Predominance of methanogens over methanotrophs in rewetted fens characterized by high methane emissions

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Abstract. The rewetting of drained peatlands alters peat geochemistry and often leads to sustained elevated methane emission. Although this methane is produced entirely by microbial activity, the

distribution and abundance of methane-cycling microbes in rewetted peatlands, especially in fens, is rarely described. In this study, we compare the community composition and abundance of methane-cycling microbes in relation to peat porewater geochemistry in two rewetted fens in northeastern Germany, a coastal brackish fen and a freshwater riparian fen, with known high methane fluxes. We utilized 16S rRNA high-throughput sequencing and quantitative polymerase chain reaction on 16S rRNA, *mcrA*, and *pmoA* genes to determine microbial community composition and the abundance of total bacteria, methanogens, and methanotrophs. Electrical conductivity was more than three times higher in the coastal fen than in the riparian fen, averaging 5.3 and 1.5 mS cm^{-1}, respectively. Porewater concentrations of terminal electron acceptors varied within and among the fens. This was also reflected in similarly high intra- and inter-site variations of microbial community composition. Despite these differences in environmental conditions and electron acceptor availability, we found a low abundance of methanotrophs and a high abundance of methanogens, represented in particular by *Methanosaetaceae*, in both fens. This suggests that rapid re/establishment of methanogens and slow re/establishment of methanotrophs contributes to prolonged increased methane emissions following rewetting.

1 Introduction

Rewetting is a technique commonly employed to restore ecological and biogeochemical functioning of drained fens. However, while rewetting may reduce carbon dioxide (CO_2) emissions (Wilson et al. 2016), it often increases methane (CH_4) emissions in peatlands that remain inundated following rewetting. On a 100-year time scale, CH_4 has a global warming potential 28 times stronger than CO_2 (Myhre et al. 2013), and the factors that contribute to the magnitude and duration of increased emissions are still uncertain (Joosten et al. 2015, Abdalla et al. 2016). Thus, elucidating the dynamics of post-rewetting CH_4 exchange is of strong interest for both modelling studies and peatland management projects (Abdalla et al. 2016). Although a recent increase in rewetting projects in Germany and other European countries has prompted a number of studies of methane cycling in rewetted peatlands (e.g., Jerman et al. 2009, Hahn-Schöfl et al. 2011, Urbanová et al. 2013, Hahn et al. 2015, Vanselow-Algan et al. 2015, Zak et al. 2015, Emsens et al. 2016, Putkinen et al. 2018), the post-rewetting distribution and abundance of methane-cycling microbes

62 in rewetted fens has seldom been examined (but see Juottonen et al. 2012, Urbanová et al. 2013,
63 Putkinen et al. 2018).
64 Peat CH_4 production and release is governed by a complex array of interrelated factors including
65 climate, water level, plant community, nutrient status, site geochemistry, and the activity of
66 microbes (i.e., bacteria and archaea) that use organic carbon as energy source (Segers 1998,
67 Abdalla et al. 2016). To date, the vast majority of studies in rewetted fens have focused on
68 quantifying CH_4 emission rates in association with environmental variables such as water level,
69 plant community, and aspects of site geochemistry (Abdalla et al. 2016). Site geochemistry indeed
70 plays an important role for methanogenic communities, as methanogenesis is suppressed in
71 presence of thermodynamically more favorable terminal electron acceptors (TEAs, Blodau 2011).
72 Due to a smaller pool of more favorable electron acceptors and high availability of organic carbon
73 substrates, organic-rich soils such as peat rapidly establish methanogenic conditions post-
74 rewetting (Segers 1998, Keller and Bridgham 2007, Knorr and Blodau 2009). Despite their
75 decisive role as producers (i.e., methanogens) and consumers (i.e., methanotrophs) of CH_4 (Conrad
76 1996), only a few studies have combined a characterization of the CH_4-cycling microbial
77 community, site geochemistry, and observed trends in CH_4 production. Existing studies have been
78 conducted in oligotrophic and mesotrophic boreal fens (e.g., Juottonen et al. 2005, Yrjälä et al.
79 2011, Juottonen et al. 2012), alpine fens (e.g., Liebner et al. 2012, Urbanová et al. 2013, Cheema
80 et al. 2015, Franchini et al. 2015), subarctic fens (Liebner et al. 2015), and incubation experiments
81 (e.g., Jerman et al. 2009, Knorr and Blodau 2009, Urbanová et al. 2011, Emsens et al. 2016).
82 Several studies on CH_4-cycling microbial communities have been conducted in minerotrophic
83 temperate fens (e.g., Cadillo-Quiroz et al. 2008, Liu et al. 2011, Sun et al. 2012, Zhou et al. 2017),
84 but these sites were not subject to drainage or rewetting. Direct comparisons of *in situ* abundances
85 of methanogens and methanotrophs in drained versus rewetted fens are scarce (Juottonen et al.
86 2012, Putkinen et al. 2018), and the studied sites, so far, are nutrient-poor fens with acidic
87 conditions.

While studies of nutrient-poor and mesotrophic boreal fens have documented post-rewetting CH_4 emissions comparable to or lower than at pristine sites (Komulainen et al. 1998, Tuittila et al. 2000, Juottonen et al 2012), studies of temperate nutrient-rich fens have reported post-flooding CH_4 emissions dramatically exceeding emissions in pristine fens (e.g., Augustin and Chojnicki 2008, Hahn et al. 2015). These high emissions typically occur together with a significant dieback in vegetation, a mobilization of nutrients and electron acceptors in the upper peat layer, and increased availability of dissolved organic matter (Zak and Gelbrecht 2007, Hahn-Schöfl et al. 2011, Hahn et al. 2015, Jurasinski et al. 2016). High CH_4 fluxes may continue for decades following rewetting, even in bogs (Vanselow-Algan et al. 2015). Hence, there is an urgent need to characterize CH_4-cycling microbial communities and geochemical conditions in rewetted minerotrophic fens. In this study, we therefore examined microbial community composition and abundance in relation to post-flooding geochemical conditions in two rewetted fens in northeastern Germany. In both fens, CH_4 emissions increased dramatically after rewetting, to over 200 g C m^{-2} a^{-1} (Augustin and Chojnicki 2008, Hahn-Schöfl et al. 2011, Hahn et al. 2015, Jurasinski et al. 2016). Average annual CH_4 emissions have decreased in both fens since the initial peak (Franz et al. 2016, Jurasinski et al. 2016). Nevertheless, fluxes remained higher than under pre-flooding conditions (*ibid.*), and higher than in pristine fens (Urbanová et al. 2013, Minke et al 2016). In the Hütelmoor in 2012, average CH_4 emissions during the growing season were 40 g m^{-2} (Koebsch et al. 2015). In Zarnekow, average CH_4 emissions were 40 g m^{-2} for the year 2013 (Franz et al. 2016). In comparison, a recent review paper (Abdalla et al. 2016) estimated an average flux of 12 ± 21 g C m^{-2} a^{-1} for pristine peatlands.

We expected patterns in microbial community composition would reflect the geochemical conditions of the two sites and hypothesized a high abundance of methanogens relative to methanotrophs in both fens. We also expected acetoclastic methanogens, which typically thrive in nutrient-rich fens (Kelly et al. 1992, Galand 2005), to dominate the methanogenic community in both fens.

2 Methods

2.1 Study sites

The nature reserve "Heiligensee and Hütelmoor" ('Hütelmoor' in the following, approx. 540 ha, 54°12'36.66" N, 12°10'34.28" E), is a coastal, mainly minerotrophic fen complex in Mecklenburg-Vorpommern (NE Germany) that is separated from the Baltic Sea by a narrow (~100 m and less) dune dike (Fig. 1a and b). The climate is temperate in the transition zone between maritime and continental, with an average annual temperature of 9.1 °C and an average annual precipitation of 645 mm (data derived from grid product of the German Weather Service, reference climate period: 1981–2010). Episodic flooding from storm events delivers sediment and brackish water to the site (Weisner and Schernewski 2013). The vegetation is a mixture of salt-tolerant macrophytes, with dominant to semi-dominant stands of *Phragmites australis*, *Bolboschoenus maritimus*, *Carex acutiformis*, and *Schoenoplectus tabernaemontani.* The dominating plants are interspersed with open water bodies that are colonized by *Ceratophyllum demersum* in summer (Koch et al. 2017). Intense draining and land amelioration practices began in the 1970s, which lowered the water level to 1.6 m below ground surface and caused aerobic decomposition and concomitant degradation of the peat (Voigtländer et al. 1996). The upper peat layer varies in depth between 0.6 and 3 m and is highly degraded, reaching up to H10 on the von Post humification scale (Hahn et al. 2015). Active draining ended in 1992, but dry conditions during summertime kept the water table well below ground surface (Schönfeld-Bockholt et al. 2005, Koebsch et al. 2013) until concerns of prolonged aerobic peat decomposition prompted the installation of a weir in 2009 at the outflow of the catchment (Weisner and Schernewski 2013). After installation of the weir, the site has been fully flooded year-round with an average water level of 0.6 m above the peat surface, and annual average CH_4 flux increased ~186-fold from 0.0014 ± 0.0006 kg CH_4 m^{-2} a^{-1} to 0.26 ± 0.06 kg CH_4 m^{-2} a^{-1} (Hahn et al. 2015).

