



*Supplement of*

## **Microbial activity, methane production, and carbon storage in Early Holocene North Sea peats**

**Tanya J. R. Lippmann et al.**

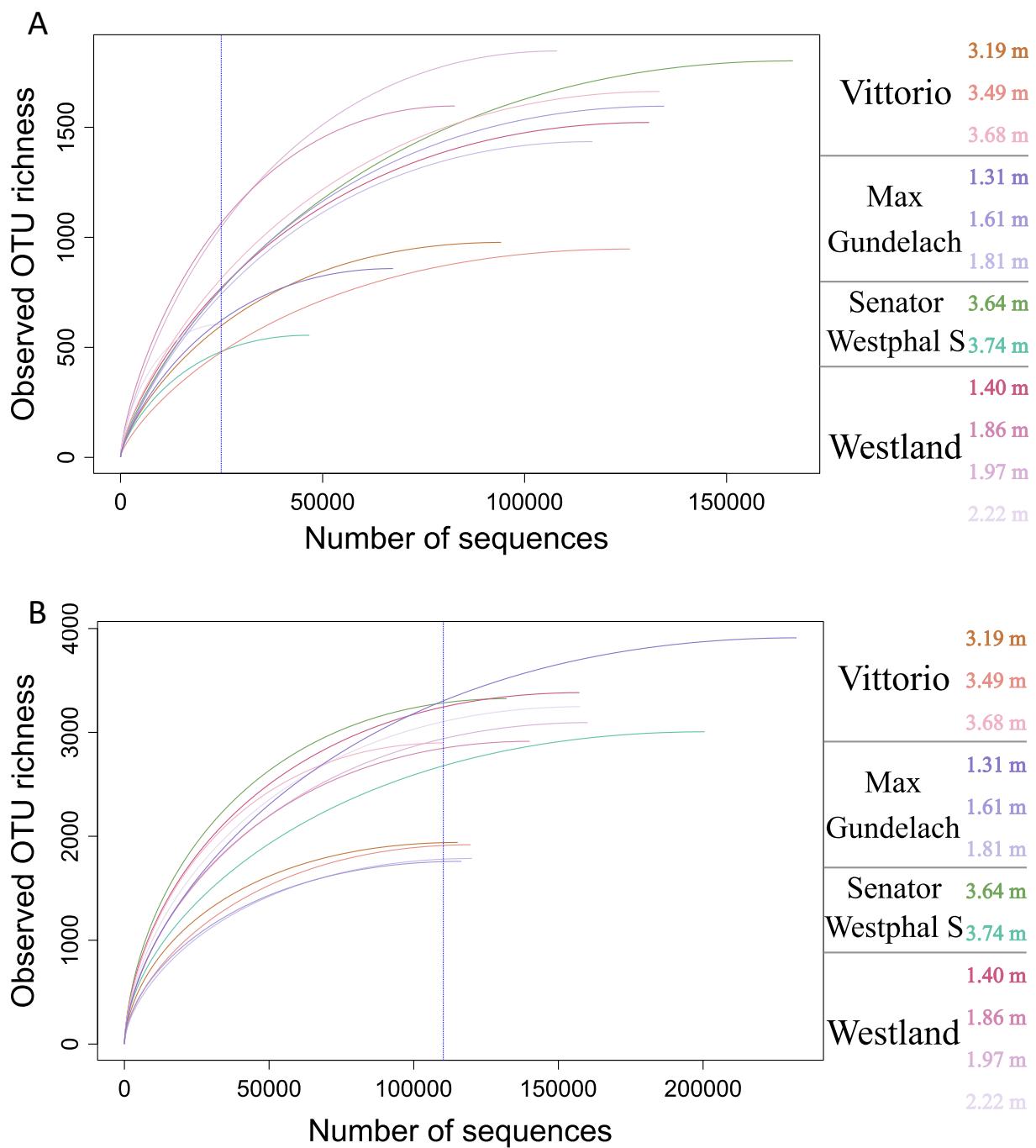
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## Supplementary Materials

**Table S1. The analyses performed on each core, at each site of this study.** An overview of the different analysis of each core is shown in the columns. Each row represents one core and shading delineates sites. "Y" is indicative of the analysis performed. The sites are named after nearby shipwrecks as documented on EMODnet (portal.emodnet-bathymetry.eu). An asterisk, "\*", next to the site name indicates that peat was not recovered at this site. All core photographs will be searchable using the Lab Code at [www.dinoloket.nl](http://www.dinoloket.nl).

