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Supplement of

Metagenomic insights into the metabolism of microbial communities that mediate iron and methane cycling in Lake Kinneret iron-rich methanic sediments

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S1. Supplementary Methods

Method S1: Analysis of microbial diversity in slurry incubations based on the 16S rRNA gene amplicon sequencing. Total genomic DNA was extracted from duplicate samples of the slurries using the MoBio Power Soil DNA isolation kit (MoBio Laboratories, Solana Beach, CA). Genomic DNA was eluted using 80 µl of elution buffer and stored at −20°C. Duplicates of 16S rRNA gene fragments were joined together and amplified by PCR using a Biometra T Gradient thermocycler (Biometra, Göttingen, Germany) for MiSeq sequencing. Linkered primers that were used are 41F/806R for bacteria: (CS1-341F: 5'-ACACTGACGACATGGTTCTACACCTACGGGAGGCAGCAG, CS2-806R:5'-TAC-GGTAGCAGAGACTTGGTCTGGACTACHVGGGTWTCTAAT) and Ar915- Ar1386 for archaea (CS1_Ar915F:5'-ACACTGACGACATGGTTCTACAAGGAATTGGCGGGGGAGCAC, CS2_Ar1386R: TACGG-TAGCAGAGACTTGGTCTGCGGTGTGTGCAAGGAGC) for an 16S rRNA genes. All primer sets were used in PCR amplification in parallel with Dream Taq (Fermentas, Lithuania). From PCR protocol initial denaturation step of 2 min at 95°C was followed by 30 cycles of the following incubation pattern at 95°C for 20 s, 52/59°C for 20 s for bacteria or archaea, respectively, and 56°C for 65 s. A final extension at 65°C for 7 min completed the reaction.

Illumina MySeq sequencing of the PCR products was performed at DNA Services (DNAS) Facility (Research Resources Center University of Illinois at Chicago). Demultiplexed paired-end reads were analyzed using QIIME2 V2019.7 (Rideout et al. 2018). Reads were truncated based on quality plots, checked for chimeras, merged and grouped into amplicon sequence variants (ASVs) with DADA2 (Callahan et al. 2016), as implemented in QIIME2. A Naïve-Bayes classifier trained on the Silva 132 full 99%-clustered 16S rRNA sequences. Representative sequences were aligned with MAFFT (Katoh and Standley 2013), masked, and trees were generated using FastTree (Price et al. 2009), as implemented in QIIME2. Downstream statistical analyses and plotting were performed in R (R Core Team 2018), using libraries phyloseq (McMurdie and Holmes 2013), ampvis2 (Andersen et al. 2018) and ggplot2 (Wickham 2009).