The study site polder Zarnekow ('Zarnekow' in the following, approx. 500 ha, 53°52'31.10" N, 12°53'19.60" E) is situated in the valley of the River Peene in Mecklenburg-Vorpommern (NE Germany, Fig. 1a and c). The climate is slightly more continental compared to the Hütelmoor, with a mean annual precipitation of 544 mm and a mean annual temperature of 8.7 °C (German Weather Service, meteorological station Teterow, 24 km southwest of the study site; reference period 1981–2010). The fen can be classified as a river valley mire system consisting of spring mires, wider percolation mires, and flood mires along the River Peene. Drainage and low-intensity agricultural use began in the eighteenth century when land-use changed to pastures and grassland. This was intensified by active pumping in the mid-1970s. Due to land subsidence of several decimeters, after rewetting (October 2004) water table depth increased to 0.1–0.5 m above peat surface. The upper horizon is highly decomposed (0–0.3 m), followed by moderately decomposed peat to a depth of 1 m and a deep layer of slightly decomposed peat up to a maximum depth of 10 m. The open water bodies are densely colonized by *Ceratophyllum* spp. and *Typha latifolia* is the dominant emergent macrophyte (Steffenhagen et al. 2012). Following flooding, CH_4 flux rates increased to ~0.21 kg m^{-2} a^{-1} (Augustin and Chojnicki 2008). No pre-rewetting CH_4 flux data were available for the Zarnekow site, but published CH_4 flux rates of representative drained fens from the same region have been shown to be negligible, and many were CH_4 sinks (Augustin et al. 1998).

2.2 Collection and analysis of peat cores and porewater samples

Peat and porewater samples were collected at four different locations (n=4) in Hütelmoor (October 2014) and at five locations (n=5) in Zarnekow (July 2015) and spanned a distance of 1,200 m and 250 m, respectively, to cover the whole lateral extension at each site (Fig. 1b and c). Sampling depths in the Hütelmoor were 0-5, 5-10, 10-20, 20-30, 30-40, and 40-50 cm below the peat surface, except for core numbers 1 and 4 where samples could only be obtained up to a depth of 10-20 and 30-40 cm, respectively. Sampling depths in Zarnekow were 0-5, 25-30, and 50-55 cm below the peat surface. Previous work at Zarnekow has revealed little variation in peat properties with depth (e.g., Zak and Gelbrecht 2007), hence, a lower depth resolution in Zarnekow cores was chosen for

this study. Peat cores were collected with a Perspex liner (ID: 60 mm, Hütelmoor) and a peat auger (Zarnekow). In order to minimize oxygen contamination, the outer layer of the peat core was omitted. Subsamples for molecular analysis were immediately packed in 50 ml sterile Falcon tubes and stored at -80 °C until further processing.

Pore waters in the Hütelmoor were collected with a stainless-steel push-point sampler attached to a plastic syringe to recover the samples from 10 cm depth intervals. Samples were immediately filtered with 0.45 µm membrane sterile, disposable syringe filters. Pore waters in Zarnekow were sampled with permanently installed dialysis samplers consisting of slotted polypropylene (PP) pipes (length: 636 mm, ID: 34 mm) surrounded with 0.22 µm polyethersulfone membrane. The PP pipes were fixed at distinct peat depths (surface level, 20 and 40 cm depth) and connected with PP tubes (4x6 mm IDxAD). Water samples were drawn out from the dialysis sampler pipes with a syringe through the PP tube. Due to practical restrictions in accessibility and sampling, permanent dialysis samplers could not be installed at the desired locations in the Hütelmoor, resulting in the different sampling techniques described above.

At both sites, electrical conductivity (EC), dissolved oxygen (DO), and pH were measured immediately after sampling (Sentix 41 pH probe and a TetraCon 325 conductivity measuring cell attached to a WTW multi 340i handheld; WTW, Weilheim). In this paper, EC is presented and was not converted to salinity (i.e., psu), as a conversion would be imprecise for brackish waters. A simplified equation for conversion can be found in Schemel (2001). Headspace CH_4 concentrations of porewater samples were measured with an Agilent 7890A gas chromatograph (Agilent Technologies, Germany) equipped with a flame ionization detector and a Carboxen PLOT Capillary Column or HP-Plot Q (Porapak-Q) column. The measured headspace CH_4 concentration was then converted into a dissolved CH_4 concentration using the temperature-corrected solubility coefficient (Wilhelm et al. 1977). Isotopic composition of dissolved CH_4 for Hütelmoor was analyzed using the gas chromatography-combustion-technique (GC-C) and the gas chromatography-high-temperature-conversion-technique (GC-HTC). The gas was directly

injected in a Gas Chromatograph Agilent 7890A, CH_4 was quantitatively converted to CO_2, and the $\delta^{13}C$ values were then measured with the isotope-ratio-mass-spectrometer MAT-253 (Thermo Finnigan, Germany). The $\delta^{13}C$ of dissolved CH_4 in Zarnekow was analyzed using a laser-based isotope analyzer equipped with a small sample isotope module for analyses of discrete gas samples (cavity ring down spectroscopy CRDS; Picarro G2201-I, Santa Clara, CA, USA). Calibration was carried out before, during and after analyses using certified standards of known isotopic composition (obtained from Isometric Instruments, Victoria, BC, Canada, and from Westfalen AG, Münster, Germany). Reproducibility of results was typically +/- 1 ‰. In the presence of high concentrations of hydrogen sulfide interfering with laser-based isotope analysis, samples were treated with iron(III) sulfate to oxidize and/or precipitate sulfide. For both sites, sulfate and nitrate concentrations were analyzed by ion chromatography (IC, Thermo Fisher Scientific Dionex) using an Ion Pac AS-9-HC 4 column, partly after dilution of the sample. Dissolved metal concentrations were analyzed by ICP-OES (iCAP 6300 DUO, Thermo Fisher Scientific). Accuracy and precision were routinely checked with a certified CASS standard as previously described (Kowalski et al. 2012).

For the incubation experiments, peat cores were collected from Zarnekow in March 2012 using a modified Kajak Corer with a plexiglass tube. The intact cores were placed in a cool box and immediately transported to the Leibniz-Institute of Freshwater Ecology and Inland Fisheries in Berlin where they were sectioned into a total of 12 samples. Fresh, surficial organic sediment (0-10 cm depth, 6 individual samples) was separated from the bulk peat (10-20 cm depth, 6 individual samples) and the samples were placed in 60 ml plastic cups. The cups were filled completely and closed with air-tight caps to minimize oxygen contamination. The samples were then express-shipped (< 24 hours) to the lab at the Netherlands Institute of Ecology for immediate processing and analysis. For CH_4 production incubations, 5 g of material and 10 ml of nitrogen (N_2)-flushed MilliQ water was weighed into three (n=3) 150 mL flasks for both surficial organic sediment and bulk peat. The flasks were capped with rubber stoppers, flushed with N_2 for approximately one

hour, then incubated stationary at 20°C in the dark. For CH_4 oxidation incubations, 5 g of fresh material and 10 ml of MilliQ water was weighed into three 150 mL flasks for both surficial organic sediment and bulk peat. The flasks were capped with rubber stoppers and 1.4 ml of pure CH_4 was added to obtain a headspace CH_4 concentration of approximately 10,000 ppm. Incubations were performed in the dark at 20°C on a gyratory shaker (120 rpm). For all incubations, headspace CH_4 concentration was determined using a gas chromatograph equipped with a flame ionization detector on days 1, 3, 5, and 8 of the incubation. Potential CH_4 production and oxidation rate were determined by linear regression of CH_4 concentration over all sampling times.

2.3 Gene amplification and phylogenetic analysis

Genomic DNA was extracted from 0.2–0.3 g of duplicates of peat soil per sample using an EurX Soil DNA Kit (Roboklon, Berlin, Germany). DNA concentrations were quantified with a Nanophotometer P360 (Implen GmbH, München, DE) and Qubit 2.0 Fluorometer (Thermo Fisher Scientific, Darmstadt, Germany). Polymerase chain reaction (PCR) amplification of bacterial and archaeal 16S rRNA genes was performed using the primer combination of S-D-Bact-0341-b-S-17/S-D-Bact-0785-a-A-21 (Herlemann et al. 2011) and S-D-Arch-0349-a-S-17/S-D-Arch-0786-a-A-20 (Takai and Horikoshi 2000), respectively, with barcodes contained in the 5'-end. The PCR mix contained 1x PCR buffer (Tris•Cl, KCl, $(NH_4)_2SO_4$, 15 mM $MgCl_2$; pH 8.7) (QIAGEN, Hilden, Germany), 0.5 µM of each primer (Biomers, Ulm, Germany), 0.2 mM of each deoxynucleoside (Thermo Fisher Scientific, Darmstadt, Germany), and 0.025 U μl^{-1} hot start polymerase (QIAGEN, Hilden, Germany). PCR samples were kept at 95 °C for 5 min to denature the DNA, with amplification proceeding for 40 cycles at 95 °C for 1 min, 56 °C for 45 s and 72 °C for 90 s; a final extension of 10 min at 72 °C was added to ensure complete amplification. PCR products were purified with a Hi Yield Gel/PCR DNA fragment extraction kit (Süd-Laborbedarf, Gauting, Germany). To reduce amplification bias, PCR products of three individual runs per sample were combined. PCR products of different samples were pooled in equimolar

concentrations and compressed to a final volume of 10 µl with a concentration of 200 ng μl^{-1} in a vacuum centrifuge Concentrator Plus (Eppendorf, Hamburg, Germany).

