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Methanogenesis by CO_2 reduction dominates lake sediments with different organic matter compositions

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Supplementary tables

Table S1. Geochemical characterization and microbial activities at the deltaic (DS) and the profundal sites (PS) in Lake Geneva. Upper part: The amounts of methane, acetate and dissolved inorganic carbon (DIC) in the sediment column represent depth-integrated values based on concentrations profiles (0-30 cm). Similarly, rates of methanogenesis from bicarbonate (MGR_{DIC}) and acetate (MGR_{Ac}), and acetate oxidation (AOR) represent depth-integrated turnover rates. Lower part: Mean values for DIC and total organic carbon (TOC) concentrations, MGR_{DIC}, the stable carbon isotopic composition of porewater CH_4 ($\delta^{13}C$-CH_4), DIC ($\delta^{13}C$-DIC) and TOC ($\delta^{13}C$-TOC), the fractionation factor α_C calculated from the difference between corresponding $\delta^{13}C$-CH_4 and $\delta^{13}C$-DIC values, as well as the nitrogen isotopic signature ($\delta^{15}N$) and C/N values for sediments at the two sampling sites.

	Profundal Site (PS)	**Deltaic Site (DS)**
Depth integrated amounts and rates (0-30 cm)		
CH_4 (mmol m^{-2})	914	976
DIC (mmol m^{-2})	1964	3490
Acetate (mmol m^{-2})	0.8	2.7
MGR_{DIC} (mmol m^{-2} d^{-1})	15.5	12.1
MGR_{Ac} (mmol m^{-2} d^{-1})	0.2	0.6
MGR (mmol m^{-2} d^{-1})	15.7	12.7
AOR (mmol m^{-2} d^{-1})	0.1	0.2
Mean values (n=15) ± s.e.m. (standard error of mean).		
DIC (mM)	6.9 ± 0.4	12.2 ± 1.0
MGR_{DIC} (µmol cm^{-3} d^{-1})	52.4 ±10.0	45.6 ± 9.7
$\delta^{13}C$-CH_4 (‰)	-74.0 ±0.15	-67.0 ±0.6
$\delta^{13}C$-DIC (‰)	1.39 ± 0.8	-0.47 ± 0.9
α_C	1.081 ± 0.001	1.071 ± 0.001
TOC (%)	1.1 ± 0.1	0.6 ± 0.1
$\delta^{13}C$-TOC (‰)	-26.7 ± 0.3	-26.1 ± 0.2
C/N	7.9 ± 0.3	10.7 ± 0.2

Table S2. Fractional abundances of the 65 identified organic compounds in sediment samples of the two Lake Geneva sediment cores (PS and DS), as analyzed by Py-GC/MS.

