										1					20	
	$^{13}CH_4$+PCA+hematite	2		1	10									1					20	
	$^{13}CH_4$+clay	2		1											1				20	Clay was added on day 43, and the bottles were flushed again with N_2. $^{13}CH_4$ was added again on day 51.
	Killed+$^{13}CH4$+black coffee	1		1									23	1					20	
	Killed+$^{13}CH_4$+PCA+hematite	1		1	10														20	
Bromoethanesulfonate (BES)	$^{13}CH_4$+hematite	2	9	1	10														20	
	$^{13}CH_4$+hematite+BES	2	9	1	10												20		20	
Acetylene	$^{13}CH_4$+hematite	4	1	0.5	10														20	
	$^{13}CH_4$+hematite+acetylene	2	1	0.5	10													120	20	Acetylene was injected to each bottle at different time point doring the experiment.
	Killed+$^{13}CH_4$+hematite	2	1	0.5	10														20	
No electron acceptor	No additions	3																	20	
	$^{13}CH_4$	3		1															20	

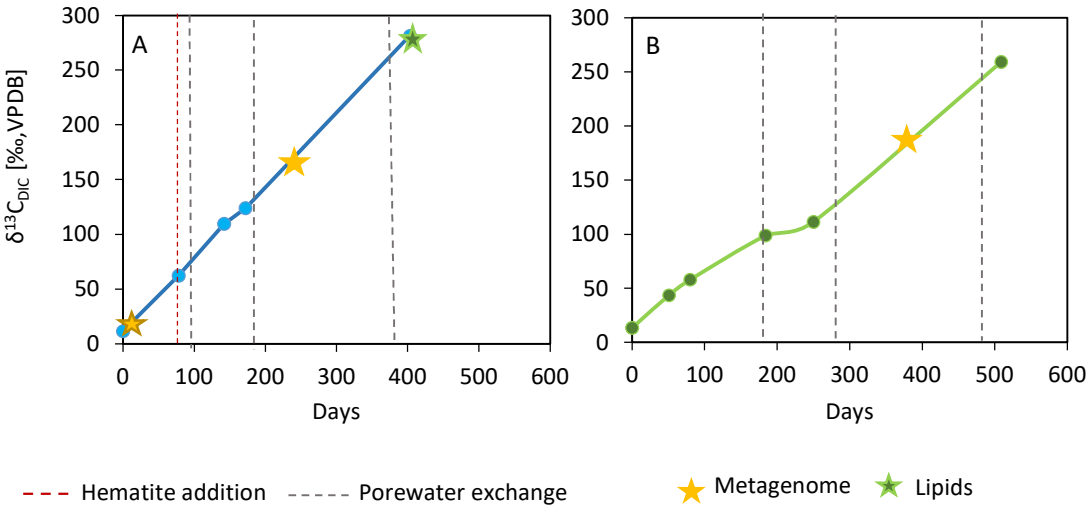

Figure S1: The change of the $\delta^{13}C_{DIC}$ value of two 1:1 sediment:porewater long-term incubations amended with ^{13}C-CH_4 (1.5-2 mL) with time: (A) set up in November 2017 with hematite addition, and (B) set up in August 2017 without any addition. Sampling time for different biological analyses (metagenome, qPCR 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.

Table S3: Abundance and taxonomy of selected archaea and bacteria in the long-term incubations and the freshly collected sediment incubations (bioreactor and batch), based on read mapping to metagenome-assembled genomes (MAGs). Binscore indicates the genome quality (0-1, DAStool).

