Dear editor,

We thank you and the reviewers for your helpful comments and the constructive suggestions which have resulted in a much-improved manuscript overall. Below you can find our full response to each point raised (section A), the list of relevant changes made (section B), as well as the marked-up version of the revised manuscript (section C). We hope that with our edits we successfully addressed each point raised by the reviewers. Please be aware that the specific line numbers we mention in our responses to the reviewers refer to the final, non-marked up manuscript.

All the best
Viktoria Unger

A) Point by point response to reviewers

Response to Anonymous Referee #1

Review of the Biogeosciences Discuss. Paper ”Predominance of methanogens over methanotrophs contributes to high methane emissions in rewetted fens by Wen et al. The authors present high throughput sequencing and qPCR data of microbial communities of two rewetted fens in northern Germany. Next to the microbial analyses the pore water chemistry, dissolved methane and the isotopic signal of the methane C was analyzed. The paper is well written but hampers in the experimental design and some missing analyses. First of all there are no datasets or samples available which connect the rewetting treatment to a control or a pre-disturbance measurement. With pre-disturbance we can argue the existence of the drained fen performance or even the performance of the fen before drainage. So to what can the results be compared? I thought that the lateral scanning of the fens by different sampling points could explain this but actually the data is not discussed in this sense. At least at Huettelmoor the gradient goes away from the dam.

We agree, it would indeed be nice, to have pre-rewetting data. However, there were no pre-rewetting peat samples available to compare our microbial data to. For this reason, we performed an extensive literature search comparing the published geochemical and microbial characteristics of drained versus rewetted fens to the fens in this study, and we

are confident that is a valid approach for discussing post-rewetting conditions. Having pre-rewetting data to compare to is, unfortunately, very rare for temperate, restored fens; thus, we rather discuss the post-rewetting conditions of the two fens and highlight differences among drained versus rewetted fens using information that is published and available. In our discussion, in lines 444-447, we now highlight that this was not a study of rewetting effects, but rather a characterization of post-rewetting conditions with conclusions drawn from in-depth literature analysis.

The data is also not discussed to the methane fluxes of the different sampling points. For Huettelmoor they exist because there have been chamber measurements which should match quite close to the H1-4 cores if they are not exactly at the same spot. I also wonder why no potential activity measurement was performed to assess the activity of methane production and oxidation. Can this still be performed because it would give much information which is not told by the community analyses of gene copy numbers.

You are correct our approach does not allow a detailed consideration of different sampling plots. However, our aim was not to present plot-scale interpretations but rather discuss the ongoing high methane fluxes on a larger scale. The location of available chamber measurements do not match the locations for the analysis presented here, thus we consider emissions on an ecosystem- rather than plot-level scale.
For this study we did not perform incubations to determine rates of production or oxidation. Methane production is indeed high for both fens and this can also be inferred from the persistently high dissolved methane concentrations we present in this study and from unpublished earlier anaerobic incubations. In more detail, incubations were performed with Zarnekow peat and these data have been added to the manuscript as supplemental information. We have added an additional author (Paul Bodelier) as he has provided us with the incubation data. The data show that besides methane production the potential for efficient methane oxidation also exists. Incubations provide ideal conditions for the organisms, and thus overestimate actual in situ methane oxidation. Specifically, in methane oxidation incubations excess of oxygen is available for methanotrophs which opposes in situ conditions in both fens where methane oxidation is overprinted by other processes.

Unfortunately, no methane oxidation incubation data are available for the Hütelmoor because earlier attempts to measure methane oxidation in this site have failed.

Next I miss in the qPCR approach the measurement for Archaea. Why has this not been measured.

In our study we were seeking for microbial controls for ongoing high emissions of methane in the two studied fens and consequently sought the ratio between methanotrophs and methanogens using qPCR. We further wanted to assess the relative contribution of both groups with regard to total bacteria and archaea and therefore performed deep sequencing using the Illumina platform. With this we could already answer our initial question. Seeking a final proof for our qPCR analysis we also quantified total bacteria with qPCR. The ratio of methanotrophs to total bacteria based on qPCR is very much in line with the sequencing results supporting the robustness of our qPCR assays. The quantification of total archaea using qPCR was thus not necessary for answering our initial questions. Finally, as the reviewer may be aware of, primer- or probe-based quantifications of total archaea targeting their 16S rRNA gene is often hampered through co-amplification of bacteria given the large sequence similarities. In summary, we refrained from qPCR data for the archaeal community since it does not add to the presentation of our major finding(s).

In the MM section the authors should tell which depths have been sampled at each site. I can see the depths in the Figs BUT they need to be told in the MM.

As suggested the sampling depths were added to the text in the materials and methods section in lines 159-163.

You have also to discuss in the Ms why the depth sampling was so different between fens and within the Huettelmoor fen. Probably the fens were never mentioned to be published together otherwise the sampling would be convergent.

As suggested, we have altered the text in lines 163-165 and 176-178 to explain why the sampling and depth resolution was different between the fens. We'd like to emphasize that

the data were indeed collected with comparison of the two fens in mind. The reason for the difference in sampling depth is that previous studies from Zarnekow show that the peat stratigraphy is much less variable than the stratigraphy at the Hütelmoor. Difference in porewater sampling methods was due to accessibility and sampling difficulty: the permanent porewater dialysis samplers could not be installed at the sampling locations in the Hütelmoor.

In the MM section I miss the sample n AND I want to point out that you have not replicated your study design. In my opinion this is a harsh critique. Taking two within replicates for DNA extraction is not the same you should have two to three adjacent lines.

You are correct, sample n should be given. We have added the sample n to our revised manuscript in the materials and methods section in lines 157-158. With this study, our aim was not to argue for differences between the sampling points within the fens, but to seek differences and similarities among both fens. In this regard, we have four (n=4) and five replicates (n=5), respectively.

In the Intro and Discussion it is stressed that elevated methane emissions after rewetting is dangerous. I doubt that. First the dried peatland lost a lot of CO2 due to peat degradation and the onset of methane emission after restoration is a hint that peat formation starts to accelerate again and this process fixes more C than it loses. There is scientific literature around this and you may bring this into your discussion.

We did not intend to state that methane emissions after rewetting are "dangerous" but in the flooded peatlands we know they are elevated and also for a quite substantial duration. It was generally assumed in rewetting projects that peat methane production returns to near neutral levels within several years but flooded hypertrophic fens might behave differently. This question puzzled us for quite some time, now but the data we present here may solve a part of that puzzle in that one reason could be the disproportionately low abundance of methanotrophs compared to other microbes. We have adjusted the text in the introduction in lines 53-57 and in the conclusion in lines 505-507 so it does not imply that methane emissions are solely a negative phenomenon.

In the MM I do not see if the rewetted Huettelmoor water table is 0.6 m above or below peat surface (line 126).

As suggested, the text was adjusted in line 135 to indicate that water level was 0.6 m above the peat surface.