S2. Supplementary Figures

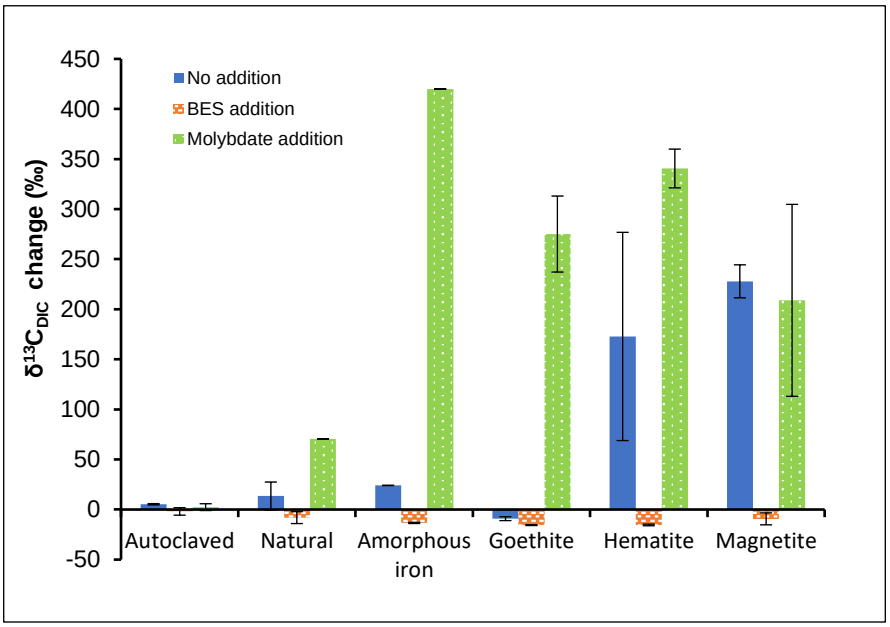

Figure S1: Net change in $\delta^{13}C_{DIC}$ values in Bar-or et al.2017 slurry incubations after 470 days. Solid blue: without inhibitor addition; Fence red: inhibition of methanogenesis by BES addition; Dot green: inhibition of sulfate reduction by molybdate addition. Analysis of DNA 16S rRNA genes was performed for all of these incubations and the untreated sediments. The following treatments: Natural (without additions), Amorphous iron with the addition of molybdate and the hematite (without additions) treatment were sequenced for metagenome analysis. After Bar-Or et al. 2017

Figure S2: The relative abundance of bacteria (top 35) at the order level in Bar-Or et al. 2017 study, based on amplicon sequencing of the 16S rRNA genes, including (a) and excluding (b) the major contaminants.

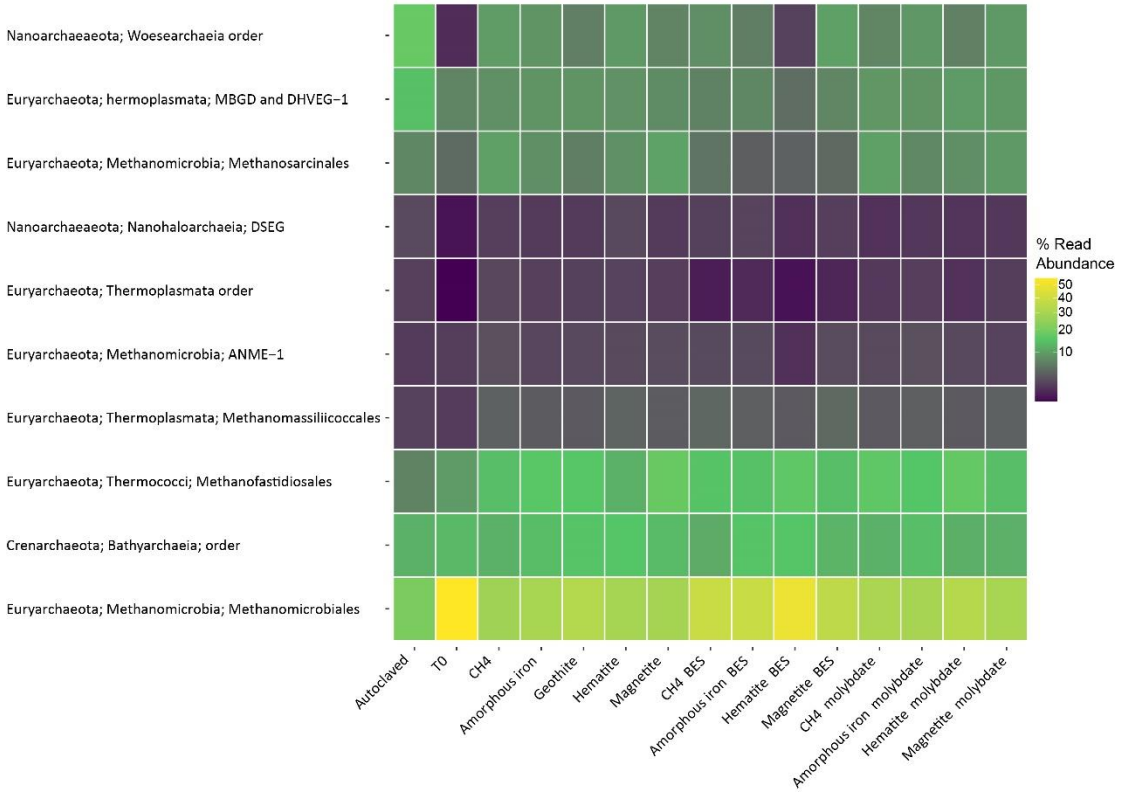

Figure S3: The relative abundance of Archaea (top 10) at the order level in Bar-Or et al. 2017 study based on amplicon sequencing of the 16S rRNA genes.

