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Title

Congruent changes in microbial community dynamics and ecosystem methane fluxes following natural drought in two restored fens

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VU and SL formulated the research questions and study design. VU, SL, FK, and GJ performed the fieldwork. VU performed the lab work. FH and SY conducted the bioinformatic analyses. FK and PG processed the eddy covariance flux data. JK and KHK provided equipment for geochemical analyses and aided in geochemical data acquisition and preparation. VU, GJ, SL,

SY, FK, and TS prepared the figures. VU prepared the original paper draft. All authors contributed to the interpretation and discussion of the data, as well as writing of the paper.

Key words

Drought; Methane; Fen; Peatland; Methanogens; Methanotrophs

Abstract

Both the frequency and intensity of drought events are expected to increase, with unresolved alterations to peatland methane cycling and the involved microbial communities. While existing studies have assessed drought effects via experimental approaches under controlled conditions, to our knowledge, no studies have examined the *in-situ* effects of natural drought in restored temperate fens. In this study, we used quantitative polymerase chain reaction (qPCR) and high throughput 16S rRNA gene amplicon sequencing of DNA and complementary DNA (cDNA) to determine the abundances and community structure of total and putatively active microbial communities following the 2018 European summer drought. Together with geochemical and methane flux data, we compared these results to a non-drought reference dataset. During drought, water level and methane flux rates decreased to a new recent minimum in both fens. This corresponded with pronounced shifts in porewater geochemistry. Microbial community composition in the drought year differed markedly, and was characterized by a greater relative and total abundance of aerobic methanotrophs, and, in one of the two sites, by a decrease in total methanogen abundance. In contrast to the non-drought reference years, type I methanotrophs were clearly more dominant than type II methanotrophs in both fens. cDNA sequencing confirmed the activity of type I methanotrophs during drought, with *Methylomonaceae* having the highest average relative abundance of bacterial cDNA transcripts. We show that changes in microbial community dynamics, porewater geochemistry, and ecosystem methane fluxes can be substantial following natural drought in restored fens, and

provide the first *in-situ* evidence from a natural drought which suggests type I methanotroph populations are more active than type II methanotrophs in response to drought effects. Type I methanotrophs may represent a key microbial control over methane emissions in restored temperate fens subject to natural drought.

1 Introduction

Peatlands, which store a substantial portion of the world's terrestrial organic carbon (approx. 550 to 1,055 Gt; Yu et al., 2010; Nichols and Peteet 2019), also emit a globally significant amount of the greenhouse gas methane (CH_4; Bastviken et al., 2011). Peatland ecosystems are particularly vulnerable to drought events, which are likely to increase in frequency and intensity in the upcoming decades (Laiho 2006; IPCC 2014). Methane in peatlands is produced metabolically by methanogenic archaea (methanogens) under water-logged, reducing conditions, when other terminal electron acceptors (TEAs) used in the breakdown of organic matter are depleted (Froelich et al., 1979; Peters and Conrad 1996; Zehnder and Stumm 1988). Methane that does not reach the atmosphere via ebullition, diffusion, or direct transport by plant aerenchyma (Joabsson et al., 1999; Baird et al., 2004) may be oxidized either aerobically or anaerobically by bacteria or archaea (methanotrophs). This mechanism, performed mainly by aerobic methanotrophs in peatlands, can reduce CH_4 emissions substantially (Yavitt et al.,1988). Drought events can decrease peatland CH_4 emissions by reducing water level and altering redox geochemistry (Roden and Wetzel 1996; Knorr and Blodau 2009; Kang et al., 2018). Associated community dynamics of methanogens and methanotrophs, however, are not well understood. *In situ* studies of fen peatlands in this respect are scarce, (Cadillo-Quiroz et al., 2008), although, compared to bogs, fens may be particularly sensitive to drying (Jaatinen et al., 2007; Peltoniemi et al., 2016).

The activity, abundance, and community structure of microbes can be altered by drought effects (Knorr et al., 2008; Kim et al., 2008; Ma et al., 2013; Potter et al., 2017). Oxidative stress can decrease rates of CH_4 production (Jasso-Chávez et al., 2015) and/or cause methanogen mortality (Morozova and Wagner 2007). This is supported by incubation studies and mesocosm experiments that show decreases in CH_4 production (Dowrick et al., 2006; Knorr and Blodau 2009) and methanogen gene abundances (Kim et al., 2008) after short term drought treatments. In two field studies of peatland archaeal communities of the Tibetan plateau, Tian et al. (2012 and 2015) found that drought caused a 10-fold decrease in archaeal abundance (of which the majority were methanogens) and altered community composition. Ma and Lu (2011) also noted a significant decrease in archaeal abundance in a rice paddy soil following short-term drought treatments, citing a decrease in methanogen abundance. In a field manipulation experiment, Peltoniemi et al. (2016) found that both warming and drying decreased methanogen abundances in boreal fens. In some studied locations, however, no changes in methanogen abundance and community structure were found (e.g. Kim et al., 2008; Peltoniemi et al., 2016), suggesting drought-induced changes to microbial community dynamics may differ among individual peatlands.

Whether aerobic methanotrophic communities can maintain function under increasing frequency and intensity of natural drought is still not clear (Ho et al., 2016a). Drought effects on aerobic methanotrophic communities in peatlands have been studied mostly in controlled incubation experiments (e.g. Henckel et al., 2001; Ma and Lu 2011; Collet et al., 2015; Ho et al., 2016a; van Kruistum et al., 2018). Some aerobic methanotrophs benefit from water level reduction and modest soil drying (Henckel et al., 2001; Ma and Lu 2011; Peltoniemi et al., 2016). Aerobic proteobacterial methanotrophs are divided into types I (gammaproteobacteria) and II (alphaproteobacteria) based on phylogenetic and biochemical distinctions (Knief 2015), and an increasing number of studies suggest a differential response to drying events among aerobic methanotroph types (Collet et al., 2015; Ho et al., 2016a; Peltoniemi et al., 2016).

Functional traits dictate microbial response to environmental changes, and determine their survival strategy (i.e., life strategy; Ho et al., 2013a). Incubation studies suggest that type I methanotrophs can adjust more readily to moisture and temperature fluctuations, and are able to rapidly increase activity and population size once suitable conditions return (Henkel et al., 2001; Bodelier et al., 2012; Ho et al., 2013a, b; Pan et al., 2014; Collet et al., 2015; Ho et al., 2016a, b). Though ubiquitous in peatlands, type II methanotroph populations are thought to be comparatively stable under changing temperature and moisture regimes (Henckel et al., 2001; Collet et al., 2015) as a consequence of their different life strategy as stress-tolerators, compared to type I methanotrophs, which have been described broadly as competitor-ruderals (Ho et al., 2013a). This has not yet been investigated in fens under natural drought. However, in their field manipulation experiment, Peltoniemi et al. (2016) found a combination of warming and drying decreased the abundance of some type I methanotrophs, while differences in the abundance of type II methanotrophs could only be explained by depth, and not by warming and/or drying treatments.

The 2018 European drought (Hanel et al., 2018) provided the opportunity to examine microbial community and CH_4 emission dynamics in a natural setting. According to the European Centre for Medium-Range Weather Forecasts, the near-surface air temperature anomaly from April to August 2018 in Europe was greater than in any year since 1979, with the Baltic Sea region experiencing the highest anomalies (Magnusson et al., 2018). Data from the National Oceanic and Atmospheric Administration place 2018 as the warmest summer in Europe on record since 1910 (NOAA Global Climate Report 2020). From April to September 2018, monthly precipitation averaged across Germany was 25.6 % (April) to 52.7 % (July) less than the 1981-2010 reference period average (Deutscher Wetterdienst 2020). Such drastic deviations are likely to be reflected in fen CH_4 cycling and the involved microbial communities, but most existing studies are experimental (e.g. Kim et al., 2008; Tian et al., 2015; Peltoniemi et al., 2016) or do not examine methanogens and methanotrophs specifically (e.g. Tian et al., 2012).

Total microbial, methanogen, and methanotroph community dynamics have not yet been studied together with CH_4 emissions and porewater geochemistry in restored fens affected by natural drought. Therefore, during the 2018 European summer drought, we collected peat and porewater samples from two previously studied fens in Northeastern Germany. We analyzed peat microbial community structure and relative abundances using 16S rRNA gene sequencing, and determined total microbial, methanogen, and bacterial methanotroph abundances using quantitative PCR (qPCR). We include porewater geochemical analyses, as well as ongoing CH_4 flux and water level measurements, and compared this dataset to a similar dataset from non-drought years preceding 2018 (Wen et al., 2018). In the drought year only, we employed reverse transcription of total RNA and subsequent sequencing of complementary DNA (cDNA) to develop a community profile of active microbes. We aimed to elucidate methanogen and methanotroph community dynamics associated with decreasing CH_4 emissions in restored fens affected by natural drought. Along with a decrease in CH_4 flux rates, we hypothesized a decrease in the abundance of methanogens, as well as shifts in microbial community composition, with a greater representation of aerobic taxa during drought, specifically methanotrophs. Furthermore, we expected the community profile of microbes active during drought to be dominated by aerobic taxa.

2 Materials and Methods

2.1 *Study sites*

For a detailed description of the study sites, see Wen et al. (2018). Briefly, the Hütelmoor is a coastal minerotrophic fen of approx. 360 ha located at the Baltic Sea in NE Germany (Fig. 1a and b). In the past, the fen received brackish water inflows from occasional storm surges, though the last brackish water intrusion event happened more than 20 years ago, in 1995. Since then, the brackish sulfate (SO_4^{2-}) pool of the upper soil horizons has been fully depleted, so that contemporary conditions for CH_4 cycling correspond to those of freshwater peatlands (Koebsch et al., 2019; Wen et al., 2018). Dominant vegetation at the site includes *Phragmites australis, Bolboschoenus maritimus, Carex acutiformis, and Schoenoplectus tabernaemontani*. The second site, Zarnekow, is a riparian fen of approx. 500 ha, located in the valley of the river Peene in NE Germany (Fig. 1a and c). The dominant emergent macrophyte is *Typha latifolia*. Both fens were drained for agricultural purposes and were restored in 2005 (Zarnekow) and 2010 (Hütelmoor) by permanently raising water levels above peat surface. After rewetting, average CH_4 fluxes increased dramatically (Augustin and Chojnicki 2008; Hahn et al., 2015; Franz et al., 2016; Wen et al., 2018). The porewater pH at both sites ranges from slightly acidic to circumneutral (~6–8). Electrical conductivity in the Hütelmoor averages 5.3 mS cm^{-1} compared to 1.5 mS cm^{-1} in Zarnekow.

Figure 1 here, to be printed in color

Figure 1. Location of the study sites in Northeastern Germany (a) and sampling locations in the coastal fen, the Hütelmoor (b), and the riparian fen, Zarnekow (c). Two sampling points were located in each of the wet unvegetated (WU) and dry unvegetated (DU) subsites. Aerial images were taken in May 2018, before average water levels decreased in the fens.

2.2 *Peat and porewater collection (drought year)*

Peat and porewater samples for the drought year were collected on August 30 and September 12, 2018 in the Hütelmoor and Zarnekow, respectively. At this time, the average water level in both fens was visibly lower, nevertheless, the distinct microtopography led to a patchy mosaic of dry spots, and small ponds with water depths ~10 cm or less. Samples were collected in both the dry and wet subsites to assess whether spatial patterns in hydrology led to larger differences in microbial community dynamics and geochemistry than drought. Sampling was conducted in non-vegetated sites to exclude the potential influence of plants and rhizosphere effects on CH_4 cycling and involved microorganisms. In each subsite, duplicate peat cores were collected for analyses of microbial community composition and concentrations of dissolved CH_4 and carbon dioxide (CO_2). Surface samples were collected for microbial analyses only. A third and fourth core (one from each subsite) were collected for bulk density and porewater geochemical analyses, respectively. All peat cores were semi-cylindrical (5 cm width x 50 cm depth) and were collected with a Russian D-corer (De Vleeschouwer et al., 2010). The cores were split into 10 cm depth sections and the depth sections 0-10, 20-30, and 40-50 cm were analyzed for this study.