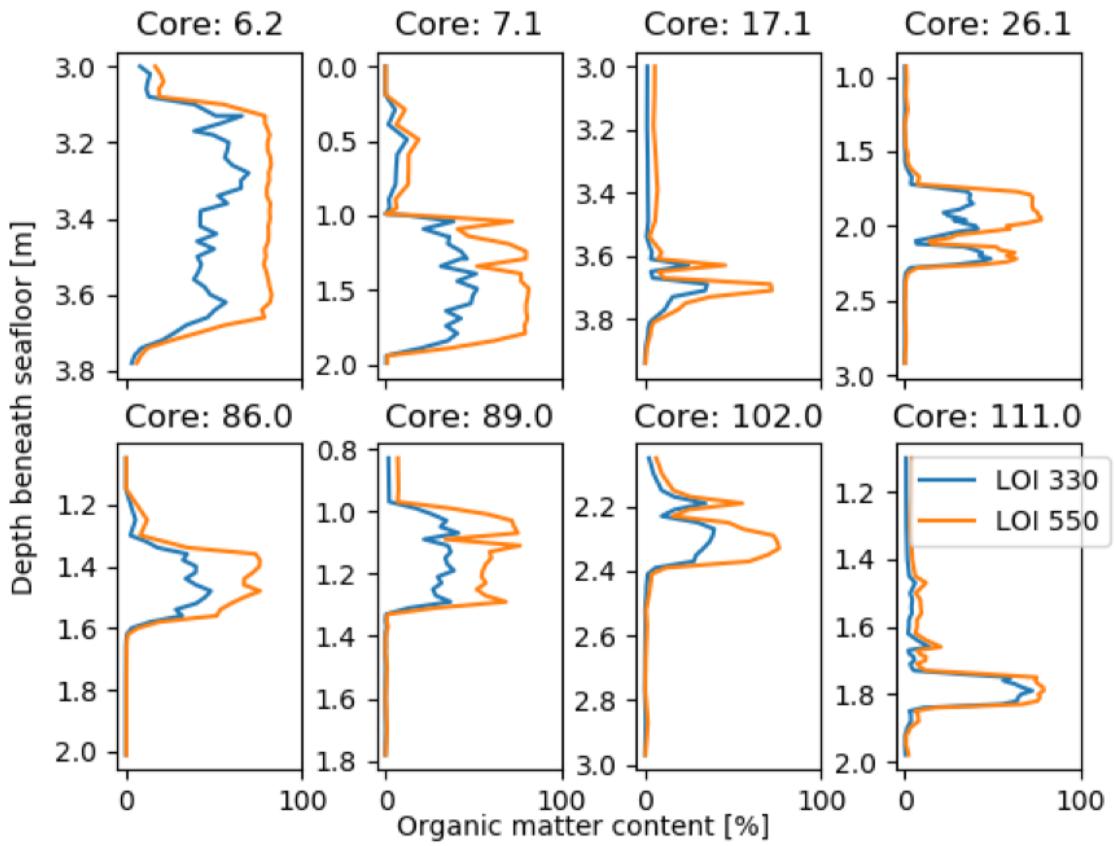
Site Name	Area	Core Name	DINOloket code	Depth of Peat Layer	Latitude	Longitude	Depth Seafloor (m)	Peat Layer Thickness (m)	CH <sub>4</sub>	Pore Water	Microbial Sequencing	Microbial Activity	Loss On Ignition	14C Dating	Plant Macrofossils
TX24	IJmuiden	14	BQ070309	1.57	52° 32.20176'	4° 0.07968'	26.81	0.19	Y	-	Y	-	-	-	-
Theodor	IJmuiden	18	BQ070311	2.52	52° 31.43544'	4° 2.23164'	25.66	0.40	Y	-	-	-	-	-	-
Easting Down*	Brown Bank	41	BP080097	N/A	52° 32.14668'	3° 23.78778	32.37	N/A	Y	-	-	-	-	-	-
Leda	Brown Bank	46	BP100076	-	52° 28.90746	3° 4.55616'	34.48	0.19	Y	-	-	-	-	-	-
Stormvogel*	Frisian Front	67	-	N/A	53° 27.86196	4° 16.25832	27.00	N/A	Y	-	-	-	-	-	-
Vittorio	Vlieland	6_2	BL120234	3.19	53° 20.0796'	4° 51.06282	18.70	0.53	-	Y	Y	-	Y	-	-
Vittorio Max	Vlieland	6_4	BL120236	3.13	53° 20.09718	4° 51.0702'	18.70	0.53	Y	-	-	-	-	-	-
Gundelach Max	Vlieland	7_1	BL120237	1.20	53° 20.38158	4° 51.60276	18.90	0.85	-	Y	Y	-	Y	Y	Y
Gundelach	Vlieland	7_3	BL120239	1.50	53° 20.38788	4° 51.61068	18.80	0.85	Y	-	-	-	-	-	-
U21	Vlieland	13_1	BF160059	-	54° 8.13726'	4° 7.76970'	46.00	0.10	-	Y	-	-	-	-	-
U21	Vlieland	13_3	BF160061	2.60	54° 8.13900'	4° 7.76406'	45.90	0.10	Y	-	-	-	-	-	-
Senator	Vlieland	16_1	BL020084	-	53° 57.91302	4° 26.57268'	40.00	0.10	-	Y	-	-	-	-	-
Westphal SW	Vlieland	16_3	BL020086	1.42	53° 57.91134'	4° 26.57544'	40.00	0.10	Y	-	-	-	-	-	-
Westphal SW	Vlieland	17_1	BL020087	3.59	53° 55.41432	4° 30.55746'	38.90	0.08	-	Y	Y	-	Y	-	-
Westphal S	Vlieland	17_3	BL020089	3.50	53° 55.41144	4° 30.55410'	39.40	0.08	Y	-	-	-	-	-	-
Mahren S	Vlieland	24_1	BL050113	-	53° 44.50896	4° 29.93766'	35.20	0.09	-	Y	-	-	-	-	-
Mahren S	Vlieland	24_3	BL050115	3.25	53° 44.51268	4° 29.93994'	35.10	0.09	Y	-	-	-	-	-	-
Mahren SW	Vlieland	25_1	BL050116	-	53° 44.66952	4° 29.91900'	34.90	-	-	-	-	-	-	-	-
Westland	Vlieland	26_1	BL050118	1.68	53° 45.41142	4° 25.74564'	36.80	0.88	-	Y	Y	-	Y	-	-
Westland	Vlieland	26_3	BL050120	1.97	53° 45.41568	4° 25.74798'	37.00	0.88	Y	-	-	-	-	-	-
Westland S	Vlieland	28_1	BL050123	-	53° 40.22754	4° 25.45656'	33.80	0.88	-	Y	-	-	-	-	-
Dorthea Deep SW	Doggerbank	83	BA090022	0.71	55° 36.57720	3° 57.42582'	37.70	0.07	Y	-	-	-	-	-	-
Dorthea Shallow SW	Doggerbank	86	BA090024	1.32	55° 36.56184	3° 57.43230'	37.24	0.30	Y	-	-	Y	Y	-	-
Dorthea SSW	Doggerbank	89	BA090026	0.99	55° 36.74568	3° 57.03786	37.51	0.34	Y	-	-	Y	Y	-	-
Dorthea NNW	Doggerbank	97	BDEU0708	2.33	55° 44.69892	3° 46.30440'	44.42	0.10	Y	-	-	-	-	-	-
Dorthea NW	Doggerbank	102	BDEU0705	2.19	55° 47.04174	3° 41.14170'	51.26	0.16	Y	-	-	Y	Y	-	-
Fredricksborg NE	Doggerbank	111	BA050024	1.74	55° 49.48110	3° 26.42682'	59.56	0.13	Y	-	-	Y	Y	-	Y
Fredricksborg NE	Doggerbank	112	-	2.74	55° 49.48926	3° 26.39760'	59.41	0.13	-	-	-	-	-	Y	-
Fredricksborg NW	Doggerbank	114	BA050025	2.06	55° 49.49064	3° 26.40180'	59.92	0.34	Y	-	-	-	-	-	-
Darci's site*	Doggerbank	163	-	N/A	55° 18.38796	4° 5.42544'	43.8	N/A	Y	-	-	-	-	-	-