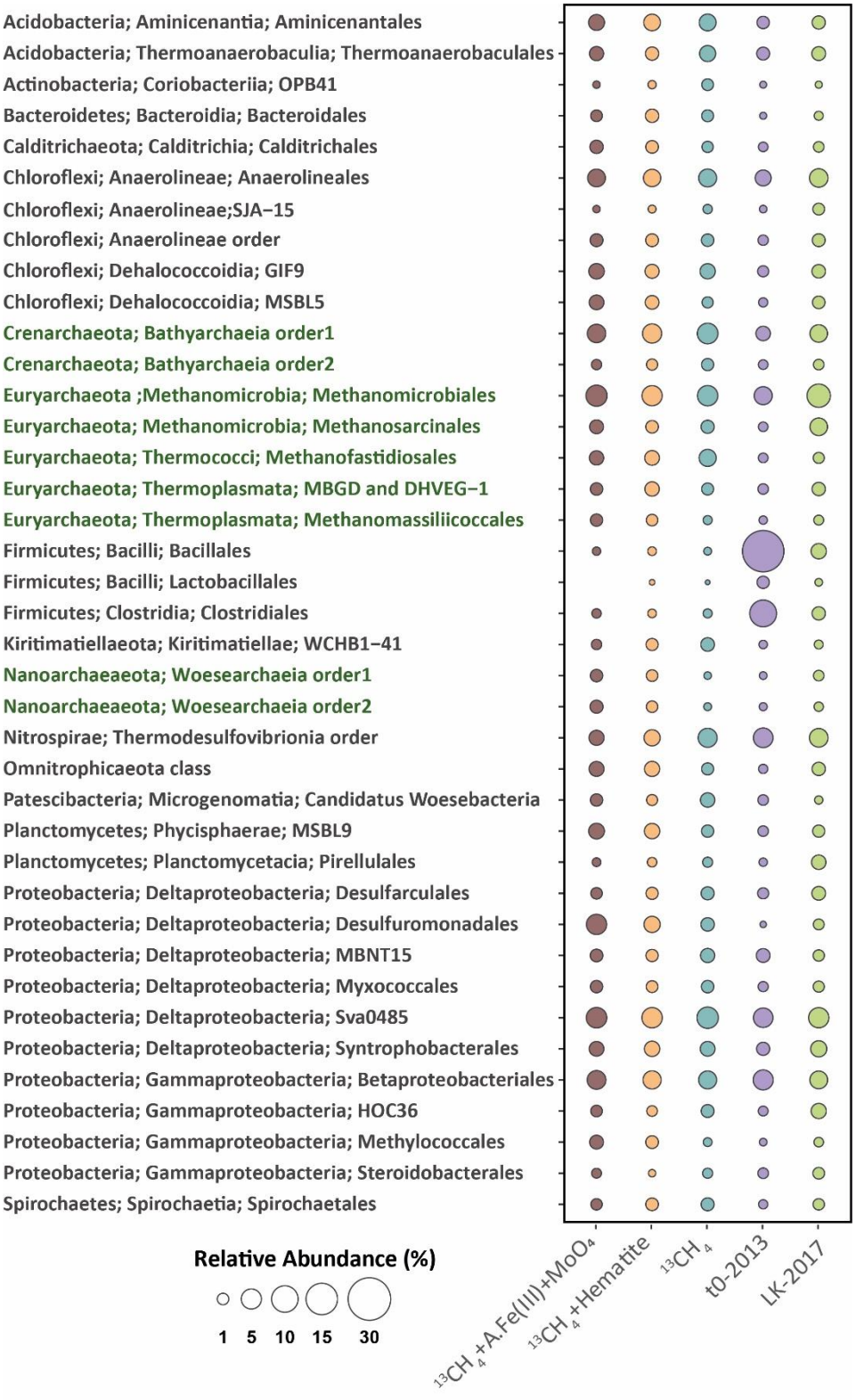

Figure S4: Relative abundance of Bacteria (black) and Archaea (green) at the order level in all five metagenomics libraries based on the mapping of metagenomic reads to the Silva132 database of the small subunit rRNA sequences. Contamination of common laboratory bacteria, such as Firmicutes and Clostridia are seen in sample t0-2013. Lineages <1%, which account together for 20-32% of the microbial community, were removed from the display.