Illumina sequencing was performed by GATC Biotech AG using 300 bp paired-end mode and a 20% PhiX Control v3 library to counteract the effects of low-diversity sequence libraries. Raw data was demultiplexed using an own script based on CutAdapt (Martin 2011). Ambiguous nucleotides at sequence ends were trimmed and a 10% mismatch was allowed for primer identification, whereas barcode sequences needed to be present without any mismatches and with a minimum Phred-Score of Q25 for each nucleotide. After sorting, overlapping paired-end reads were merged using PEAR [Q25, p 0.0001, v20] (Zhang et al. 2014). The orientation of the merged sequences was standardized according to the barcode information obtained from demultiplexing. Low-quality reads were removed using Trimmomatic [SE, LEADING Q25, TRAILING Q25, SLIDINGWINDOW 5:25; MINLEN 200] (Bolger et al. 2014). Chimeric sequences were removed using USEARCH 6.1 and the QIIME-script identify_chimeric_seqs.py (Caporaso et al. 2010). Pre-processed sequences were taxonomically assigned to operational taxonomic units (OTUs) at a nucleotide sequence identity of 97% using QIIME's pick_open_reference_otus.py script and the GreenGenes database 13.05 (McDonald et al. 2012) as reference. The taxonomic assignment of representative sequences was further checked for correct taxonomical classification by phylogenetic tree calculations in the ARB environment referenced against the SILVA database (https://www.arb-silva.de) version 119 (Quast et al. 2013). The resulting OTU table was filtered for singletons, OTUs assigned to chloroplasts or mitochondria, and for low-abundance OTUs (below 0.2% within each sample). Archaeal and bacterial samples were processed separately while only OTUs that were assigned to the respective domain were considered for further analysis. For archaea, a total of 6,844,177 valid sequences were obtained, ranging from 60,496 to 398,660 in individual samples. These sequences were classified into 402 OTUs. For bacteria, a total of 2,586,148 valid sequences were obtained, ranging from 22,826 to 164,916 in individual samples. These sequences were classified into 843 OTUs. The OTU tables were then collapsed at a higher

taxonomic level to generate the bubble plots. The 16S rRNA gene sequence data have been deposited at NCBI under the Bioproject PRJNA356778. Hütelmoor sequence read archive accession numbers are SRR5118134-SRR5118155 for bacterial and SRR5119428-SRR5119449 for archaeal sequences, respectively. Zarnekow accession numbers are SRR6854018-SRR6854033 and SRR6854205-SRR6854220 for bacterial and archaeal sequences, respectively.

2.4 qPCR analysis

Quantitative polymerase chain reaction (qPCR) for the determination of methanotrophic and methanogenic functional gene copy numbers and overall bacterial 16S rRNA gene copy numbers was performed via SybrGreen assays on a Bio-Rad CFX instrument (Bio-Rad, Munich, Germany) with slight modifications after Liebner et al. (2015). The functional methanotrophic *pmoA* gene was amplified with the primer combination A189F/Mb661 (Kolb et al. 2003) suitable for detecting all known aerobic methanotrophic Proteobacteria. Annealing was done at 55 °C after a 7-cycle-step touchdown starting at 62 °C. The functional methanogenic *mcrA* gene was amplified with the *mlas/mcrA*-rev primer pair (Steinberg and Regan 2009) with annealing at 57 °C. The bacterial 16S rRNA gene was quantified with the primers Eub341F/Eub534R according to Degelmann et al. (2010) with annealing at 58 °C. Different DNA template concentrations were tested prior to the qPCR runs to determine optimal template concentration without inhibitions through co-extracts. The 25 µl reactions contained 12.5 µl of iTaq universal Sybr Green supermix (Bio-Rad, Munich, Germany), 0.25 µM concentrations of the primers, and 5 µl of DNA template. Data acquisition was always done at 80 °C to avoid quantification of primer dimers. The specificity of each run was verified through melt-curve analysis and gel electrophoresis. Only runs with efficiencies between 82 and 105% were used for further analysis. Measurements were performed in duplicates. The ratio of methanogens to methanotrophs was determined based on gene abundances of *mcrA* and *pmoA*. The marker gene for the soluble monooxygenase, *mmoX*, was neglected due to the absence of *Methylocella* in the sequencing data (Fig. 4).

2.5 Data visualization and statistical analysis

All data visualization and statistical analysis were done in R (R Core Team). The taxonomic relative abundances across samples were visualized through bubble plots with the R package ggplot2 (Wickham 2009). Differences in microbial community composition were visualized with 2-dimensional non-metric multidimensional scaling (NMDS) based on Bray-Curtis distances. The NMDS ordinations were constructed using R package vegan (Oksanen et al. 2017). An environmental fit was performed on the ordinations to determine the measured geochemical parameters that may influence community composition. The geochemical data were fitted to the ordinations as vectors with a significance of $p < 0.05$. Depth profiles were constructed with the porewater geochemical data, as well as with the microbial abundances, to elucidate depthwise trends and assess whether differences in microbial community and abundances among the two fens are related to differences in their respective geochemistry.

3 Results

3.1 Environmental characteristics and site geochemistry

The two rewetted fens varied substantially in their environmental characteristics (e.g., proximity to the sea) and porewater geochemistry (Fig. 2, Tables 1 and 2). EC was more than three times higher in Hütelmoor than in Zarnekow, averaging 5.3 and 1.5 mS cm^{-1}, respectively. Mean values of pH were approximately neutral (6.5 to 7.0) in the upper peat profile and comparable in both fens until a depth of about 30 cm where pH decreased to ~6 in the Hütelmoor. Concentrations of the TEAs nitrate and sulfate were lower in Zarnekow and near zero in the pore water at all depths, while nitrate and sulfate were abundant in the upper and lower peat profile in Hütelmoor at ~1.5 to 3.0 mM and ~4 to 20 mM, respectively (Fig. 2). Iron concentrations were higher in the Hütelmoor pore water, while manganese concentrations were higher in Zarnekow pore water. Dissolved oxygen concentrations in the upper peat profile (i.e. 0 to 25 cm depths) were much higher in Hütelmoor than in Zarnekow (Fig. 2). Here DO concentrations averaged ~0.25 mM until a depth of 15 cm at which they dropped sharply, reaching concentrations slightly below 0.05 mM

320 at 25 cm. In Zarnekow, DO concentrations did not exceed 0.1 mM and varied little with depth.
321 Regarding geochemical conditions, Hütelmoor core (HC) 1 differed from all other Hütelmoor
322 cores and was more similar to Zarnekow cores. In HC 1 – the core taken nearest to potential
323 freshwater sources (Fig. 1b) – pore water EC and DO concentrations were lower while pH was
324 slightly higher than in all other Hütelmoor cores. Moreover, this was the only Hütelmoor core
325 where nitrate concentrations were below detection limit (0.001mM) (Fig. 2). In all cores we found
326 high concentrations of dissolved CH_4 that varied within and among fens and were slightly higher
327 in Zarnekow pore water. Stable isotope ratios of $\partial^{13}C\text{-}CH_4$ (Fig. 2) in the upper peat (approx.
328 −59‰) suggest a predominance of acetoclastic methanogenesis, with a shift to hydrogenotrophic
329 methanogenesis around −65‰ in the lower peat profile. Additionally, the observed shifts toward
330 less negative $\partial^{13}C\text{-}CH_4$ values in the upper peat layer, as in HC 1 and HC 2, could indicate partial
331 oxidation of CH_4 occurred (Chasar et al. 2000).

332 **3.2 Community composition of bacteria and archaea**

333 Bacterial sequences could be affiliated into a total of 30 bacterial phyla (Fig. 3). Among them,
334 Proteobacteria, Acidobacteria, Actinobacteria, Chloroflexi, Nitrospirae and Bacteroidetes were
335 present in all samples. With mean relative abundance of 48%, Proteobacteria was the most
336 abundant phylum. Some taxa (e.g., Verrucomicrobia, Atribacteria (OP9), and AD3) were present
337 only in Hütelmoor. Variation in community composition was larger in Hütelmoor samples than in
338 Zarnekow. Within Proteobacteria, the alpha subdivision was the most dominant group, having
339 contributed 26.7% to all the libraries on average (Fig. 4). The family *Hyphomicrobiaceae*
340 dominated the Alphaproteobacteria, and was distributed evenly across samples, but missing in the
341 surface and bottom peat layers in HC 2. In addition, methanotrophs were clearly in low abundance
342 across all samples, representing only 0.06% and 0.05% of the bacterial community in Hütelmoor
343 and Zarnekow, respectively. Of the few methanotrophs that were detected, type II methanotrophs
344 (mainly *Methylocystaceae*) outcompeted type I methanotrophs (mainly *Methylococcaceae*) in the
345 community, while members of the genus *Methylocella* were absent (Fig. 4).

