

Interactive comment on "Microtopography matters for CH₄ formation in a peat soil: a combined inhibitor and ¹³C study" *by* Johannes Krohn et al.

Anonymous Referee #2

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This manuscript examined the CO2 and CH4 production potentials in a depth profile at two different microforms in an oligotrophic fen, and attempted to characterize the dominant methanogenesis pathway through the use of two different approaches. The influence of microtopography on GHG production and emission has been well investigated by numerous studies. It is well known that both GHG production and the dominance of acetoclastic methanogenesis would decrease with peat depth. The authors need to place this study in proper context and point out the novel findings in light of the previous work done.

The methodology used in this study appears to have a number of flaws, which would adversely affect the reliability of data collected and validity of the findings generated. For example, the authors suggested that oxygen might be present in the glass jars after N2 flushing, leading to suppression of CH4 production in the controls. The soil was not

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flooded in the anaerobic incubation, and the addition of small volume of BES to soil samples without mixing raise concern about the degree of completion of the intended inhibition. Low CH4 concentrations also led to a number of samples being failed to be analyzed for 13C with IRMS, resulting in a limited sample size.

The manuscript also suffers from a lack of some essential information on site conditions and research methodology for readers to interpret the results. Some methods were not applied consistently (e.g. sampling period), and more detailed explanations/justifications are required. Overall, the discussion of this manuscript is not strong, and is over-speculative without strong support of ancillary data. Given this research site has been quite well studied with regards to GHG dynamics, relevant literature and/or data should be included for a more elaborate interpretation of the results.

Below are some specific comments on the manuscript:

Title – Only CH4 formation is mentioned, but CO2 production actually constitutes a considerable portion of the manuscript. The title should be revised to better indicate the actual contents covered in the manuscript.

L27-28 – Please specify the difference in % contribution.

L39 – "release" and "fluxes" are redundant. Use either "potential of releasing large CO2 and CH4…" or "potential of large CO2 and CH4 fluxes…".

L40 – Add "CO2 and CH4" between "both" and "are".

L47-55 – Include a more detailed review of previous findings on the effects of microtopography on peatland GHG dynamics here.

L48 – Do you mean "depressed hollows"?

L60 – Give the full term of SOM before using abbreviation.

L67 - A bit confusing to write "metals (Fe)" as Fe is just one of the many metals that exist in nature.

L93-94 – Replace "more wet hollows" with "wetter hollows". The deeper peat layers in both hummocks and hollows were inundated – how would that affect your hypothesis?

L97-98 – This hypothesis needs to be revised such that it could be tested scientifically – specify which pathway would be dominant in hummocks and hollows. The authors hypothesize here that the difference will be caused by substrate quality, yet the quality of peat in the two sites has not been adequately characterized in this study.

L106-108 – Given that the site has three main microforms, why the authors have decided to include only two microforms in this study but not all three for more comprehensive investigation? In another paper by the same group of researchers, three microforms were included in the analysis (Lozanovska et al., 2016). Moreover, some important site information (e.g. average water table, dominant plant community, etc.) associated with the two microforms should also be added for proper interpretation of results.

L111 – Any reasons for choosing these 5 depths for investigation? Given that the water table at hollow is rather shallow, the upper peat profile should be studied in greater resolution as this is the zone where the hydrologic regime is most distinctively different between the two microforms?

L112 – The meaning of "middle 10 cm section" is not clear. For example, for the depth of 15 cm, do the authors sample from the layer of 10-20 cm to represent this specific depth?

L123-128 – Why was gas production measured by sampling over a relatively short period of \sim 2 hours for 8 times throughout the whole study period? What was the rationale of determining production rate over such a short period, compared to determining an overall production rate over the whole study period by sampling gas periodically? Also, why were sampling frequency and duration differ between hummocks and hollows? How many jars exactly have been treated with BES, and those without as controls? Reasons should be given to support the chosen methodology.