Peat samples for microbial analyses were collected using sterile equipment, placed in 15 ml Falcon Tubes and immediately placed in a dry-shipper that was pre-cooled with liquid nitrogen. Samples were stored in the lab at -80 °C for approximately one week until nucleic acid extraction. For dissolved CH_4 and CO_2 concentrations and their $^{13}C/^{12}C$ isotopic composition, a 3 ml peat "plug" was collected with a tip-cut 3 ml syringe from each depth section and placed in a 20 ml vial containing 5M NaCl solution. The vials were closed immediately with butyl rubber stoppers and aluminum crimp caps with no headspace, and then stored upside down until processing. After collecting a peat plug of a known volume for bulk density analysis (again using a tip-cut syringe), peat samples for porewater geochemical analyses were immediately packed in air-tight aluminum bags and sealed with plastic clips. Upon returning to the lab several hours later, the aluminum bags were flushed with nitrogen gas, completely degassed using a vacuum pump, heat-sealed, and stored at 4ºC until analysis.

Sampling during the drought year could not be conducted at the exact same time points as sampling in the non-drought year due to the inability to anticipate the onset and duration of drought events. However, previous work in the fens, and similar fens from the same region, revealed little variation in key parameters considered in the present study among late summer and early autumn. Monthly porewater sampling campaigns conducted June through November (2015) in both fens showed minimal variation in dissolved oxygen, SO_4^{2-}, nitrate (NO_3^-), and nitrite (NO_2^-) concentrations. The porewater data were published in Wen et al. (2018) but were not presented in a monthly fashion. Additionally, preliminary (unpublished) research in the Hütelmoor showed no significant differences in qPCR-based methanogen and methanotroph abundances among the summer and autumn seasons. No such data are available for Zarnekow, however, given that site conditions typically do not change drastically between July and mid-September, any temporal influence should be negligible compared to the effects of the extreme drought. Finally, Wang et al. (2021) found no seasonal variation in prokaryotic microbial community composition in other coastal and riparian fen systems in northeastern Germany.

2.3 *Sample and data collection (non-drought year)*

A detailed description of the study design and methods from the non-drought year is provided in Wen et al. (2018), but a summary of this, and an explanation how the data were applied in this study, are provided here. Peat and pore water were collected in four locations in the Hütelmoor and five locations in Zarnekow in October 2014 and July 2015, respectively. At the time of sampling, the sampling locations were fully inundated since rewetting. From this timepoint, two sampling locations from each fen were selected to compare to the data collected in the drought year. This was done to ensure that data from similar locations in the fens were compared.

In both the drought and non-drought years, peat collection methods for microbial analyses were conducted with the same equipment and analyzed with the same protocols. Porewater collection differed between the drought and non-drought years. In the non-drought year, porewater was collected from permanently installed porewater dialysis samplers using a syringe. In the drought year, peat samples were collected, from which porewater was extracted using a hydraulic pore water press. Dissolved CH_4 and dissolved CO_2 concentrations were determined using the same equipment, while concentrations of TEAs and isotopic analyses were conducted on different equipment. Sequencing data from the non-drought year were initially analyzed and published using OTU clustering methods (Wen et al., 2018). For the present study, amplicon sequence variant methods were employed, and sequencing data from the non-drought year were therefore reanalyzed accordingly. RNA extraction, reverse transcription, and subsequent cDNA sequencing were conducted in the drought year only.

2.4 *Water level and methane flux measurements*

Water level in Zarnekow was derived with a sonic ranging sensor SR50A (Campbell Scientific, Canada) that measured the distance from a fixed platform down to the water surface. The distance measured by the SR50A was corrected for temperature effects and converted to water levels relative to the sediment surface. Water level in the Hütelmoor was derived from pressure transducers using a HOBO 13-Foot Depth Titanium Water Level Data Logger (Onset, Bourne, USA) after barometric pressure correction, and was referenced to the mean surface level. In this study, water levels below the peat surface are denoted with a negative sign.

Methane fluxes were measured with the eddy covariance approach, which provides a continuous time series of half-hourly gas fluxes on ecosystem scale. In the Hütelmoor, the measurement setup comprised of two open-path infrared gas analyzers (LI-7500 and LI-7700, both LI-COR, Lincoln, NE, USA), and a three-dimensional sonic anemometer (CSAT3, Campbell Scientific, Logan, UT, USA) for wind velocities and sonic temperature. All signals were recorded by a CR3000 Micrologger (Campbell Scientific, Logan, UT) with a scan rate of 10 Hz. In Zarnekow, the setup for CH_4 flux measurements comprised of an open-path CH_4 analyzer (LI-7700, LI-COR, Lincoln, NE, USA) and a closed-path Fast Greenhouse Gas Analyzer (FGGA EP, Los Gatos Research). The sonic anemometer is a Gill HS-50 (Gill, Lymington, Hampshire, UK) and raw data were recorded with a LI-7550 digital data logger system (LI-COR Biogeosciences, Lincoln, NE, USA) at 20 Hz in half-hourly files. Though instrumentation and configuration differed slightly between both sites, each of the measurement setups is well in line with the default practice used for eddy covariance-determined greenhouse gas flux measurements (for details see Koebsch et al. 2020). Half-hourly net CH_4 fluxes were processed with the software EddyPro version 6.0.0 (LI-COR, Lincoln, NE, USA). Data gaps were imputed with the same artificial neural network approach for both sites.

2.5 *Bulk density and porewater chemistry*

Water content was determined by dividing the oven dry weight of a given peat sample over the wet weight. Bulk density (g dry weight cm^{-3}) of each depth section was calculated by dividing the oven dry weight of a peat sample by the initial volume. Porewater for geochemical (pH and anion) analyses was extracted from the peat samples using an IODP-style titanium pore water squeezer (Manheim 1966) in a 22-ton hydraulic press (Carver, Wabash, USA). The extractors were fitted with 0.22 μm pore size filters. Anions (NO_2^-, NO_3^-, and SO_4^{2-}) were quantified on an ion chromatograph system with an S5200 sample injector, a 3.0 mm×150 mm SykroGel A 01 column, a S3115 conductivity detector (all SYKAM Chromatographie Vertriebs GmbH, Germany), and a SeQuant SAMS anion IC suppressor (Merck KGaA, Germany). The eluent (6 mM Na_2CO_3 and 90 μM NaSCN) had a flow rate of 1 mL min^{-1}. Column oven temperature was 50 °C, and the injection volume was 50 μl.

2.6 *Dissolved methane and carbon dioxide*

In the lab, a 3 mL gas headspace was created in each porewater sample vial by addition of ultrapure helium using two sterile syringes. After being allowed to equilibrate for two weeks (upside down to avoid gas leakage) samples were analyzed on a 7890A gas chromatograph (GC) system (Agilent Technologies, Germany) equipped with a flame ionization detector and a Carboxen PLOT Capillary Column or HP-Plot Q (Porapak-Q) column. Injection volume was 250 μL. Dissolved CH_4 and CO_2 concentrations were calculated and converted to micromolar values from the equation $\frac{G*H}{T*R*V*P} * 1000$, where G = headspace gas mole fraction (ppm), H = headspace volume (3 mL), T = absolute temperature (301.15 °K), R = universal gas constant (0.082 L·atm·K^{-1}·mol^{-1}), V = peat volume (3 mL), and P = peat porosity. Porosity was calculated from the measured bulk density.

2.7 *Isotopic composition of methane and carbon dioxide*

The $^{13}C/^{12}C$ isotopic composition of CH_4 and CO_2 was determined using cavity ring-down spectroscopy (CRDS; PICARRO G2201-i PICARRO Instruments, Sunnyvale, CA, United States) coupled to a Small Sample Isotope Module (SSIM) in order to measure small gas sample volumes (~20 ml). To avoid interference with hydrogen sulfide present in samples, 1 ml of a saturated Zn-acetate solution was added to precipitate hydrogen sulfide as ZnS. As the measurement range of the instrument is 300-2000 ppm for CO_2 and 2.5-2000 ppm CH_4, small gas samples from headspace vials were taken (100 µL) and analyzed on a GC equipped with FID and TCD (GC 2010, Shimadzu, Kyoto, Japan) to determine a suitable dilution in synthetic air for isotope measurements. The calibration for CH_4 was performed using a working standard of 1000 ppm (-42.48 ‰) and four standards of 2500 ppm (-38.30, -54.45, -66.50 and -69.00 ‰). CO_2 calibration was performed using a standard of 1000 ppm with known isotopic signature of -31.07 ‰ and dilution of pure CO_2 with signatures of -27.10 and -4.55 ‰, respectively. All gas standards had been calibrated against reference materials from IAEA (RM8562) using elemental analysis coupled to isotope ratio mass spectrometry (EA 3000, Eurovector, Redavalle, Italy; Horizon, NU Instruments, Wrexham, UK) or were provided from Air Gas (Air Liquide, Plumsteadville, PA, USA) or from Isometric Instruments (GASCo, Victoria, BC, Canada) with certificates. Isotope values are expressed in units of per mill (‰) in the typical δ-notation vs. V-PDB.

2.8 *DNA extraction and sequencing*

Genomic DNA was extracted from 150 to 200 mg of peat soil per sample using an EurX GeneMATRIX soil DNA Purification Kit. DNA concentrations were quantified using a Nanophotometer P360 (Implen GmbH, Munich, Germany). Polymerase chain-reaction (PCR) analysis of bacterial and archaeal 16S rRNA genes was performed as detailed in Wen et al. (2018). Primer combinations for archaea were S-D-Arch- 0349-a-S-17/S-D-Arch-0786-a-A-20 (Takai and Horikoshi 2000) and S-D-Bact-0341-b-S-17/S-D- Bact-0785-a-A-21 (Herlemann et al., 2011) for bacteria. The targeted amplicons were a length of 400 for bacteria and 406 for archaea. Illumina HiSeq sequencing was performed by Eurofins Genomics using 300 bp paired-end mode and a 20 % PhiX Control v3 library to offset the effects of low-diversity sequence libraries.

2.9 *RNA extraction and cDNA synthesis*

Total RNA was extracted using the Qiagen RNeasy PowerSoil Total RNA kit. The resulting RNA was purified using a TURBO DNA-free kit (Invitrogen). Complete digestion of DNA was verified using RNA as non-template control in conventional 16S rRNA PCR reactions as described in Liebner and Svenning (2013). Quality of the RNA was checked with an Agilent 2100 Bioanalyzer (Agilent Technologies, US). Reverse Transcriptase polymerase chain reaction was conducted on total RNA according to in house protocol. First, 10 µl sterile distilled water, 1µl 10mM dNTP mix (Invitrogen), 1µl pd(N)$_6$ Random Hexamer (GE Healthcare), and 1µl of sample were combined in a PCR tube on ice (final volume 13 µl). The mixture was heated at 65°C for 5 minutes in a PCR machine (Bio-Rad) and immediately chilled on ice. The contents of the tubes were spun down and the following reagents were then added: 1 µl sterile distilled water, 1 µl M DTT, 1 µl Superscript III Reverse Transcriptase (Invitrogen), and 4 µl 5 x First Strand Buffer. The reagents were mixed gently by pipetting up and down, and were incubated first at 25°C for 5 minutes (because random primers were used), then 50°C for 60 minutes, and finally deactivated by heating at 70°C for 15 minutes. The resulting cDNA was then used as a template for PCR amplification. PCR amplification was performed on bacterial and archaeal cDNA using the same primer combinations as mentioned above.