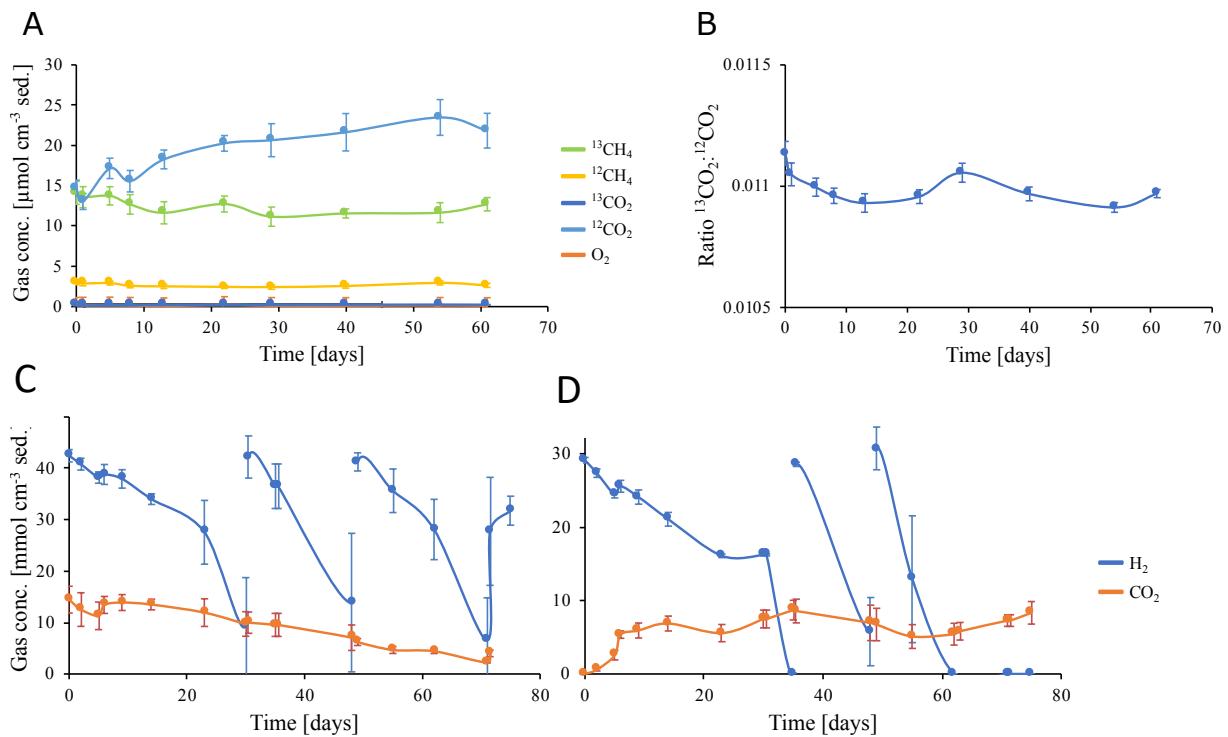
**Figure S1.** **(A)** Rarefaction curves from the archaeal reads and **(B)** from the bacterial reads. Singletons were removed. The blue vertical line represents lowest number of sequences found in sample “Core 26 – 2.22 m” for archaea and “Core 6 – 3.58 m” for bacteria. Archaeal datasets were rarefied to 24,925 reads; bacterial datasets were rarefied to 110,236 reads.