		Counts per million reads						Taxonomy (Genome Taxonomy Database)					
Bin ID	binscore	1:1 Incubation with hematite	1:1 Incubation with hematite (t0)	Bioreactor with hematite (151st day) a	Bioreactor with hematite (151st day) b	Bioreactor with hematite (t0)	Incubation with freshly collected sediment and hematite (470th day)	Domain	Phylum	Class	Order	Family	Genus
Archaea MAG1	0.57	1716	711	6336	6441	5826	4101	Archaea	Crenarchaeota	Bathyarchaeia	B26-1	BA1	
Archaea MAG2	0.74	36813	20115	39005	39498	41982	32868	Archaea	Crenarchaeota	Bathyarchaeia	B26-1	UBA233	20-14-0-80-47-9
Archaea MAG3	0.76	3859	3234	1602	1563	1565	3260	Archaea	Crenarchaeota	Bathyarchaeia	TCS64	TCS64	UBA8941
Archaea MAG4	0.72	4689	4879	7032	6713	9478	7407	Archaea	Halobacterota	Methanomicrobia	Methanomicrobiales	Methanofollaceae	
Archaea MAG5	0.74	20311	40522	9547	9813	11884	21123	Archaea	Halobacterota	Methanomicrobia	Methanomicrobiales	Methanoregulaceae	Methanoregula
Archaea MAG6	0.42	7529	8385	4026	4154	8684	12015	Archaea	Halobacterota	Methanosarcinia	Methanotrichales	Methanotrichaceae	Methanothrix
Archaea MAG7	0.57	12393	11097	8154	7975	11292	10578	Archaea	Halobacterota	Methanosarcinia	Syntrophoarchaeales	ANME-1a	
Archaea MAG8	0.87	7412	3807	8067	8432	8330	7557	Archaea	Thermoplasmatota	E2	DHVEG-1	DHVEG-1	SM1-50
Archaea MAG9	0.43	1693	2220	1530	1513	1749	2681	Archaea	Thermoplasmatota	Thermoplasmata_A	Methanomassiliicoccales		
Bacteria MAG1	0.86	17218	8327	11568	11988	13613	12701	Bacteria	Chloroflexota	Dehalococcoidia	GIF9	AB-539-J10	RBG-13-51-36
Bacteria MAG2	0.72	9784	13545	13088	13863	15732	13024	Bacteria	Desulfobacterota	BSN033	BSN033	UBA1163	UBA1163
Bacteria MAG3	0.82	8951	12496	4625	4626	5904	6310	Bacteria	Desulfobacterota	Desulfobacteria	Desulfatiglanales	HGW-15	
Bacteria MAG4	0.80	5043	3505	803	773	1700	1526	Bacteria	Desulfobacterota	Desulfomonilia	UBA1062	UBA1062	
Bacteria MAG5	0.69	3385	5474	635	645	826	1687	Bacteria	Desulfobacterota	Syntrophia	Syntrophales	UBA2185	UBA2185
Bacteria MAG6	0.64	1732	2831	1964	1955	2063	3678	Bacteria	Desulfobacterota	Syntrophobacteria			
Bacteria MAG7	0.90	1	3	10956	10978	1	1135	Bacteria	Desulfuromonadota	Desulfuromonadia	Desulfuromonadales	UBA2197	UBA2197
Bacteria MAG8	0.98	4074	10706	278	277	185	1131	Bacteria	Desulfuromonadota	Desulfuromonadia	Geobacterales	Geobacteraceae	
Bacteria MAG9	0.46	2581	4719	5430	5156	6950	4206	Bacteria	MBNT15	MBNT15	MBNT15	MBNT15	
Bacteria MAG10	0.82	3728	4647	2680	2591	2187	3841	Bacteria	Methylomirabilota	Methylomirabilia	Methylomirabilales	2-02-FULL-66-22	
Bacteria MAG11	0.81	8831	23576	10360	11040	12901	14794	Bacteria	Nitrospirota	Thermodesulfovibrionia	Thermodesulfovibrionales	SM23-35	
Bacteria MAG12	1.00	2	4	27	27	1	1236	Bacteria	Proteobacteria	Gammaproteobacteria	Methylococcales	Methylomonadaceae	Methylomonas
Bacteria MAG13	0.31	1	3	26	28	1	17	Bacteria	Proteobacteria	Gammaproteobacteria	Burkholderiales	Methylophilaceae	Methylotenera

Table S4: 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
K15864	*nirS*	54	32	28	26
K00370	*narG*/*narZ*/*nxrA*	139	82	75	43
K00371	*narH*/*narY*/*nxrB*	82	40	34	16

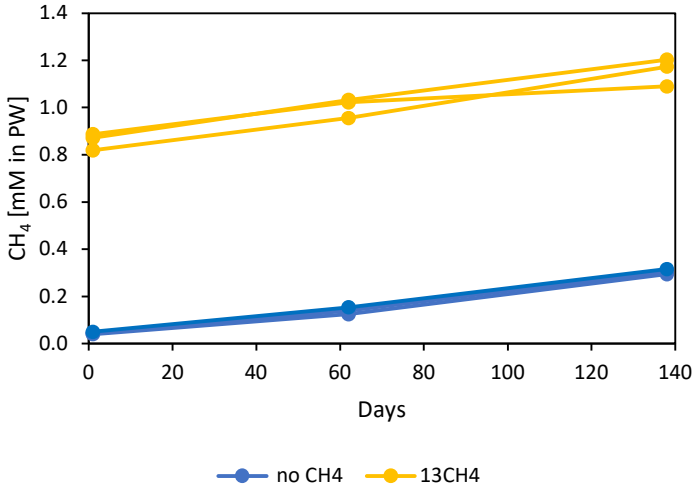

Figure S2: The change of CH_4 concentrations with time of a representative pre-incubated slurry experiment, showing apparent net methanogenesis with rate of 2 µM day^{-1}.