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L130-132 – What exactly was meant by "comparatively effective"? Give more concrete figures. Why the BES concentration was chosen to be 1 mM? A higher concentration might ensure a more complete inhibition and thus be more conservative. Was there any disadvantage of using a higher concentration? Only 2 ml of BES was added to 15 g of soils – was this small volume enough to ensure saturation and hence complete inhibition? There was no shaking or other measures to ensure a good mix of soil and inhibitor neither.

L134-135 – No difference in CH4 production initially after BES amendment – does that mean no inhibitory effect? Why measurements only began 9 days after BES addition? Not sure about the linkage here.

L141-142 – What was the dilution rate? What exactly were the "suitable concentrations" chosen? Why was the number of measurements different between microforms? Further justification is needed.

L155 – The ratio is weight to volume?

L155-163 – Why did the authors only measure dissolved N but not other chemical species? Why did the authors use H2O for extraction, but not the common reagent KCI used in soil analysis? How was total dissolved N be determined? By acid digestion?

L169 – What was this "weighting", and how was it exactly done? Please give more information.

L179 – Give the mean and p values.

L191-192 – Why would CH4 production increase over time? This was not addressed in the discussion. One would expect CH4 production to decline with time as substrates become increasingly depleted.

L194-196 – If there was indeed oxygen left in the jar after N2 bubbling, then perhaps the whole experimental setup was flawed? If this is true, then I would expect CH4 production to be underestimated in the BES treatment as well?

L216 – I do not think it is appropriate to put "0" for concentrations under the detection limit – should use "trace" to represent instead. Was there significant difference in N concentration among depths or microforms?

L230 – Did the Becker et al study measure in situ CO2 emission from soil surface rather than CO2 production? If so, then the reported values would not be comparable with those of production determined in this study.

L234 – What were the "differences in SOM properties"? If hollows had more labile C (not sure if this was the case as no details were provided), one would expect CH4 production should be higher there?

L237-240 – Please further explain this sentence. How would the presence of aerobe and facultative anaerobe in hollows lead to the same CO2 production rate between the two microforms? I could not see a clear linkage here.

L276-280 – There was no mentioning of measuring these elements/compounds in the methodology section – please add these missing information. Why did the authors analyze total Fe, S and NH4 contents, but not the alternative electron acceptors (e.g. NO3, SO42-) that have been widely known to play a role in governing CH4 dynamics? That would give more meaningful analysis, and the Lozanovska et al (2016) paper published by their group should be relevant here. Figure 6 should first be presented in the results section.

L288-289 – Not sure if other studies involving delta 13C-CH4 have the same issue with the low concentration of CH4 for analysis?

L292-293 – Again cannot see the linkage – why inhibition by BES was not selective when the hydrogenotrophic pathway was dominant before BES addition?

L295 - Replace typo "culturs" with "cultures"

L314-316 – Why would the hydrogenotrophic pathway be more dominant in hollows? Were there any possible causes to account for this biogeochemical difference? Would

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there be differences in the amount and quality of root exudates from peatland vegetation, for example?

Figure 2 – Should show the data points and error bars for the control also. Y-axis should be "CH4 production rate" rather than "CH4 rate". Would CH4 production in the control for hummock at 50 cm depth on day 63 be an artefact? If this data point is removed, CH4 rate actually had little change over the whole incubation period.

Figure 5 – Soil N should be expressed in per unit mass of soil?

Figure 6 – Why only three depths were chosen for presentation here? The N size seems quite small for establishing relationships between the two variables. Was the fitted line statistically significant?

Reference Lozanovska, I., Kuzyakov, Y., Krohn, J., Parvin, S., & Dorodnikov, M. (2016). Effects of nitrate and sulfate on greenhouse gas emission potentials from microformderived peats of a boreal peatland: A 13C tracer study. Soil Biology and Biochemistry, 100, 182-191.

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