In the Results of the MM statistical chapter I miss information of have many sequences were retrieved. How many OTUs were obtained and the bubble data is generated on and how many observations.

For archaea, a total of 6844177 valid sequences were obtained, ranging from 60496 to 398660 in individual samples. These sequences were classified into 402 OTUs. Then the OTU table was collapsed at higher taxonomic level to generate the bubble plot. For bacteria, a total of 2586148 valid sequences were obtained, ranging from 22826 to 164916 in individual samples. These sequences were classified into 843 OTUs. Then the OTU table was collapsed at higher taxonomic level to generate the bubble plot. This information was added to the materials and methods section in lines 244-249.

In lines 201-202 is something I do not understand. Three PCR products of the same sample were combined. OK but why. But the next sentence says PCR products of different samples were pooled...???

The samples were pooled to reduce amplification bias. We adjusted the text in lines 221-223 so that it is clear why the samples were pooled.

On lines 273-276 give the percentage of Methanotrophs out of the total. You tell them in the discussion. This so, because you present for Methanogens this data on line 280.

As suggested, in our revised manuscript we added the specific methanotroph abundances to the results section in lines 322-324.

Looking at the Figs you have no real depth separation in your measured variables at Zarnekow. WHY?

As detailed above, we have provided an explanation for the different sampling depths and lower depth resolution in Zarnekow in the methods section. (163-165 and 176-178)

For the end; line 78-80 states wrong: there are more publications to the theme Reumer et al. 2018. Impact of peat mining, and restoration on methane turnover potentials and methane-cycling microorganisms in a northern bog. Applied and Environmental Microbiology 84, 3 e02218-17. https://doi.org/10.1128/AEM.02218-17. Putkinen et al. 2018. Recovery of methane turnover and the associated microbial communities in restored cut-away peatlands is strongly linked with increasing Sphagnum abundance. Soil Biology & Biochemistry 116: 110-119.

Thank you for suggested citations. We have adjusted the text accordingly and added the reference there and throughout the manuscript.

Response to Anonymous Referee #2

The study of Wen et al on the "Predominance of methanogens .... in rewetted fans" is very well written and presented in a clear way. In this study the abundance and community structure of methanogenic and methanotrophic microorganisms in two rewetted fans is related to geochemical parameters. However, the study has in my opinion to major drawbacks: In the title and within the text the authors refer to methane emissions of the two fens, however no data on methane emission are presented. With so many authors involved there certainly should be data on this important factor?? The relation / explanation how the environmental parameters influence the abundance or community structure of the methane related organisms is not convincing; it seems to be rather biased. I know it is not easy to explain microbial patterns with geochemical ones, but I would suggest a more serious statistic here.

With regards to the first drawback mentioned by the reviewer, we agree that the paper would profit from including actual methane emission data. To this end, we have added the

most recent published values for average methane flux rates for both fens to the revised manuscript to lines 104-108.

With regards to the second major drawback mentioned by the reviewer, we believe 2-dimensional non-metric multidimensional scaling (NMDS) is a robust statistical method. The environmental fit to the NMDS is a statistical approach based on a Monte Carlo permutation that shows which variables are significantly related to the community structure of the microorganisms. For this reason, we feel that additional statistics are not necessary to support our overall conclusions based on the NMDS. We performed correlation analyses on methanotroph abundance versus oxygen and dissolved methane concentrations, however the relationships were not significant. Nevertheless, we failed to mention this in the original manuscript and have adjusted the text in lines 364-365.

Line 201 "PCR products of three individual runs per sample were combined." – why this?

The PCR products were combined to reduce amplification bias. A short phrase was added to the manuscript to make this clear to the reader in line 221.

Line 292 "I suggest to start the results section with the geochemical description of the study site"

As suggested, we have changed the order of the results sections and now describe the site geochemistry before the microbial data.

Line 295 "as you refer later in the discussion to salinity, it would be nice to have these values converted to PSU, for comparison with other studies"

We agree that it would be better to have salinity values for comparability. However, for brackish waters the calculation is unreliable as salinity in low-salt waters is not well-defined. This is an issue that is unresolved among hydrogeologists and chemical oceanographers alike, thus conversions from EC to psu are generally not performed for brackish systems. To

retain the integrity of our results, we instead provide a widely-used reference (Schemel 2001) for the readers in line 183 for those wishing to make the conversion.

Line 322-323 “I do not think that "depth" is a suitable parameter here. It should be seen as envelopping parameter which is characterized in itself by NO3, SO4, O2 ...Also it makes figure 6 rather confusing. Maybe you could try to do the analysis without "depth", by pooling all the data? also, the parameter "site" could be omitted....

We did not want to include depth as a parameter but rather as a proxy for other parameters. We think including depth is important as it may stand for a proxy for other parameters which were not measured in this study. We further believe that site is an important parameter here as comparison of the two fens is a main point of the paper. The inclusion of site in the NMDS reinforces our findings that both geochemistry and microbial community composition were much more variable in the Hütelmoor than in Zarnekow.

Line 347 “where does the emission data come from? Are there any data available??”

Data on methane exchange was recorded in both fens by us and other colleagues with chambers and eddy covariance in the past and still today. Since we have no measurements that are directly associated with the core samplings and the porewater sampling used here, we first decided to go without CH4 exchange data. As two reviewers have criticized this point, we have updated the manuscript to include the most recent publish values for methane fluxes from the two fens in lines 104-108.

Line 362 “thus CH4 concentration had no influence?? At least for the methanotrophs this should be an important factor. If not, this should at least be stated so”

You are right, a correlation between methanotrophs and CH4 concentrations is an important factor. According to our analyses CH4 concentrations did not correlate with methanotroph abundance nor with the abundance of other microbes. Because many studies have found it to be an important factor influencing methanotroph populations, we should have

nevertheless mentioned that we found no correlation in our study. Therefore, we now mention this in lines 364-365.

Line 365 “for comparison it would be nice to have EC converted to salinity”

We agree that it would be better to have salinity values for comparability. However, for brackish waters the calculation is unreliable as salinity in low-salt waters is not well-defined. This is an issue that is unresolved among hydrogeologists and chemical oceanographers alike, thus conversions from EC to psu are generally not performed for brackish systems. We would therefore suggest that we present our original EC data, which is more scientifically sound, but provide the information needed for conversion from EC to psu for the reader (in the methods section).

Line 380 “I do not see any significant shifts in the figure, but only a scatter of data ....”

The sentence was adjusted in lines 408-411 to help the reader follow the specifically mentioned shift in the isotopic data of HC 1. The delta signature of HC 1 (open circles) shifts from ~-65 to ~60 which could be the result of oxidation processes.

Line 401 “however, it is not clear to me, why the abundance of methanotrophs is so low. Shifting O2 regimes should be no problem, as this is often the case in other environments, tidal sediments...”

