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Biogeosciences Discussions

Interactive comment on "Microbial activity, methane production, and carbon storage in Early Holocene North Sea peats" *by* Tanya J. R. Lippmann et al.

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Thank you for your comments and constructive suggestions. We provide a response to each comment below.

Reviewer 1: Rhiannon Mondav (Referee) rhiannon.mondav@ebc.uu.se Received and published: 28 November 2020

Reviewer 1 General comments: The authors have done a lot of work creating datasets to characterise the geochemistry, biogeochemistry, botany, microbial community, and carbon of submerged peats from the southern North Sea. Marine sediments are an

understudied aspect of C cycle and climate and specifically the area of the North sea investigated here with its sandwiched layer of peat. The carbon pool of the region is estimated and potential emissions calculated. The potential for biological conversion of this carbon pool to CH4 is analysed via microbial taxonomic survey and incubations. This study shows a decoupling between CH4 production and C storage and posits that there is threat of re-coupling.

In general, the ideas are clearly defined though a little more work connecting ideas within paragraphs in the introduction and connecting the theory presented in the introduction to what was done in the study will assist readers in following the work. Further, adding explanations and justification for method choices will also assist readers. It is currently unclear why certain data was collected and analyses done. Some of the datasets are underutilized. Analyses connecting the different datasets could increase the value of the manuscript (MS) to a variety of audiences. There are a few instances in the discussion that reference data or results not detailed in the results section.

R: We will include extra sentences to more clearly connect ideas in the introduction. We feel that throughout the paper, we use the theory to justify the study design, the methods, and to quantitatively compare and discuss our results in the discussion. We better explain our reasons why the macrofossils and DNA analysis was performed in this way. It is true that our datasets do not all receive an equal focus in this paper. Firstly, some data are more indicative of the ecosystem dynamics than others and secondly, some data is more novel than others. For this reason, this paper focuses on the microbiology analyses, CH4 analyses, and macrofossil analyses. We discuss the possibility of statistically comparing data in a later specific comment. We will remove L622-624 from the discussion because it does not add insightful knowledge, "Present-day Northern peatlands have been estimated to store 547,000 Tg C, over a surface area of 4,000,000 km2 (Yu et al., 2010). The calculated amount of total carbon stored in North Sea basal-peat per km2 is lower due to the decomposition of carbon over time."

Reviewer 1 Specific comments: Figure 1A states that the southern North sea was inundated due to anthropogenic caused sea level rise. Is this a typo?

R: We would like to change the 3rd panel of Fig 1 to “North Sea basin (present day conditions, human-induced climate warming, rising sea levels)”. We will also change Fig 1 caption from “tsites” to “sites”.

Reviewer 1: Figure 1C would be great if it was even higher resolution covering just the section sampled ie the rectangle in 1B, with sample locations marked. Maybe even the location of the ‘special’ samples coded in a different colour or symbol. Just to help the readers visualize what was done.

R: We’ve added a panel that shows the sample sites. Figure 1 caption: Peats submerged beneath the North Sea region of study. (A) Schematic of the evolution of processes that led to the conversion from the Pleistocene land surface to the buried marine peat sediments as they occur today. (B) The sampling area location within the context of Western Europe, (C) the sampling areas, and (D) the sampling sites in the North Sea, coloured according to the area names, plotted in C. B, C, and D were generated using Python’s Basemap module and the background map image uses NASA’s Earth Observatory’s Blue Marble: Next Generation.

Reviewer 1: The methods section, especially the computational description needs more detail (or citations) and should include versions of software used and parameters chosen. -Why was sequencing and culture carried out on different samples/cores? It would make more sense to survey the community that was the base for incubations. Please provide justification for this choice. -I also ask for justification for choice of 60âŮęC annealing temperature for the initial amplification. Please also check the citation for the bacterial reverse primer it is the same paper as for the forward. -I could not find the deposited sequence data as there was no listing in Genbank found for the BioProject identifier. -Why was a qPCR carried out? -2.6.3 why is cloning mentioned in the subtitle? Was cloning done? Please provide method, results, and justification for

using this method. And integrate into results, discussion and conclusion. -The link to the core data also is not yet working.

R: -We have added detailed information on software versions and parameters in the materials and methods section. -Regarding the annealing temperature: the primers were tested in an annealing temperature gradient experiment before, and 60 degrees was determined as the optimal temperature. -There are indeed two papers for the two different primers: Herlemann et al. (2011) ISME J and Klindworth et al. (2013). Nucleic Acids Res -Indeed the sequence data is not yet available in Genbank, since the data deposit will be openly available upon publication. -The cloned 16S rRNA gene fragments were used as a standard in the qPCR. We have now included a respective citation where it is described how the respective plasmids were obtained. We have also included additional information on software analysis and qPCR efficiency. -Unfortunately, the cores from the first sampling expedition did not provide enough material to perform both sequencing and the incubation experiments. Therefore, we chose to divide the experiments over the different sites in order to obtain the maximum amount of information possible, while taking the experimental constraints into consideration. -We carried out a quantitative PCR to investigate the relative abundance of bacteria and archaea in these samples. This is especially relevant for microorganisms in the methane cycle, since all methanogenic microorganisms are found in the archaeal domain. Therefore, the qPCR results provide an indication of the relative contribution of methanogenic archaea in these ecosystems.

