Response to Reviewer 1

Thank you for your comments and constructive suggestions. We feel these comments were very helpful in improving the manuscript. We provide a response (in blue text) to each comment below.

Reviewer 1:

Rhiannon Mondav (Referee)
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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 CH_4 is analysed via microbial taxonomic survey and incubations. This study shows a decoupling between CH_4 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 intro- duction 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 have made some edits to the introduction to more clearly connect ideas.

We considered your point regarding justification of the study. We include a paragraph at L123 that clearly links the theory and our study aims and design.

We better explain our reasons why the macrofossils and DNA analysis was performed in this way at L215 of the methods.

It is true that not all datasets 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, CH_4 analyses, and macrofossil analyses.

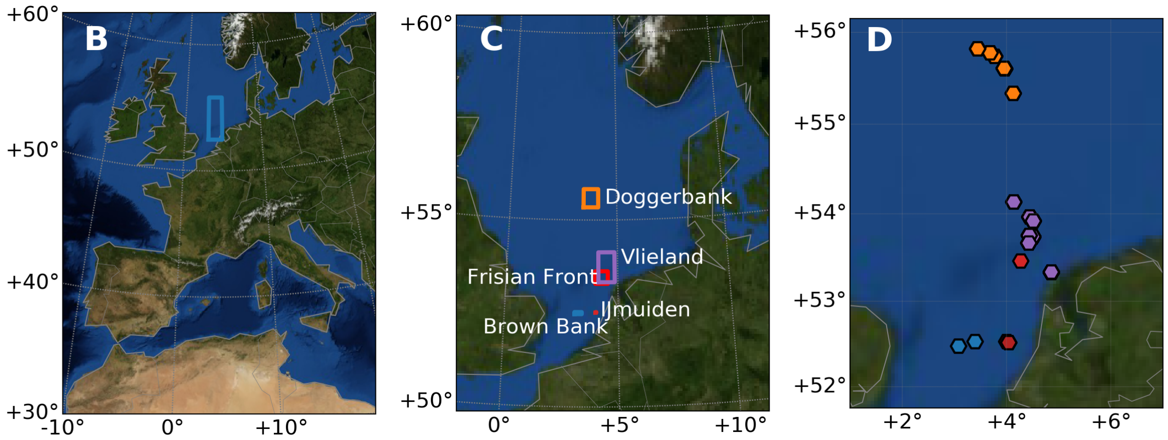
We discuss the possibility of statistically comparing data in a reply to a specific comment.

We have removed L622-624 from the discussion because it does not contribute insightful knowledge.

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: To clarify this, we have changed the heading to read, 'The submersion of north sea peatlands and the current microbial habitats'. We have changed the caption of the 3rd panel to, "North Sea basin present day conditions (human-induced climate warming, rising sea levels)".

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 and adjusted the figure caption, accordingly.



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 60◦C 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 section 2.6 of 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 explicitly state this in the final paragraph of the introduction (L123-L133) and in the first paragraph of the methods (L135-141).

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 men- tioned 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 multi-variable statistical comparison is useful and considered this point very seriously. As a result, we did perform a PCA analysis. In the end, we have decided not to include this in the final manuscript. We include the results here for your interest. We have removed and reduced comparing relationships between variables. Considering the highly heterogenous nature of peat OM, and micro-organisms, our sample size is not adequate to deduce relationships or processes.

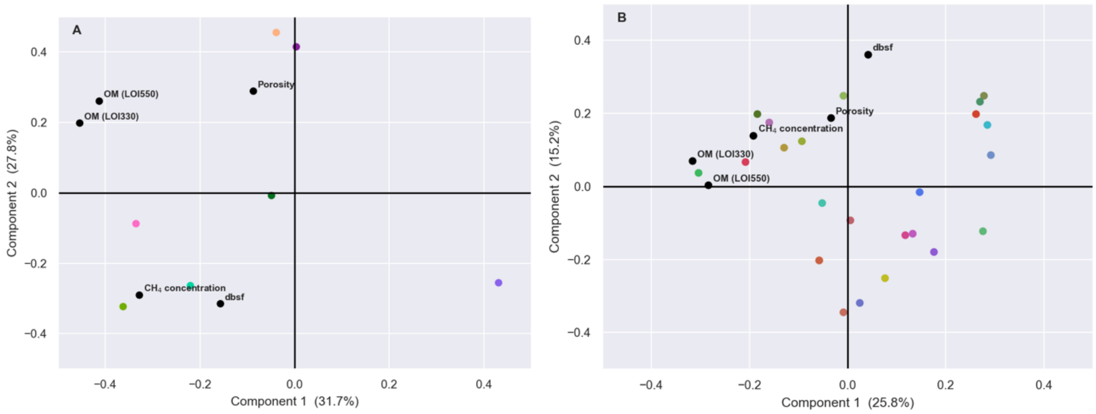


***Figure description**: Principal component analyses calculated using species' abundance, CH_4 concentration, porosity, OM content (LOI330, LOI550) and depth beneath seafloor (dbsf), and site. PC1 loadings (x-axis) are plotted against the PC2 loadings (y-axis) for **A.** archeal species' abundance, **B.** bacterial species' abundance. The coloured dots represent microbial species and uses the legend of Fig. 5b.*

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: We have discussed this in section '4.1 Origins of this newly measured CH4 store'.