2.10 *Taxonomic analyses*

Cutadapt (Martin 2011) was used to demultiplex the NGS libraries. Primer sequences were detected while allowing for a maximum error rate of 10 % whereas sample barcodes were not allowed to show any sequencing error while having a high-quality score (Q25). The DADA2 pipeline (Callahan et al., 2016) was applied to process the sample sequence libraries by truncating (250 bp forward reads; 200 bp reverse reads) and filtering read sequences. A library-specific error model was generated and used for dereplication, for sample inference and for the merging of read pairs. Resulting sequences were required to have a minimum length of 200 bp and their orientation was standardized by calculating the hamming distance of the sequences and their reverse complements. De novo chimera removal was applied to the resulting sequence table. The resulting amplicon sequence variants (ASVs) were assigned to the SILVA taxonomy (v132) (Quast et al., 2013) by applying vsearch (Rognes et al., 2016) as implemented in the QIIME2 pipeline (Bolyen et al 2018). In order to account for different sequencing depths, the relative abundances of the ASVs were used for the visualization and comparison of the microbial communities.

2.11 *Quantification of 16S rRNA, mcrA, and pmoA gene copy numbers*

Quantitative polymerase chain reaction (qPCR) was performed on a Bio-Rad CFX instrument (Bio-Rad, Munich, Germany) using the SYBR green method for determination of 16S *rRNA* (total bacterial), *mcrA* (methanogenic), and *pmoA* (bacterial methanotrophic) gene copy numbers. Technical replicates were performed in triplicate. A detailed description of the procedure can be found in Wen et al. (2018). *Methylocella* spp. were not present in the sequencing data and the marker gene *mmoX was* therefore excluded from qPCR analysis.

2.12 *Statistical methods and data visualization*

All statistics and data visualization were done using R (R Core Team 2019). The Mann-Whitney test was employed to examine differences in geochemical parameters and microbe abundances in drought versus non-drought years, as well as subsites. To assess whether the 2018 drought led to a significant reduction in CH_4 flux rates, daily individual CH_4 fluxes across the years 2015–2018 were compared using Tukey's honest significance (Tukey's HSD) test. This analysis was conducted for CH_4 flux rates over (i) the entire year, (ii) a subperiod of March–November, and (iii) a subperiod of September–December. The full results of the analyses are provided in the supplemental data (Fig. S1). Means, maximums, and sums of CH_4 fluxes for each time period were calculated and are also provided in the supplemental material (Table S1, S2). Additionally, correlation analyses were performed for CH_4 fluxes and water level, precipitation, and air temperature. For air temperature, a fitted exponential model was constructed, and correlation coefficients were determined. NMDS ordinations were constructed using the function metaMDS of R package vegan (Oksanen et al., 2019) to visualize dissimilarity in microbial community composition at the family level among subsites and before and after drought. Bubble plots were constructed at the family level to examine differences in relative abundances of methanogens and methanotrophs between drought and non-drought conditions, as well as to visualize the active communities of both groups. In the bubble plots, family level is presented in order to remain consistent with previously published sequencing data from the two sites.

2.13 *Accession numbers*

The 16S rRNA gene sequence data from the non-drought year were deposited at EBI under the BioProject PRJNA356778. The Hütelmoor sequence read archive accession numbers are SRR5118134 - SRR5118155 and SRR5119428 - SRR5119449 for bacterial and archaeal sequences, respectively. The Zarnekow accession numbers are SRR6854018 - SRR6854033 and SRR6854205 - SRR6854220 for bacterial and archaeal sequences, respectively. Raw sequencing data from the drought year are available at the European Nucleotide Archive under

BioProject accession number PRJEB38162 and sample accession number ERS4542720-ERS4542857.

3 Results

3.1 *Methane emissions and water level*

Methane emissions and water level dynamics were similar among the fens in the drought year (Fig. 2). Water levels were high in April 2018, at 0.6 and 0.8 m above the surface in the Hütelmoor and Zarnekow, respectively (Fig. 2c and d). However, water levels dropped steadily from May on and reached surface level between end of July/beginning of August. At this time, CH_4 emissions in both fens peaked at 0.90 (Hütelmoor) and 0.87 (Zarnekow) g m^{-2} d^{-1}, then decreased sharply afterwards (Fig. 2a and b). For the rest of the year, CH_4 emissions remained low with median fluxes of 0.02 and 0.04 g m^{-2} d^{-1}, respectively. Although water levels rose above ground surface in November, CH_4 emissions were lower than the same time period of the three previous years. This held particularly for the Hütelmoor, where median November CH_4 fluxes reached only 3% of the magnitude of the previous 3 reference years.

Figure 2 here -to be printed in black and white

Figure 2. Annual trends in eddy covariance-determined methane flux rates (a and b) and water level (c and d) for the Hütelmoor and Zarnekow. Grey areas represent the range of measured values for the three years prior to the drought year (2015 - 2017), while the solid black line represents the drought-year average (2018). Dotted lines indicate the time of sampling in the drought year.

Methane flux rates in the drought year differed from all other years in both fens ($p < 0.05$) only when comparing the subperiod of September to December 2018 (Supplemental data, Fig S1) when the drought effects were most prominent (Fig. 2). When considering the entire year, CH_4 emissions in the drought year were significantly lower compared to 2015 and 2016, in the Hütelmoor only, with no statistically significant differences in Zarnekow.

Figure 3 here - to be printed in color

Figure 3. Fitted exponential models for CH_4 flux rates (g m^{-2} day^{-1}) versus air temperature (°C) in the Hütelmoor (a) and Zarnekow (b) fen peatlands in NE Germany. Solid lines represent average values, while shaded areas delineate 95% confidence intervals. Orange and blue colors correspond to the time periods of January – August and September – December in the three years prior to the drought (2015-2017), respectively. Red and black colors correspond to the time periods of January – August and September – December in the drought year (2018), respectively. Calculated correlation coefficients are shown for each time period within the panels.

Methane fluxes did not correlate with precipitation or water level (data not shown), but there was a distinct exponential temperature response (Fig. 3), which weakened during and after the drought. Correlation coefficients calculated for September – December in 2018, the time period during and immediately after the drought, were lower (Fig. 3).

3.2 *Porewater geochemistry*

Dissolved SO_4^{2-} concentrations were higher during drought in both fens ($p = 0.02$, Table 1), while NO_3^- concentrations did not differ among the drought and non-drought years ($p > 0.05$). In contrast to the non-drought year, where NO_3^- was only detected in a few surface samples, NO_3^- was detected in nearly every sample during drought. Dissolved CH_4 concentrations were significantly lower in both fens during the drought (Table 1). Dissolved CO_2/CH_4 ratios were higher in the dry subsites than wet subsites, ranging from 31 to 1,912 and 1 to 18 in dry and wet subsites respectively (Table 1). $\delta^{13}C\text{-}CH_4$ values ranged from -67.5‰ to -52.5‰ Slightly higher $\delta^{13}C\text{-}CH_4$ values were measured in the dry subsites of Hütelmoor (-52.5‰) and Zarnekow (-56.7‰) at the 20-30 and 40-50 cm depths, respectively. Except for the 0-10 cm depth in the Hütelmoor and 40-50 cm depth in Zarnekow, where $\delta^{13}C\text{-}CO_2$ values were -13.3‰ and -14.8‰, respectively, $\delta^{13}C\text{-}CO_2$ values in the dry subsites were around -20‰. In the wet subsites, $\delta^{13}C\text{-}CO_2$ values were higher (Table 1). Gas concentrations were too low to determine isotopic values for depths of 0 to 40 cm in the dry subsites in Zarnekow, and isotopic values for the 40 to 50 cm depth section in Zarnekow could only be obtained for one core (Table 1).

Table 1. Total microbial, methanogen, and methanotroph abundances as well as geochemical parameters for dry and wet subsites during the 2018 summer drought. Values shown are averages calculated from duplicate cores.

	Depth	16S rRNA	*mcrA*	*pmoA*	Dissolved CH_4	Dissolved CO_2	CO_2/CH_4	$\delta^{13}C-CH_4$	$\delta^{13}C-CO_2$	NO_3^-	NO_2^-	SO_4^{2-}
	cm	copies g dry peat^{-1}	copies g dry peat^{-1}	copies g dry peat^{-1}	µM	µM		V-PDB	V-PDB	mM	mM	mM
Hütelmoor Dry	Surface	3.6 x 10^{10}	6.5 x 10^{8}	4.8 x 10^{7}	-	-	-	-	-	-	-	-
	0-10	2.0 x 10^{10}	7.2 x 10^{8}	2.7 x 10^{7}	8	1194	121	-60.9	-21.0	6.20	-	28.5
	20-30	1.2 x 10^{10}	2.0 x 10^{8}	1.1 x 10^{7}	12	1147	94	-52.5	-21.6	-	-	19.0
	40-50	1.3 x 10^{10}	1.0 x 10^{8}	9.2 x 10^{6}	6	739	69	-63.3	-19.6	3.51	2.75	35.7
Hütelmoor Wet	Surface	2.2 x 10^{10}	1.5 x 10^{9}	1.2 x 10^{8}	-	-	-	-	-	-	-	-
	0-10	3.3 x 10^{10}	8.7 x 10^{8}	7.8 x 10^{7}	248	1818	5	-64.6	-13.3	0.03	0.09	0.60
	20-30	2.6 x 10^{10}	3.0 x 10^{6}	2.7 x 10^{7}	80	777	17	-66.3	-19.8	0.12	0.15	0.44
	40-50	8.9 x 10^{9}	4.2 x 10^{6}	7.5 x 10^{6}	35	1231	18	-67.5	-20.9	3.57	0.22	38.6
Zarnekow Dry	Surface	2.0 x 10^{10}	1.6 x 10^{8}	3.6 x 10^{7}	-	-	-	-	-	-	-	-
	0-10	2.2 x 10^{10}	2.4 x 10^{8}	1.5 x 10^{7}	0.4	1072	1912	-	-	0.09	0.12	1.49
	20-30	1.8 x 10^{10}	1.2 x 10^{8}	2.3 x 10^{7}	4	1080	276	-	-	0.19	0.20	1.16
	40-50	7.3 x 10^{9}	4.5 x 10^{7}	8.5 x 10^{6}	27	666	31	-56.7	-14.8	0.10	0.07	0.33
Zarnekow Wet	Surface	1.8 x 10^{9}	1.2 x 10^{8}	1.0 x 10^{7}	-	-		-	-	-	-	-
	0-10	3.7 x 10^{11}	6.1 x 10^{7}	8.5 x 10^{6}	538	332.4	1	-62.5	-10.2	-	-	0.65
	20-30	3.2 x 10^{10}	4.4 x 10^{7}	7.1 x 10^{6}	382	651.0	1	-65.0	-10.8	0.53	0.38	33.90
	40-50	5.5 x 10^{10}	4.9 x 10^{6}	2.9 x 10^{6}	226	667.4	6	-62.9	-13.2	-	-	2.25

3.3 *16S rRNA, mcrA, and pmoA gene copy numbers*

During drought, total microbial abundance based on 16S *rRNA* gene copy numbers ranged from 1.8×10^9 to 3.7×10^{11} and was—compared to the non-drought year—slightly higher in Zarnekow ($p = 0.03$), but did not differ in the Hütelmoor (Table 1). Total methanogen abundance based on *mcrA* gene copy numbers ranged from 3.0×10^6 to 1.5×10^9 copies g dry peat^{-1} and was significantly lower in Zarnekow during drought ($p = 9.8 \times 10^{-5}$), but similar among the drought and non-drought years in the Hütelmoor ($p = 0.4$). Total bacterial methanotroph abundance based on *pmoA* gene copy numbers, which ranged from 2.9×10^6 to 1.2×10^8 copies g dry peat^{-1}, was significantly higher in the Hütelmoor during the drought ($p = 2.5 \times 10^{-2}$) but did not differ in Zarnekow ($p = 0.15$). Bacterial methanotroph abundances were significantly higher in the dry subsites in Zarnekow ($p = 0.007$), but no other significant differences in gene abundances were found among dry and wet subsites.