Organic compounds	Fractional abundance (%)	
	Profundal sediments (PS)	**Deltaic sediments (DS)**
	a.v. ± sd (min – max)	a.v. ± sd (min – max)
***n*-alkanes**		
n-C12:0	**0.6** ± 0.1 (0.5 - 0.8)	**1.1** ± 0.2 (0.9 - 1.4)
n-C15:0	**1.3** ± 0.1 (1.2 - 1.4)	**0.8** ± 0.5 (0.0 - 1.3)
n-C17:0	**1.3** ± 0.2 (1.1 - 1.5)	**0.5** ± 0.4 (0.0 - 0.9)
n-C20:0	**0.7** ± 0.3 (0.3 - 0.9)	**0.3** ± 0.3 (0.0 - 0.6)
n-C23:0	**0.6** ± 0.3 (0.3 - 0.9)	**0.2** ± 0.2 (0.0 - 0.6)
n-C24:0	**0.4** ± 0.3 (0.0 - 0.7)	**0.3** ± 0.3 (0.0 - 0.7)
n-C25:0	**0.2** ± 0.3 (0.0 - 0.7)	**0.3** ± 0.5 (0.0 - 1.2)
n-C27:0	**0.2** ± 0.2 (0.0 - 0.4)	**0.1** ± 0.3 (0.0 - 0.7)
***n*-alkenes**		
n-C10:1	**1.0** ± 0.2 (0.8 - 1.3)	**1.0** ± 0.3 (0.8 - 1.4)
n-C13:1	**1.4** ± 0.2 (1.2 - 1.7)	**0.9** ± 0.7 (0.1 - 1.5)
n-C15:1	**1.7** ± 0.2 (1.3 - 1.9)	**0.8** ± 0.5 (0.2 - 1.3)
n-C16:1	**2.0** ± 1.1 (0.3 - 3.0)	**0.9** ± 0.6 (0.2 - 1.6)
n-C17:1	**1.6** ± 0.8 (0.3 - 2.4)	**0.6** ± 0.4 (0.1 - 1.1)
n-C18:1	**2.3** ± 0.9 (0.9 - 3.1)	**0.5** ± 0.4 (0.0 - 1.1)
n-C20:1	**1.2** ± 0.7 (0.1 - 1.8)	**0.3** ± 0.3 (0.0 - 0.8)
n-C22:1	**1.0** ± 0.5 (0.3 - 1.6)	**0.5** ± 0.7 (0.0 - 1.8)
n-C24:1	**0.2** ± 0.3 (0.0 - 0.6)	**0.1** ± 0.2 (0.0 - 0.5)
Carbohydrates		
2-butenal	**0.8** ± 0.5 (0.0 - 1.5)	**3.3** ± 0.8 (2.2 - 4.5)
3-methyl-butenal	**2.0** ± 0.5 (1.4 - 2.8)	**0.5** ± 0.2 (0.3 - 0.8)
3(2H)-furanone	**1.2** ± 0.4 (0.8 - 1.8)	**1.3** ± 0.7 (0.1 - 1.9)
3-furaldehyde	**1.6** ± 0.2 (1.4 - 2.0)	**2.0** ± 0.1 (1.8 - 2.2)
2-furaldehyde	**7.7** ± 0.9 (6.9 - 8.9)	**9.3** ± 0.7 (8.5 - 10.1)
2-Cyclopenten-1-one	**2.4** ± 0.4 (2.0 - 2.8)	**2.9** ± 0.7 (2.3 - 4.2)
5-methyl -2(3H)-furanone	**0.4** ± 0.1 (0.3 - 0.5)	**0.8** ± 0.3 (0.3 - 1.1)
4-Cyclopentene-1,3-dione	**0.48** ± 0.04 (0.42 - 0.53)	**0.9** ± 0.1 (0.7 - 1.1)
2-Cyclopenten-1-one, 2-methyl-	**1.5** ± 0.2 (1.4 - 1.9)	**1.7** ± 0.5 (1.3 - 2.5)
2-acetyl-furan	**0.8** ± 0.1 (0.7 - 1.0)	**1.1** ± 0.1 (0.9 - 1.3)
Methyl-2-furaldehyde	**0.8** ± 0.2 (0.7 - 1.2)	**1.3** ± 0.2 (1.0 - 1.4)
2(3H)-furanone	**1.4** ± 0.3 (1.2 - 1.9)	**1.1** ± 0.6 (0.0 - 1.6)
Methyl-2-furaldehyde	**4.7** ± 0.5 (4.1 - 5.5)	**4.0** ± 0.4 (3.6 - 4.6)
3-methyl-2-cyclopenten-1-one	**1.5** ± 0.2 (1.4 - 1.8)	**1.6** ± 0.1 (1.5 - 1.7)
2,3 dihydro-benzofuran	**1.0** ± 0.6 (0.0 - 1.5)	**1.6** ± 1.0 (0.0 - 2.4)
N compounds		
Methyl-pyrrole	**1.1** ± 0.2 (0.8 - 1.3)	**1.0** ± 0.2 (0.9 - 1.3)
Pyridine	**9.1** ± 1.1 (7.4 - 10.0)	**8.8** ± 1.4 (7.5 - 11.1)
Pyrrole	**0.4** ± 0.0 (0.3 - 0.4)	**0.4** ± 0.2 (0.0 - 0.5)
Pyrrole	**0.6** ± 0.2 (0.4 - 0.8)	**0.3** ± 0.0 (0.2 - 0.3)
2-methyl-pyridine	**1.7** ± 0.4 (1.2 - 2.4)	**4.3** ± 0.8 (3.4 - 5.4)
Methyl-pyrrole	**3.6** ± 0.4 (3.0 - 4.0)	**3.2** ± 0.6 (2.4 - 4.0)
Methyl-pyrrole	**0.3** ± 0.1 (0.2 - 0.5)	**0.3** ± 0.2 (0.1 - 0.5)
3/4-methyl-pyridine	**0.7** ± 0.3 (0.5 - 1.2)	**0.7** ± 0.2 (0.4 - 1.0)
Dimethyl-pyrrole	**0.9** ± 0.3 (0.7 - 1.5)	**1.2** ± 0.4 (0.8 - 1.8)
Pyrrole-2,5-dione (maleimide)	**1.1** ± 0.1 (0.9 - 1.2)	**0.7** ± 0.2 (0.4 - 0.8)
Benzonitrile	**1.1** ± 0.2 (0.8 - 1.3)	**4.0** ± 0.9 (2.9 - 5.3)
2-acetyl-pyrrole	**1.3** ± 0.1 (1.2 - 1.5)	**0.7** ± 0.6 (0.0 - 1.3)
Benzeneacetonitrile	**3.6** ± 0.2 (3.3 - 4.0)	**3.4** ± 1.9 (0.0 - 4.8)