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: We have added the following to L681 in the discussion: "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 CH4 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 long-held hypothesis that methane CH4 is stored...." is changed to, "… the long-held hypothesis that CH4 is stored..." We have changed '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. We have changed this to: "Large carbon stores in the presence of methanogens but in the absence of methanotrophs hold the potential to be metabolised into methane gas..." We have corrected 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'

Response to Reviewer 2

We thank the reviewer #2 for their considered and constructive comments on this manuscript. They have been very helpful in improving the manuscript. We provide a response (in blue text) to each comment below.

Reviewer 2:

Anonymous Referee #2
Received and published: 19 February 2021

Reviewer 2 Note from reviewer: I do not have expertise in the experimental elements of this manuscript, thus my critique of microbial activity, gene sequencing and methane pro- duction is limited. General comments This manuscript documents a significant carbon store in the North Sea during the last glacial-interglacial transition, with experiments to understand the precise microbial activity and methane production/potential. The authors use gene-based sequencing to understand the microbial community struc- ture and to explore the role and potential of peat microbial communities in carbon (methane) cycling. A key contribution of this work is quantifying these peats via observations/measurements and incubation experiments to determine the carbon stor- age potential with implications for better understanding the role of peat deposits in the global carbon budget. This manuscript is well written and contributes important knowl- edge for better understating the role of buried peats in the carbon cycle. Two overall suggestions (1) keep discussion and result separate. For example, “capped by either shallow marine clay or sands” should perhaps move the ‘shallow marine’ interpretation to the discussion section. There are countless other examples of discussion embedded into the results section, for example starting at L477-L484. This would help with the organization of the manuscript. Also (2) perhaps place more emphasis on the global implications of this work for the carbon budget ie. How much does this change our estimates of carbon stores? What are the potentials for this carbon to be released in the future? Are there any other regions where a similar peat has been deposited?

R:

(1) We have revised the results section to keep this to a minimum.

L358: We have edited the paragraph at L358 to reflect that this description is observational. It is now at L365. We also changed the subheading to ‘3.1 Basal peats vary in thickness, formed on Pleistocene sands, capped by marine clays.’

We have moved L361-364, “The basal-peat developed due to rising groundwater as a result of the postglacial sea-level rise and was capped by rapidly deposited clays and in some instances, stratified sand deposits.” to section, ‘4.1 Origins of this newly measured CH4 store’ in the discussion.

Moved L390-L393 to L653 of the discussion and reword to: The highest CH4 concentrations were measured at the Vittorio site, the site of the second thickest peat layer. However, we did not find evidence that the thickness of the basal peat was linked to CH4 concentrations, as both thick and thin peat layers harboured both high and low concentrations (Fig. 3).”

Moved L477-483 to section 4.6 the discussion.

We removed L409-L412 because this is repeated in section 4.6 of the discussion.

In a similar spirit, we moved L170-L174 (methods), “Peat was recovered at all sites, except Easting Down, Stormvogel, and Darci’s site.“ to L369 of the results section.

(2) *We have placed our C and CH4 estimates in relation to other global accounting estimates. We have added the following to L657, “The 741 Tg-C stored in these submerged peats is equivalent to 70% of the 1,030 Tg-C stored in Dutch peatlands today (Erkens et al., 2016), or 2.4% of the 30,600 Tg-C stored in the globe’s largest peatland C storage facility, the Congo Basin complex (Dargie et al., 2017). This C has the potential to be released into the overlying water column in the occurrence of a marine seep, that could be either naturally initiated or an outcome of fossil fuel extraction (Schneider von Deimling et al., 2015).” We have quantified the CH4 budget in relation to global CH4. We have added the following to L628, “For comparison with global* CH_4 *inventories, the estimated 0.411 Tg* CH_4 *present in these submerge sediments is equivalent to almost one quarter of the annual biogenic oceanic* CH_4 *emissions (2 Tg-*CH_4 yr^{-1}*) (Saunois et al., 2020a), 1 month of the global growth of atmospheric* CH_4 *that occurred during the years, 2000-2009 (5.8Tg* yr^{-1}*), or 1.5 weeks of the global atmospheric* CH_4 *growth that occurred in 2017 (16.8 Tg* yr^{-1}*) (Saunois et al., 2020a).”*

Reviewer 2 Specific comments
Reviewer 2 L70 – “ice sheets reaching as far south as the Doggerbank area were subjected to strong glacio-isostatic adjustment” – ice sheets were subject to GIA? Or the earth was subject to GIA? please clarify.
TL: Thank you for picking this up. We have edited the sentence to, “During the Late Pleistocene and Early Holocene, strong glacio-isostatic adjustments (GIA) resulted in isostatic subsidence of the North Sea basin (Hijma et al., 2012; Vink et al., 2007) and, combined with rapid melting of polar ice sheets, high rates of sea level rise, up to 1-2 cm yr-1 (Hijma and Cohen, 2019), gave rise to paludification, peatland development and later peatland submersion (now basal peats).”