Reviewer 1: I believe that the physical, chemical, botanical, and radiocarbon dating (others?) were all done in order to establish how and when the peats formed and maybe what quality of carbon they hold. A large portion of the MS describes sampling, testing, physical qualities of the cores so it would be worth stating why these attributes were analysed as I did not notice this explicitly stated anywhere. Providing justification and motivation for choices will help the reader (who is unlikely to have the same level of expertise as the authorship team) to understand the work.

R: Thank you for your interest. We believe that this is articulated in the final paragraph of the introduction, “To provide a better understanding of the basal-peat ecosystem submerged beneath the North Sea, and its role in the CH4 cycle, we measure in situ CH4 concentrations and sediment organic matter content. Further, plant macrofossil analysis was performed to determine plant community composition and describe the micro-organismal habitat. 16S rRNA gene amplicon sequencing was performed to determine microbial diversity, and batch incubations were conducted to investigate actual and potential microbial CH4 cycle activity in the submerged peat deposits.”

Reviewer 1: Published literature documents both co-occurrence and (spatial and temporal) separation of methanogenesis and methanotrophy. There is also substantial literature on the ANME archaea which I did not notice specifically and clearly mentioned in this MS. Here are some randomly chosen non-exhaustive examples for your consideration: https://sfamjournals.onlinelibrary.wiley.com/doi/10.1111/1462-2920.13096 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5104750/ https://pubmed.ncbi.nlm.nih.gov/30664670/ https://aem.asm.org/content/74/13/3985

R: While we agree with the referee that the topic of ANME activity and the interactions of ANME/aerobic methanotrophs and methanogens is a highly interesting research topic, we do not see the need to incorporate this into our manuscript. Both our incubation experiments as well as our amplicon study confirm that neither ANME methanotrophs nor aerobic bacterial methanotrophs were present in our samples. Therefore, adding additional literature on ANME/methanogen co-occurrence remains speculative. We have discussed the absence of methanotrophs in our discussion and conclusion.

Reviewer 1: I would love to see greater use made of the core chemical data. Perhaps a multivari- ate approach comparing microbial community membership to chemistry would be very interesting and tie together major components of the data presented here. Similarly, there a few places in the MS where the C, CH4, or organic content of the peats is referred to in comparison to microbes but I did not notice a statistical analysis to back up any comparison. This could add value to the MS.

R: We agree that a statistical comparison would substantiate discussion. We will include the following PCA figures for the archaea and bacterial populations.

L603 of results: “Principal Component Analysis (PCA) was used to describe the relationships between the large number of sampling methods. Each principal component (PC) is an uncorrelated linear combination of variables that maximises variance and the PC loads represent the relative contributions of the original variables to the PCs. PCA was calculated for archaeal (Fig. 6a) and bacterial (Fig. 6b) species abundance separately.

The arachael PC1 and PC2 account for 59% (PC1 accounts for 32.8% and PC2 accounted for 26.0%) of the total data variability of all variables included in this analysis. PC1 loadings showed that Marine Benthic Group is anti-correlated with OM content (labelled LOI330, LOI550). I.e. Marine Benthic Group B population abundance was greater in samples with lower OM content. PC2 loadings indicated Bathyarachaeia and Methanoregulaceae population abundance are anti-correlated with high methane concentrations and Lokiarchaeia population abundance.

The bacterial PC1 and PC2 accounted for 43% (PC1 accounts for 26.7% and PC2 accounted for 15.8%) of the total data variability of all variables included in this analysis. There were clear groupings indicated by both the PC1 and PC2 loadings. Methane concentration did not have strong (anti)correlation with any variable in any PC. JS1 was negatively correlated with Sprochaetales, SBR1031, Pla1 lineage, and Pirellulales in PC1 loadings.“

Figure caption: Principal component analyses calculated using species’ abundance, CH4 concentration (‘CH4_porewater’), latitude, porosity,OM content (LOI330, LOI550) and depth beneath seafloor (‘dbsf_m’). PC1 loadings (x-axis) are plotted against the PC2 loadings (y-axis) for A. archeal species’ abundance, B. bacterial species’ abundance.