**Table S2. Pore water data** analyzed by inductive coupled plasma-optical emission spectrometry (ICP-OES) for aluminium (Al), calcium (Ca), iron (Fe), potassium (K), magnesium (Mg), manganese (Mn), sodium (Na), phosphorus (P), sulfur (S), silicon (Si), and zinc (Zn) in parts per million (ppm). Pore water data were analyzed by continuous flow analysis (CFA) for nitrate ( $\text{NO}_3^-$ ), ammonium ( $\text{NH}_4^+$ ) and phosphate ( $\text{PO}_4^{3-}$ ) in parts per million (ppm). AVG values show average values, STDEV values show standard deviation of the mean depth below sea floor (dbsf), ND: not detected, ADL: above detection limit. AVG values show average values, STDEV values show standard deviation of the mean. The site names of all cores are given in Table S1.

Core	dbsf	Al	Ca	Fe	K	Mg	Mn	Na	P	S	Si	Zn	$\text{NO}_3^-$	$\text{NH}_4^+$	$\text{PO}_4^{3-}$
6	3	0.1	627	0.0	411	863	0.8	7337	2.3	243	17.8	0.0	1.3	2004.2	14.3
	3.1	0.0	556	0.0	1359	767	0.6	7062	0.4	239	18.1	0.0	1.4	160.0	1.6
	3.4	ND	559	0.0	947	854	0.9	7612	2.6	275	17.7	0.0	1.3	391.8	3.3
	3.6	ND	471	0.2	3502	570	0.5	4947	1.5	303	14.4	0.2	1.2	48.7	0.9
	AVG	0.1	553	0.1	1555	764	0.7	6739	1.7	265	17.0	0.1	1.3	651.2	5.0
7	STDEV	0.0	64	0.1	1355	136	0.2	1216	1.0	30	1.8	0.1	0.1	913.3	6.3
	1.58	ND	367	ND	2539	759	0.5	6344	2.2	334	14.1	ND	1.0	76.7	1.0
	1.68	0.1	492	ND	612	974	0.7	8493	2.0	436	17.6	0.0	1.1	428.2	3.6
	1.78	0.1	410	ND	2249	776	0.5	6800	1.9	334	14.2	0.0	3.5	192.3	1.1
	1.88	ND	558	ND	1036	1029	0.5	9442	2.9	435	20.2	0.0	1.6	64.1	0.9
	1.98	ND	528	0.0	419	942	0.5	8596	1.7	386	16.9	0.0	1.5	382.8	2.4
	2.08	ND	550	ND	854	982	0.3	8880	2.2	399	16.3	0.0	1.5	60.3	0.7
	AVG	0.1	484	0.0	1285	910	0.5	8093	2.2	387	16.5	0.0	1.7	200.7	1.6
	STDEV	0.0	79	0.0	889	114	0.1	1231	0.4	46	2.3	0.0	0.9	166.6	1.2
	3.45	0.0	340	0.0	647	1063	0.1	ADL	2.9	593	14.1	0.0	0.1	55.6	1.3
17	3.55	0.0	344	0.0	429	1080	0.0	ADL	2.9	595	12.4	0.0	0.1	56.8	1.3
	3.65	ND	353	0.0	552	1100	0.1	8317	3.0	597	12.1	0.0	0.1	27.0	0.6
	3.75	0.0	346	0.0	743	1068	0.1	8415	2.7	589	11.6	0.0	0.1	17.2	0.3
	3.85	0.0	359	0.0	521	1119	0.1	8434	5.6	623	11.9	0.0	0.1	44.9	0.9
	AVG	0.0	348	0.0	579	1086	0.1	8388	3.4	600	12.4	0.0	0.1	40.3	0.9
26	STDEV	0.0	8	0.0	121	23	0.0	63	1.2	14	1.0	0.0	0.0	17.6	0.4
	1.67	ND	327	0.0	958	1124	0.1	9196	1.7	570	10.0	0.0	1.1	293.6	2.5
	1.77	0.0	305	0.0	413	1073	0.1	ADL	1.6	559	9.5	0.0	0.2	436.3	2.8
	1.86	0.0	297	ND	419	1054	0.1	8007	1.5	534	9.2	0.0	0.9	427.8	3.3
	1.96	0.0	295	0.0	446	1037	0.1	ADL	1.5	535	9.3	0.0	0.4	454.8	3.2
	2.06	ND	308	ND	1008	1076	0.1	8297	1.8	539	10.3	ND	0.4	69.1	0.6
	2.44	0.0	323	0.0	492	1096	0.1	8973	1.5	610	10.7	ND	0.3	27.6	0.3
	2.53	ND	324	0.1	1704	1087	0.1	8871	1.9	570	9.7	ND	0.4	160.3	1.1
	2.59	ND	337	0.0	516	1152	0.1	9492	1.7	623	10.2	0.0	0.6	19.1	0.2
	2.69	0.4	119	0.3	5312	358	0.0	2819	2.7	169	5.1	ND	0.2	113.3	0.9
AVG	0.1	293	0.1	1252	1006	0.1	7951	1.8	523	9.3	0.0	0.5	222.4	1.6	
	STDEV	0.1	67	0.1	1581	246	0.0	2319	0.4	136	1.7	0.0	0.3	182.2	1.3



**Figure S2:** The organic matter content across the depth profile of the four sites where 16S rRNA gene-based analyses were performed (top row) and the four sites where microbial activity studies were performed (bottom row). Note the varying scale on the y-axis, which highlights the different depths of the cores below the seafloor. The site names of all cores are given in Table S1.

