The authors have done a lot of excellent work both responding to reviewers comments that will assist readers to understand their work. It is nicely rounded and very well placed into context.

We thank the reviewer for the constructive feedback on our manuscript. Please find below an answer to the points for consideration.

I have a few points for consideration on the manuscript.
1. The authors have an incorrect reference for the Bacterial reverse primer. Please see the following quotes from the Klindworth paper cited for the reverse primer:
"Per sample, two separate PCR reactions were performed in order to test two bacterial primer pairs for 16S rDNA amplification. Primer pairs were: (i): S-D-Bact-0341-b-S-17, 5′-CCTACGGGNGGCWGCAG-3′ (32), and S-D-Bact-0785-a-A-21, 5′-GACTACHVGGGTATCTAATCC-3 (32);"
"32
Herlemann D.P.R., Labrenz M., Juergens K., Bertilsson S., Wani ek J.J., Anderrson A.F.. Transition in bacterial communities along the 2000 km salinity gradient of the Baltic Sea, ISME J, 2011, vol. 5 (pg. 1571-1579)"

We thank the reviewer for carefully checking this. The first publication of this primer is in Klindworth et al., 2013. We have not altered our in-text reference.

We agree with you that Klindworth et al., 2013 references this primer using Herlemann et al. 2011. However, we found no mention of S-D-Bact-0785-a-A-21, 5′-GACTACHVGGGTATCTAATCC-3 within Klindworth et al., 2013.

2. In previous work by the authors in which these primers were tested they used a temperature gradient PCR to select 60° C. It is not stated what the authors observed at 60°C that helped them choose this temperature. Could the authors please include this information in the methods as it is relevant to the interpretation of the results and might help others in their lab work.

We have included at L250: "The annealing temperature of 60°C was selected because we observed intense bands of the correct amplicon and no observable primer dimers at this temperature."

3. Generally an annealing temperature of +/- 5° from the mean Tm of the primer pair, and as low as possible to achieve amplification is chosen. Typically this particular primer pair is run with annealing temperatures between 48°C and 56°C. The use of a higher annealing temperature could increase amplification bias reducing the phylogenetic range of template DNA amplified. Please carefully phrase any statements about the absence of detection of any clade as the absence could, more than usual, be due to amplification bias.
-https://academic.oup.com/femsec/article/60/2/341/584515
-https://academic.oup.com/nar/article/41/1/e1/1164457#119410076
-http://cshprotocols.cshlp.org/content/2009/4/pdb.ip66.short?casa_token=h2nXrvzrHDIAAAAA:4IzeOXuwzgz48-voKEUGXXMXq7jPlHPUUAkmR8WSgcMNN3B79NXvMPf_nkNgxkTC7SbgD5TJQ5I

We agree that primer biases are inevitable with amplicon based studies, also due to primer mismatches or not binding primers due to suboptimal PCR conditions for certain microbial clades.

We have here chosen the PCR conditions based on the observation on the specific DNA samples. To further highlight this we have added the sentences to the section on amplicon sequencing at line 271: "We want to stress that biases associated with PCR-based amplicon studies can result in an observed absence of specific microbial clades and results need to be interpreted with care."

4. Please provide standard details of the PCR e.g. volumes, concentrations etc of reactants and template, or provide a reference where these details are given.

We have added these details to the section 2.6.2 Amplicon sequencing and analysis (Line 242): "The total PCR reaction volume was 25 μL with 1 μL forward and 1 μL reverse primer (20 μM), 1 μL template, 12,5 μL 2x concentrated SYBR™ Green PCR Master Mix (ThermoFisher, Carlsbad, CA, USA) and 9,5 μL sterile MilliQ."

5. I have trouble reconciling the NMDSs in fig S7 which show a random placement of OTUs and figure 6 which shows that there is not just a difference in archaea and bacterial assemblages between sites but that differences can be seen at family and higher taxonomic levels and so should be very obvious at OTU level. Please provide details of how the NMDS were plotted as this difference seems strange. The statement that no difference in structure was observed according to the NMDSs while an accurate description of what is plotted (though because sites are not plotted on the NMDSs the statement is only partially supported), does not fit with the other analyses which show clear differences at sites in communities and different depths.