346 Within the archaeal community, Bathyarchaeota were mostly dominating over Euryarchaeota (Fig.
347 5). The MCG group (mainly the order of pGrfC26) in Bathyarchaeota prevailed across all samples
348 but was especially abundant in HC 2 samples. In addition to Bathyarchaeota, methanogenic
349 archaea were important, and on average contributed 30.6% to the whole archaeal community.
350 Among the methanogens, acetoclastic methanogens were more abundant in most of the samples
351 and *Methanosaetaceae* (24.8%) were the major component. They were present in most samples
352 and much more dominant than *Methanosarcinaceae* (2.0%). Hydrogenotrophic methanogens, such
353 as *Methanomassiliicoccaceae* (1.6%), *Methanoregulaceae* (1.2%) and *Methanocellaceae* (0.6%),
354 albeit low in abundance, were detected in many samples. Hütelmoor samples displayed greater
355 variability in archaeal community composition compared to Zarnekow samples. The putative
356 anaerobic methanotrophs of the ANME-2D (Raghoebarsing et al. 2006) clade occurred in patchy
357 abundance with dominance in single spots of both sites. In HC 1 they represented a mean relative
358 abundance of 40.9% of total archaeal reads but were almost absent in all other Hütelmoor cores.
359 In Zarnekow core (ZC) 3, ANME-2D represented up to approximately 30% of all archaea but were
360 otherwise low in abundance.

361 **3.3 Environmental drivers of microbial community composition**

362 Bacterial and archaeal population at both peatland sites showed distinct clustering (Fig. 6) with
363 similarly high intra- and inter-site variations but greater overall variation in community
364 composition in the Hütelmoor. Community composition varied much more strongly in HC 2 than
365 in any other core (Fig. 6). Bacterial communities in HC 1 were more similar to communities in all
366 Zarnekow cores than in other Hütelmoor cores (Fig. 6a). The archaeal community in HC 1 was
367 more similar to Zarnekow cores as well (Fig. 6b). Environmental fit vectors suggest pH, oxygen
368 and alternative TEA availability as important factors influencing microbial community
369 composition. The EC vector suggests the importance of brackish conditions in shaping microbial
370 communities in the Hütelmoor (Fig. 6a - c).

371 **3.4 Total microbial and functional gene abundances**

Quantitative PCR results show that in both fens, *mcrA* abundance is up to two orders of magnitude greater than *pmoA* abundance (Fig. 7, Tables 1 and 2). Gene copy numbers of *mcrA* are overall higher and spatially more stable in Zarnekow than in Hütelmoor. Total microbial abundance declined with depth more strongly in Hütelmoor than in Zarnekow (Fig. 7). There was a pronounced decrease in microbial abundances at 20 cm depth in the Hütelmoor. For example, 16S rRNA gene and *pmoA* gene copy numbers in deeper samples (below 20 cm depth) are one order of magnitude lower than in upper samples on average, while the *mcrA* gene abundance are approximately two orders of magnitude lower. Hütelmoor samples also exhibited larger heterogeneity in terms of abundances than Zarnekow samples. Contrary to previous studies, methanotroph abundance did not correlate with dissolved CH_4 or oxygen concentrations.

4 Discussion

4.1 Fen geochemistry and relations to microbial community composition

The rewetting of drained fens promotes elevated CH_4 production and emission, which can potentially offset carbon sink benefits. Few studies have attempted to link microbial community dynamics and site geochemistry with observed patterns in CH_4 production and/or emission in rewetted fens, while such data are crucial for predicting long-term changes to CH_4 cycling (Galand et al. 2002, Yrjälä et al. 2011, Juottonen et al. 2012). In this study, we show that CH_4-cycling microbial community composition is related to patterns in site geochemistry in two rewetted fens with high CH_4 emissions, high methanogen abundances, and low methanotroph abundances. Our results suggest that high methanogen abundances concurrent with low methanotroph abundances are characteristic of rewetted fens with ongoing high CH_4 emissions. Thus, we present microbial evidence for sustained elevated CH_4 emissions in mostly inundated rewetted temperate fens.

The environmental conditions and associated geochemistry of the two rewetted fens were largely different. Depth profiles of porewater geochemical parameters show the fens differed in EC throughout the entire peat profile, while pH and concentrations of alternative TEAs differed at

certain depths. In general, concentrations of TEAs oxygen, sulfate, nitrate, and iron were higher in the Hütelmoor. In Zarnekow, geochemical conditions varied little across the fen and along the peat depth profiles (Fig. 2). As expected, the geochemical heterogeneity was reflected in microbial community structure in both sites, suggesting the importance of environmental characteristics and associated geochemical conditions as drivers of microbial community composition (Figs. 2, 3, 4, 6). The NMDS ordinations (Fig. 6) show large variation in archaeal and bacterial community composition in the coastal brackish fen, and much less variation in the freshwater riparian fen. Environmental fit vectors (Fig. 6) suggest that salinity (indicated by the EC vector), pH, oxygen and alternative TEA availability are the most important measured factors influencing microbial communities in the two fens. Patterns in microbial community composition have previously been linked to salinity (e.g., Chambers et al. 2016), pH (e.g., Yrjälä et al. 2011), and TEA availability in peatlands (e.g., He et al. 2015).

Comparing the geochemical depth profiles (Fig. 2) with the relative abundance of bacteria and archaea (Figs. 3 and 4) provides a more complete picture of the relationships between microbial communities and site geochemistry, particularly with respect to TEA utilization. While the porewater depth profiles suggest there is little nitrate available for microbial use in HC 1, the relative abundance plot for Archaea showed that this core was dominated by ANME-2D. ANME-2D were recently discovered to be anaerobic methanotrophs that oxidize CH_4 performing reverse methanogenesis using nitrate as an electron acceptor (Haroon et al. 2013). However, ANME-2D has also been implicated in the iron-mediated anaerobic oxidation of methane (Ettwig et al. 2016), and the HC 1 site showed slightly higher total iron concentrations. The relevance of ANME-2D as CH_4 oxidizers in terrestrial habitats is still not clear. Rewetting converts the fens into widely anaerobic conditions, thus providing conditions suitable for the establishment of anaerobic oxidation of methane, but this has yet to be demonstrated in fens. The patchy yet locally high abundance of ANME-2D both in Hütelmoor and in Zarnekow suggests an ecological relevance of this group. Shifts towards less negative $\delta^{13}C$-CH_4 signatures in the upper peat profile, for example,

424 from -65 to -60‰ in HC 1 (where ANME-2D was abundant), may indicate that partial oxidation
425 of CH_4 occurred, but we could only speculate whether or not ANME-2D are actively involved in
426 this CH_4 oxidation.
427 Although TEA input may be higher in the Hütelmoor, here, methanogenic conditions also
428 predominate. This finding contrasts the measured oxygen concentrations in the upper peat profile,
429 as methanogenesis under persistently oxygenated conditions is thermodynamically not possible.
430 However, seasonal analysis of oxygen concentrations in both sites suggests highly fluctuating
431 oxygen regimes both spatially and temporary (data not shown). Such non-uniform distribution of
432 redox processes has already been described elsewhere, in particular for methanogenesis (Hoehler
433 et al. 2001, Knorr et al. 2009). It is possible that oxygen levels in both fens are highly variable,
434 allowing for both aerobic and anaerobic carbon turnover processes. Recent studies from wetlands
435 also show that methanogenesis can occur in aerobic layers, driven mainly by *Methanosaeta*
436 (Narrowe et al. 2017, Wagner 2017), which were detected in a high abundance in this study (Fig.
437 5). Further, oxygen may not necessarily be available within aggregates entailing anaerobic
438 pathways and thus, the existence of anaerobic microenvironments may also partially explain the
439 seemingly contradictory co-occurrence of oxygen and the highly abundant methanogens.
440 Anaerobic conditions are also reflected by the extensive and stable occurrence of the strictly
441 anaerobic syntrophs (e.g., *Syntrophobacteraceae*, *Syntrophaceae*) in most samples, even in the top
442 centimeters. This suggests that syntrophic degradation of organic material is taking place in the
443 uppermost layer and the fermented substances are readily available for methanogens. As
444 geochemistry and microbial community composition differ among the sites in this study, it is thus
445 notable that a similarly high abundance of methanogens, and low abundance of methanotrophs
446 was detected in both fens. The dominance of methanogens implies that readily available substrates
447 and favorable geochemical conditions promote high anaerobic carbon turnover despite seasonally
448 fluctuating oxygen concentrations in the upper peat layer.
449 **4.2 Low methanotroph abundances in rewetted fens**