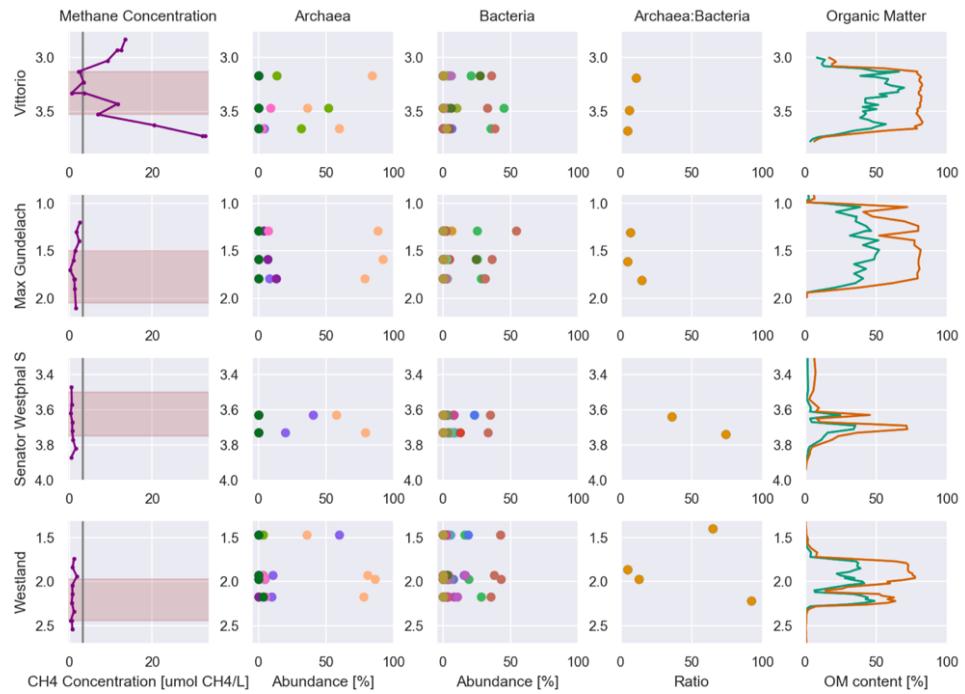


**Figure S3. Incubation assays.** (A, B) Anaerobic methanotrophic activity in batch incubation assays of peat slurries, and (C, D) methanogenic activity in batch incubation assays of peat slurries. (A) data on  $^{13}\text{C}$ - and  $^{12}\text{C}$ - $\text{CH}_4$ ,  $\text{CO}_2$  and  $\text{O}_2$  levels for sulfate-dependent anaerobic oxidation of methane. (B) ratio of  $^{13}\text{C}-\text{CO}_2$  to  $^{12}\text{C}-\text{CO}_2$ . (C)  $\text{H}_2$  and  $\text{CO}_2$  data for the hydrogenotrophic methanogenic incubations. Gas concentrations are given in mmol per

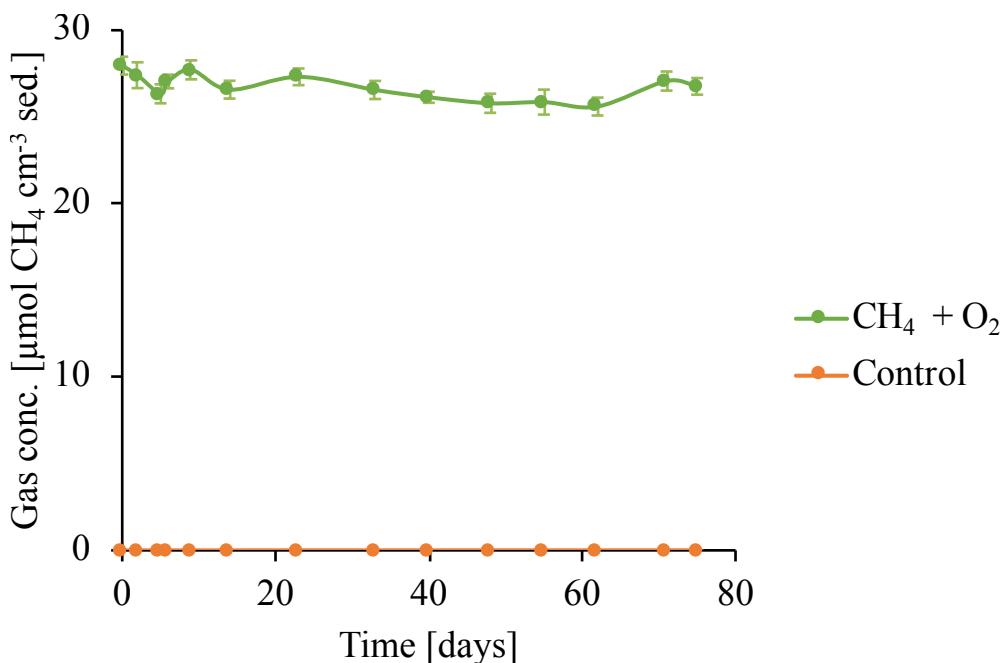
cm<sup>3</sup> of original peat sediment. New doses of H<sub>2</sub> were added at 30 and 49 days of incubation. (D) H<sub>2</sub> and CO<sub>2</sub> data for the methanol-dependent hydrogenotrophic methanogenic incubations. New doses of H<sub>2</sub> were added at 35 and 49 days of incubation. At 63 days of incubation, a second dose of 10 mM MeOH was added. For all graphs, data points represent the average of triplicate measurements on triplicate incubations. Error bars indicate the standard deviation of the mean.