The argument for the low abundance of methanotrophs was strengthened in the revised manuscript. Specifically, in lines 461-477 we suggest that competition for oxygen with heterotrophic organisms rather than fluctuations in oxygen are likely a reason for the low abundance of methanotrophs. In fact, our data support this as our bubble plot for bacteria shows hyphomicrobiaceae dominated the bacterial community, a family of which the large majority are aerobic heterotrophs.

Line 402 “The heading is not suitable here, as you only discuss the low abundance of methantrophs here. Data on methane emissions would be helpful here....”

The authors agree that the headline was not suitable. We thus changed the headline in line 434 to better represent the section – "Low methanotroph abundances in rewetted fens"

Line 423 "but these disturbances in O2 regime would be also inhibitory for the methanogens on the other side...."

Though recent studies show that methanosaeta, which was the most abundant methanogen in this study, thrive even in oxic layers, it is also likely additional factors are affecting the methanotroph populations that were not thoroughly discussed. In our revised manuscript we expand on the discussion regarding the absence of methanotrophs. Specifically, we suggest in lines 461-477 that competition by heterotrophs which also utilize oxygen may ultimately be preventing methanotroph establishment.

Line 428 "what about methane availability??"

Substrate (i.e. methane) availability has indeed been shown to correlate with methanotroph populations. We have added this to our revised manuscript in lines 364-365 as previously mentioned. As mentioned above, in our study methanotroph abundance and methane concentrations did not correlate, though. Also, methane concentrations in the pore water were high throughout all sites so the availability of methane is unlikely to constrain methanotroph abundance in the two rewetted fens of our study.

Response to Anonymous Referee #3

Wen et al. address microbial controls of high methane emission after re-wetting in two temperate peatlands with contrasting geochemistry. There is very little information available on microbiology of re-wetted peatlands, so as the first study of re-wetted non-acidic fens, this study is very welcome. The manuscript is clearly written and easy to follow. The molecular analyses for microbes have been carried out with care (testing for sample inhibition in qPCR, pooling three different PCR products to reduce amplification bias, checking the taxonomic affiliations of OTUs in ARB). This is not a study of rewetting effects, because no samples from before re-wetting or from a non- rewetted control site are available. However, in addition to

providing much needed information on re-wetted peatlands, the results contain some interesting details such as the strikingly patchy distribution of ANME-2d.

My biggest concern is that the main result is based on comparison of two different qPCR assays (mcrA vs. pmoA). Such a direct comparison of values assumes nearly absolute quantification, which is not realistic for environmental samples (different limitations in coverage for each primer pair etc). Comparisons of values of one assay between samples, on the other hand, do not rely on this assumption in the same way. The previous examples of pristine wetlands used as support (l. 413-421, 450-452) similarly rely on comparisons of two different qPCR assays. If/when these studies have used different methods and primers as this study, the comparisons become even more problematic, even when made at the broad level of orders of magnitude. I do not disagree with the overall conclusion that high numbers of methanogens the most likely reason for the high methane fluxes, but I would strongly recommend addressing this limitation in the discussion and modifying the text on l. 404-421 and elsewhere, including the title of the manuscript. Maybe strengthening the interpretation of microbial community results in relation to geochemistry could provide an alternative main message.

Please find our reply to these concerns below.

In addition, I am wondering about the role of methanotrophs in completely inundated peat and in the water layer. It is very much expected that methanotrophic activity would be low considering that in both sites the sampled peat was inundated. The optimal peat layer for methanotrophs where both methane and oxygen are readily available is largely missing (which the authors do address in the end of the manuscript). However, such conditions could be present in the water layer. I realise the water layer is out of the scope of this study, but are there reasons to exclude it from discussion or assume it plays no role in methane oxidation?

It is indeed possible that oxidation may be occurring in the water column. It is true, however, that the water column was beyond the scope of this manuscript. Recent, preliminary data for Zarnekow show methanotrophs in high abundance associated with ceratophyllum in the water column (unpublished data, still in progress). Nevertheless, even if oxidation is occurring in the water column in these two sites it is clearly not significant enough to keep

methane concentrations and emissions low as demonstrated by the flux data (added) in the revised manuscript. This is now mentioned in the discussion in lines 477-480.

Minor comments:

1. l. 190-193 Did the primers contain sequencing adapters and barcodes or were they added later?

Yes, the primers contained barcodes. A phrase was added to the manuscript in line 213 to denote that the primers contained barcodes in the 5'-end.

2. l. 234-235 Please remove the word 'all' from 'suitable for detecting all aerobic methanotrophic Proteobacteria' or change to 'all known' or similar (we cannot assume to be able to detect the full diversity).

As suggested we have changed the text in line 260 to instead say "all known aerobic methanotrophic Proteobacteria".

3. l. 318-319, l. 360-361 The Hütelmoor samples show higher within-site variation, but the samples were also taken much further apart from each other. Could this not explain the larger variation? On l. 360-361, the sentence could be understood to suggest the difference is due to brackish vs. freshwater.

Though in the study the Zarnekow samples were taken closer together, we know from previous work at the site that there is indeed less variation across the Zarnekow peatland (e.g. Zak and Gelbrecht 2007). Thus, taking the cores further apart in Zarnekow in this study would not have resulted in greater variation in our measured variable.

4. l. 360 Please change 'significant' to another word because no statistical testing was carried out for differences of community composition.

The phrase 'significant variation' was changed to 'large variation' in line 388 in the revised version of the manuscript.

5. l. 415 I do not think it is possible to compare PCR-based relative abundances between different studies, unless the studies used completely identical methods and equipment. Was this the case with Liebner et al. 2015?

It is an inevitable limitation that the methods and equipment of different studies are not completely identical. This is not only a limitation of our work but a general issue for meta-studies. All comparisons regarding bacterial, methanotrophic and methanogenic abundance are based on universal primer combinations of the respective groups. The primers we used for the bacterial 16S rRNA and mcrA genes of this study are identical with the primers used in Liebner et al. 2015. With regards to pmoA, both studies used universal primer combinations including identical forward primers, but as a result of initial testing different reverse primers. Further, the same qPCR technology was used. In addition, we compared the ratio of methanogenic to methanotrophic abundances and the fraction of methanotrophs in relation to the total bacterial community based on two independent methods, namely qPCR and sequencing, instead of the direct methanogenic or methanotrophic abundances. This kind of 'normalization' mitigates the bias of different experiments and makes the results more reasonable and reliable. As suggested, we now discuss this potential limitation in our revised manuscript in lines 454-460.
Further, we have revised the title of our manuscript. Our revised manuscript title is as follows, "Predominance of methanogens over methanotrophs in rewetted fens characterized by high methane emissions".