Methanogens (mainly *Methanosaetaceae*) dominated nearly all of the various niches detected in this study, while methanotrophs were highly under-represented in both sites (Figs. 3 and 4). Functional and ribosomal gene copy numbers not only show a high ratio of methanogen to methanotroph abundance (Fig. 7), irrespective of site and time of sampling, but also a small contribution of methanotrophs to total bacterial population in both sites. Methanotrophs constitute only ~0.06% of the total bacterial population in the Hütelmoor and ~0.05% at Zarnekow. It should be noted that in this study we measured only gene abundances and not transcript abundances, and the pool both of active methanogens and methanotrophs was likely smaller than the numbers presented here (Freitag and Prosser 2009, Freitag et al. 2010, Cheema et al. 2015, Franchini et al. 2015). Also, as we were unable to obtain microbial samples from before rewetting, a direct comparison of microbial abundances was not possible. This was therefore, not a study of rewetting effects. For this reason, we performed an exhaustive literature search on relevant studies of pristine fens. Compared to pristine fens, we detected a low abundance of methanotrophs. Liebner et al. (2015), for example, found methanotrophs represented 0.5% of the total bacterial community in a pristine, subarctic transitional bog/fen palsa, while *mcrA* and *pmoA* abundances were nearly identical. In a pristine Swiss alpine fen, Liebner et al. (2012) found methanotrophs generally outnumbered methanogens by an order of magnitude. Cheema et al. (2015) and Franchini et al. (2015) reported *mcrA* abundances higher than *pmoA* abundances by only one order of magnitude in a separate Swiss alpine fen. In the rewetted fens in our study, *mcrA* gene abundance was up to two orders of magnitude higher than *pmoA* abundance (Fig. 7). Due to inevitable differences in methodology and equipment, direct comparisons of absolute gene abundances are limited. Therefore, only the abundances of methanotrophs relative to methanogens and relative to the total bacterial community were compared, rather than absolute abundances. We are confident that this kind of 'normalization' can mitigate the bias of different experiments and allows a comparison of sites. Further, all primers and equipment used in this study were identical to those used by Liebner et al. (2012, 2015), making the comparison more reliable.

As most methanotrophs live along the oxic-anoxic boundary of the peat surface and plant roots therein (Le Mer and Roger 2001), the low methanotroph abundances in both fens could be explained by disturbances to this boundary zone and associated geochemical pathways following inundation. In rewetted fens, a massive plant dieback has been observed along with strong changes in surface peat geochemistry (Hahn-Schöfl et al. 2011, Hahn et al. 2015). In addition to substrate (i.e. CH_4) availability, oxygen availability is the most important factor governing the activity of most methanotrophs (Le Mer and Roger 2001, Hernandez et al. 2015). The anoxic conditions at the peat surface caused by inundation may have disturbed existing methanotrophic niches, either directly by habitat destruction, and/or indirectly by promoting the growth of organisms that are able to outcompete methanotrophs for oxygen. Heterotrophic organisms, for example, have been shown to outcompete methanotrophs for oxygen when oxygen concentrations are greater than 5 μM (van Bodegom et al. 2001). Our microbial data support this conclusion, as *Hyphomicrobiaceae,* most of which are aerobic heterotrophs, was the most abundant bacterial family in both fens. Incubation data from Zarnekow (Fig. S1) show that the CH_4 oxidation potential is high, however incubations provide ideal conditions for methanotrophs and thus only potential rates. It is likely that, *in situ,* the activity of methanotrophs is overprinted by the activity of competitive organisms such as heterotrophs. It is also possible that methane oxidation may occur in the water column above the peat surface, but this was beyond the scope of this study. Nevertheless, it is low enough that methane production and emissions remain high, as demonstrated by the high dissolved CH_4 concentrations and ongoing high fluxes.

Comparable studies have so far been conducted in nutrient-poor or mesotrophic fens where post-rewetting CH_4 emissions, though higher than pre-rewetting, did not exceed those of similar pristine sites (e.g., Yrjälä et al. 2011, Juottonen et al. 2005, Juottonen et al. 2012). Nevertheless, there is mounting evidence linking CH_4-cycling microbe abundances to CH_4 dynamics in rewetted fens. Juottonen et al. (2012), for example, compared *pmoA* gene abundances in three natural and three rewetted fens and found them to be lower in rewetted sites. The same study also measured a lower

abundance of *mcrA* genes in rewetted sites, which was attributed to a lack of available labile organic carbon compounds. In peatlands, and especially fens, litter and root exudates from vascular plants can stimulate CH_4 emissions (Megonigal et al. 2005, Bridgham et al. 2013, Agethen and Knorr 2018), and excess labile substrate has been proposed as one reason for substantial increases in CH_4 emissions in rewetted fens (Hahn-Schöfl et al. 2011). Future studies should compare pre- and post-rewetting microbial abundances along with changes in CH_4 emissions, plant communities, and peat geochemistry to better assess the effect rewetting has on the CH_4-cycling microbial community.

5 Conclusion

Despite a recent increase in the number of rewetting projects in Northern Europe, few studies have characterized CH_4-cycling microbes in restored peatlands, especially fens. In this study, we show that rewetted fens differing in geochemical conditions and microbial community composition have a similarly low abundance of methanotrophs, a high abundance of methanogens, and an established anaerobic carbon cycling microbial community. Comparing these data to pristine wetlands with lower CH_4 emission rates, we found that pristine wetlands have a higher abundance of methanotrophs than measured in the fens in this study, suggesting the inundation and associated anoxia caused by flooding may disturb methanotrophic niches and negatively affect the ability of methanotrophic communities to establish. The abundances of methane producers and consumers are thus suggested as indicators of continued elevated CH_4 emissions following the rewetting of drained fens. Management decisions regarding rewetting processes should consider that disturbances to methanotrophic niches is possible if rewetting leads to long-term inundation of the peat surface.

Competing interests

The authors declare that they have no conflict of interest.

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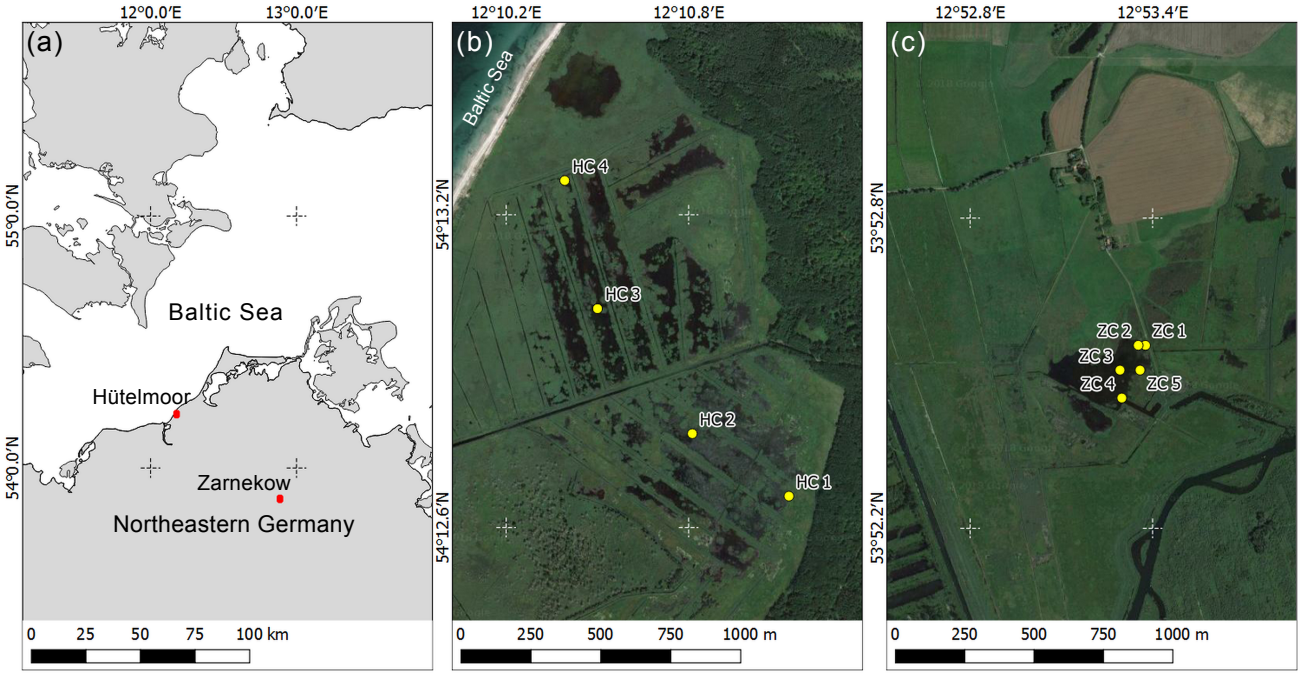


Figure 1: Location of study sites in northeastern Germany (a) and sampling locations within sites (b) Hütelmoor and (c) Zarnekow. Maps b) and c) are drawn to the same scale. Image source: (a) QGIS, (b) and (c) Google Earth via QGIS OpenLayer Plugin. Imagery date: August 9, 2015.