We agree that the OTU figures do not provide a clear difference. This is mainly attributed to the fact that an OTU analysis does not necessarily visualize a difference in e.g. microbial family, which can be interpreted in taxonomic analysis. In addition, as stated in the materials and methods as well as in the legend description the OTU representation does not include rare taxa and is therefore only a coarse first overview of the population. We have therefore also decided to only supply these very rough overviews of the data in the supplementary data and to not draw any major conclusions on the data.

6. Multiple contradictions were found in the following lines. Could the authors please fix these statements regarding the presence/absence of methanotrophs and methanogens so they are consistent.
Line 497-8 "Molecular analysis showed that both methanogens and methanotrophs were present at all four assessed sites Fig 6"
Line 666 "No aerobic or anaerobic methanotrophic prokaryotes were found in these peat deposits"
Line 610-611 "We conclude that in the observed absence of methanogenic and methanotrophic microbial populations, the in situ CH4 observed in this study are trapped pockets of millennia old CH4."
Line 672-4 "The absence of methanotroph activity is congruent with their absence in the results of 16S rRNA gene
amplicon sequencing and confirms that methanotrophic species are most likely not present or active in this
environment."
Line 780-782 "Whilst the source of CH4 remains unconfirmed, we conclude that in the observed absence of methanogenic and methanotrophic microbial populations, the in situ CH4 observed in this
study are trapped pockets of millennia old CH4."
Other contradictions:
Line 600-601 "Further, microbial analyses show that neither aerobic or anaerobic methanotrophic

prokaryotes were activated by oxic or anoxic incubations. Therefore, we did not observe processes where biogenic CH4 may have been produced in the present day."

Thank you for carefully reading these lines. Indeed, no methanotrophs were found in the data (not in the activity data and no molecular signatures), and this was wrongfully stated in some sentences. It is our intention to highlight the observed absence of active methanogenic and methanotrophic populations. This we have stressed in the lines below. We have changed this in the relevant sentences as follows (see words highlighted in bold for additions and strikethroughs for deletions):

Line 497-8 "Molecular analysis showed that ~~both methanogens and methanotrophs~~ **methanogens** were present at all four assessed sites Fig 6"

Line 600-601 "Further, microbial **activity-based** analyses show that neither aerobic or anaerobic methanotrophic prokaryotes were activated by oxic or anoxic incubations. Therefore, we did not observe processes indicating biogenic CH4 may have been produced in the present day."

Line 620-621 "We conclude that in the observed absence of **active** methanogenic and methanotrophic
microbial populations, the in situ CH4 observed in this study are trapped pockets of millennia old CH4."

Line 675 "No aerobic or anaerobic methanotrophic prokaryotes were found in these peat deposits"

Line 672-4 "The absence of methanotroph activity is congruent with their absence in the results of 16S rRNA gene amplicon sequencing and confirms that methanotrophic species are most likely not present or active in this environment."

Line 780-782 "Whilst the source of CH4 remains unconfirmed, we conclude that in the observed **indicated** absence of **active** methanogenic and methanotrophic microbial populations, the in situ CH4 observed in this
study are **most likely** trapped pockets of millennia old CH4."

7. Methanosarcinales are mentioned in the discussion but not in the results.
BTW: There are several recent studies showing the release of methane by bacteria and others.
-https://www.nature.com/articles/ngeo2837
-https://www.nature.com/articles/s41564-017-0091-5
-https://advances.sciencemag.org/content/6/3/eaax5343?utm_source=TrendMD&utm_medium=cpc&utm_campaign=TrendMD_1

Indeed we did not observe Methanosarcinales-sequences in the data. We have changed the sentence to indicate that Methanosarcinales are the most probably subject, but not observed in our data: "In contrast to H_2/CO_2 and acetate, methylated compounds are a non-competitive methanogenic substrate that is metabolized by *Methanosarcinales*. However, we did surprisingly not observe presence of this methanogenic family in our data."

We are indeed aware of the studies focusing on methane release by bacteria. However, on the bigger picture in these ecosystems it is unlikely that these micro-organisms do play a role (ecosystem fully anoxic, absence of labile organic matter). We therefore did not further speculate on potential roles of these micro-organisms in this ecosystem specifically.