Reviewer 1: I have concerns about the NMDSs presented. My reading of the

manuscript is that there were 12 samples sequenced. On an NMDS where the samples are mapped onto species space there should therefore only be 12 dots. Please provide details of computational methods used so that what has been plotted on the NMDS is under- stood. For an example of the level of method detail required and correct plotting of an NMDS see e.g. https://www.pnas.org/content/115/47/11994

R: We have corrected the legends of the NMDS plots to properly explain the procedure. The NMDS plots here are based on the OTUs that were pre filtered. OTUs that only occur once per sample (on average for the total amount of samples: OTUs with 12 or less occurrences were removed). The dots in these plots thus represent OTUs.

Reviewer 1: The MS states early on that it looks at C storage and CH4 seepage/accumulation etc. Please check the MS for typos 'CH4 storage' or do you have evidence that CH4 is trapped in the peat? Is that why the term CH4 storage is used? I would have guessed that the CH4 currently in the peat deposits can bubble up through the overlying clay and sands into the water column where (depending on factors that might be worth listing) it is consumed by methanotrophs in the water column or emitted to the atmosphere. This could make an interesting discussion point for this MS.

R: CH4 ebullition is possible. Based on our observations, we do not understand it to be a widespread occurrence in this environment. We propose to include the following text into the discussion of the CH4 budget (L626 onwards): "There exists two potential hypotheses explaining the presence of CH4. Firstly, the CH4 observed here was produced some time ago and in the absence of active methanotrophs, has been trapped by the overlying sediment layer. The observed clay layer overlying the peat is sufficiently dense to prevent CH4 outgassing. Our results show that neither aerobic or anaerobic methanoptrophic prokaryotes were activated by oxic or anoxic incubations.

Alternatively, the observed CH4 concentrations were produced by methanogens in the present day. Our incubation activity studies show that whilst the methanogenic community could be revived within a two week window, methanogens were not observed

to be active in the present day (Fig. 5). Therefore, it is likely that trapped pockets of old methane have been observed here. This supports previous non-in situ seismic studies, showing trapped methane pockets in the sedimentary peat layer beneath the North Sea but contradicts the hypothesis that this methane is produced in the present day."

Reviewer 1: There is a statement in the abstract and conclusion that the C in the peats could be converted to CH4 under other circumstances. What other circumstances? Your MS shows and states that the remaining C is not accessible to methanogens so what would make it available? This would be an interesting discussion point.

R: "Methanotrophs have the potential to be activated in the presence of additional CH4. Such additional CH4 may occur due to emission caused by leakage from fossil fuel extraction, which has occurred in the local area previously (Schneider von Deimling et al. 2015). Upon activation, methanotrophs would have the potential to consume both the newly added and existing methane sources."

Reviewer 1: Technical corrections: -Community structure was not studied. Community member- ship was, please change this throughout MS.

R: The term 'community structure' is commonly used in the literature to describe the results of amplicon-based sequencing studies. For this reason, we also use this term in our manuscript.

Reviewer 1: -There is a mix of 'methane' and 'CH4' throughout the MS please pick one.

R: Thank you for picking this up. L612: "The findings confirm the long-held hypothesis that methane CH4 is stored...." is changed to, "The findings confirm the long-held hypothesis that CH4 is stored..." We will change 'methane' to 'CH4' except for where it occurs in a heading or at the start of a sentence.

Reviewer 1: -Ln 779 methanogenic bacteria – is this a typo?

R: Thank you for spotting this. "Large carbon stores in the presence of methanogens

but in the absence of methanotrophs hold the potential to be metabolised into methane gas...” We will correct any other instances.

Reviewer 1: -‘activity assay’ refers to e.g testing catalase activity in a lab. This study documents incubations not activity assays. Please be careful about using the word ‘activity’ (in- cluding in the title) throughout the MS

R: To avoid potential confusion we have changed the occurrences of ‘activity assays’ with ‘incubations’

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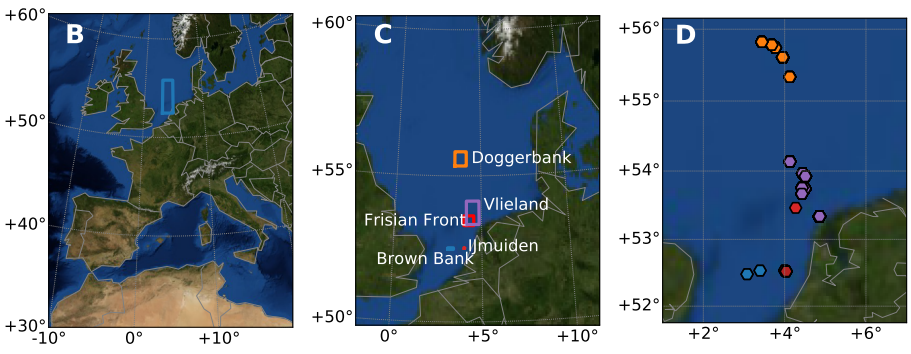

Fig. 1.