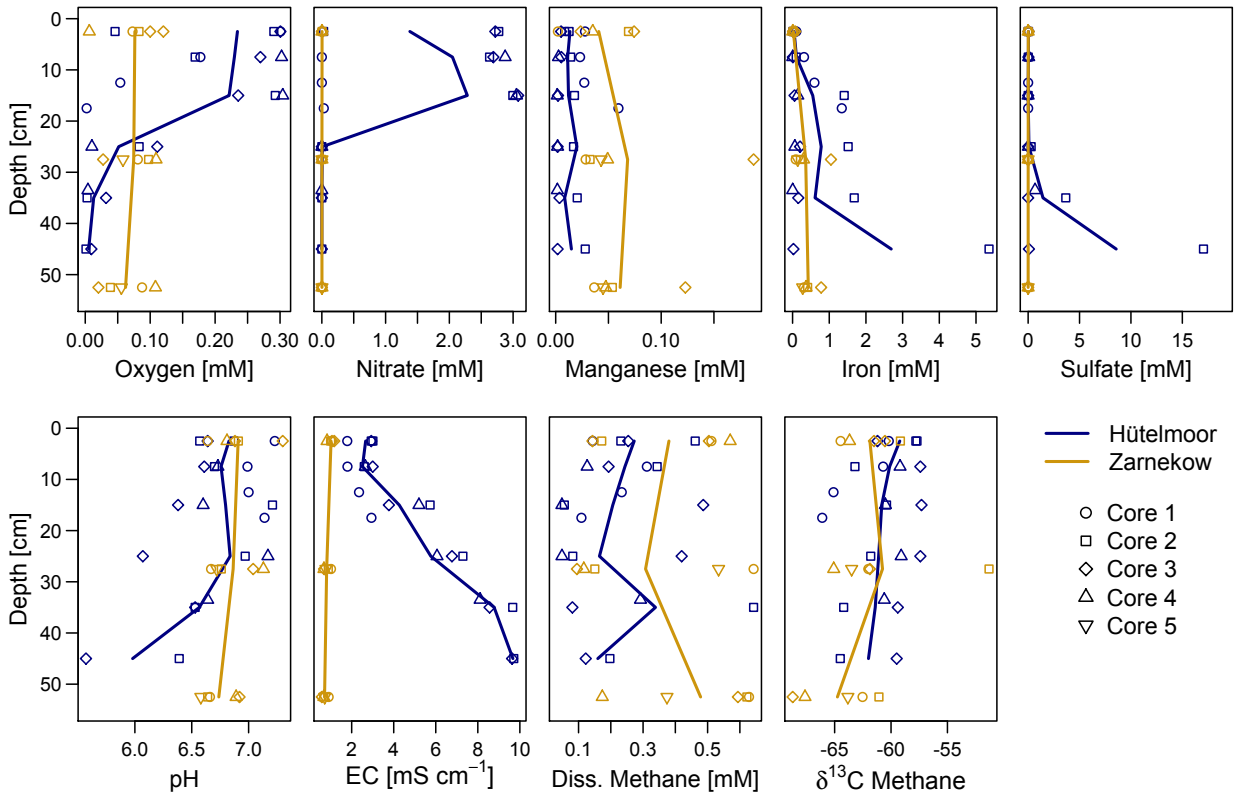


Figure 2: Depth profiles of oxygen, nitrate, total iron, manganese, and sulfate (upper panels), and profiles of pH, EC, dissolved methane, and the isotopic signature of methane-bound carbon in both study sites. Solid lines connect the respective means of individual wetlands (n=4 for Hütelmoor and n=5 for Zarnekow).

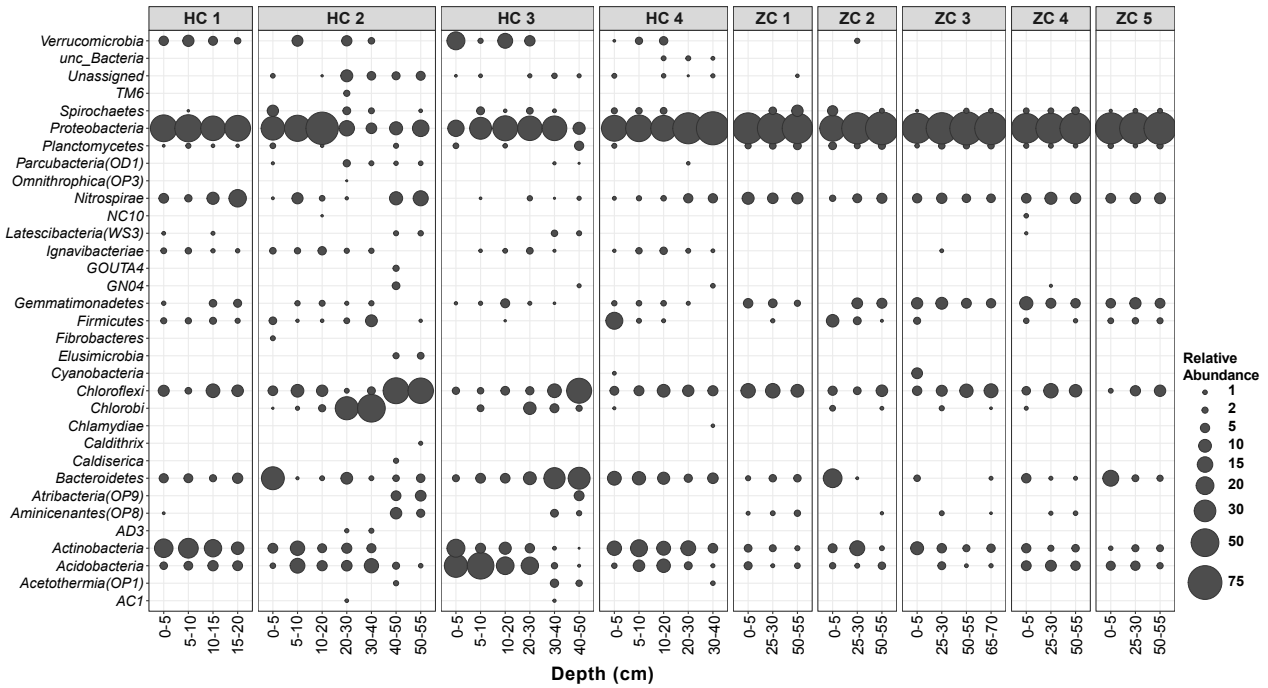


Figure 3: Relative abundances of different bacterial lineages in the study sites. Along the horizontal axis samples are arranged according to site and depth. The rank order along the vertical axis is shown for the phylum level.

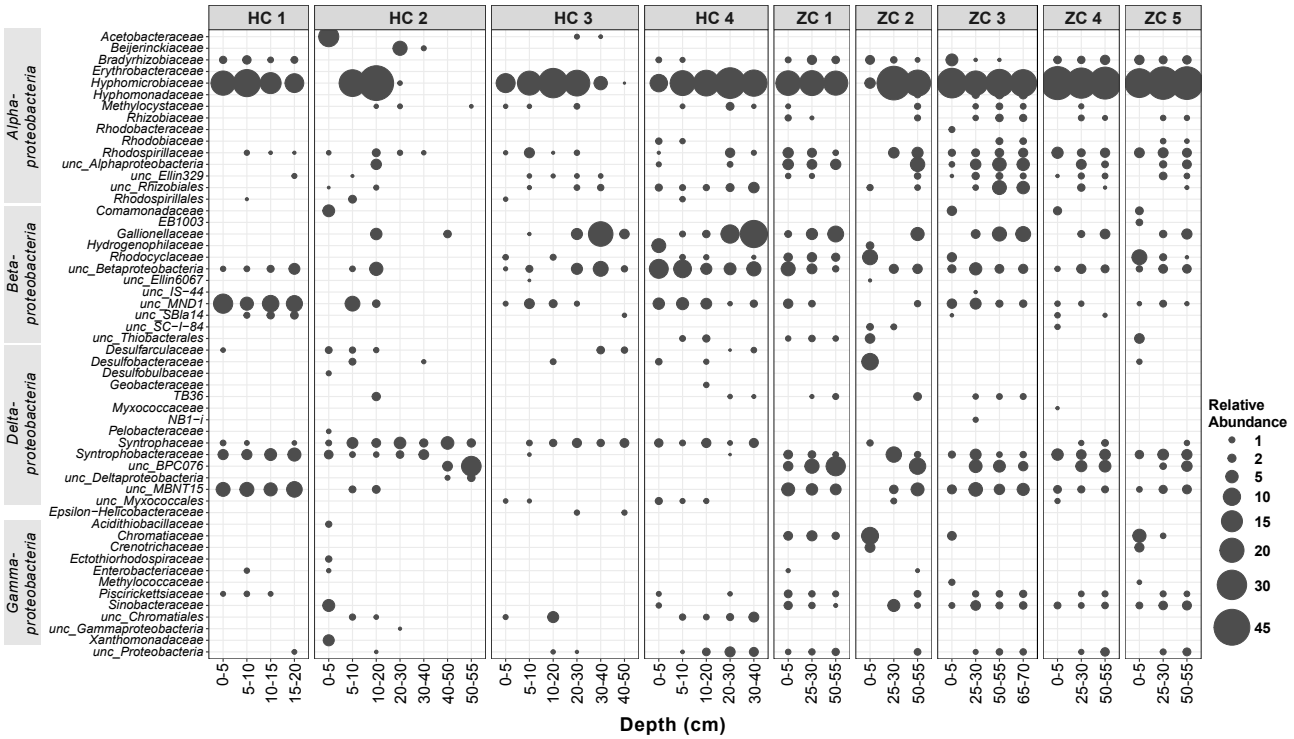


Figure 4: Relative abundances of Proteobacteria phyla in the study sites. Along the horizontal axis samples are arranged according to site and depth. The rank order along the vertical axis is shown for the family level. If an assignment to the family level was not possible the next higher assignable taxonomic level was used.