Reviewer 2 L95 – “task of measuring CH4 stores remains challenging” – why is this the case?
TL: We have changed L95 to: “Despite extensive efforts to map these submerged peatland ecosystems (Treat et al., 2019; Xu et al., 2018), basal peats remain hard to reach, meaning accessing and measuring CH4 stores remains challenging, limiting in situ measurements, (Dean et al., 2018).”

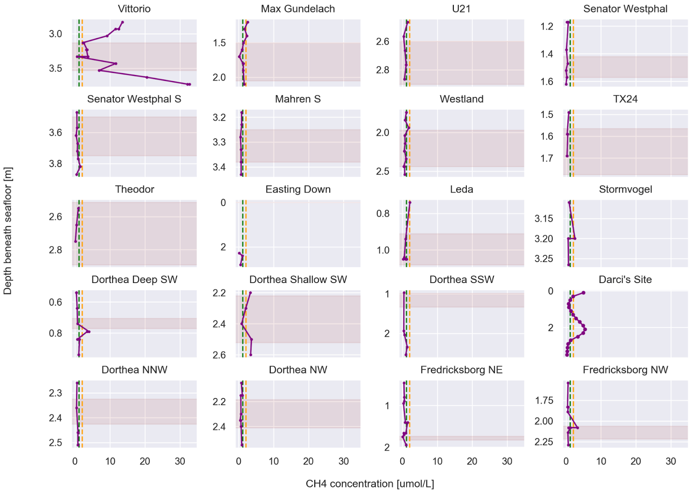
Reviewer 2 L145 – why were these sites chosen for microbial sequencing? This is unclear. Do they provide good spatial coverage that is representative of the region?
R: We chose to divide the experiments over the different sites in order to obtain the maximum amount of information possible, while taking the experimental constraints into consideration. Due to limitations in the available sample amounts we were not able to carry out both the incubation studies and the amplicon sequencing on the cores of the first sampling expedition. We clarified this in L216-L220: “Four cores in the southern North Sea were selected for 16S rRNA amplicon sequencing, and 4 cores from the Doggerbank area were selected for microbial activity studies. 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 microbial experiments over multiple sites in order to obtain the maximum amount of information possible, whilst taking the experimental constraints into consideration.”

Reviewer 2 L213 – same as above. why were these particular sites chosen for microbial sequencing? This is unclear.
R: As above.

Reviewer 2 L325-327 – this mixture of high/low sampling resolution and high/low taxonomic resolution is interesting. Perhaps an extra line on why this technique was chosen?
R: We have explained this in L332-337: “The Max Gundelach site was analysed with low sample resolution but high taxonomic resolution, showing the main peat components as well as an overview of the less abundant taxa. As the less abundant taxa were, in this research, not highly relevant we

analysed the Fredericksborg NE site with high sample resolution but low taxonomic resolution, showing only the main peat components. The sites can be compared based on the main peat components."

Reviewer 2 L391 – " the thickness of the peat layer does not appear to play a determining role in CH4 concentrations, as both thick and thin peat layers harboured both high and low CH4 concentrations" – this is an interesting finding of this work, with implications for carbon modelling of paleo-peatlands. Is it possible to show this graphically? A quick plot showing thickness vs. CH4 concentration?
R: We have adjusted Figure 3, the methane depth profiles, to include the peat thickness.



Reviewer 2 L420 – The header suggests that this section will contain information on plant macrofossil communities, but there is no such information here.
R: Thisisnowsection 3.3 and we changed the heading to, 'Peatland establishment and cessation'. Section 3.3 includes the following subsections, consistent with the Biogeosciences guidelines: '3.3.1 Local vegetation succession in the southern North Sea' and '3.3.2 Local vegetation succession in the mid North Sea at Doggerbank'.

Reviewer 2 L489 – given the high spatial vari- ability in peat thickness, I would expect to see (large?) errors on this estimate. What uncertainties were incorporated into this calculation and how do they impact the result- ing error?
R: We have included this sentence and formula at L188 of the methods, "The minimum and maximum peat thicknesses provide uncertainty estimates of the total C and CH4 pools, respectively.

Reviewer 2 L614 – what is meant by "from a similar period"? is this referring strictly to the time interval, or the sequence of events (SL rise) that would cause these peats to be buried?
R: We have removed this sentence.

Fig. 1 – Does the 3rd panel "North Sea basin" refer to present-day condi- tions? It might be worth clarifying. Fig. 1 caption – "The distribution of tsites within this sampling area" – sites?
R: To clarify this, we have changed the heading to read, 'The submersion of North Sea peatlands and the current microbial habitats'. We have changed the caption of the 3rd panel to, "North Sea basin present day conditions (human-induced climate warming, rising sea levels)". Fig 1 caption should indeed be, "sites" and not "tsites". Thank you for picking this up.

We have used a new citation that has been included in the reference list:
Dargie, G., Lewis, S., Lawson, I. et al. Age, extent and carbon storage of the central Congo Basin peatland complex. Nature 542, 86–90 (2017). https://doi.org/10.1038/nature21048