Supplement of

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

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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.

| Site Name | Area | Core Name | DINOloket code | Depth of Peat Layer (m) | Peat Layer Thickness (m) | Depth Seafloor (m) | Longitude | Latitude | Peat Water | Microbial Sequencing | Microbial Activity | Loss On Ignition | 14C Dating | Plant Macrofossils |
|-----------|------|-----------|----------------|------------------------|--------------------------|-------------------|------------|----------|------------|-------------------|------------------|------------------|------------|---------------------|
| TX04      | Ijmuiden | 14 | RO070309 | 1.57 52°32.20174' 4°0.07968' 26.81 0.19 Y - Y - - - - |
| Theodor   | Ijmuiden | 18 | RO070311 | 2.52 52°31.43544' 4°2.23164' 25.66 0.40 Y - - - - - -|
| Easting Down* | Brown Bank | 41 | BP080097 | N/A 52°32.14668' 4°23.78778' 32.37 N/A Y - - - - - -|
| Leda      | Brown Bank | 46 | BP000076 | 52°28.90746' 4°4.55616' 34.48 0.19 Y - - - - - -|
| Stormvogel* | Frisian Front | 67 | - | N/A 53°27.86196' 4°16.25832' 27.00 N/A Y - - - - - -|
| Vittorax  | Vlieland | 6.2 | BL120234 | 3.19 53°20.0796' 4°51.06282' 18.70 0.53 Y Y Y - - - -
| Vittorax  | Vlieland | 6.4 | BL120236 | 3.13 53°20.09719' 4°51.0702' 18.70 0.53 Y - - - - - -|
| Gundelach | Vlieland | 7.1 | BL120237 | 1.20 53°20.38158' 4°51.60276' 18.90 0.85 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°8.13907' 4°7.76400' 45.90 0.10 Y - - - - - -|
| Senator  | Westphal SW | 16.1 | BL020084 | 53°57.91602' 4°26.57268' 40.00 0.10 Y - - - - - -|
| Senator  | Westphal SW | 16.3 | BL020086 | 1.42 53°57.91134' 4°26.57544' 40.00 0.10 Y - - - - - -|
| Senator  | Westphal S | 17.0 | BL020087 | 1.59 53°55.41612' 4°30.55746' 38.90 0.08 Y Y Y - - - -
| Senator  | Westphal S | 17.3 | BL020088 | 2.50 53°55.41144' 4°30.55410' 39.40 0.08 Y - - - - - -|
| Mahren S | Vlieland | 24.3 | BL050113 | 53°44.50896' 4°29.93766' 35.20 0.09 Y - - - - - -|
| Mahren S | Vlieland | 24.4 | BL050115 | 2.32 53°44.52168' 4°29.93994' 35.10 0.09 Y - - - - - -|
| Mahren SW | Vlieland | 25.1 | BL050116 | 53°44.66952' 4°29.9100' 34.90 - - - - - - - - - - |
| Westland | Vlieland | 26.1 | BL050118 | 1.68 53°45.41424' 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 - - - - - -|
| Christmas deep SW | Doggerbank | 83 | BA090022 | 0.71 55°36.57720' 3°57.42582' 37.70 0.07 Y - - - - - -|
| Christmas deep SW | Doggerbank | 86 | BA090024 | 1.32 55°36.56184' 3°57.43230' 37.24 0.30 Y Y - - Y Y - -
| Dorthea SSW | Doggerbank | 89 | BA090026 | 0.99 55°36.74568' 3°57.03786' 37.51 0.34 Y Y Y - - - -
| Dorthea NW | Doggerbank | 97 | BDE07068 | 2.33 55°44.69892' 3°46.30440' 44.42 0.10 Y - - - - - -|
| Dorthea NW | Doggerbank | 102 | BDE070705 | 2.19 55°47.04154' 3°41.14170' 51.26 0.16 Y Y Y - - - -|
| Fredricksson NE | Doggerbank | 111 | BA050024 | 1.74 55°49.48110' 3°26.42682' 59.56 0.13 Y Y Y - - Y Y -
| Fredricksson NE | Doggerbank | 112 | - | 1.74 55°49.48926' 3°26.39760' 59.41 0.13 - - - - - - Y Y -
| Fredricksson NW | Doggerbank | 114 | BA050025 | 2.06 55°49.49006' 3°26.40180' 59.92 0.34 Y - - - - - - Y Y -
| Daro's site* | Doggerbank | 163 | - | N/A 55°18.38796' 4°5.42544' 43.8 N/A Y Y Y Y Y Y Y Y Y Y Y Y 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 (NO₃⁻), ammonium (NH₄⁺) and phosphate (PO₄³⁻) 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  | Mg  | Mn  | P   | S   | Si  | Zn  | NO₃⁻ | NH₄⁺ | PO₄³⁻ |
|------|------|-----|-----|-----|-----|-----|-----|-----|-----|-----|------|------|-------|
| 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  | 1.58 | ND  | 367 | ND  | 259 | 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| 766 | 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  |
| 7    | 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  |
|      | 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  |
| STDEV| 0.0  | 8   | 0.0 | 121 | 23  | 0.0 | 63  | 1.2 | 14  | 1.0  | 0.0  | 0.0   | 17.6  | 0.4  |
| 17   | 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 | ND  | 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 | 1056| 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 $^{13}$C- and $^{12}$C-CH$_4$, CO$_2$ and O$_2$ levels for sulfate-dependent anaerobic oxidation of methane. (B) ratio of $^{13}$C-CO$_2$ to $^{12}$C-CO$_2$. (C) H$_2$ and CO$_2$ data for the hydrogenotrophic methanogenic incubations. Gas concentrations are given in mmol per
cm$^3$ of original peat sediment. New doses of H$_2$ were added at 30 and 49 days of incubation. (D) H$_2$ and CO$_2$ data for the methanol-dependent hydrogenotrophic methanogenic incubations. New doses of H$_2$ 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 |
|------------------|--------|--------|---------|---------|
| Bathyarchaeia    | 84.23  | 36.10  | 59.54   | 38.21   |
| Marine Benthic Group D and DHVEG-1 | 0.00  | 0.00  | 4.26    | 0.00    |
| Lokarchaeia      | 13.42  | 51.59  | 31.65   | 59.73   |
| Methanoregulaceae| 0.00  | 0.00  | 4.26    | 0.00    |
| Methanomassiliicoccaceae | 0.00 | 9.05 | 2.66   | 0.00  |
| Alistarchaeia    | 0.00  | 0.00  | 0.00    | 0.00    |
| Other            | 2.35  | 3.25  | 1.86    | 0.85    |