Benzenepropanenitrile	**1.6** ± 0.2 (1.4 - 1.9)	**1.9** ± 0.1 (1.7 - 2.0)
Indole	**1.4** ± 0.2 (1.2 - 1.6)	**0.6** ± 0.6 (0.0 - 1.1)
Methyl-indole	**1.7** ± 1.1 (0.5 - 2.7)	**1.1** ± 0.6 (0.1 - 1.5)
Diketodipyrrole	**1.1** ± 0.2 (0.8 - 1.3)	**1.0** ± 0.2 (0.9 - 1.3)
Alkanenitrile	**9.1** ± 1.1 (7.4 - 10.0)	**8.8** ± 1.4 (7.5 - 11.1)
Phenols and lignin oligomers		
Phenol	**0.3** ± 0.1 (0.2 - 0.4)	**0.7** ± 0.2 (0.4 - 1.0)
3/4- methyl-phenol	**3.6** ± 0.3 (3.3 - 4.0)	**5.5** ± 0.9 (4.6 - 7.1)
Dimethyl-phenol	**2.6** ± 0.2 (2.3 - 3.0)	**4.3** ± 0.3 (3.9 - 4.7)
4-vinyl-guaiacol	**0.3** ± 0.0 (0.2 - 0.3)	**0.5** ± 0.2 (0.2 - 0.7)
(poly)aromatics		
Benzene	**2.9** ± 0.3 (2.5 - 3.2)	**2.2** ± 0.8 (1.5 - 3.5)
Toluene	**6.7** ± 1.4 (5.8 - 9.2)	**3.3** ± 0.4 (2.6 - 3.7)
Benzaldehyde	**0.8** ± 0.2 (0.6 - 1.0)	**1.3** ± 0.4 (0.9 - 2.0)
Benzeneacetaldehyde	**1.4** ± 0.2 (1.2 - 1.7)	**1.2** ± 0.2 (1.0 - 1.5)
1-propynyl-benzene or indene	**0.8** ± 0.0 (0.8 - 0.9)	**1.9** ± 0.4 (1.5 - 2.6)
Acetyl-benzene	**0.7** ± 0.1 (0.6 - 0.9)	**2.0** ± 0.9 (1.2 - 3.4)
Methyl-1H-Indene or methyl-(propynyl)-benzene	**1.1** ± 0.0 (1.0 - 1.1)	**0.7** ± 0.4 (0.0 - 1.1)
ethyl-dimethyl-benzene	**0.4** ± 0.1 (0.3 - 0.5)	**0.7** ± 0.1 (0.5 - 0.9)
2,3-dihydro-inden-1-one	**1.0** ± 0.1 (1.0 - 1.1)	**0.4** ± 0.3 (0.0 - 0.6)
Benzene C17	**0.5** ± 0.1 (0.4 - 0.6)	**0.3** ± 0.2 (0.0 - 0.5)
Benzene C18	**0.3** ± 0.2 (0.0 - 0.5)	**0.1** ± 0.1 (0.0 - 0.2)