3.4 *Overall community composition of bacteria and archaea*

According to the NMDS ordination, bacterial community composition based on 16S *rRNA* gene sequencing differed among the fens in both the drought and non-drought years (Fig. 4). In the drought year, bacterial community composition differed from the non-drought year in Zarnekow. In the Hütelmoor, surface sample bacterial community composition clearly differed among the drought and non-drought year, but there was some similarity among the deeper samples. The patterns of differentiation were not consistent among the fens, with community composition diverging in Zarnekow and converging in the Hütelmoor. Expectedly, surface samples showed the strongest differentiation in community composition among drought and non-drought years. Archaea community composition also differed among the fens at both time points (Fig. 4). Community composition converged strongly in the Hütelmoor following drought, but there was little overall difference among the dry and wet subsites and time points. In Zarnekow, archaeal community composition during drought clearly differed from the non-drought year. Notably, the relative abundances of the phyla Actinobacteria and Firmicutes were higher in both fens during drought (data not shown).

Figure 4 here, to be printed in color

Figure 4. NMDS ordinations showing bacterial (a) and archaeal (b) community composition in the non-drought year (pre WU) and the wet and dry subsites of the drought-year (post WU and post DU, respectively). Points represent distinct microbial communities, with the darkest points representing the microbial community composition at depth (40 – 50 cm), and the lightest points representing surface sample communities. Polygons indicate clusters within the respective time points. The dotted and dashed polygons refer to samples from the drought-year, while the solid polygons refer to the non-drought year. Blue highlights samples from the Hütelmoor (HM) and yellow highlights samples from Zarnekow (ZN).

3.5 *Methanotroph community composition and relative abundances based on 16S rRNA gene sequencing and cDNA sequencing*

In contrast to the non-drought year, the type I aerobic methanotrophs of the order Methylococcales clearly dominated the methanotrophic community during drought. Type I methanotrophs of Methylococcales were detected in all but four samples, and represented an average of 1.1 % of total bacterial community, with a maximum of 3 % in dry subsites, and 6 % in wet subsites. In particular, there was a pronounced increase in the distribution and relative abundance of family *Methylomonaceae,* but *Methylococcaceae* were also detected in a greater number of samples in the drought year (Fig. 5 a and b). The distribution and abundance of type II methanotrophs within *Beijerinckiaceae* was similar among the drought and non-drought years (Fig. 5 a and b). *Methylomonas* was the most abundant genus, followed by *Crenothrix*, *Methylobacter, Methylococcus*, “*Candidatus* Methylospira”, and *Methyloparacoccus*. Type II methanotrophs were represented mainly by *Methylocystis*, and were only a small portion of the *Beijerinckiaceae* taxa detected, making up only 0.1 % of bacterial community or less.

Methylomirabilaceae, the family containing proposed anaerobic methanotroph (ANME) "*Candidatus* Methylomirabilis" (*Ca*. Methylomirabilis), was detected in many samples from both time points (Fig. 5 a and b), and was represented almost exclusively by the uncultured group Sh765B-TzT-35. *Ca*. Methylomirabilis was detected in deeper peat (20–50 cm depth) at both time points in Zarnekow where it represented 0.01 to 0.05 % of bacterial community, but was low in abundance (≤ 0.05 %) and not detected in the Hütelmoor (data not shown).

Figure 5 here, to be printed in color

Figure 5. Bubble plots showing the relative abundances (in %) of aerobic methanotrophs (blue), methanogens (red), and ANME (orange) for the non-drought (a) and drought years (b and c). Plots a and b were constructed from sequenced DNA in the non-drought and drought year, respectively, while plot c was constructed from sequenced cDNA from the drought-year only. The abundances shown for aerobic methanotrophs are calculated relative to total bacterial community, while the abundances of methanogens and ANME are calculated relative to total archaeal community. Sequencing data are shown for the family level. Other groups besides methanogens and methanotrophs that may be classified within the shown groups are not included in this chart.

Along with other aerobic bacteria (e.g. *Arenicellaceae* and *Nitrosomondaceae*), type I methanotrophs (mostly *Methylomonaceae*) were active during drought, and dominated the active community profile (Supplemental data, Fig. S3). Within the bacterial community, type I methanotrophs of *Methylomonaceae* had the highest average (8 %) and maximum (26 %) relative abundance of all methanotrophs (Fig. 5 c). Type II methanotrophs (*Methylocystis*) were active in just five samples from both fens, with a maximum relative abundance of 0.09 % of bacteria. *Ca*. Methylomirabilis was detected in just two deeper samples in Zarnekow. However, *Methylomirabilaceae,* and in particular group Sh765B-TzT-35 (data not shown), represented a significant portion of the cDNA-determined community (Fig. 5 c).

In the DNA-based community profile, the archaeal ANME family *Methanoperedencaceae* was detected in nearly all depths in both fens, but was relatively most abundant in deeper peat (Fig. 5 a and b). This family was represented entirely by the anaerobic methanotroph "*Candidatus* Methanoperedens nitroreducens" (*Ca*. M. nitroreducens). At depths of 20 to 50 cm, "*Ca*. M. nitroreducens" represented up to 2 % of archaea in the Hütelmoor and up to 18 % in Zarnekow. In the active community profile, "*Ca*. M. nitroreducens" was detected in one sample from Hütelmoor at 40 – 50 cm, where it represented 0.2 % of archaeal cDNA sequences. In Zarnekow, it was detected at 40 – 50 cm in both cores from the dry subsites with relative abundances of 0.4 and 4 % (Fig. 5). This was additionally the only location and depth where "*Ca*. Methylomirabilis" (group Sh765B-TzT-35) cDNA sequences were detected. The organism is understood to use NO_2^- produced by "*Ca*. M. nitroreducens" via anaerobic CH_4 oxidation coupled to NO_3^- reduction (Ettwig et al., 2010; Haroon et al., 2013).

3.6 *Methanogen community composition and relative abundances based on DNA and cDNA sequencing*

In the drought year, methanogens remained the most abundant archaeal group, along with Crenarchaeota (Supplemental data, Fig. S4). During drought, *Methanosaetaceae* accounted for 23 – 97 % of archaeal community in the DNA-based, which was similar to relative abundances measured in the non-drought year (Fig. 5 a and b). The acetoclastic methanogens *Methanosaetaceae* (also known as *Methanothrix*) dominated the archaeal community, along with the metabolically diverse *Methanosarcinaceae*, the H_2/formate-utilizing *Methanoregulaceae,* and the hydrogenotrophic *Methanomassiliicoccaceae* (Supplemental data, Fig. S4). This was also reflected in the active community profiles (Fig. 5 c; Supplemental data, Fig. S5). Taxa that were detected in the DNA-based community profiles were not always detected in the active community profiles at similar depths (this was true for both bacteria and archaea), suggesting the presence of inactive microorganisms (Fig. 5 a – c, Supplemental data, Figs. S2–S5).

4 Discussion

4.1 *Drought methane emissions and site conditions*

In both fens, a substantial peak in CH_4 emissions occurred in August of the drought year, as water level approached the peat surface. The magnitude of the peak exceeded CH_4 emission rates of the previous 3 years (Fig. 2). Previous studies have reported a brief pulse in CH_4 emissions associated with water level reduction in peatlands, likely because of degassing due to reduced hydrostatic pressure (Moore et al., 1990; Dinsmoore et al., 2009) and/or increasing peat temperatures associated with the heat wave, as warming can enhance CH_4 production significantly as long as fen peats are moist (Turetsky et al., 2008). In the present study, CH_4 emissions correlated strongly with temperature, but not water level or precipitation (Fig. 3). Once water levels reached below the peat surface, CH_4 emissions were diminished (below 0.2 g CH_4 m^{-2} d^{-1}), and a decoupling of CH_4 emissions and temperatures occurred, as indicated by calculated correlation coefficients (Fig. 3). This is likely due to the increasing importance of a combination of other factors in regulating CH_4 emissions from the investigated sites, such as oxygen intrusion related to increased diffusivity upon water level decline, and changes in microbial community dynamics.

Despite increasing water levels in autumn, CH_4 emissions remained low in both fens (Fig. 2), and were significantly lower in the time period of September to December, compared to the previous 3 years (Supplemental data, Fig. S1, Tables S1, S2), signifying a biogeochemical legacy of draining. This could be explained by drought-induced reoxidation of alternative TEAs (Estop-Aragonés et al., 2013; Clark et al., 2020). During drought, we measured higher SO_4^{2-} concentrations and detected NO_3^- at greater depths and in a larger number of samples compared to the non-drought year. With these (and other) alternative electron acceptors, various other microorganisms may outcompete methanogens for substrates (Kristjansson and Schönheit 1983; Scholten et al., 2002; Gao et al., 2019), effectively inhibiting methanogenesis. In the Hütelmoor, the drought-related decrease in CH_4 emissions led to lower yearly CH_4 emissions in 2018, compared to the years 2015 and 2016, while no differences were found in annual CH_4 budgets in Zarnekow (Supplemental data, Fig. S1, Tables S1, S2). This nevertheless highlights the potential for drought events to lead to significant reduction in CH_4 emissions, and may translate to changes in CH_4 budgets on a broader scale (e.g., regional budgets). However, compilation of data from various sites is needed to venture into regional or global predictions, as highlighted by the contrasting results from our two study sites.

At the time of sampling during the drought year, CH_4 emissions had already dropped to a new recent minimum, and the shifting site conditions were evident in the geochemical data. During drought we measured (mostly in the dry subsites) significantly lower dissolved CH_4 concentrations, high dissolved CO_2/CH_4 ratios, as well as more negative $\delta^{13}C\text{-}CO_2$ values (Table 1). High CO_2/CH_4 ratios indicate CO_2 formation from other metabolic processes such as aerobic decomposition, reduction of alternative TEAs, or CH_4 oxidation (Corbett et al., 2013; Holmes et al., 2015; Gao et al., 2019), while CO_2/CH_4 ratios close to one are associated with predominantly methanogenic conditions (Keller et al., 2009). CO_2 produced from non-methanogenic pathways should have a $\delta^{13}C\text{-}CO_2$ signature closer to the parent organic matter, as observed in the dry subsites or during drought (with two exceptions, Table 1), while CO_2 produced via methanogenesis is enriched in ^{13}C, as observed under wet conditions (Boehme et al., 1996 ; Corbett et al., 2013 ; Table 1). With respect to the measured $\delta^{13}C\text{-}CO^2$ values, contribution of older CO_2 and CO_2 formed at depth cannot be excluded. Nevertheless, together, the geochemical data show a shift in redox conditions and associated dominating metabolic pathways, and underscore the more oxidized site conditions under drought. This is supported by our taxonomic dataset that shows a shift toward the dominance of aerobic bacteria. Additionally, the relative abundances of phyla Actinobacteria and Firmicutes were higher during drought (data not shown). Studies of the root-associated microbiome of grasses (Naylor et al., 2017) and rice (Santos-Medellín et al., 2017) have also noted an increase in the relative abundance of these groups following experimental drought manipulations.

Though average water level decrease was not as dramatic in Zarnekow during the drought (Fig. 2), we conclude the drought-induced water level reduction was a major contributor to the patterns observed in this study. In the non-drought years, average water level was at or above the peat surface by September (the time of sampling in the drought year). During the drought year, water level remained below the surface late into autumn. The heat wave during the drought year probably exacerbated the effects of the water level decrease, as widespread drying out of the peat surface was observed, in contrast to previous years. A separate study of the same sites showed that water level decrease in both fens stressed the established vegetation, and led to changes in vegetation composition and atmospheric carbon exchange (Koebsch et al. 2020). Similarities in CH_4 emission trajectories (Fig. 2), porewater geochemistry (Table 1), and aspects of microbial community composition among the two fens during the drought year further support the conclusion that water level decrease led to the observed patterns.