**Table S3. Relative abundance percentages for the archaeal and bacterial taxonomic groups.** The maximum taxonomy depth for archaea is on family level, and for bacteria on class level. Taxonomic groups with < 2% abundance are grouped in 'Other', and quantification of archaeal and bacterial 16S rRNA gene copy number per gram of sediment. The site names of all cores are given in Table S1.

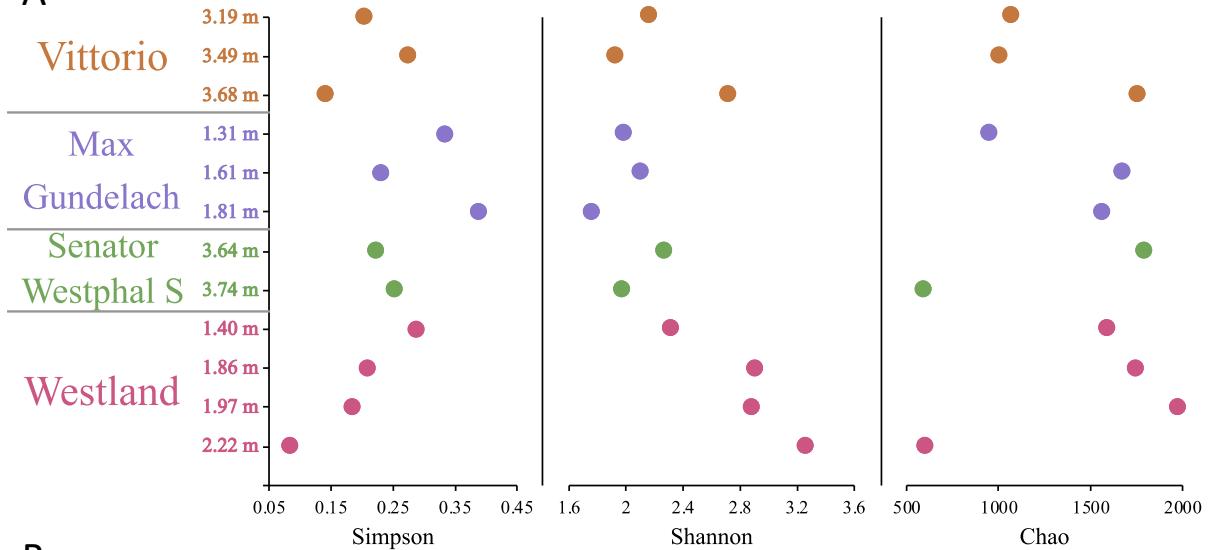
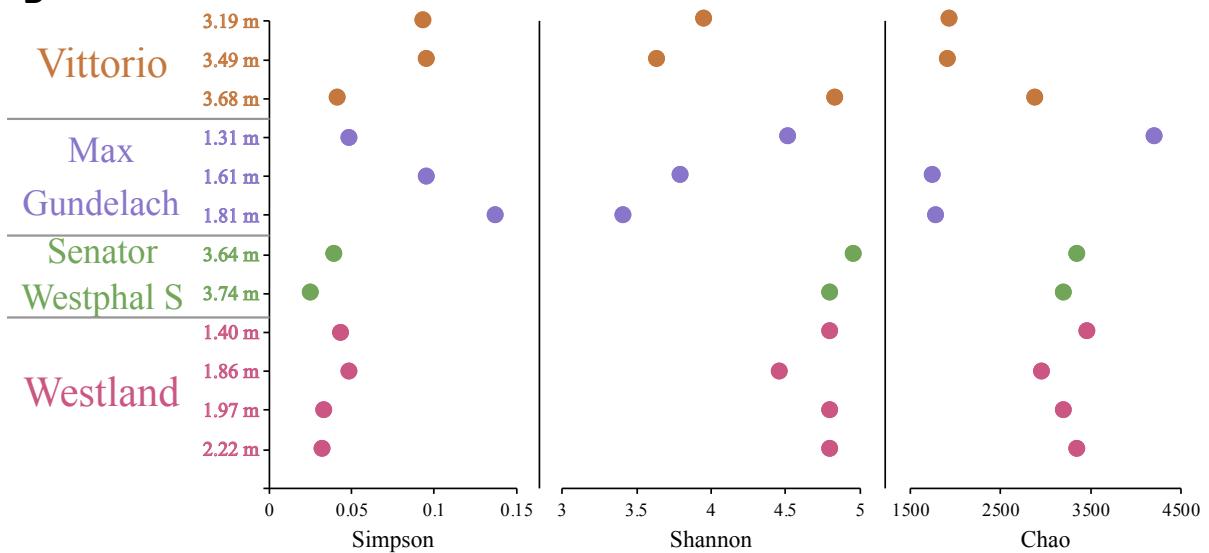
Taxonomy Archaea	Core 6			Core 7			Core 17		Core 26			
	3.19 m	3.49 m	3.68 m	1.31 m	1.61 m	1.81 m	3.64 m	3.74 m	1.4 m	1.86 m	1.97 m	2.22 m
Bathyarchaeia	84.23	36.10	59.54	88.38	92.20	78.72	57.56	78.90	35.86	80.73	86.18	77.70
Marine Benthic Group D and DHVEG-1	0.00	0.00	4.26	0.00	0.00	7.96	40.30	19.73	59.73	10.54	4.50	9.91
Lokiarchaeia	13.42	51.59	31.65	0.00	0.00	0.00	0.00	0.00	3.56	3.49	4.10	0.00
<i>Methanoregulaceae</i>	0.00	0.00	0.00	3.82	6.72	13.17	0.00	0.00	0.00	0.00	0.00	0.00
<i>Methanomassilicoccaceae</i>	0.00	9.05	2.66	7.25	0.00	0.00	0.00	0.00	0.00	3.06	4.35	4.87
Aliiarchaeia	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	3.39
Other	2.35	3.25	1.89	0.55	1.08	0.15	2.14	1.37	0.85	2.17	0.87	4.13
<b>Taxonomy Bacteria</b>												
JS1	20.80	45.14	35.18	25.34	25.23	28.20	7.75	8.35	15.79	15.76	18.94	28.33
Aerophobetes	0.00	0.00	4.76	2.27	0.00	0.00	8.08	5.36	4.16	15.71	4.45	8.21
Aminicenatales	0.00	0.00	6.31	2.37	0.00	0.00	23.02	0.00	18.34	0.00	0.00	0.00
MSB.5B2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	2.11	0.00	4.78	0.00
GIF9	0.00	0.00	0.00	0.00	0.00	0.00	0.00	6.88	0.00	16.33	2.06	10.46
H3-93	0.00	9.98	0.00	0.00	0.00	0.00	2.07	0.00	0.00	0.00	0.00	0.00
TA06	0.00	0.00	0.00	2.01	0.00	0.00	0.00	0.00	0.00	3.25	7.68	0.00