[1] Schellekens et al. (2009)

Table S3. TOC-normalized concentrations of selected lipid biomarkers and their $\delta^{13}C$ (in ‰ vs. V-PDB) in profundal (PS) and deltaic (DS) sediments of Lake Geneva. N-alkanes with 15-35 carbon atoms were used for the determination of average chain lengths (ACL), and chain lengths of 21-35 carbon atoms were used for computing the carbon preference index (CPI) (Bray and Evans 1961).

Lipid biomarkers		Sediment samples at PS					Sediment samples at DS				
		0-2	4-6	10-12	18-20	28-30	0-2	4-6	10-12	18-20	28-30
Concentrations (µg/g TOC)											
Fatty acids	C16:1w7	201	328	103	69	42	293	1250	1188	278	134
	C16:1w5	111	167	116	59	40	85	64	82	45	31
	C16:0	1827	2985	2516	2102	1947	1316	1016	1365	700	515
	C18:1w9	459	656	479	337	282	606	434	548	355	203
	C18:1w7	178	287	256	185	162	146	94	105	65	52
	C24:0	429	573	1538	1825	1634	217	219	375	246	219
	C26:0	226	377	1207	1045	993	134	130	248	159	142
	C28:0	119	285	626	437	447	119	89	203	130	102
Alcohols	Phytol	1013	1271	1413	2106	1301	365	522	516	388	159
	Cholesterol	384	385	319	252	185	340	287	223	114	48
	Brassicasterol	179	207	76	147	79	83	128	86	72	28
n-alkanes	C17	9	8	30	13	17	6	11	13	10	8
	C23	14	21	39	37	30	13	11	14	10	11
	C25	22	58	54	42	26	25	24	30	24	19
	C27	40	66	79	59	40	69	63	82	62	44
	C29	50	74	105	88	75	77	68	83	66	50
	C31	48	69	78	73	57	66	60	73	61	48
	C33	18	23	28	27	23	24	23	27	23	17
ACL		27.5	27.5	26.8	27.2	27.1	27.9	27.8	27.7	28	27.6
CPI		4.5	5.1	4.2	4.3	3.6	5	4.1	5.2	5.7	4.8
Stable carbon isotopic compositions (in ‰ vs. V-PDB)											
Fatty acids	C16:1w7	-39.6	-40.0	-29.0	-30.6	-30.5	-33.5	-29.6	-41.1	-38.7	-31.2
	C16:1w5	-54.3	-52.8	-32.6	-35.0	-33.0	-33.8	-39.9	-39.2	-36.5	-30.4
	C16:0	-32.3	-32.5	-25.7	-27.2	-28.4	-30.7	-31.3	-32.7	-29.5	-29.4
	C18:1w9	-30.2	-29.6	-25.8	-24.9	-25.7	-30.2	-31.5	-31.3	-29.5	-27.4
	C18:1w7	-35.7	-33.2	-28.7	-28.9	-28.3	-37.8	-33.8	-34.3	-34.1	-32.8
	C24:0	-27.9	-29.3	-24.5	-22.8	-22.6	-28.9	-31.3	-30.3	-29.3	-28.0
	C26:0	-26.5	-26.9	-23.4	-22.5	-23.5	-30.7	-31.8	-29.9	-29.6	-29.5
	C28:0	-29.7	-30.1	-28.5	-27.0	-27.3	-30.9	-32.1	-31.0	-31.6	-31.3
Alcohols	Phytol	-25.5	-25.5	-25.5	-20.0	-21.5	-24.5	-24.3	-23.8	-22.3	-20.9
	Cholesterol	-27.1	-27.1	-27.1	-21.8	-19.6	-26.1	-25.2	-25.7	-26.3	-25.3
	Brassicasterol	-30.6	-30.6	-30.6	-33.6	-29.6	-28.4	-43.4	-28.0	-28.9	-27.0
n-alkanes	C17	-27.4	-41.8	-36.1	n.a.	-35.2	-37.4	-38.6	-39.1	-40.0	-37.3
	C23	-28.5	-32.0	-30.5	-32.4	-29.9	-35.1	-30.5	-30.1	-29.9	-31.3
	C25	-31.7	-29.3	-31.8	-29.0	-31.0	-33.5	-32.7	-31.9	-31.0	-32.7
	C27	-33.7	-32.6	-33.5	-32.7	-31.9	-34.2	-32.6	-33.1	-33.7	-33.5
	C29	-35.4	-34.3	-33.7	-35.1	-35.1	-35.4	-33.5	-34.3	-35.1	-34.7
	C31	-35.4	-33.8	-34.8	-35.1	-34.9	-36.1	-34.0	-34.9	-35.3	-35.0
	C33	-35.3	-33.1	-34.7	-35.8	-34.7	-36.1	-32.9	-34.5	-35.1	-35.0