| Taxonomy Bacteria | Core 6 | Core 7 | Core 17 | Core 26 |
|-------------------|--------|--------|---------|---------|
| JS1               | 20.80  | 45.14  | 35.18   | 15.79   |
| Aerophobetes      | 0.00  | 0.00  | 4.76    | 4.16    |
| Aminicincantales  | 0.00  | 0.00  | 6.31    | 18.34   |
| MSB 5B2           | 0.00  | 0.00  | 0.00    | 2.11    |
| GIF9              | 0.00  | 0.00  | 0.00    | 0.00    |
| H3-93            | 0.00 | 9.98 | 0.00   | 0.00   |
| TA06              | 0.00  | 0.00  | 0.00    | 3.25    |
| Anaerolineales    | 0.00  | 0.00  | 6.30    | 0.00    |
| GIF3              | 4.26  | 0.00  | 0.00    | 5.50    |
| SBR1031           | 0.00  | 0.00  | 0.00    | 4.17    |
| Spirochaetales    | 0.00  | 0.00  | 0.00    | 2.09    |
| FW22              | 0.00  | 0.00  | 0.00    | 0.00    |
| Zixibacteria      | 3.47  | 4.49  | 0.00    | 0.00    |
| Desulfuraeates    | 0.00  | 0.00  | 0.00    | 3.02    |
| MSBL5             | 0.00  | 3.72  | 0.00    | 2.35    |
| Bacteroidales     | 2.50  | 0.00  | 2.56    | 0.00    |
| Napoli-4B-65      | 5.75  | 2.60  | 2.68    | 0.00    |
| Dehalococcoida    | 27.42 | 5.95  | 3.50    | 3.10    |
| Pla1_lineage      | 0.00  | 0.00  | 0.00    | 0.00    |
| WCHB1-81          | 0.00  | 0.00  | 0.00    | 0.00    |
| Pirellulales      | 0.00  | 0.00  | 0.00    | 2.10    |
| Phycisphaerales   | 0.00  | 0.00  | 0.00    | 0.00    |
| MSBL9             | 0.00  | 2.38  | 0.00    | 0.00    |
| Other             | 35.80 | 32.61 | 38.13   | 42.48   |

| Quantification copy no. | Archaeal | 4.20 | 9.13 | 3.50 | 2.60 | 3.81 | 9.77 | 9.55 | 6.70 | 7.43 | 2.58 | 2.14 | 9.04 |
|-------------------------|----------|------|------|------|------|------|------|------|------|------|------|------|------|
|                         | E+06     | E+08 | E+07 | E+06 | E+06 | E+06 | E+07 | E+07 | E+07 | E+07 | E+07 | E+07 | E+07 |
| Quantification copy no. | Bacterial| 3.90 | 1.67 | 7.39 | 3.06 | 6.63 | 6.70 | 2.84 | 6.07 | 1.14 | 5.28 | 1.60 | 9.84 |
|                         | E+05     | E+06 | E+06 | E+05 | E+06 | E+05 | E+05 | E+05 | E+06 | E+06 | E+06 | E+06 | E+06 |
| Ratio Archaea:Bacteria | 10.8:1   | 5.5:1 | 4.7:1 | 7.1:1 | 5.4:1 | 14:1 | 36:2 | 73:9:1 | 85:4:1 | 4.9:1 | 13.3:1 | 91.9:1 |
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 CH4 produced per cm3 of original peat sediment over the course of 75 days. Substrates: methane and oxygen for aerobic methane oxidation (CH4 + O2) 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.
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.