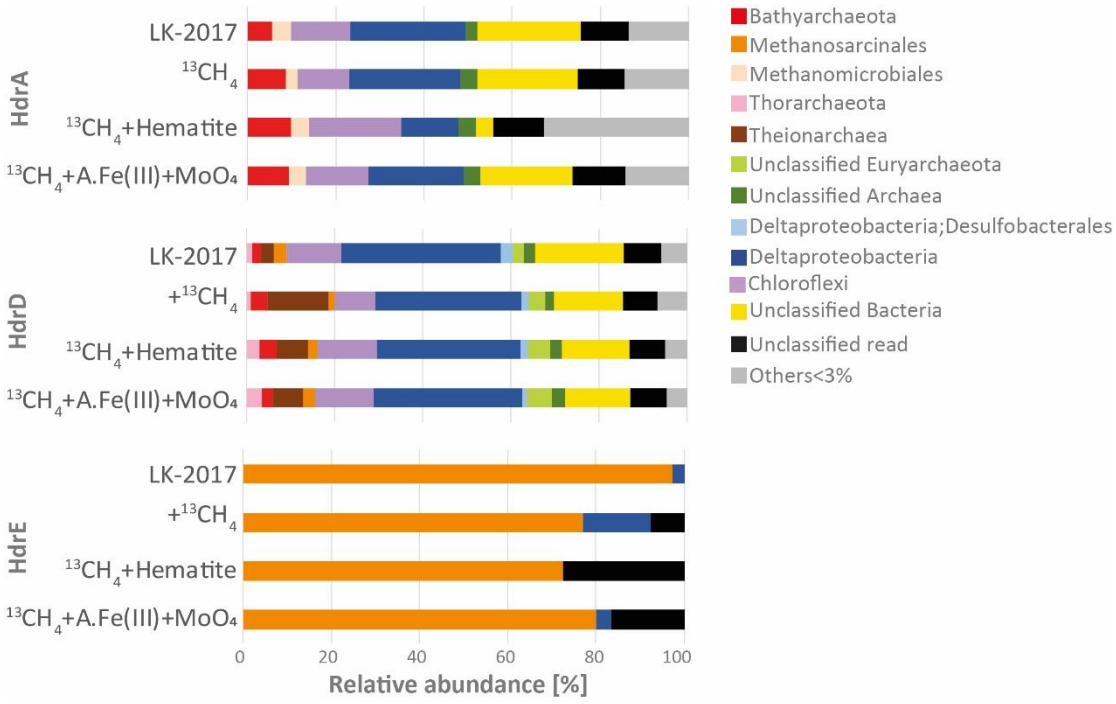

Figure S5: Phylogenetic diversity of HdrA, D and E subunits of the heterodisulfide reductase. Phylogenetic assignments are based on BLAST mapping against the RefSeq database. Taxonomic classifications at the highest level possible (up to the Order level) are shown. A.Fe(III)+Mo= amorphous iron and molybdate.

S3. Supplementary Tables

Table S1: The various treatments in all slurry incubations, from Bar-Or et al. 2017.

Slurry label	amorphous iron (0.1g)	goethite (0.1g)	hematite (0.1g)	magnetite (0.1g)	BES (0.5ml)	molybdate (0.5ml)
Autoclaved after:$^{13}CH_4$+all iron minerals	X X	X X	X X	X X		
Autoclaved after:$^{13}CH_4$+all iron minerals+BES	X X	X X	X X	X X	X X	
Autoclaved after:$^{13}CH_4$+all iron minerals+molybdate	X X	X X	X X	X X		X X
$^{13}CH_4$						
$^{13}CH_4$+BES					X X	
$^{13}CH_4$+amorphous iron	X X					
$^{13}CH_4$+amorphous iron+BES	X X				X X	
$^{13}CH_4$+goethite		X X				
$^{13}CH_4$+goethite+BES		X X			X X	
$^{13}CH_4$+hematite			X X			
$^{13}CH_4$+hematite+BES			X X		X X	
$^{13}CH_4$+magnetite				X X		
$^{13}CH_4$+magnetite+BES				X X	X X	
$^{13}CH_4$+molybdate						X X
$^{13}CH_4$+amorphous iron+molybdate	X X					X X
$^{13}CH_4$+goethite+molybdate		X X				X X
$^{13}CH_4$+hematite+molybdate			X X			X X
$^{13}CH_4$+magnetite+molybdate				X X		X X