n.a., not available.

Table S4. Coverage of the primer pair 515F-Y/926R for methanogen sequences in the SILVA database (SSU-138, RefNr) assessed by the TestPrime tool (Klindworth et al. 2013) (https://www.arb-silva.de/search/testprime/). Methanogens detected in this study are indicated in bold.

Taxa			**Coverage**	Accessions	Match	Mismatch
Order	Family	Genus	**(%)**			
Methanobacteriales	**Methanobacteriaceae**	***Methanobacterium***	**88.8**	**216**	**191**	**24**
Methanobacteriales	**Methanobacteriaceae**	***Methanobrevibacter***	**91.1**	**1030**	**938**	**92**
Methanobacteriales	Methanobacteriaceae	*Methanosphaera*	77.8	198	154	44
Methanobacteriales	Methanothermaceae	*Methanothermus*	100	2	2	0
Methanobacteriales	Methanothermobacteriaceae	*Methanothermobacter*	85.1	47	40	7
Methanococcales	Methanocaldococcaceae	*Methanocaldococcus*	96.3	27	26	1
Methanococcales	Methanococcaceae	*Methanococcus*	92.9	28	26	2
Methanococcales	Methanococcaceae	*Methanothermococcus*	88.9	9	8	1
Methanococcales	Methanococcaceae	*Methanotorris*	35.7	14	5	9
Methanopyrales	Methanopyraceae	*Methanopyrus*	0	9	0	9
Methanomicrobiales	Methanocorpusculaceae	*Methanocalculus*	88.2	34	30	4
Methanomicrobiales	Methanocorpusculaceae	*Methanocorpusculum*	79.1	45	34	9
Methanomicrobiales	Methanomicrobiaceae	*Methanoculleus*	86.1	204	174	28
Methanomicrobiales	Methanomicrobiaceae	*Methanofollis*	95.5	22	21	1
Methanomicrobiales	Methanomicrobiaceae	*Methanogenium*	90.6	32	29	3
Methanomicrobiales	Methanomicrobiaceae	*Methanolacinia*	100	6	6	0
Methanomicrobiales	Methanomicrobiaceae	*Methanomicrobium*	76.9	39	30	9
Methanomicrobiales	Methanomicrobiaceae	*Methanoplanus*	100	5	5	0
Methanomicrobiales	**Methanoregulaceae**	***Methanolinea***	**87.6**	**99**	**85**	**12**
Methanomicrobiales	**Methanoregulaceae**	***Methanoregula***	**81.7**	**186**	**152**	**34**
Methanomicrobiales	**Methanoregulaceae**	***Methanosphaerula***	**100**	**4**	**4**	**0**
Methanomicrobiales	**Methanospirillaceae**	***Methanospirillum***	**90.3**	**115**	**102**	**11**
Methanotrichales	**Methanotrichaceae**	***Methanothrix***	**83.2**	**564**	**466**	**94**
Methanosarciniales	Methanosarcinaceae	*Methanimicrococcus*	88.2	34	30	4
Methanosarciniales	Methanosarcinaceae	*Methanococcoides*	82.2	45	37	8
Methanosarciniales	Methanosarcinaceae	*Methanohalobium*	100	4	4	0
Methanosarciniales	Methanosarcinaceae	*Methanohalophilus*	89.7	39	35	4
Methanosarciniales	Methanosarcinaceae	*Methanolobus*	83.3	66	55	11
Methanosarciniales	Methanosarcinaceae	*Methanomethylovorans*	85.7	49	42	7
Methanosarciniales	Methanosarcinaceae	*Methanosalsum*	100	3	3	0
Methanosarciniales	**Methanosarcinaceae**	***Methanosarcina***	**81.3**	**251**	**196**	**45**