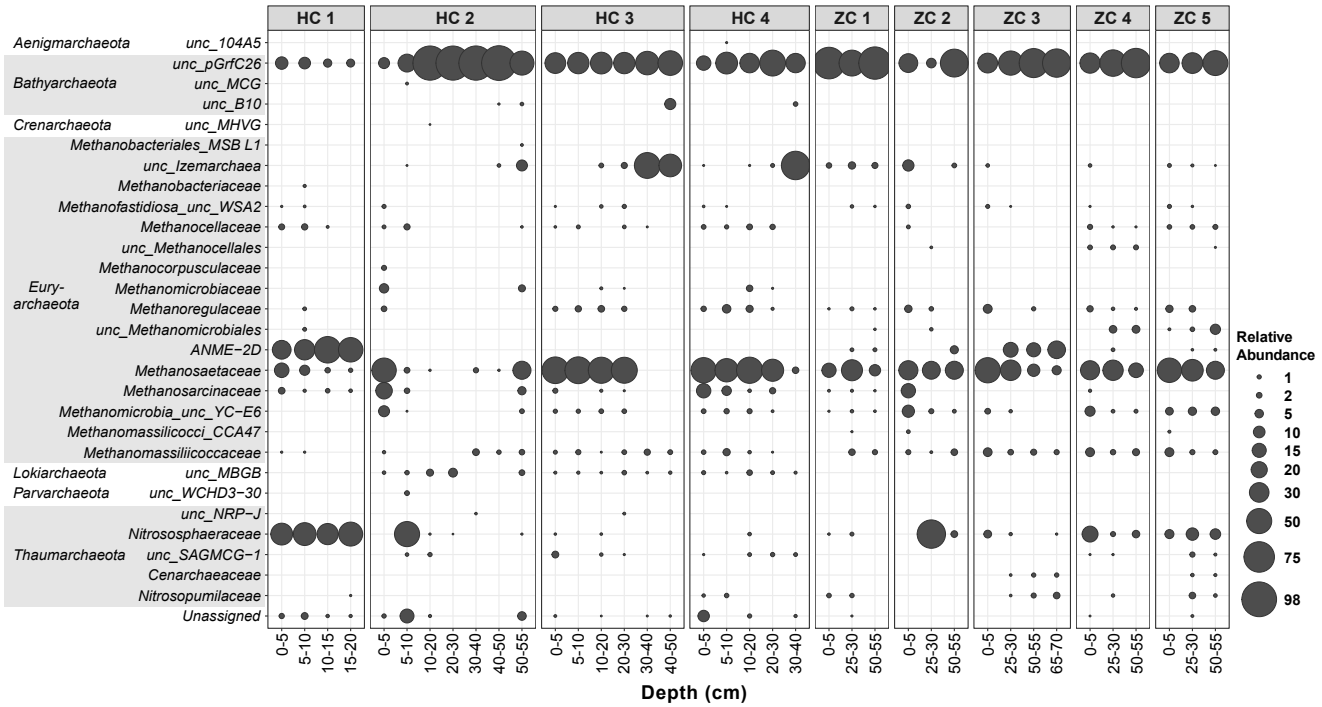


Figure 5: Relative abundances of different archaeal lineages in the study sites. Along the horizontal axis samples are arranged according to site and depth. The rank order along the vertical axis is shown for the family level. If an assignment to the family level was not possible, the next higher assignable taxonomic level was used.

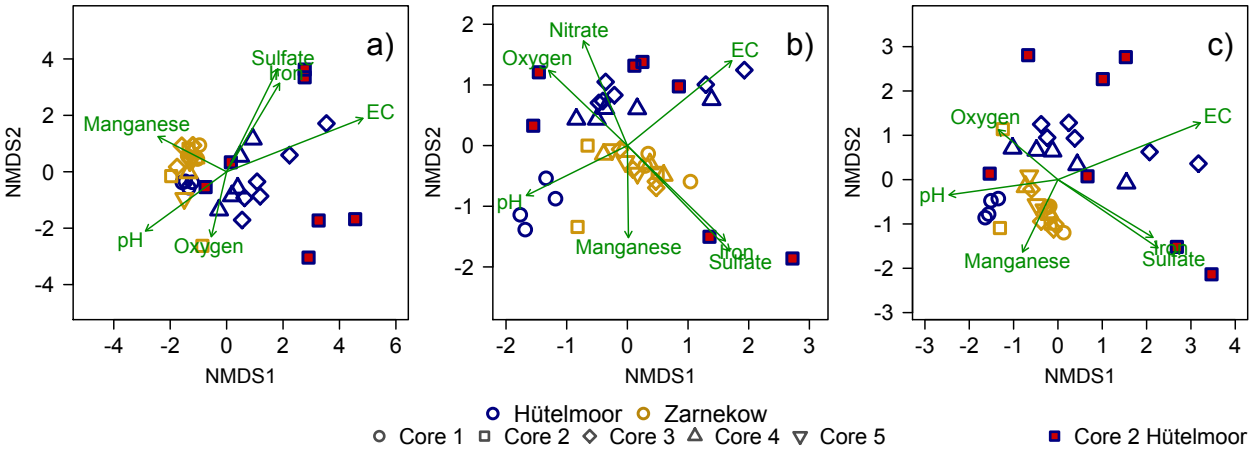


Figure 6: NMDS plots showing (a) bacterial, (b) archaeal, and (c) microbial (bacterial plus archaeal) community composition across the nine peat cores. The point positions represent distinct microbial communities, with the border colors of the symbols referring to the study sites and their shapes representing the core number. HC 2 symbols are highlighted with red fill to emphasize the large variation in microbial community within the core. Environmental fit vectors with a significance of $p < 0.05$ are shown in green.

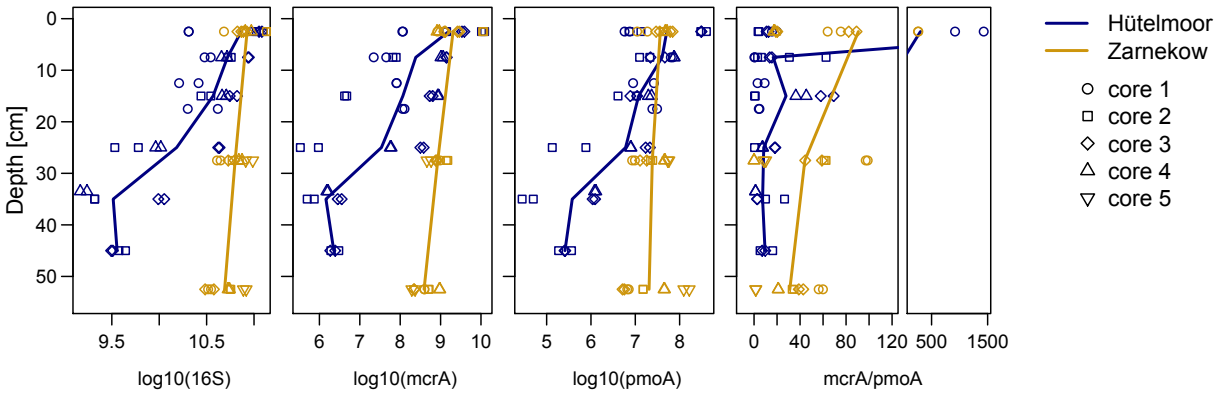


Figure 7: Depth distribution of qPCR abundances for total microbial (16S), methanogen (*mcrA*), methanotroph (*pmoA*), and ratio of *mcrA* to *pmoA* gene copy numbers in both sites. Microbial abundances were designated as numbers of gene copies per gram of dry peat soil. Duplicate measurements per depth section are shown against sampling depth using log-transformed values. Solid lines indicate mean abundances for individual wetlands (n=4 for Hütelmoor and n=5 for Zarnekow). Note that the plot at the right was split into two plots to capture very high *mcrA*/*pmoA* ratios in the upper peat layer.