Anaerolineales	0.00	0.00	0.00	6.30	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
GIF3	4.26	0.00	0.00	0.00	0.00	0.00	3.64	8.44	5.50	0.00	4.98	2.95
SBR1031	0.00	0.00	0.00	0.00	0.00	0.00	0.00	8.67	4.17	0.00	0.00	0.00
Spirochaetales	0.00	0.00	0.00	0.00	2.03	0.00	0.00	12.44	2.09	2.14	0.00	0.00
FW22	0.00	0.00	0.00	0.00	2.09	0.00	0.00	0.00	0.00	0.00	0.00	2.24
Zixibacteria	3.47	0.00	4.49	0.00	4.69	2.83	0.00	0.00	0.00	0.00	3.05	3.02
Desulfarculales	0.00	0.00	0.00	2.32	3.01	2.06	2.52	5.19	3.02	2.18	4.16	3.60
MSBL5	0.00	3.72	0.00	0.00	0.00	0.00	7.61	0.00	2.35	3.68	3.98	3.71
Bacteroidales	2.50	0.00	2.56	3.07	2.26	2.81	0.00	4.55	0.00	0.00	0.00	0.00
Napoli-4B-65	5.75	2.60	2.68	0.00	0.00	2.18	2.20	0.00	0.00	0.00	2.87	0.00
Dehalococcoidia	27.42	5.95	3.50	0.00	24.39	30.69	3.10	0.00	0.00	3.10	0.00	0.00
Pla1_lineage	0.00	0.00	0.00	0.00	0.00	0.00	2.88	3.24	0.00	0.00	0.00	0.00
WCHB1-81	0.00	0.00	0.00	2.17	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Pirellulales	0.00	0.00	0.00	0.00	0.00	0.00	2.10	3.50	0.00	0.00	0.00	0.00
Phycisphaerales	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	2.34
MSBL9	0.00	0.00	2.38	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Other	35.80	32.61	38.13	54.15	36.29	31.24	35.04	33.38	42.48	37.86	43.03	35.14
Quantification copy no.	Archaeal	4.20 E+06	9.13 E+06	3.50 E+06	2.60 E+07	3.61 E+06	9.77 E+06	9.55 E+07	6.70 E+07	7.43 E+07	2.58 E+07	2.14 E+07
Quantification copy no.	Bacterial	3.90 E+05	1.67 E+06	7.39 E+06	3.66 E+06	6.63 E+05	6.70 E+05	2.64 E+06	9.07 E+05	1.14 E+06	5.28 E+06	1.60 E+05
<b>Ratio Archaea:Bacteria</b>	<b>10.8:1</b>	<b>5.5:1</b>	<b>4.7:1</b>	<b>7.1:1</b>	<b>5.4:1</b>	<b>14.6:1</b>	<b>36.2:1</b>	<b>73.9:1</b>	<b>42.48</b>	<b>37.86</b>	<b>43.03</b>	<b>35.14</b>