Supplementary figures

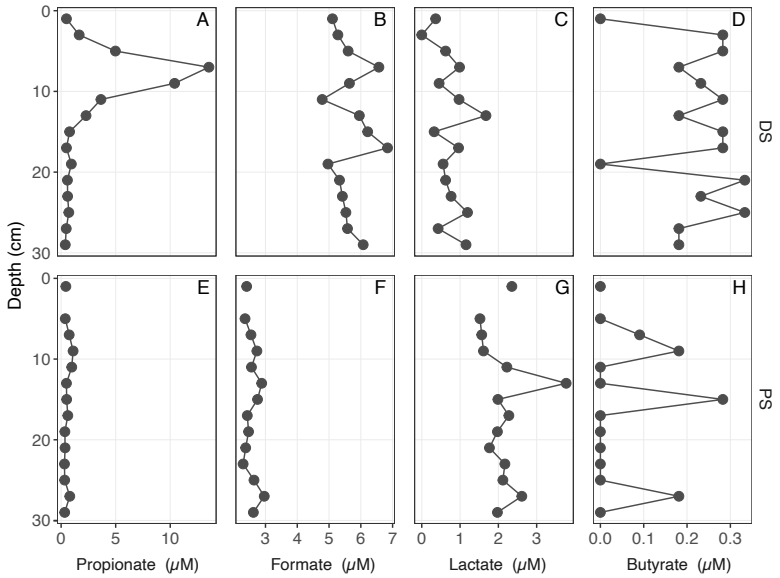

Fig. S1. Concentration profiles of volatile fatty acids (VFA) including propionate (A, E), formate (B, F), lactate (C, G) and butyrate (D, H) in the sediment porewater of the deltaic (DS) and the profundal site (PS) in Lake Geneva.

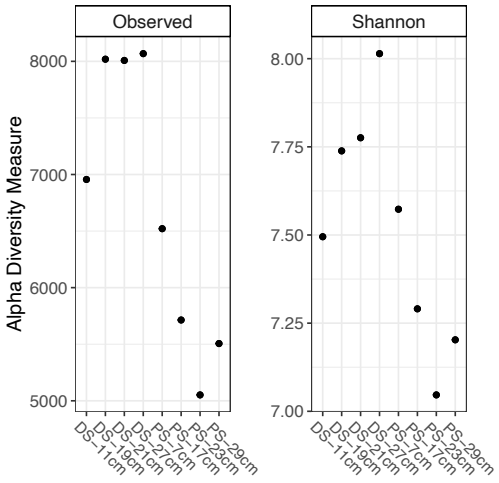

Fig. S2. Alpha diversity measures with the most common indices (Observed and Shannon), showing the microbial community richness of individual sediment samples at the profundal site (PS) and the deltaic site (DS) in Lake Geneva.