B) List of relevant changes made in the manuscript

- In our discussion, in lines 444-447, we now highlight that this was not a study of rewetting effects, but rather a characterization of post-rewetting conditions with conclusions drawn from in-depth literature analysis
- The sampling depths were added to the text in the materials and methods section in lines 159-163
- The text was altered in lines 163-165 and 176-178 to explain why the sampling and depth resolution was different between the fens

- The sample n was added to our revised manuscript in the materials and methods section in lines 157-158

- The text was adjusted in the introduction in lines 53-57 and in the conclusion in lines 505-507 so it does not imply that methane emissions are solely a negative phenomenon.

- The text was adjusted in line 135 to indicate that water level was 0.6 m above the peat surface

-Details on the numbers of archaeal and bacterial sequences obtained were added to the materials and methods section in lines 244-249

-The text in lines 221-223 was adjusted so that it is clear why the samples were pooled

-The specific methanotroph abundances were added to the results section in lines 322-324

-An explanation was provided for the different sampling depths and lower depth resolution in Zarnekow in the methods section (163-165 and 176-178)

-Putkinen et al. 2018 was added as an additional reference for rewetted fens

-The most recent published values for methane emissions in the two fens was added to the manuscript in lines 104-108

-The order of the results section was changed: Geochemical data are now presented first, before the microbial data

-A reference was added to line 183 that contains a simplified converstion equation for EC to psu for readers who would like to make the conversion for comparability with other studies

-Lines 364-365 now state that no correlation was found between methanotrophs and methane or oxygen concentrations

-The argument for low methanotroph abundances was strengthened in the discussion in lines 461-477

-The possibility that methane oxidation may be occurring in the water column is acknowledged in lines 477-480

-Lines 213 now states that primers contained barcodes in the 5'-end

-Line 260 now states "all known aerobic methanotrophic Proteobacteria" rather than just "all"

- The phrase 'significant variation' was changed to 'large variation' in line 388 in the revised version of the manuscript

C) Marked up revised manuscript

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

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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 rDNA high-throughput sequencing and quantitative polymerase chain reaction on 16S rDNA, *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 often 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

Deleted: The factors that contribute to the magnitude and duration of this increase are still uncertain (Joosten et al. 2015, Abdalla et al. 2016). On a 100-year time scale CH_4 has a global warming potential 28 times stronger than CO_2 (Myhre et al. 2013); thus, increased CH_4 emissions could potentially offset the benefit of decreased CO_2 emissions (Jurasinski et al. 2016).

in rewetted fens has seldom been examined (but see Juottonen et al. 2012, Urbanová et al. 2013, Putkinen et al. 2018).

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

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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. Therefore, in this study, we 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 annual 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.

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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 of the fens were CH_4 sinks (Augustin et al. 1998).

2.2 Collection 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 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).

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

234 deoxynucleoside (Thermo Fisher Scientific, Darmstadt, Germany) and 0.025 U μl^{-1} hot start
235 polymerase (QIAGEN, Hilden, Germany). PCR samples were kept at 95 °C for 5 min to denature
236 the DNA, with amplification proceeding for 40 cycles at 95 °C for 1 min, 56 °C for 45 s and 72
237 °C for 90 s; a final extension of 10 min at 72 °C was added to ensure complete amplification. PCR
238 products were purified with a Hi Yield Gel/PCR DNA fragment extraction kit (Süd-Laborbedarf,
239 Gauting, Germany). To reduce amplification bias, PCR products of three individual runs per
240 sample were combined. PCR products of different samples were pooled in equimolar
241 concentrations and compressed to a final volume of 10 µl with a concentration of 200 ng μl^{-1} in a
242 vacuum centrifuge Concentrator Plus (Eppendorf, Hamburg, Germany).
243 Illumina sequencing was performed by GATC Biotech AG using 300 bp paired-end mode and a
244 20% PhiX Control v3 library to counteract the effects of low-diversity sequence libraries. Raw
245 data was demultiplexed using an own script based on CutAdapt (Martin 2011). Ambiguous
246 nucleotides at sequence ends were trimmed and a 10% mismatch was allowed for primer
247 identification, whereas barcode sequences needed to be present without any mismatches and with
248 a minimum Phred-Score of Q25 for each nucleotide. After sorting, overlapping paired-end reads
249 were merged using PEAR [Q25, p 0.0001, v20] (Zhang et al. 2014). The orientation of the merged
250 sequences was standardized according to the barcode information obtained from demultiplexing.
251 Low-quality reads were removed using Trimmomatic [SE, LEADING Q25, TRAILING Q25,
252 SLIDINGWINDOW 5:25; MINLEN 200] (Bolger et al. 2014). Chimeric sequences were removed
253 using USEARCH 6.1 and the QIIME-script identify_chimeric_seqs.py (Caporaso et al. 2010). Pre-
254 processed sequences were taxonomically assigned to operational taxonomic units (OTUs) at a
255 nucleotide sequence identity of 97% using QIIME's pick_open_reference_otus.py script and the
256 GreenGenes database 13.05 (McDonald et al. 2012) as reference. The taxonomic assignment of
257 representative sequences was further checked for correct taxonomical classification by
258 phylogenetic tree calculations in the ARB environment referenced against the SILVA database
259 (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. We determined the ratio of methanogens to methanotrophs 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).

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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 ~0.250 mM until a depth of 15 cm at which they dropped sharply, reaching concentrations slightly below 0.050 mM at 25 cm. In Zarnekow, DO concentrations did not exceed 0.1 mM and varied little with depth. Regarding geochemical conditions, HC 1 differed from all other Hütelmoor cores and was more similar to Zarnekow cores. In HC 1 – the core taken nearest to potential freshwater sources (Fig. 1b) – pore water EC and DO concentrations were lower while pH was slightly higher than in all other Hütelmoor cores. Moreover, this was the only Hütelmoor core where nitrate concentrations were below detection limit (0.001mM) (Fig. 2). In all cores we found high concentrations of dissolved CH_4 that varied within and among fens and were slightly higher in Zarnekow pore water. Stable isotope ratios of $\partial^{13}C$-CH_4 (Fig. 2) in the upper peat (approx. −59‰) suggest a predominance of acetoclastic methanogenesis, with a shift to hydrogenotrophic methanogenesis around −65‰ in the lower peat profile. Moreover, the observed shifts toward less negative $\partial^{13}C$-CH_4 values in the upper peat layer, as in HC 1 and HC 2, could also indicate partial oxidation of CH_4 occurred (Chasar et al. 2000).

3.2 Community composition of bacteria and archaea

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Bacterial sequences could be affiliated into a total of 30 bacterial phyla (Fig. 3). Among them, Proteobacteria, Acidobacteria, Actinobacteria, Chloroflexi, Nitrospirae and Bacteroidetes were present in all samples. With mean relative abundance of 48%, Proteobacteria was the most abundant phylum. Some taxa (e.g., Verrucomicrobia, Atribacteria (OP9), and AD3) were present only in Hütelmoor. Variation in community composition was larger in Hütelmoor samples than in Zarnekow. Within Proteobacteria, the alpha subdivision was the most dominant group, having

contributed 26.7% to all the libraries on average (Fig. 4). The family *Hyphomicrobiaceae* dominated the Alphaproteobacteria, and was distributed evenly across samples, but missing in the surface and bottom peat layers in Hütelmoor core (HC) 2. In addition, methanotrophs were clearly in low abundance across all samples, representing only 0.06% and 0.05% of the bacterial community in Hütelmoor and Zarnekow, respectively. Of the few methanotrophs that were detected, type II methanotrophs (mainly *Methylocystaceae*) outcompeted type I methanotrophs (mainly *Methylococcaceae*) in the community, while members of the genus *Methylocella* were absent (Fig. 4).