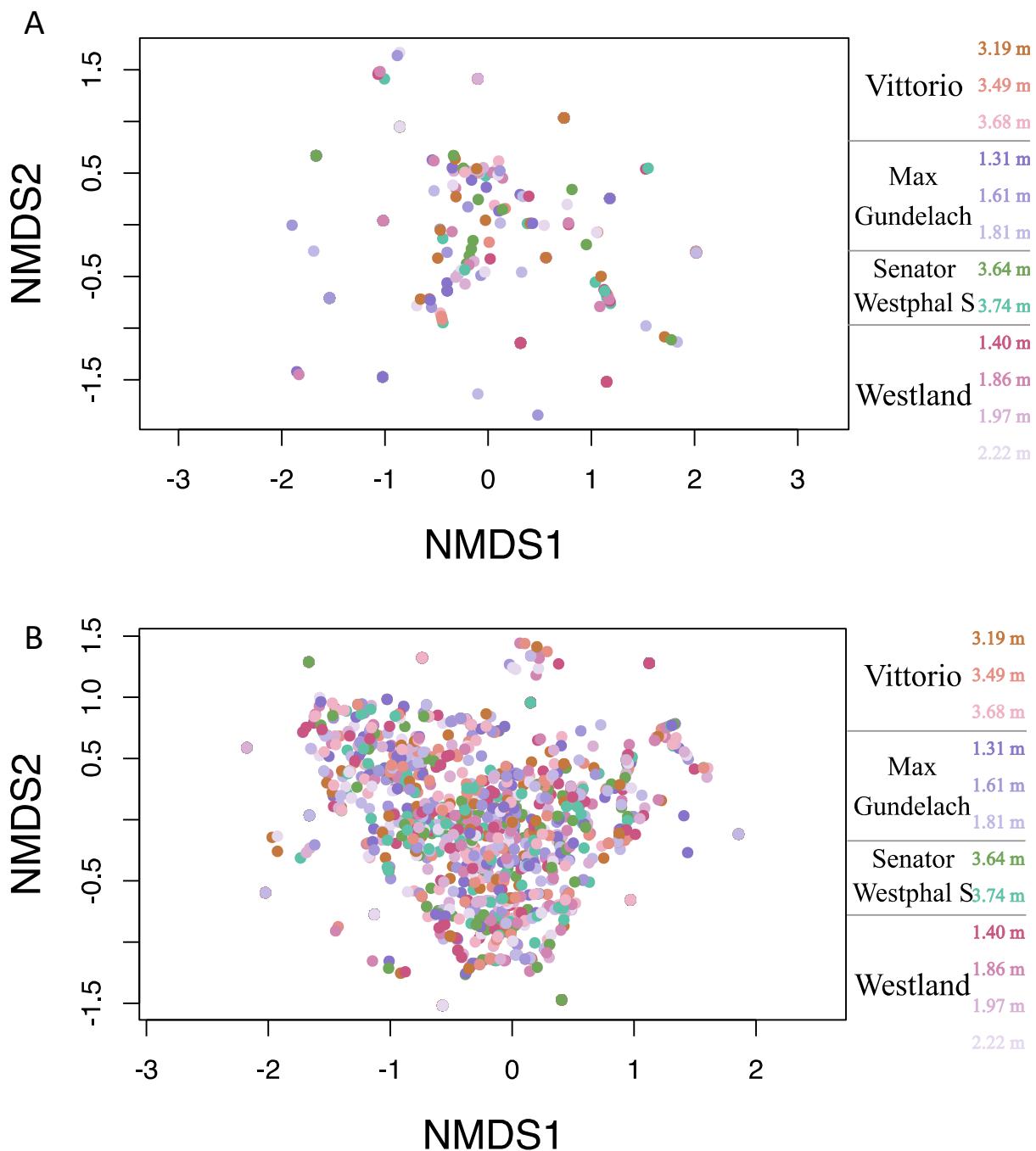
**Figure S4:** Methane concentrations, archaeal and bacterial populations, archaea to bacteria ratios, and organic matter content (orange line represents the results of LOI550, the green line represents the results of LOI330) of the four sites on which 16S rRNA gene-based phylogenetic analyses were performed: Vittorio, Max Gundelach, Senator Westphal S and Westland. For the coloured dots in the archaea and bacteria plots, please refer to the legends of archaeal and bacterial species in Fig. 6.



**Figure S5. Aerobic methane oxidation in batch incubation assays of peat slurries.** Methane contents are given as CH<sub>4</sub> produced per cm<sup>3</sup> of original peat sediment over the course of 75 days. Substrates: methane and oxygen for aerobic methane oxidation (CH<sub>4</sub> + O<sub>2</sub>) and an aerobic control without substrate amendment (Control). Data points represent the average of triplicate measurements on triplicate incubations. Error bars indicate the standard deviation of the mean.

**A****B**

**Figure S6. (A) Archaeal and (B) bacterial alpha diversity analyses.** All datasets were rarefied for the smallest dataset = 24,925 sequences for archaea and 110,236 sequences for bacteria. Average sample coverage was 98.4% for archaea and 99.6% for bacteria, respectively, which indicates that the communities were sampled deep enough.



**Figure S7.** **(A)** Non-metric multidimensional scaling (NMDS) plot for archaeal community structure and **(B)** of reads with a sequence count  $>12$ . The NMDS has a stress value of 0.045 for archaea and 0.086 for bacteria, respectively.