Table S2: Thermodynamic calculation for the feasibility of active Fe-AOM in Lake Kinneret methanic zone:

Fe-AOM

Calculation of ΔG standard state

$CH_4+8Fe(OH)_3+15H^+ \rightarrow HCO_3^- + 8Fe^{2+} + 21H_2O$

Exponent	Species name	Chemical	state	ΔG0
8	Ferrous ion	Fe_2^+	aq	-78.9
21	Water	H_2O	l	-237.2
1	Bicarbonate	HCO_3^-	aq	-586.9
8	Ferric-hydroxide prec.	$Fe(OH)_3$	s	-696.6
15	Proton	H^+	aq	0
1	Methane	CH_4	aq	-34.4
ΔG0		**-592.1**	**kJ mol^{-1}**	

Fe-AOM		
G0	-592.1	kJ mol^{-1}
T	293	K
R	0.0083	KJ/(mol*K)
Q	$7.81x10^{79}$	
lnQ	183.96	
RTlnQ	448.15	kJ mol^{-1}
ΔG	**-144**	**kJ mol^{-1}**

Acetate oxidation by ferrous iron reduction

Calculation of ΔG standard state

$Rx:CH_3COOH + 8Fe(OH)_3 + 14H^+ \rightarrow 8Fe^{2+} + 2HCO_3 + 20H_2O$

Exponent	Species name	Chemical	state	ΔG0
8	Ferrous ion	Fe^{2+}	aq	-78.9
20	Water	H_2O	l	-237.2
2	Bicarbonate	HCO_3^-	aq	-586.9
8	Ferric-hydroxide prec.	$Fe(OH)_3$	s	-696.6
14	Proton	H^+	aq	0
1	Acetic acid	CH_3COOH	aq	-396.6
ΔG0		**-579.6**	**kJ mol^{-1}**	

Acetate oxidation by ferrous iron reduction		
G0	-579.6	kJ mol^{-1}
T	293	K
R	0.0083	KJ/(mol*K)
Q	$3.91x10^{72}$	
lnQ	167.15	
RTlnQ	407.2	kJ mol^{-1}
ΔG	**-172**	**kJ mol^{-1}**

H_2 Iron reduction

Calculation of ΔG standard state

$8Fe(OH)_3 + 4H_2 + 16H^+ \rightarrow 8Fe^{2+} + 24H_2O$

Exponent	Species name	Chemical	state	ΔG0
24	Water	H2O	l	-237.2
8	Ferrous ion	Fe2+	aq	-78.9
8	Ferric-hydroxide prec.	Fe(OH)3	s	-696.6
4	Hydrogen	H2	aq	17.55
16	Proton	H+	aq	0
ΔG0		**-821.4**	**kJ 4mol H_2^{-1}**	

H_2 iron reduction		
G0	-821.4	kJ mol^{-1}
T	293	K
R	0.0083	KJ/(mol*K)
Q	$3.91x10^{105}$	
lnQ	243.13	
RTlnQ	592.31	kJ mol^{-1}
ΔG	**-229**	**kJ 4mol H_2^{-1}**

Acetoclastic methanogenesis

Calculation of ΔG standard state

$CH_3COOH \rightarrow CH_4 + CO_2$

Exponent	Species name	Chemical	state	ΔG0
1	Methane	CH_4	aq	-34.4
1	Carbon dioxide	CO_2	aq	-386
1	Acetic acid	CH_3COOH	aq	-396.6
ΔG0		**-23.8**	**kJ mol^{-1}**	

Acetoclastic methanogenesis		
G0	-23.8	kJ mol^{-1}
T	293	K
R	0.0083	KJ/(mol*K)
Q	0.5	
lnQ	-0.69	
RTlnQ	-1.69	kJ mol-1
ΔG	**-25**	**kJ mol^{-1}**

H_2 methanogenesis

Calculation of ΔG standard state

HCO_3^- + $4H_2$ + H^+ → CH_4 + $3H_2O$				
Exponent	Species name	Chemical	state	ΔG0
1	Methane	CH_4	aq	-34.4
3	Water	H_2O	l	-237.2
1	Bicarbonate	HCO_3^-	aq	-586.9
4	Hydrogen	H_2	aq	17.55
1	Proton	H^+	aq	0
ΔG0		**-229.3**	**kJ 4mol H_2^{-1}**	