4.2 *Total microbial abundances and community structure*

Differences in gene abundances and microbial community structure between the drought and non-drought years were site-specific. We expected to detect a lower total abundance of methanogens in both fens due to lowering of average water level and potential associated oxidative stress, as has been documented in previous studies of fen peat (Tian et al., 2012; Tian et al., 2015; Peltoniemi et al., 2016). In the present study, total methanogen abundance was significantly lower following drought compared to a non-drought year in the riparian fen (Zarnekow), but no difference was found in the coastal fen (Hütelmoor). Methanotroph abundance was significantly higher in the coastal fen, while in the riparian fen, total methanotroph abundance was greater only in the dry subsites sampled. This observation is likely due to differing site conditions of the fens, differing initial microbial community compositions, and/or stress history of the microbial communities within them. In a mesocosm study, Kim et al. (2008) found that short-term drought treatments decreased methanogen abundance in bog, but not in fen peats. Based on this and other evidence, they concluded that distinct microbial communities associated with different peatland types would respond differently to drought effects. Other studies echo this conclusion (Jaatinen et al., 2007; Ho et al., 2016b; Peltoniemi et al., 2016).

Environmental stress history is important in shaping microbial communities, as stress selects for taxa that are able to withstand disturbances (Ho et al., 2016b; Krause et al., 2018; van Kruistum et al., 2018). Although both fens in this study were historically drained for agricultural purposes, microbial communities in the Hütelmoor may be better adapted to environmental stress, having experienced fluctuating salinity and water levels associated with historical seawater intrusions. Baumann and Marschner (2013) suggested that microorganisms tolerant to salt stress are likely more resistant to desiccation, though the study did not examine CH_4-cycling microbes specifically. Overall, these results show that a months-long, natural drought can change the total abundance of methanogens and methanotrophs, and support previous studies that suggest abundance patterns may differ among individual peatlands.

The ordination analyses further illustrate how patterns in microbial community dynamics may vary among individual peatlands. Microbial community composition differed among the fens, as has often been reported in previous studies (Kim et al., 2008; Peltoniemi et al., 2016; Wen et al., 2018). In this study, this was true for both the drought and non-drought years. Community composition (including both wet and dry subsites) of bacteria and archaea during drought was distinct from community composition under inundated conditions, with the exception of a few deeper samples that showed some overlap, and of archaea in the Hütelmoor (Fig. 4). Based on this and the qPCR results, the archaeal community in Zarnekow appeared to be more strongly affected by the drought than in the Hütelmoor. This is in line with some long-term studies that indicate drought will alter bacterial and archaeal community composition (Tian et al., 2012; Tian et al., 2015). Other studies suggest that archaeal community composition may be more resistant to warming and/or drying effects in both the short- and long-term (Kim et al., 2008; Peltoniemi et al., 2016). Although archaea community composition converged strongly in the Hütelmoor in the drought year, there was little separation among the polygons indicating the different time points (Fig. 4 b), suggesting less change in community composition. It is worthwhile to highlight that, while changes in methanogen and methanotroph abundances and overall microbial community composition differed among the fens, CH_4 emission dynamics were ultimately similar, implying functional redundancy among microbial groups. Peltoniemi et al. (2016) reported a similar observation, when they found CH_4 emission dynamics to be similar among two fens subject to warming and drying treatments, despite differing patterns in microbial control over the emissions.

4.3 *Methanotroph relative abundances and links to laboratory studies*

According to 16S *rRNA* gene sequencing, the increase in the relative abundance of type I aerobic methanotrophs, in particular *Methylomonaceae*, was striking in both fens (Fig. 5 a and b). In the Hütelmoor, type I methanotrophs were hardly detectable in the non-drought year (Fig. 5). Previously, it was believed that type II methanotrophs may be more resistant to drought stress due to formation of desiccation-resistant spores (Whittenbury et al., 1970; Bowman 2006). However, more recent work has shown type I methanotrophs to be unexpectedly resistant to environmental stress, as well as responsive to fluctuations in temperature and water availability (Collet et al., 2015; Ho et al., 2016b), while type II methanotroph populations, though ubiquitous, are relatively stable and do not experience significant changes in population size in comparison. To our knowledge, Peltoniemi et al. (2016) were the first to provide *in situ* evidence of this in fens. They found type I methanotroph abundances were altered by warming and drying treatments, while depth was the only controlling factor over type II methanotrophs. Similarly, in our study, there was little difference in the distribution and relative abundance of type II methanotrophs between the drought and non-drought years.

This study provides the first *in situ* evidence from a natural drought that suggests type I methanotrophs are comparatively adaptive to changes in temperature and water availability, which supports earlier experimental work (Henkel et al., 2001; Ho et al., 2013a; Ho et al., 2013b; Pan et al., 2014; Collet et al., 2015; Ho et a. 2016a; Ho et al., 2016b; Peltoniemi et al., 2016). The dominance of type I methanotrophs during drought was confirmed by cDNA sequencing. The type I methanotrophs of the *Methylomonaceae* family had the highest relative amount of cDNA transcripts (Fig. 5), and were one of the most abundant bacterial groups (Supplemental data, Fig. S3). Thus, type I methanotrophs may represent a significant microbial control over CH_4 emissions in restored temperate fens subject to natural drought.

4.4 *Evidence for anaerobic methanotrophic activity*

Denitrifying anaerobic methanotrophs "*Ca*. M. nitroreducens" (archaea) and "*Ca*. Methylomirabilis" (bacteria) were detected in both the DNA- and cDNA-based community profiles at the same depths. Interestingly, at these depths, the highest $\delta^{13}C$-CH_4 values were measured (20 – 30 and 40 – 50 cm depths), and higher $\delta^{13}C$ values (approaching -50‰) in dissolved CH_4 may be indicative of CH_4 oxidation. While this is not conclusive evidence that these organisms contribute to CH_4 emission mitigation in these systems, they appear to be active at depth and may represent an additional filter for CH_4 produced in deeper peats. "*Ca*. M. nitroreducens" has been implicated in CH_4 oxidation in incubations of rice paddy soils, which typically have nigh nitrogen availability (Vaksmaa et al., 2016). Decades of agricultural use (and therefore increased nitrogen availability) during drainage could have promoted the establishment of these organisms in the fens. The role of ANME in fens warrants further investigation. In vitro studies of various peatland types suggest ANME may be capable of oxidizing a significant portion of produced CH_4 (Zhu et al., 2012; Miller et al., 2019).

5 Conclusions

This study shows that reduced CH_4 emissions in drought-affected fens are associated with multiple substantial changes in pore water chemistry and microbial community dynamics, and that some changes in microbial community dynamics may be site-specific. In the riparian fen (Zarnekow), total methanogen abundance decreased and methanotroph abundance increased in some areas, while, in the coastal fen (Hütelmoor), only total methanotroph abundances increased. Reduced CH_4 emissions in restored fens subject to natural drought are thus at least partially the result of the differential controlling patterns of methanogens and methanotrophs. However, a large increase in the relative abundance of methanotrophs, particularly type I methanotrophs, was documented in both fens. We provide the first *in situ* evidence from a natural drought that suggests type I methanotrophs are comparably more adaptive than type II methanotrophs when experiencing drought effects. The abundance of type I methanotrophs seems to be an important microbial control over CH_4 emissions in temperate fens experiencing natural drought.

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Conflict of interest:

The authors declare that they have no conflict of interest.

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Supplemental Data

(a) Hütelmoor entire year

	diff	lwr	upr	p adj
2016-2015	0.02139804	-0.01012618	0.052922262	0.3003450
2017-2015	-0.01759089	-0.04913667	0.013954885	0.4780980
2018-2015	-0.03727024	-0.06883767	-0.005702802	0.0129951
2017-2016	-0.03898893	-0.07051315	-0.007464711	0.0081546
2018-2016	-0.05866828	-0.09021417	-0.027122384	0.0000113
2018-2017	-0.01967935	-0.05124678	0.011888088	0.3769631

(b) Hütelmoor March–November

	diff	lwr	upr	p adj
2016-2015	0.02925793	-0.007592128	0.066107995	0.1729896
2017-2015	-0.01622863	-0.053078693	0.020621430	0.6690881
2018-2015	-0.04715843	-0.084008491	-0.010308368	0.0056420
2017-2016	-0.04548657	-0.082336627	-0.008636504	0.0083427
2018-2016	-0.07641636	-0.113266425	-0.039566302	0.0000007
2018-2017	-0.03092980	-0.067779859	0.005920264	0.1353221

(c) Hütelmoor September–December

	diff	lwr	upr	p adj
2016-2015	-0.03471091	-0.060411060	-0.009010766	0.0030412
2017-2015	-0.01502216	-0.040722309	0.010677986	0.4341202
2018-2015	-0.10480862	-0.130561811	-0.079055427	0.0000000
2017-2016	0.01968875	-0.006011395	0.045388899	0.1988906
2018-2016	-0.07009771	-0.095850897	-0.044344514	0.0000000
2018-2017	-0.08978646	-0.115539649	-0.064033265	0.0000000

(d) Zarnekow entire year

	diff	lwr	upr	p adj
2016-2015	0.011341698	-0.01421481	0.03689820	0.6638840
2017-2015	0.001531912	-0.02404207	0.02710589	0.9986975
2018-2015	0.004518714	-0.02105527	0.03009269	0.9687702
2017-2016	-0.009809786	-0.03536629	0.01574672	0.7567516
2018-2016	-0.006822984	-0.03237949	0.01873352	0.9022117
2018-2017	0.002986802	-0.02258718	0.02856078	0.9905834

(e) Zarnekow March–November

	diff	lwr	upr	p adj
2016-2015	0.016120751	-0.01441631	0.04665781	0.5259329
2017-2015	0.003008888	-0.02752817	0.03354595	0.9942829
2018-2015	0.005851478	-0.02468558	0.03638854	0.9606520
2017-2016	-0.013111863	-0.04364892	0.01742520	0.6866694
2018-2016	-0.010269273	-0.04080633	0.02026779	0.8227794
2018-2017	0.002842589	-0.02769447	0.03337965	0.9951669

(f) Zarnekow September–December

	diff	lwr	upr	p adj
2016-2015	-0.012644704	-0.02747245	0.0021830366	0.1250960
2017-2015	-0.011054778	-0.02588252	0.0037729626	0.2201220
2018-2015	-0.027880208	-0.04270795	-0.0130524675	0.0000100
2017-2016	0.001589926	-0.01323781	0.0164176668	0.9926169
2018-2016	-0.015235504	-0.03006324	-0.0004077633	0.0413954
2018-2017	-0.016825430	-0.03165317	-0.0019976893	0.0188336

Figure S1: Full results of the Tukey's Honest Significance test, conducted on daily eddy covariance-determined CH_4 fluxes (g m^{-2} d^{-1}) from a coastal fen (Hütelmoor: a, b, and c) and a riparian fen (Zarnekow: d, e, and f) in northeastern Germany. The analysis was conducted for three distinct time periods within the years 2015–2017 (non-drought years) and 2018 (a drought year). The three time periods analyzed include: the entire year, March-November, and September-December. *p*-values below 0.05 indicate significantly different CH_4 fluxes among the time periods.