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

3.3 Environmental drivers of microbial community composition

Bacterial and archaeal population at both peatland sites showed distinct clustering (Fig. 6) with similarly high intra- and inter-site variations but greater overall variation in community

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The two rewetted fens varied substantially in their environmental characteristics (e.g., proximity to the sea) and porewater geochemistry (Fig. 5, Tables 1 and 2). Electrical conductivity was more than three times higher in Hütelmoor than in Zarnekow, averaging 5.3 and 1.5 mS cm^{-1}, respectively. Mean pH was approximately neutral (6.5 to 7) in the upper peat profile and comparable in both fens until a depth of about 30 cm where pH was ~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. 5). 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. 5). Here DO concentrations averaged ~0.250 mM until a depth of 15 cm at which they dropped sharply, reaching concentrations slightly below 0.050 mM at 25 cm. In Zarnekow, DO concentrations did not exceed 0.1 mM and varied little with depth. Regarding geochemical conditions, HC 1 was distinct from all other Hütelmoor cores and more similar to Zarnekow cores. In HC 1 – the core taken nearest to potential freshwater sources (Fig. 1b) – pore water EC and DO concentrations were lower while pH was slightly higher than all other Hütelmoor cores. Moreover, this was the only Hütelmoor core where nitrate concentrations were undetectable (Fig. 5). Dissolved CH_4 concentrations were high, varied within and among fens and were slightly higher in Zarnekow pore water. Stable isotope ratios of $\partial^{13}C$-CH_4 (Fig. 5) in the upper peat (approx. −59‰) suggest a predominance of acetoclastic methanogenesis, with a shift to hydrogenotrophic methanogenesis around −65‰ in the lower peat profile. Also, shifts toward less negative $\partial^{13}C$-CH_4 values in the upper peat layer, as in HC 1 and HC 2, could indicate partial oxidation of CH_4 occurred (Chasar et al. 2000

composition in the Hütelmoor. Community composition varied much more strongly in HC 2 than in any other core (grey dashed-line polygon in Fig. 6). Bacterial communities in HC 1 were more similar to communities in all Zarnekow cores than in other Hütelmoor cores (Fig. 6a). The archaeal community in HC 1 was more similar to Zarnekow cores as well (Fig. 6b). Overall, the influence of depth on microbial community was evident, especially in the Hütelmoor where the differences were more pronounced. Environmental fit vectors suggest pH, oxygen and alternative TEA availability as important factors influencing microbial community composition. The EC vector suggests the importance of brackish conditions in shaping microbial communities in the Hütelmoor (Fig. 6a - c).

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. Very 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

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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, from -65 to -60‰ in HC 1 (where ANME-2D was abundant), may indicate that partial oxidation of CH_4 occurred, but we could only speculate whether or not ANME-2D are actively involved in this CH_4 oxidation. Although TEA input may be higher in the Hütelmoor, here, methanogenic conditions also predominate. This finding contrasts the measured oxygen concentrations in the upper peat profile, as methanogenesis under persistently oxygenated conditions is thermodynamically not possible. However, seasonal analysis of oxygen concentrations in both sites suggests highly fluctuating oxygen regimes both spatially and temporary (data not shown). Such non-uniform distribution of redox processes has already been described elsewhere, in particular for methanogenesis (Hoehler et al. 2001, Knorr et al. 2009). It is possible that oxygen levels in both fens are highly variable, allowing for spatially decoupled aerobic and anaerobic carbon turnover processes. Recent studies from wetlands also show that methanogenesis can occur in aerobic layers, driven mainly by *Methanosaeta* (Narrowe et al. 2017, Wagner 2017), which were detected in a high abundance in this study (Fig. 5). Further, oxygen may not necessarily be available within aggregates entailing anaerobic pathways and thus, the existence of anaerobic microenvironments may also partially

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explain the seemingly contradictory co-occurrence of oxygen and the highly abundant methanogens. Anaerobic conditions are also reflected by the extensive and stable occurrence of the strictly anaerobic syntrophs (e.g., *Syntrophobacteraceae*, *Syntrophaceae*) in most samples, even in the top centimeters. This suggests that syntrophic degradation of organic material is taking place in the uppermost layer and the fermented substances are easily available for methanogens. As geochemistry and microbial community composition differ among the sites in this study, it is thus notable that a similarly high abundance of methanogens, and low abundance of methanotrophs was detected in both fens. The dominance of methanogens implies that readily available substrates and favorable geochemical conditions promote high anaerobic carbon turnover despite seasonally fluctuating oxygen concentrations in the upper peat layer.

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, so that 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

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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.

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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 generally have a higher abundance of methanotrophs than measured in the fens in this study, suggesting the inundation and associated anoxia caused by flooding disturbs methanotrophic niches and may negatively affect the ability of methanotrophic communities to establish. The abundances of methane producers and consumers are thus suggested as important drivers for 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.

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Competing interests

The authors declare that they have no conflict of interest.

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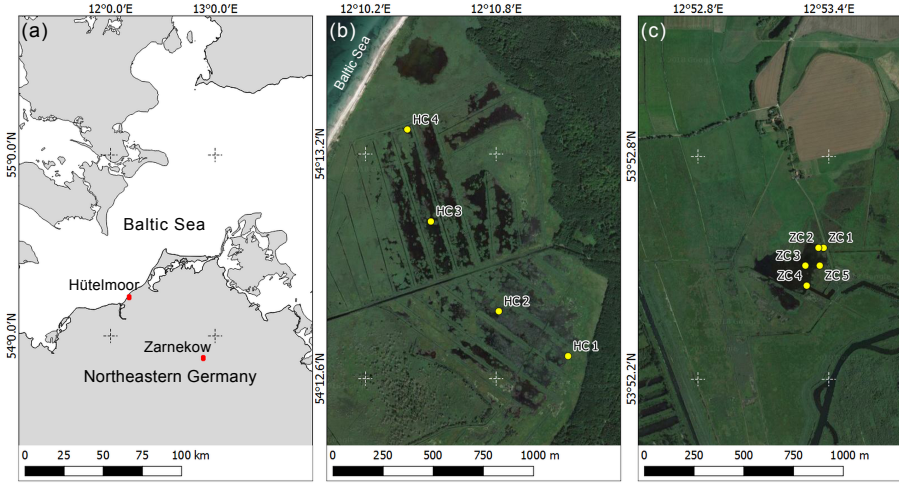

Figure 1: Location of study sites in northeastern Germany (a) and sampling locations within sites (b) Hütelmoor and (c) Zarnekow. Maps b) and c) are drawn to the same scale. Image source: (a) QGIS, (b) and (c) Google Earth via QGIS OpenLayer Plugin. Imagery date: August 9, 2015.