H_2 methanogenesis

Calculation of dG standard state

$CO2^-$ + $4H^{2+}$ → $CH4$ + $2H_2O$				
Exponent	Species name	Chemical	state	ΔG0
1	Methane	CH4	aq	-34.4
2	Water	H2O	l	-237.2
1	Carbon dioxide	CO2	aq	-386
4	Hydrogen	H2	aq	17.55
ΔG0		**-193.0**	**kJ 4mol H_2^{-1}**	

H2 methanogenesis

G0	-229.3	kJ mol^{-1}
T	293	K
R	0.0083	KJ/(mol*K)
Q	$5x10^{31}$	
lnQ	72.99	
RTlnQ	177.81	kJ mol^{-1}
ΔG	**-51**	**kJ 4mol H_2^{-1}**

H_2 meth +Fe-AOM	-195	kJ mol^{-1}
H_2 Fe red	-229	kJ mol^{-1}
Ac meth + Fe-AOM	-169	kJ mol^{-1}
Ac Fe red	-172	kJ mol^{-1}

Concentrations in the sediment (After Sivan et al., 2011; Adler, 2016)			
		mM	M
DIC conc. Used for CO_2	HCO_3^-	10	0.01
Measured	Fe^{2+}	0.1	0.0001
	CH_4	0.5	0.0005
	reac Fe(III)	200.0	0.2
pH=7	H_+	1.00E-04	1.00E-07
	H_2	0.01	0.00001
Measured	acetate	0.01	0.00001
	water	1	1

ΔG standard state			
Hydrogen	H_2	aq	17.55 kJ mol^{-1}
Ferric-hydroxide prec.	$Fe(OH)_3$	s	-696.6 kJ mol^{-1}
Ferrous ion	Fe^{2+}	aq	-78.9 kJ mol^{-1}
Proton	H^+	aq	0 kJ mol^{-1}
Carbon dioxide	CO_2	aq	-386 kJ mol^{-1}
Ferric ion	Fe^{3+}	aq	-4.6 kJ mol^{-1}
Water	H_2O	l	-237.2 kJ mol^{-1}
Methane	CH_4	aq	-34.4 kJ mol^{-1}
Bicarbonate	HCO_3^-	aq	-586.9 kJ mol^{-1}

S4. Electronic Supplementary Databases

S.DB.1| Microbial composition of Lake Kinneret sediments and slurry incubations based on SILVA (V132) database -Taxonomic classification (up until genus level) and abundance (in percentage) based on metagenomic reads to SILVA (V132) database. Eukaryote, Chloroplast, Mitochondria sequences were removed before normalization. https://doi.org/10.6084/m9.figshare.12933863.v1

S.DB.2| Microbial composition of Lake Kinneret sediments and slurry incubations based on MAR (MARine) database -Microbial abundance based on read mapping to MAR (MARine) database of prokaryotic genomes. https://doi.org/10.6084/m9.figshare.11800875.v3

S.DB.3| Abundance and classification of genes in Lake Kinneret sediments and slurry incubations based on KEGG orthology (in units of counts per million (CPM). https://doi.org/10.6084/m9.figshare.12933893.v1

S.DB.4| ANME2d Multiheme c-type cytochromes (MHC)sequences used as a query for BLASTing against Lake Kinneret sediment metagenome. https://doi.org/10.6084/m9.figshare.12933905.v1

S.DB.5| Abundance (in units of counts per million (CPM)) of genes encoding for F420:methanophenazine dehydrogenase complex (*fpoABCDHIJKLMNO*) in Lake Kinneret sediments and slurry incubations. https://doi.org/10.6084/m9.figshare.12933908.v1

S.DB.6| Metagenomic hits (amino acid sequences) for methanogenesis related enzymes -FwdC/FmdC, Ftr, Mch , MtrA, Mer, Mtd, McrA. https://doi.org/10.6084/m9.figshare.13091126.v2

S.DB.7| Metagenomic hits (amino acid sequences) for extracellular electron transfer related enzymes - MHC, OmcS and PilA. https://doi.org/10.6084/m9.figshare.13092821.v1

S.DB.8| Metagenomic hits (amino acid sequences) for heterodisulfide reductase subunits A, D and E. https://doi.org/10.6084/m9.figshare.13092842.v1

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