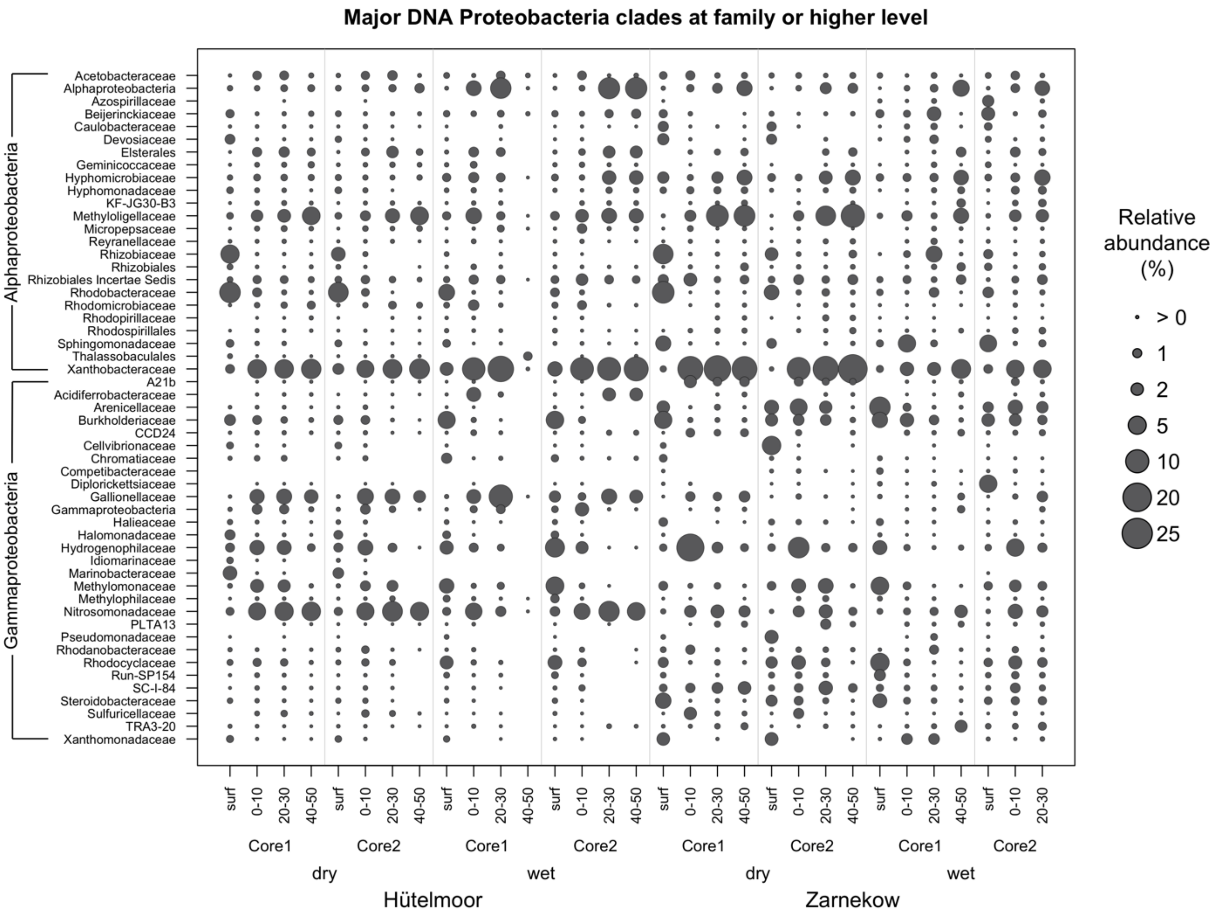


Figure S2. Bubble plots showing relative abundance of major bacterial families during the 2018 summer drought based on 16s rRNA gene sequencing. Samples are arranged along the x-axis according to site (HM and ZN for the Hütelmoor and Zarnekow, respectively) and depth section in cm (surf = surface samples) given as a range. The Abbreviations DU and WU denote samples from dry unvegetated and wet unvegetated subsites, respectively. Taxa were placed at the next possible higher taxonomic level if assignment to the family level was not possible.

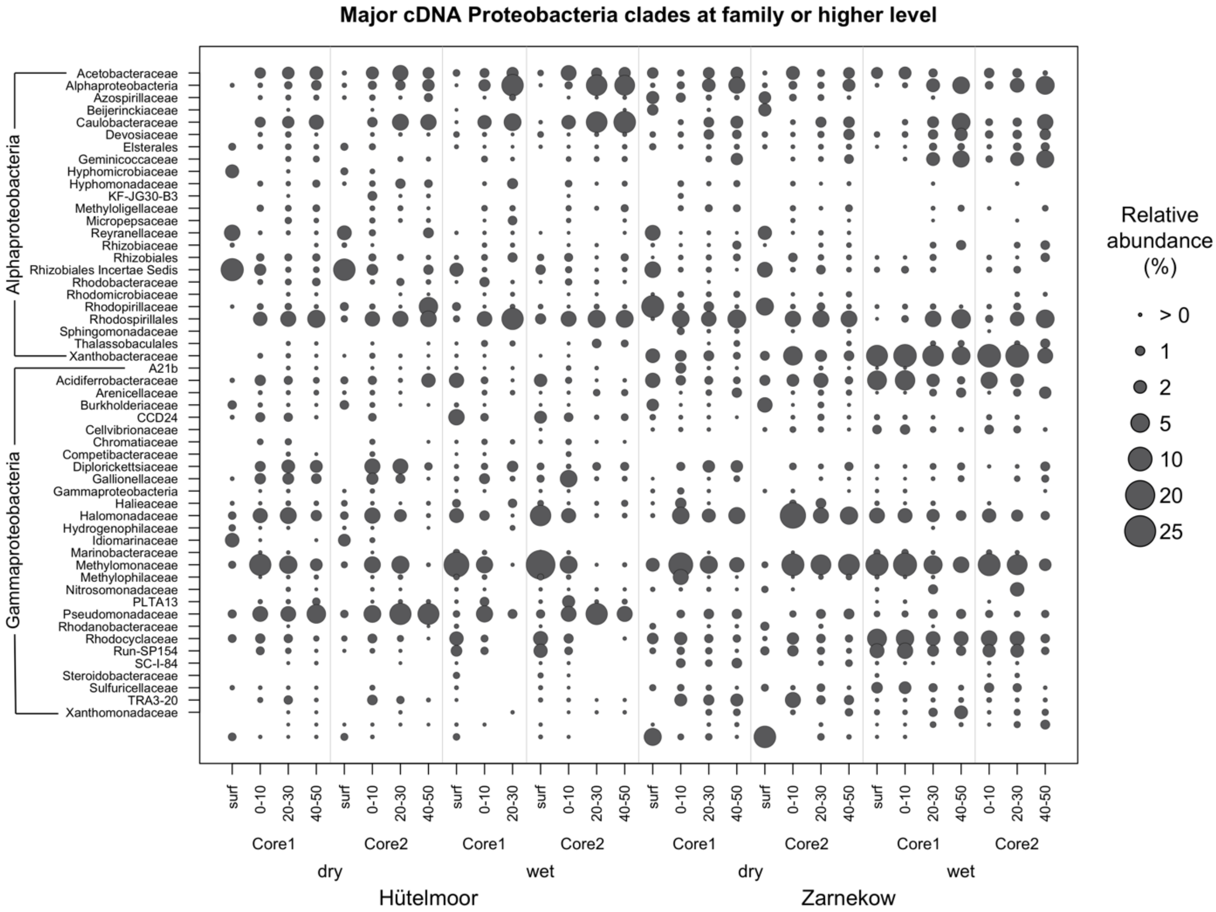


Figure S3. Bubble plots showing relative abundance of major active bacterial families during the 2018 summer drought based on cDNA sequencing. Samples are arranged along the x-axis according to site (HM and ZN for the Hütelmoor and Zarnekow, respectively) and depth section in cm (surf = surface samples) given as a range. The Abbreviations DU and WU denote samples from dry unvegetated and wet unvegetated subsites, respectively. Taxa were placed at the next possible higher taxonomic level if assignment to the family level was not possible.

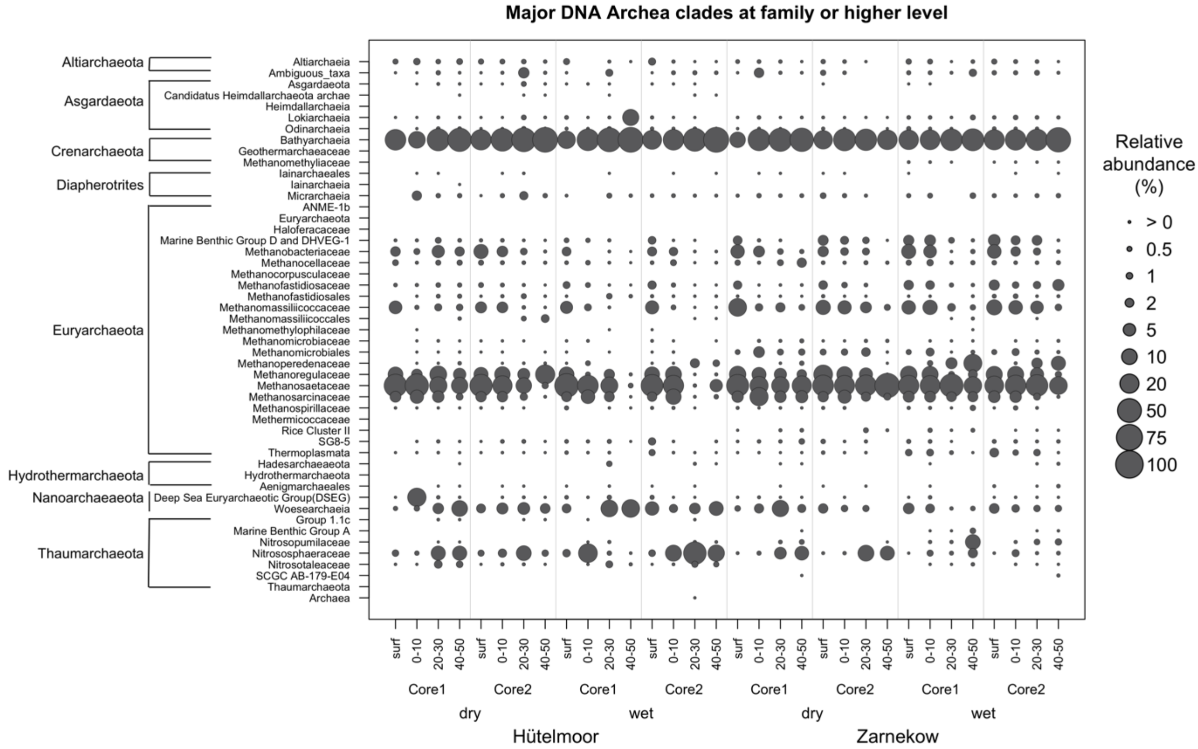


Figure S4. Bubble plots showing relative abundance of major archaeal families during the 2018 summer drought based on 16s rRNA gene sequencing. Samples are arranged along the x-axis according to site (HM and ZN for the Hütelmoor and Zarnekow, respectively) and depth section in cm (surf = surface samples) given as a range. The Abbreviations DU and WU denote samples from dry unvegetated and wet unvegetated subsites, respectively. Taxa were placed at the next possible higher taxonomic level if assignment to the family level was not possible.