Table S5: Experimental data of the representative pre-incubated slurry experiment and the calculated slopes representing the methanogenesis rates along the experiment time. Slope a represent the rate between the first two time points (1-62), and slope b represent the rate between the second and the third time points (62-138).

				Methanogenesis rate	
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_4	0.040	0.124	0.294	1.382	2.239
no CH_4	0.042	0.139	0.306	1.598	2.191
no CH_4	0.049	0.154	0.315	1.710	2.126
$^{13}CH_4$	0.887	1.022	1.090	2.201	0.901
$^{13}CH_4$	0.872	1.033	1.203	2.637	2.242
$^{13}CH_4$	0.820	0.956	1.174	2.238	2.858

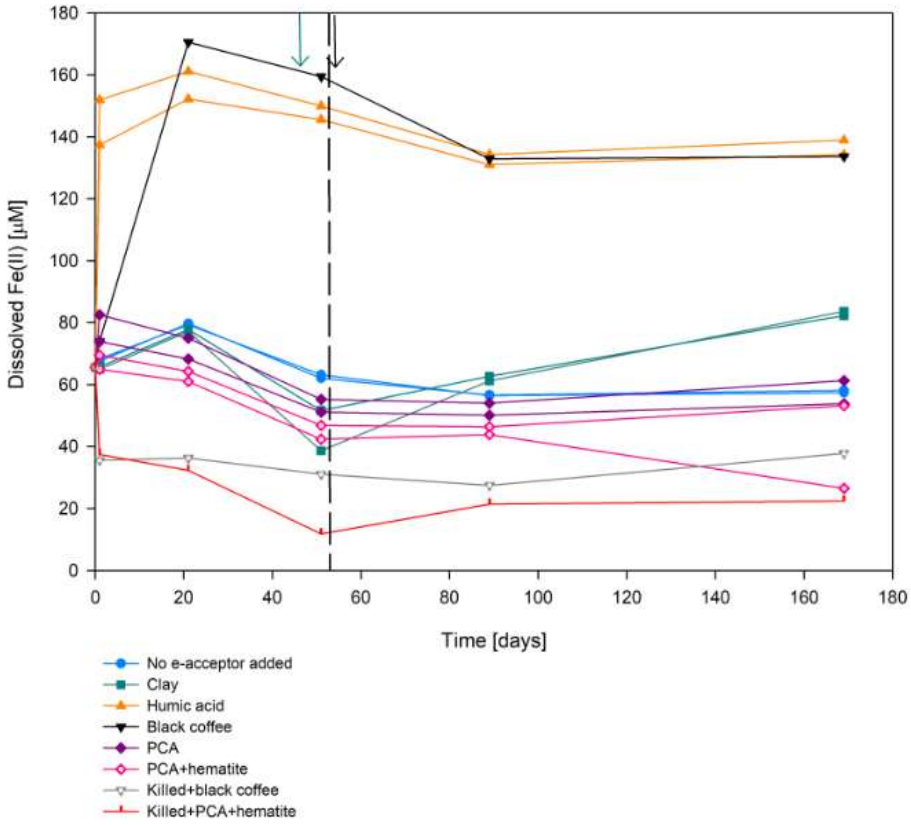

Figure S3: The change of dissolved Fe(II) in the long-term pre-incubated slurry experiments with time with the addition of clay, natural humic substances, black coffee and PCA. The green arrow represents the time clay was added to the specific bottles and those bottles flushed with N_2, the dashed line represents the time the rest of the bottles were flushed, and the black arrow represents the time $^{13}CH_4$ was added again.

Table S6: Comparison of methanogenesis rates and mcrA copy numbers of incubation experiments from the methanic zone in Lake Kinneret and the SE Mediterranean sediments.

Site	Methanogenesis rate [µM/day]	mcrA copy no.	Sediment type	Dilution	Reference
Lake Kinneret methanic zone	1.6	$1x10^6$	Pre-incubated sediment	1:3	This work
	2.3	$1x10^6$	Pre-incubated sediment slurries with $^{13}CH_4$	1:3	This work
SE Mediterranean methanic zone	0.75	$3x10^5$	Slurry incubation experiment with $^{13}CH_4$	1:4	Yorshensky, 2019