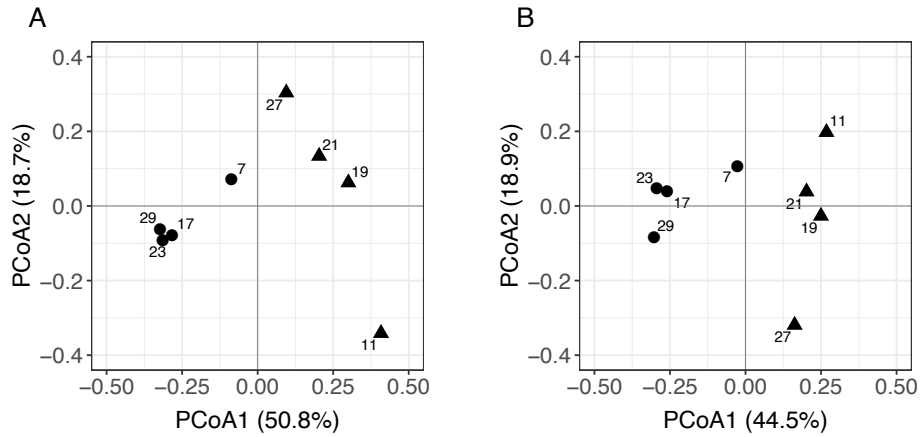

Fig. S3. Principal coordinate analysis (PCoA) plots showing the community differences of (A) archaea and (B) bacteria between sediment samples from deltaic (DS, filled triangles) and profundal (PS, filled circles) sediments in Lake Geneva. The analysis was performed on a Bray-Curtis dissimilarity matrix of rarefied read abundances of 16S rRNA gene sequences.

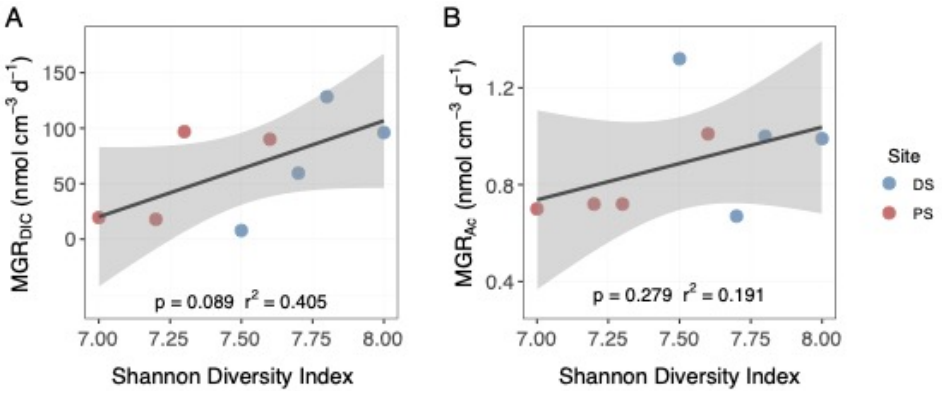

Fig. S4. Correlation of hydrogenotrophic (A) and acetoclastic methanogenesis (B) with microbial diversity in sediments of the deltaic (DS, blue circles) and the profundal site (PS, orange circles) in Lake Geneva.