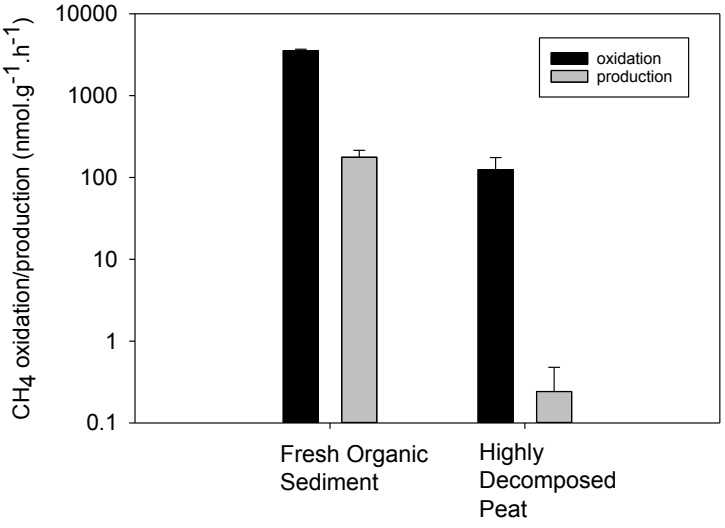
Table 1: Environmental conditions, geochemical conditions, and microbial abundances in peat cores from the Hütelmoor, a coastal minerotrophic in northeastern Germany. Environmental conditions are described by pH and EC (electrical conductivity). Geochemical parameters shown are dissol methane (CH_4) concentrations, the isotopic signature of methane-bound carbon ($\partial^{13}C–CH_4$), and concentrations of terminal electron acceptors wh are denoted with their respective chemical abbreviations. Microbial abundances here represent the mean value of subsamples for each depth sec (n=2). nd = not detected.

Core, depth	pH	EC	$\partial^{13}C–CH_4$	Dissolved CH_4	O_2	NO_3^-	Fe	Mn	SO_4^{2-}	16S	*mcrA*	*pmoA*
cm		mS cm^{-1}				mM						gene copies g dry
HC 1, 0–5	7.2	1.79	-60.2	0.14	0.30	nd	0.10	0.03	0.03	$2.04x10^{10}$	$1.15x10^{08}$	$6.60x10^{06}$
5–10	7.0	1.80	-60.7	0.31	0.18	nd	0.31	0.02	0.01	$3.25\ x10^{10}$	$3.36x10^{07}$	$6.68x10^{07}$
10–15	7.0	2.35	-65.1	0.23	0.05	nd	0.60	0.03	nd	$2.11x10^{10}$	$8.12x10^{07}$	$1.76x10^{07}$
15–20	7.1	2.94	-66.1	0.11	nd	0.03	1.34	0.06	nd	$3.08x10^{10}$	$1.21x10^{08}$	$2.76x10^{07}$
HC 2, 0–5	6.9	3.01	-57.8	0.46	0.05	0.03	0.03	0.01	nd	$1.10x10^{11}$	$1.13x10^{10}$	$1.03x10^{07}$
5–10	6.7	2.60	-63.2	0.34	0.17	2.63	0.10	0.01	0.01	$5.51x10^{10}$	$7.27x10^{07}$	$1.69x10^{07}$
10–20	7.2	5.73	-60.4	0.06	0.29	3.00	1.41	0.02	nd	$3.13x10^{10}$	$4.47x10^{06}$	$7.32x10^{06}$
20–30	7.0	7.29	-61.8	0.08	0.08	nd	1.51	0.02	0.29	$4.71x10^{09}$	$6.41x10^{05}$	$4.50x10^{05}$
30–40	6.5	9.66	-64.2	0.64	nd	nd	1.68	0.02	3.66	$2.09x10^{09}$	$6.21x10^{05}$	$3.90x10^{04}$
40–50	6.4	9.71	-64.5	0.20	nd	nd	5.35	0.03	17.1	$4.09x10^{09}$	$2.47x10^{06}$	$2.75x10^{05}$
HC 3, 0–5	6.6	2.93	-57.7	0.23	0.29	2.77	0.11	0.01	0.04	$1.10x10^{11}$	$1.34x10^{09}$	$3.51x10^{08}$
5–10	6.6	3.00	-57.4	0.19	0.27	2.69	0.01	0.01	0.03	$8.72x10^{10}$	$1.40x10^{09}$	$3.42x10^{07}$
10–20	6.4	3.77	-57.3	0.49	0.24	3.08	0.05	nd	nd	$6.08x10^{10}$	$5.86x10^{08}$	$9.35x10^{06}$
20–30	6.1	6.77	-57.4	0.42	0.11	nd	0.20	nd	nd	$4.26x10^{10}$	$3.48x10^{08}$	$1.92x10^{07}$
30–40	6.5	8.56	-59.4	0.08	0.03	nd	0.16	nd	nd	$1.05x10^{10}$	$3.20x10^{06}$	$1.17x10^{06}$
40–50	5.6	9.36	-59.5	0.12	0.01	nd	0.02	nd	0.08	$3.18x10^{09}$	$2.16x10^{06}$	$2.58x10^{05}$
HC 4, 0–5	6.6	2.93	-61.2	0.25	0.30	2.72	0.02	0.01	0.04	$1.17x10^{11}$	$3.63x10^{09}$	$3.09x10^{08}$
5–10	6.7	2.65	-59.2	0.13	0.30	2.87	0.01	nd	0.05	$4.87x10^{10}$	$1.09x10^{09}$	$7.51x10^{07}$
10–20	6.6	5.20	-60.5	0.05	0.30	3.05	0.14	nd	nd	$4.85x10^{10}$	$8.71x10^{08}$	$2.15x10^{07}$
20–30	7.2	6.06	-59.1	0.05	0.01	nd	0.06	nd	0.02	$9.78x10^{09}$	$5.82x10^{07}$	$7.91x10^{06}$
30–40	6.6	8.11	-60.6	0.29	nd	nd	0.09	nd	0.67	$1.60x10^{09}$	$1.58x10^{06}$	$1.25x10^{06}$

Table 2: Environmental conditions, geochemical conditions, and microbial abundances in peat cores from Zarnekow, a freshwater minerotrophic in northeastern Germany. Environmental conditions are described by pH and EC (electrical conductivity). Geochemical parameters shown are dissol methane (CH_4) concentrations, the isotopic signature of methane-bound carbon ($\partial^{13}C–CH_4$), and concentrations of terminal electron acceptors wh are denoted with their respective chemical abbreviations. Microbial abundances here represent the mean value of subsamples for each depth sec (n=2). nd = not detected.

Core, depth	pH	EC	$\partial^{13}C–CH_4$	Dissolved CH_4	O_2	NO_3^-	Fe	Mn	SO_4^{2-}	16S	*mcrA*	*pmoA*
cm		mS cm^{-1}		mM						gene copies g d		
ZC 1, 0–5	6.64	1.03	-64.5	0.51	0.07	0.001	0.007	0.002	0.002	$6.33x10^{10}$	$1.02x10^{09}$	1.49x10
25–30	6.67	1.14	-62.0	0.64	0.08	0.001	0.087	0.028	0.003	$4.25x10^{10}$	$8.96x10^{08}$	9.14x10
50–55	6.66	1.31	-62.5	0.63	0.09	0.005	0.310	0.037	0.002	$3.40x10^{10}$	$3.97x10^{08}$	6.85x10
ZC 2, 0–5	6.91	1.00	-59.2	0.17	0.08	0.004	0.012	0.069	0.007	$1.43x10^{11}$	$1.14x10^{10}$	4.35x10
25–30	6.76	1.29	-51.3	0.15	0.10	0.001	0.215	0.033	0.013	$6.44x10^{10}$	$1.45x10^{09}$	2.34x10
50–55	6.64	1.52	-61.1	0.62	0.04	nd	0.410	0.054	0.003	$5.64x10^{10}$	$5.10x10^{08}$	1.50x10
ZC 3, 0–5	6.88	1.17	-60.5	0.50	0.10	0.001	0.073	0.074	0.032	$7.86x10^{10}$	$2.78x10^{09}$	3.26x10
25–30	7.04	3.39	-61.9	0.10	0.03	0.002	1.046	0.188	0.003	$5.79x10^{10}$	$7.81x10^{08}$	1.55x10
50–55	6.92	3.82	-68.7	0.59	0.02	nd	0.779	0.123	0.003	$3.41x10^{10}$	$2.21x10^{08}$	5.41x10
ZC 4, 0–5	7.3	1.06	-61.5	0.14	0.12	0.010	0.013	0.024	0.035	$7.19x10^{10}$	$1.28x10^{09}$	6.53x10
25–30	7.13	1.58	-65.1	0.12	0.11	0.002	0.301	0.049	0.002	$7.19x10^{10}$	nd	4.60x10
50–55	6.89	1.51	-67.6	0.17	0.11	0.002	0.366	0.048	0.002	$5.42x10^{10}$	$9.47x10^{08}$	4.50x10
ZC 5, 0–5	6.81	0.83	-63.7	0.57	0.01	0.002	0.005	0.035	0.005	$8.73x10^{10}$	$8.73x10^{08}$	4.97x10
25–30	6.72	0.86	-63.5	0.53	0.06	0.002	0.139	0.043	0.001	$8.94x10^{10}$	$5.21x10^{08}$	5.57x10
50–55	6.58	1.00	-63.8	0.37	0.06	0.002	0.275	0.045	0.002	$8.00x10^{10}$	$2.14x10^{08}$	1.44x10

966 **Supplemental Material**



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968
969 Figure S1: Incubation data from Zarnekow, a freshwater minerotrophic fen in Northeastern Germany. Rates of methane production (n=3) and meth
970 oxidation (n=3) are shown for both fresh (surficial) organic sediment and the bulk peat.