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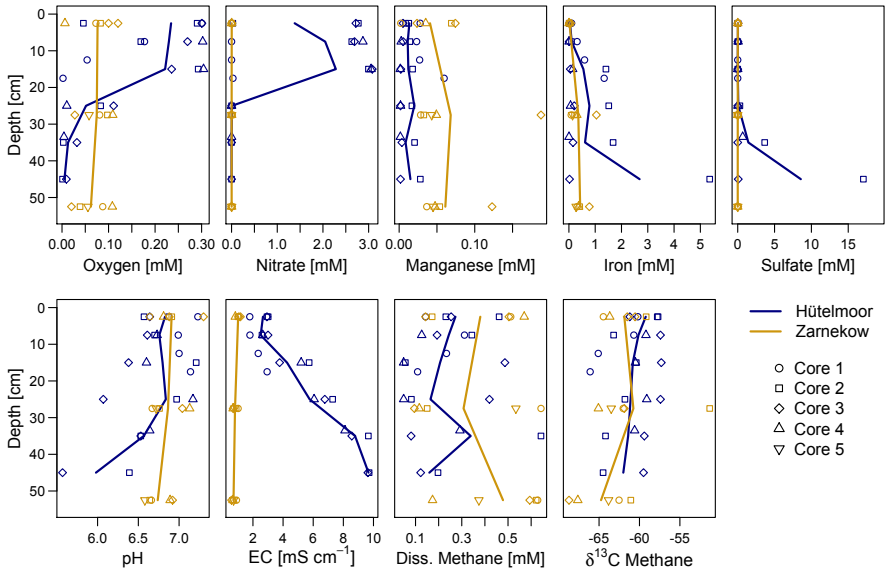

Figure 2: Depth profiles of oxygen, nitrate, total iron, manganese, and sulfate (upper panels), and profiles of pH, EC, dissolved methane, and the isotopic signature of methane-bound carbon (lower panels) in both study sites. Solid lines connect the respective means of individual wetlands (n=4 for Hütelmoor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

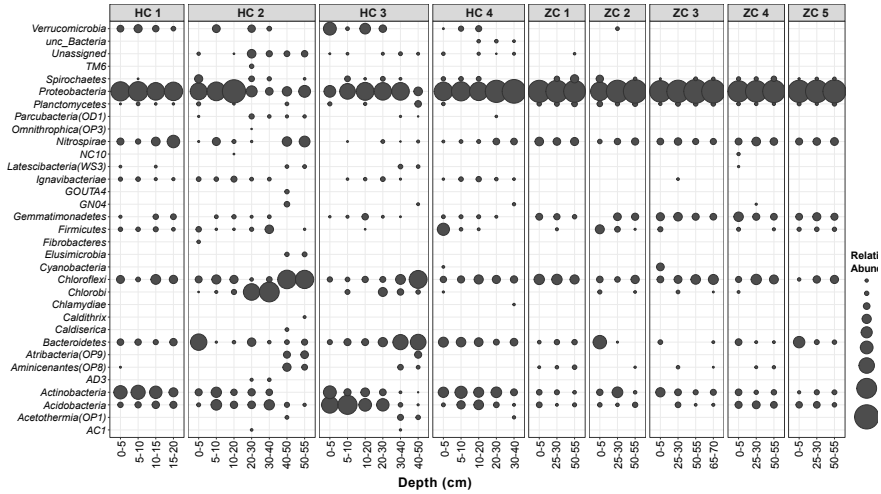

Figure 3: Relative abundances of different bacterial lineages in the study sites. Along the horizontal axis samples are arranged according to site and depth. The rank order along the vertical axis is shown for the phylum level.

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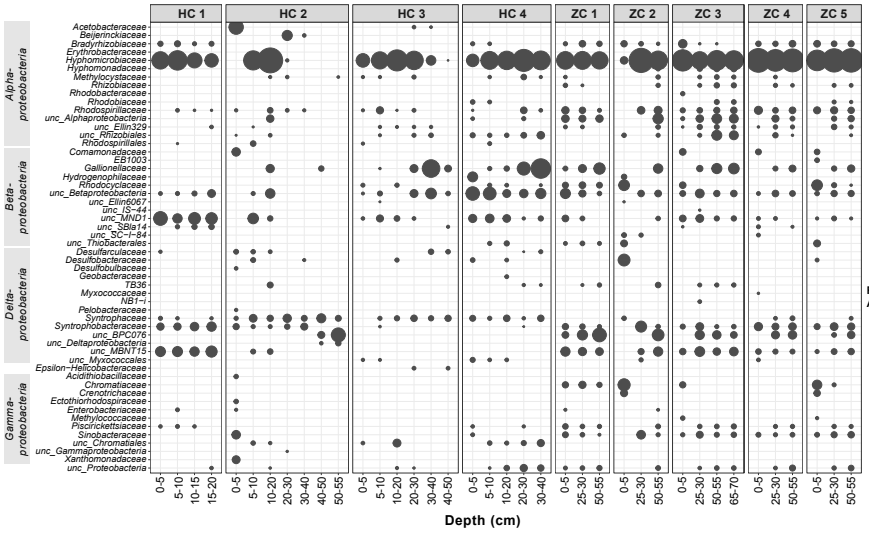

Figure 4: Relative abundances of Proteobacteria phyla in the study sites. Along the horizontal axis samples are arranged according to site and depth. The rank order along the vertical axis is shown for the family level. If an assignment to the family level was not possible the next higher assignable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

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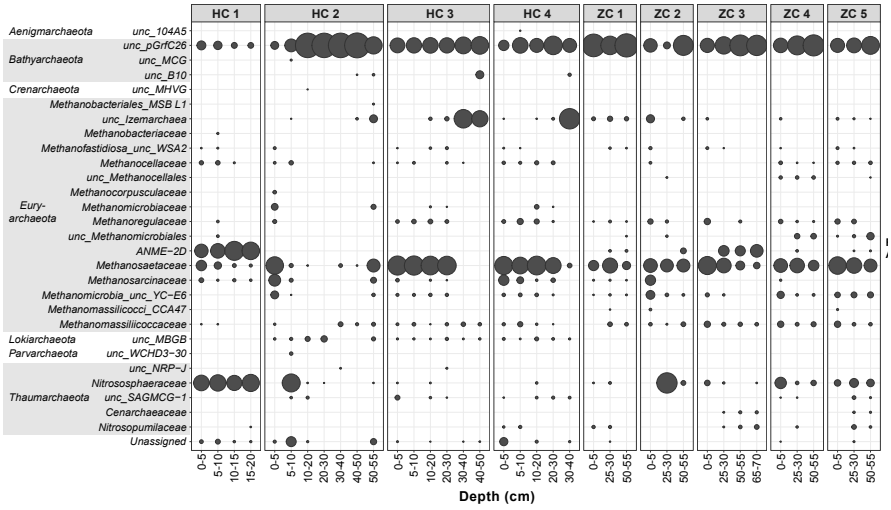

