

Interactive comment on “Predominance of methanogens over methanotrophs contributes to high methane emissions in rewetted fens” by Xi Wen et al.

Anonymous Referee #3

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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

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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.

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?

Minor comments:

1. l. 190-193 Did the primers contain sequencing adapters and barcodes or were they added later?
2. l. 234-235 Please remove the word 'all' from 'suitable for detecting all aerobic

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methanotrophic Proteobacteria', or change to 'all known' or similar (we cannot assume to be able to detect the full diversity).

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.

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

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?

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