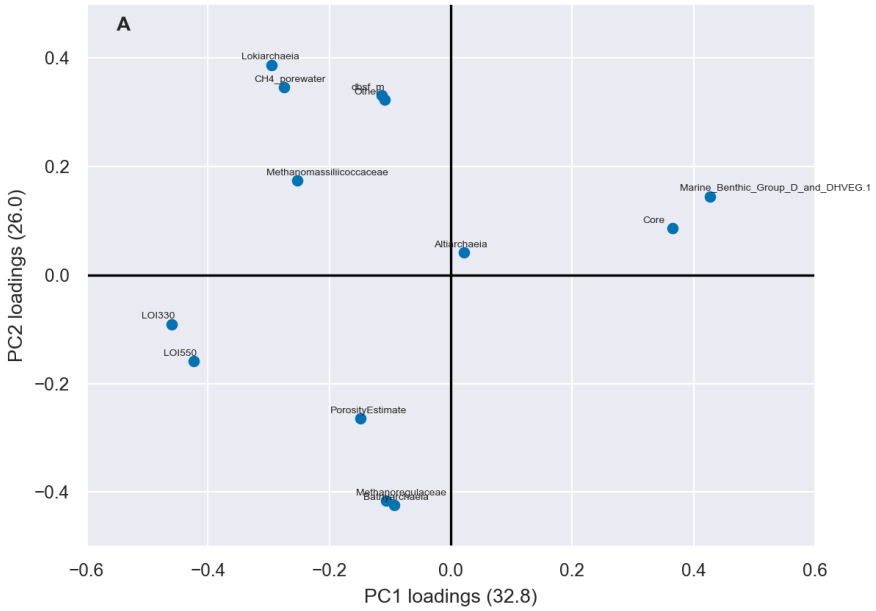
A
Lokiarchaeia
CH4_porewater
Methanomassiliicoccaceae
Marine_Benthic_Group_D_and_DHVEG.1
Core
Altiarchaeia
LOI330
LOI550
PorosityEstimate
Methanoregulaceae
0.4
0.2
0.0
−0.2
−0.4
−0.6
−0.4
−0.2
0.0
0.2
0.4
0.6
PC2 loadings (26.0)
PC1 loadings (32.8)

Fig. 2.

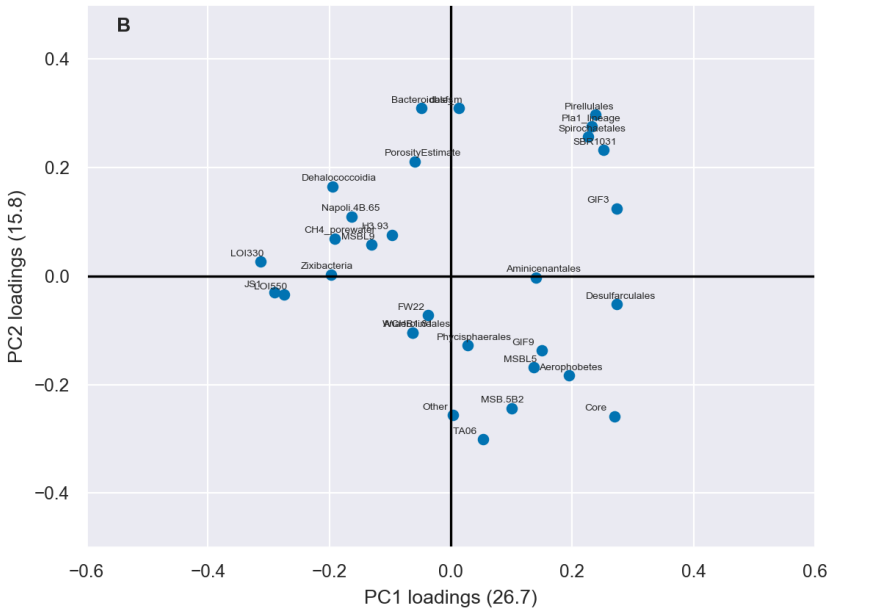
B
Pirellulales
SBR1031
PorosityEstimate
Dehalococcoidia
GIF3
Napoli.4B.65
CH4_porewater
MSBL8
LOI330
Zixibacteria
Aminicenantales
Desulfarculales
FW22
Phycisphaerales
GIF9
MSBL5
Aerophobetes
MSB.5B2
Other
Core
TA06
0.4
0.2
0.0
−0.2
−0.4
−0.6
−0.4
−0.2
0.0
0.2
0.4
0.6
PC2 loadings (15.8)
PC1 loadings (26.7)

Fig. 3.