Figure 5: Relative abundances of different archaeal lineages in the study sites. Along the horizontal axis samples are arranged according to site and depth. The rank order along the vertical axis is shown for the family level. If an assignment to the family level was not possible, the next higher assignable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

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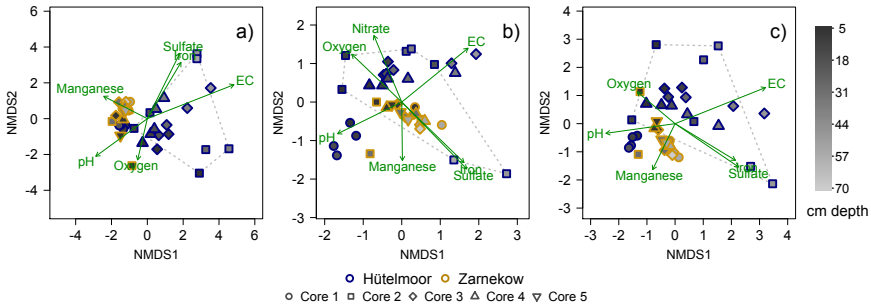

Figure 6: NMDS plots showing (a) bacterial, (b) archaeal, and (c) microbial (bacterial plus archaeal) community composition across the nine peat cores and their respective depth sections. The point positions represent distinct microbial communities, with the border colors of the symbols referring to the study sites and their shapes representing the core number. The shading indicates sample depth, with darker shades representing shallower depths, and lighter shades representing deeper depths. The dashed grey polygon highlights the large variation in microbial community composition in HC 2. Environmental fit vectors with a significance of $p < 0.05$ are shown in green.

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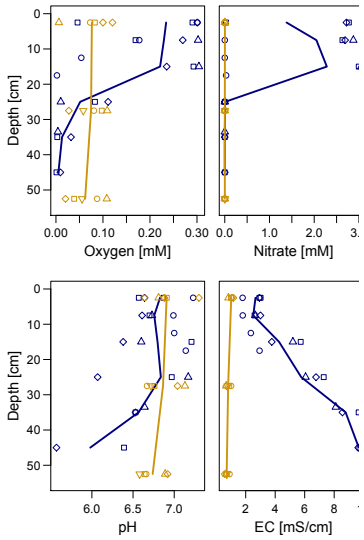

Figure 5: Depth profiles of porewater geochemistry (see x-axis labels for considered variables) in both study sites. Lines connect the respective means.¶

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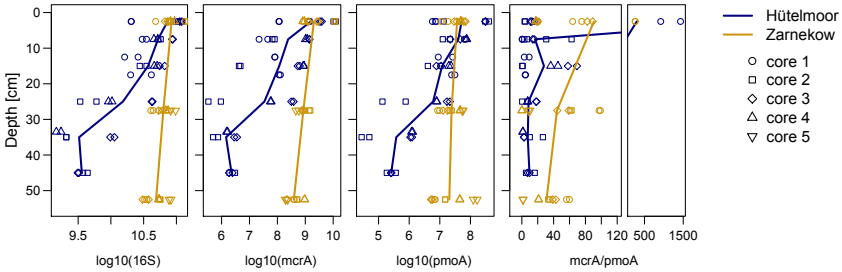

Figure 7: Depth distribution of qPCR abundances for total microbial (16S), methanogen (*mcrA*), methanotroph (*pmoA*), and ratio of *mcrA* to *pmoA* gene copy numbers in both sites. Microbial abundances were designated as numbers of gene copies per gram of dry peat soil. Duplicate measurements per depth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

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Table 1: Environmental conditions, geochemical conditions, and microbial abundances in peat cores from the Hütelmoor, a coastal minerotrophic fen in northeastern Germany. Environmental conditions are described by pH and EC (electrical conductivity). Geochemical parameters shown are dissolved methane (CH_4) concentrations, the isotopic signature of methane-bound carbon ($\partial^{13}C–CH_4$), and concentrations of terminal electron acceptors which are denoted with their respective chemical abbreviations. Microbial abundances here represent the mean value of averaged subsamples for each depth section (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*	*mcrA*/*pmoA*
cm		mS cm^{-1}				mM				gene copies g dry $peat^{-1}$			
HC 1, 0–5	7.2	1.79	-60.2	0.14	0.30	nd	0.10	0.03	0.03	$2.04x10^{10}$	$1.15x10^{08}$	$6.60x10^{06}$	17.7
5–10	7.0	1.80	-60.7	0.31	0.18	nd	0.31	0.02	0.01	$3.25\ x10^{10}$	$3.36x10^{07}$	$6.68x10^{07}$	0.51
10–15	7.0	2.35	-65.1	0.23	0.05	nd	0.60	0.03	nd	$2.11x10^{10}$	$8.12x10^{07}$	$1.76x10^{07}$	6.12
15–20	7.1	2.94	-66.1	0.11	nd	0.03	1.34	0.06	nd	$3.08x10^{10}$	$1.21x10^{08}$	$2.76x10^{07}$	4.41
HC 2, 0–5	6.9	3.01	-57.8	0.46	0.05	0.03	0.03	0.01	nd	$1.10x10^{11}$	$1.13x10^{10}$	$1.03x10^{07}$	1,170
5–10	6.7	2.60	-63.2	0.34	0.17	2.63	0.10	0.01	0.01	$5.51x10^{10}$	$7.27x10^{07}$	$1.69x10^{07}$	4.73
10–20	7.2	5.73	-60.4	0.06	0.29	3.00	1.41	0.02	nd	$3.13x10^{10}$	$4.47x10^{06}$	$7.32x10^{06}$	0.74
20–30	7.0	7.29	-61.8	0.08	0.08	nd	1.51	0.02	0.29	$4.71x10^{09}$	$6.41x10^{05}$	$4.50x10^{05}$	3.75
30–40	6.5	9.66	-64.2	0.64	nd	nd	1.68	0.02	3.66	$2.09x10^{09}$	$6.21x10^{05}$	$3.90x10^{04}$	18.3
40–50	6.4	9.71	-64.5	0.20	nd	nd	5.35	0.03	17.1	$4.09x10^{09}$	$2.47x10^{06}$	$2.75x10^{05}$	10.7
HC 3, 0–5	6.6	2.93	-57.7	0.23	0.29	2.77	0.11	0.01	0.04	$1.10x10^{11}$	$1.34x10^{09}$	$3.51x10^{08}$	3.86
5–10	6.6	3.00	-57.4	0.19	0.27	2.69	0.01	0.01	0.03	$8.72x10^{10}$	$1.40x10^{09}$	$3.42x10^{07}$	46.6
10–20	6.4	3.77	-57.3	0.49	0.24	3.08	0.05	nd	nd	$6.08x10^{10}$	$5.86x10^{08}$	$9.35x10^{06}$	63.6
20–30	6.1	6.77	-57.4	0.42	0.11	nd	0.20	nd	nd	$4.26x10^{10}$	$3.48x10^{08}$	$1.92x10^{07}$	18.2
30–40	6.5	8.56	-59.4	0.08	0.03	nd	0.16	nd	nd	$1.05x10^{10}$	$3.20x10^{06}$	$1.17x10^{06}$	2.74
40–50	5.6	9.36	-59.5	0.12	0.01	nd	0.02	nd	0.08	$3.18x10^{09}$	$2.16x10^{06}$	$2.58x10^{05}$	8.39
HC 4, 0–5	6.6	2.93	-61.2	0.25	0.30	2.72	0.02	0.01	0.04	$1.17x10^{11}$	$3.63x10^{09}$	$3.09x10^{08}$	11.7
5–10	6.7	2.65	-59.2	0.13	0.30	2.87	0.01	nd	0.05	$4.87x10^{10}$	$1.09x10^{09}$	$7.51x10^{07}$	14.5
10–20	6.6	5.20	-60.5	0.05	0.30	3.05	0.14	nd	nd	$4.85x10^{10}$	$8.71x10^{08}$	$2.15x10^{07}$	40.8
20–30	7.2	6.06	-59.1	0.05	0.01	nd	0.06	nd	0.02	$9.78x10^{09}$	$5.82x10^{07}$	$7.91x10^{06}$	7.36
30–40	6.6	8.11	-60.6	0.29	nd	nd	0.09	nd	0.67	$1.60x10^{09}$	$1.58x10^{06}$	$1.25x10^{06}$	1.27