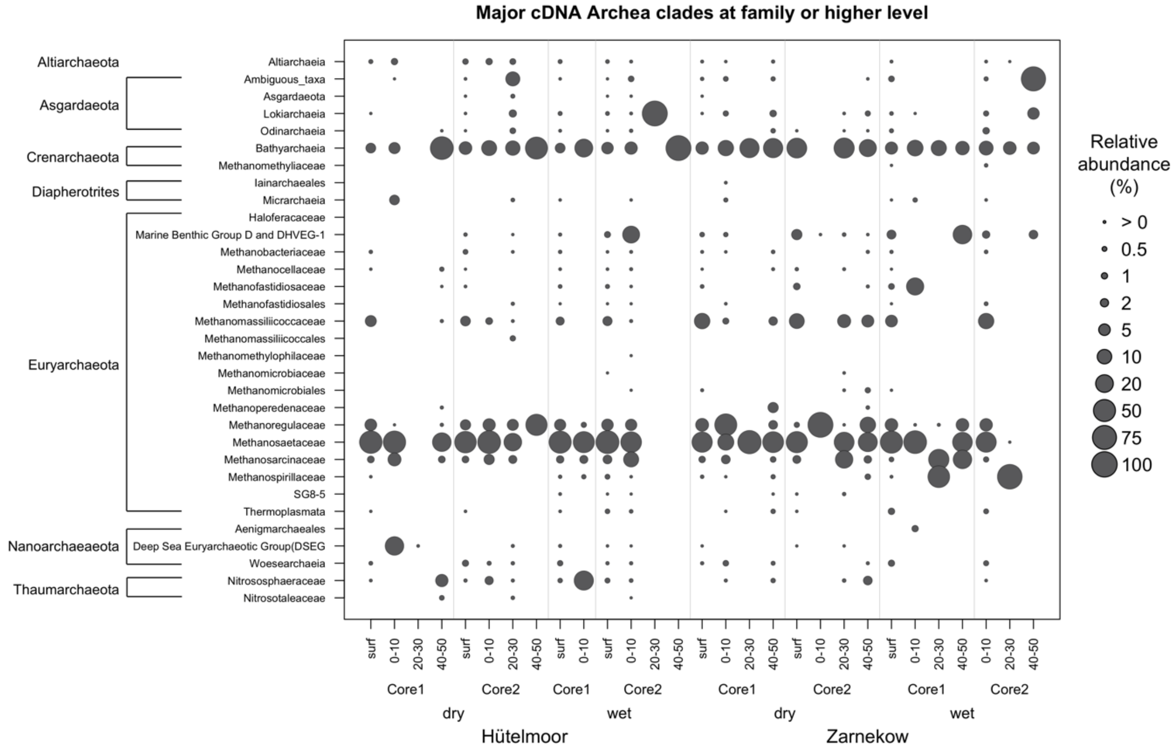


Figure S5. Bubble plots showing relative abundance of major active archaeal families during the 2018 summer drought based on cDNA sequencing. Samples are arranged along the x-axis according to site (HM and ZN for the Hütelmoor and Zarnekow, respectively) and depth section in cm given as a range (surf = surface samples). The Abbreviations DU and WU denote samples from dry unvegetated and wet unvegetated subsites, respectively. Taxa were placed at the next possible higher taxonomic level if assignment to the family level was not possible.

Table S1. Sums, means, and maximums in CH_4 emissions for the years 2015–2017 (non-drought years) and 2018 (a drought year) in the Hütelmoor, a coastal fen in northeastern Germany. Values were calculated for three distinct: the entire year, March–November, and September–December (when the drought effects were most prominent).

Year	Time period	Sum ($g\ CH_4\ m^{-2}\ year^{-1}$)	Mean ($g\ CH_4\ m^{-2}\ day^{-1}$)	Max ($g\ CH_4\ m^{-2}\ day^{-1}$)
2015	entire year	67.3	0.18	0.74
2016	entire year	75.3	0.21	0.77
2017	entire year	60.9	0.17	0.52
2018	entire year	53.6	0.15	0.90
2015	March–November	62.9	0.23	0.74
2016	March–November	71.0	0.26	0.77
2017	March–November	58.5	0.21	0.52
2018	March–November	50.0	0.18	0.90
2015	September–December	16.8	0.13	0.47
2016	September–December	12.5	0.1	0.40
2017	September–December	15.0	0.12	0.36
2018	September–December	4.0	0.03	0.19

Table S2. Sums, means, and maximums in CH_4 emissions for the years 2015–2017 (non-drought years) and 2018 (a drought year) in Zarnekow, a riparian fen in northeastern Germany. Values were calculated for three distinct time periods: the entire year, March–November, and September–December (when the drought effects were most prominent).

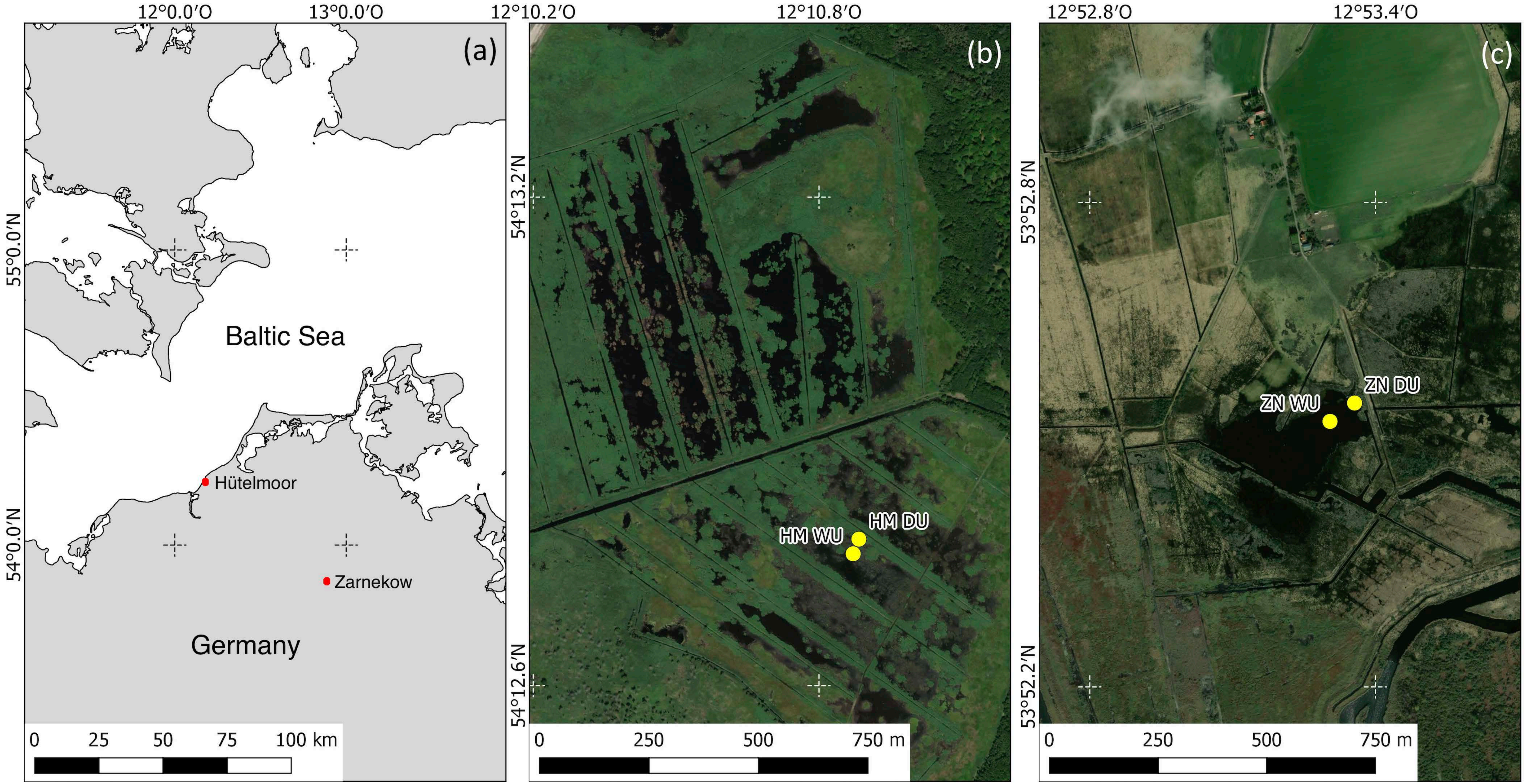
Year	Time period	Sum ($g\ CH_4\ m^{-2}\ year^{-1}$)	Mean ($g\ CH_4\ m^{-2}\ day^{-1}$)	Max ($g\ CH_4\ m^{-2}\ day^{-1}$)
2015	entire year	39.7	0.11	0.47
2016	entire year	44	0.12	0.62
2017	entire year	40.3	0.11	0.66
2018	entire year	41.4	0.11	0.86
2015	March–November	38.6	0.14	0.47
2016	March–November	43.1	0.15	0.62
2017	March–November	39.5	0.14	0.66
2018	March–November	40.2	0.14	0.86
2015	September–December	5.8	0.04	0.36
2016	September–December	4.2	0.03	0.26
2017	September–December	4.4	0.03	0.14
2018	September–December	2.4	0.02	0.09

Table S3. qPCR-determined total microbial (16S), methanogen (*mcrA*), and aerobic methanotroph (*pmoA*) gene copy numbers for the Hütelmoor, a coastal fen in northeastern Germany. *p*-values lower than 0.05 % indicate significant differences in microbial abundances among the non-drought and drought years.

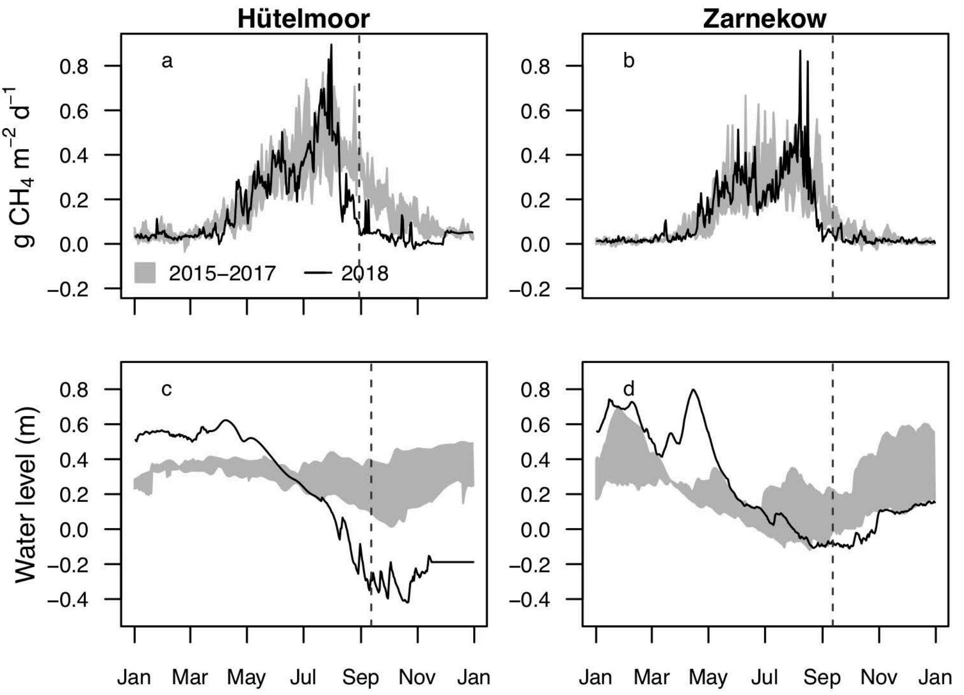
Hütelmoor	Non-drought year	Drought year	*p*-value
16S (copies g dry peat^{-1})	4.04×10^{10}	2.14×10^{10}	0.68
mcrA (copies g dry peat^{-1})	1.16×10^{9}	5.05×10^{8}	0.39
pmoA (copies g dry peat^{-1})	3.47×10^{7}	4.11×10^{7}	0.02

Table S4. qPCR-determined total microbial (16S), methanogen (*mcrA*), and aerobic methanotroph (*pmoA*) gene copy numbers for Zarnekow, a riparian fen in northeastern Germany. *p*-values lower than 0.05 % indicate significant differences in microbial abundances among the non-drought and drought years.