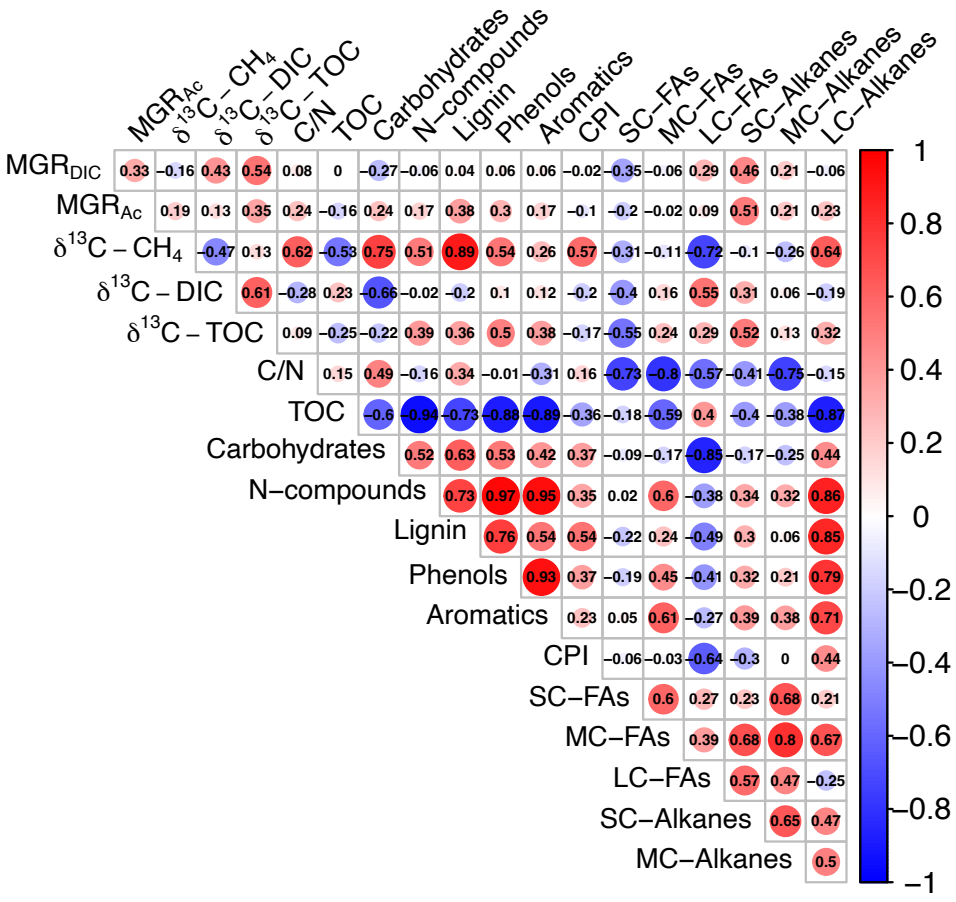

Fig. S5. Pearson correlation analysis (significance level α = 0.05) between hydrogenotrophic (MGR_{DIC}) and acetoclastic (MGR_{Ac}) methane production rates, and environmental parameters, including the carbon isotopic compositions of different carbon pools, TOC-normalized relative abundances of carbohydrates, n-compounds, n-alkenes, lignin, phenols and aromatics, and concentrations of different groups of lipid biomarkers. The numbers represent Pearson correlation coefficients, and the circles indicate significant correlation ($p < 0.05$; red: positive correlation; blue: negative correlation). CPI, carbon preference index based on $C_{21\text{-}33}$ n-alkanes; SC-FAs, short-chain fatty acids ($C_{14:0}$+$C_{16:0}$+$C_{18:0}$); MC-FAs, median-chain fatty acids ($C_{20:0}$+$C_{22:0}$); LC-FAs, long-chain fatty acids ($C_{24:0}$+$C_{26:0}$+$C_{28:0}$); SC-Alkanes, short-chain n-alkanes (C_{15}+C_{17}+C_{19}); MC-FAs, median-chain n-alkanes ($C_{23:0}$+$C_{25:0}$); LC-Alkanes, long-chain n-alkanes (C_{27}+C_{29}+C_{31}+C_{33}).

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