Table 2: Environmental conditions, geochemical conditions, and microbial abundances in peat cores from Zarnekow, a freshwater minerotrophic fen in northeastern Germany. Environmental conditions are described by pH and EC (electrical conductivity). Geochemical parameters shown are dissolved methane (CH_4) concentrations, the isotopic signature of methane-bound carbon ($\partial^{13}C–CH_4$), and concentrations of terminal electron acceptors which are denoted with their respective chemical abbreviations. Microbial abundances here represent the mean value of averaged subsamples for each depth section (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*	*mcrA*/*pmoA*
cm		mS cm^{-1}		mM						gene copies g dry $peat^{-1}$			
ZC 1, 0–5	6.64	1.03	-64.5	0.51	0.07	0.001	0.007	0.002	0.002	$6.33x10^{10}$	$1.02x10^{09}$	$1.49x10^{07}$	69.7
25–30	6.67	1.14	-62.0	0.64	0.08	0.001	0.087	0.028	0.003	$4.25x10^{10}$	$8.96x10^{08}$	$9.14x10^{06}$	98.0
50–55	6.66	1.31	-62.5	0.63	0.09	0.005	0.310	0.037	0.002	$3.40x10^{10}$	$3.97x10^{08}$	$6.85x10^{06}$	58.1
ZC 2, 0–5	6.91	1.00	-59.2	0.17	0.08	0.004	0.012	0.069	0.007	$1.43x10^{11}$	$1.14x10^{10}$	$4.35x10^{07}$	261
25–30	6.76	1.29	-51.3	0.15	0.10	0.001	0.215	0.033	0.013	$6.44x10^{10}$	$1.45x10^{09}$	$2.34x10^{07}$	61.8
50–55	6.64	1.52	-61.1	0.62	0.04	nd	0.410	0.054	0.003	$5.64x10^{10}$	$5.10x10^{08}$	$1.50x10^{07}$	34.0
ZC 3, 0–5	6.88	1.17	-60.5	0.50	0.10	0.001	0.073	0.074	0.032	$7.86x10^{10}$	$2.78x10^{09}$	$3.26x10^{07}$	85.7
25–30	7.04	3.39	-61.9	0.10	0.03	0.002	1.046	0.188	0.003	$5.79x10^{10}$	$7.81x10^{08}$	$1.55x10^{07}$	51.8
50–55	6.92	3.82	-68.7	0.59	0.02	nd	0.779	0.123	0.003	$3.41x10^{10}$	$2.21x10^{08}$	$5.41x10^{06}$	40.9
ZC 4, 0–5	7.3	1.06	-61.5	0.14	0.12	0.010	0.013	0.024	0.035	$7.19x10^{10}$	$1.28x10^{09}$	$6.53x10^{07}$	19.6
25–30	7.13	1.58	-65.1	0.12	0.11	0.002	0.301	0.049	0.002	$7.19x10^{10}$	nd	$4.60x10^{07}$	-
50–55	6.89	1.51	-67.6	0.17	0.11	0.002	0.366	0.048	0.002	$5.42x10^{10}$	$9.47x10^{08}$	$4.50x10^{07}$	21.0
ZC 5, 0–5	6.81	0.83	-63.7	0.57	0.01	0.002	0.005	0.035	0.005	$8.73x10^{10}$	$8.73x10^{08}$	$4.97x10^{07}$	17.6
25–30	6.72	0.86	-63.5	0.53	0.06	0.002	0.139	0.043	0.001	$8.94x10^{10}$	$5.21x10^{08}$	$5.57x10^{07}$	93.4
50–55	6.58	1.00	-63.8	0.37	0.06	0.002	0.275	0.045	0.002	$8.00x10^{10}$	$2.14x10^{08}$	$1.44x10^{08}$	14.9

Supplemental Material

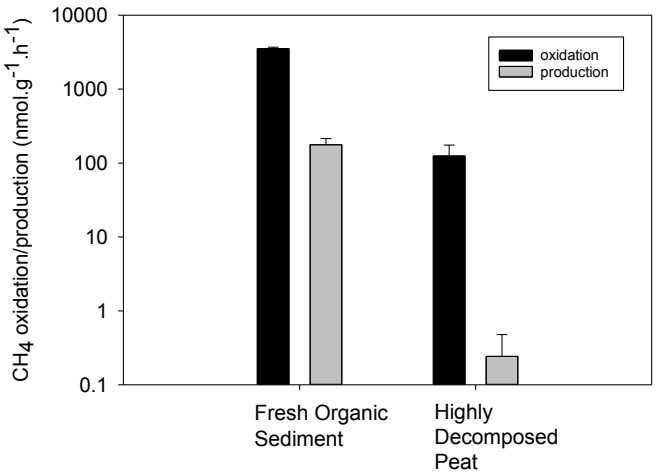

Figure S1: Incubation data from Zarnekow, a freshwater minerotrophic fen in Northeastern Germany. Rates of methane production and methane oxidation are shown for both fresh (surficial) organic sediment and the bulk peat.