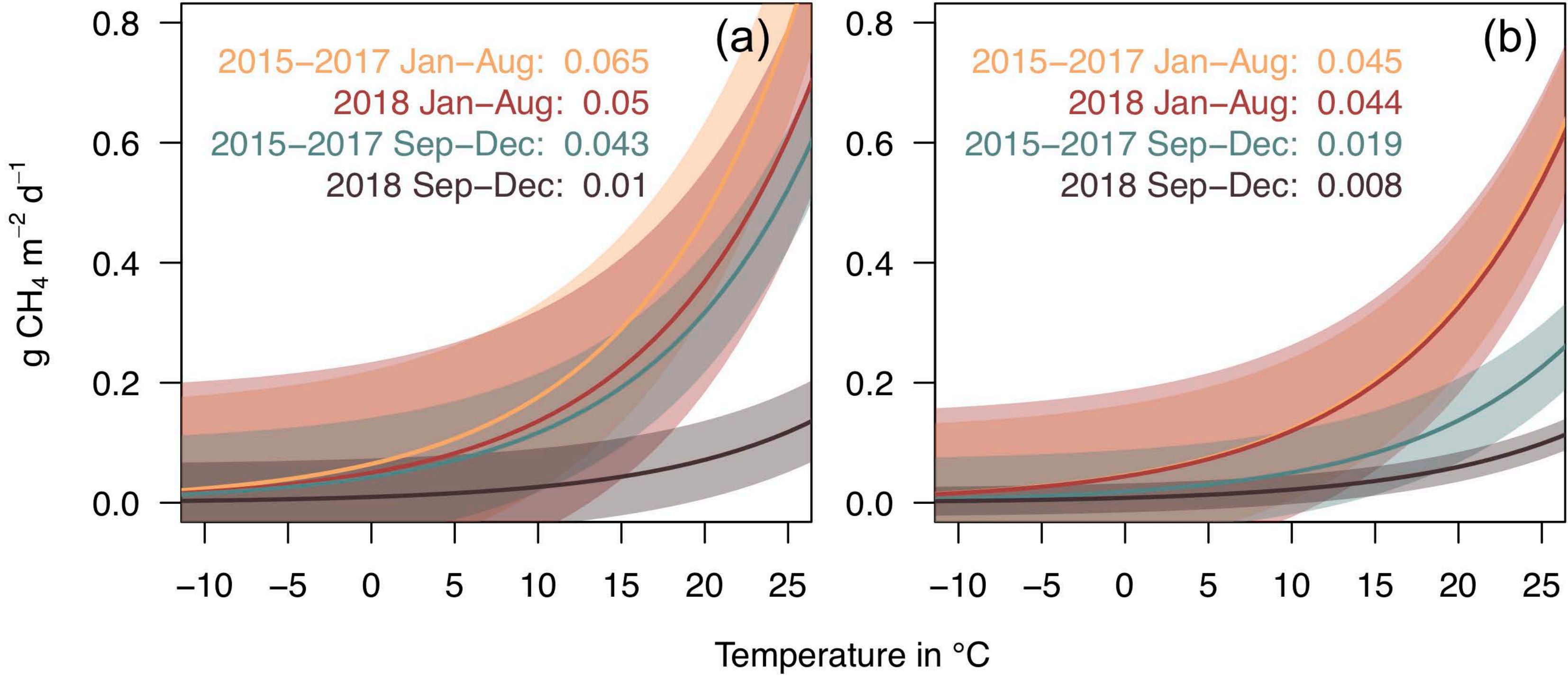
Zarnekow	Non-drought year	Drought year	*p*-value
16S (copies g dry peat^{-1})	6.23×10^{10}	6.54×10^{10}	0.03
mcrA (copies g dry peat^{-1})	8.82×10^{8}	9.94×10^{7}	9.8×10^{-5}
pmoA (copies g dry peat^{-1})	4.36×10^{7}	1.39×10^{7}	0.15



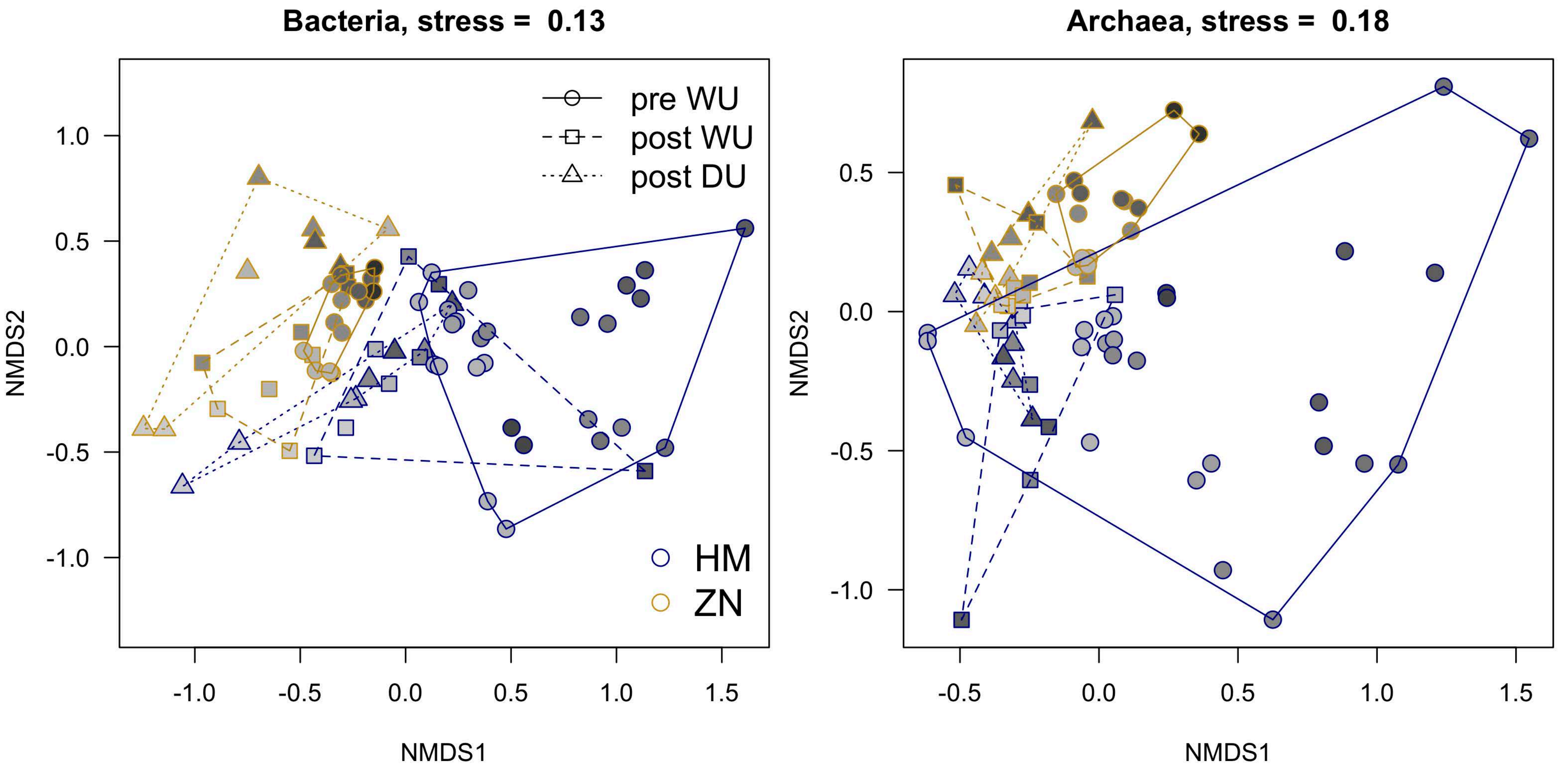
12°0.0′O
13°0.0′O
12°10.2′O
12°10.8′O
12°52.8′O
12°53.4′O
(a)
(b)
(c)
55°0.0′N
54°0.0′N
54°13.2′N
54°12.6′N
53°52.8′N
53°52.2′N
Baltic Sea
Hütelmoor
Zarnekow
Germany
HM WU
HM DU
ZN WU
ZN DU
0
25
50
75
100 km
0
250
500
750 m
0
250
500
750 m



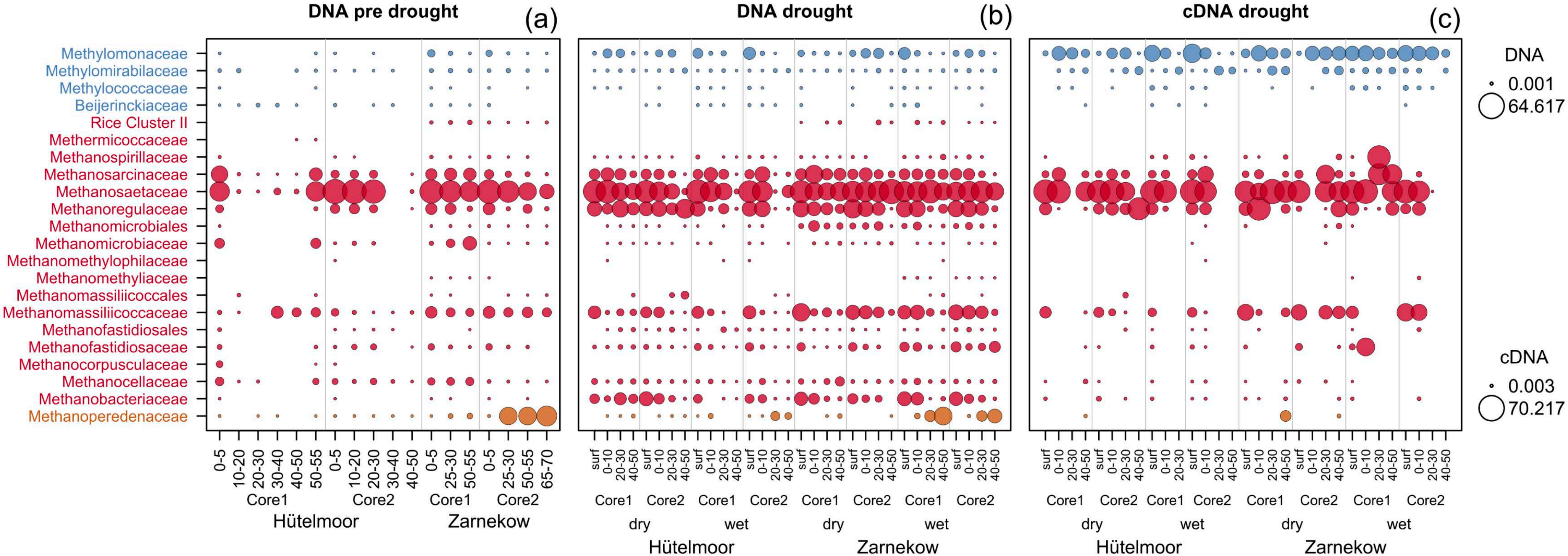
Hütelmoor
Zarnekow
a
b
c
d
g CH_4 m^{-2} d^{-1}
Water level (m)
2015−2017
2018
0.8
0.6
0.4
0.2
0.0
−0.2
−0.4
Jan
Mar
May
Jul
Sep
Nov
Jan



(a)
2015–2017 Jan–Aug: 0.065
2018 Jan–Aug: 0.05
2015–2017 Sep–Dec: 0.043
2018 Sep–Dec: 0.01
(b)
2015–2017 Jan–Aug: 0.045
2018 Jan–Aug: 0.044
2015–2017 Sep–Dec: 0.019
2018 Sep–Dec: 0.008
0.8
0.6
0.4
0.2
0.0
−10
−5
0
5
10
15
20
25
g CH4 m−2 d−1
Temperature in °C



Bacteria, stress = 0.13
Archaea, stress = 0.18
pre WU
post WU
post DU
HM
ZN
NMDS1
NMDS2
1.0
0.5
0.0
-0.5
-1.0
1.5



DNA pre drought
(a)
DNA drought
(b)
cDNA drought
(c)
Methylomonaceae
Methylomirabilaceae
Methylococcaceae
Beijerinckiaceae
Rice Cluster II
Methermicoccaceae
Methanospirillaceae
Methanosarcinaceae
Methanosaetaceae
Methanoregulaceae
Methanomicrobiales
Methanomicrobiaceae
Methanomethylophilaceae
Methanomethyliaceae
Methanomassiliicoccales
Methanomassiliicoccaceae
Methanofastidiosales
Methanofastidiosaceae
Methanocorpusculaceae
Methanocellaceae
Methanobacteriaceae
Methanoperedenaceae
0-5 10-20 20-30 30-40 40-50 50-55 0-5 10-20 20-30 30-40 40-50 0-5 25-30 50-55 0-5 25-30 50-55 65-70
Core1 Core2 Core1 Core2
Hütelmoor Zarnekow
surf 0-10 20-30 40-50
Core1 Core2 Core1 Core2 Core1 Core2 Core1 Core2
dry wet dry wet
Hütelmoor Zarnekow
DNA
0.001
64.617
cDNA
0.003
70.217