Biogeosciences Discussions

Biogeosciences Discuss., referee comment RC2
https://doi.org/10.5194/bg-2021-353-RC2, 2022

Comment on bg-2021-353

Anonymous Referee #2

Referee comment on "Genetic functional potential displays minor importance in explaining spatial variability of methane fluxes within a Eriophorum vaginatum dominated Swedish peatland" by Joel Dawson White et al., Biogeosciences Discuss., https://doi.org/10.5194/bg-2021-353-RC2, 2022

Factors that affect and/or govern methane emission from wetlands are of great interest because better understanding of the influential factors would enhance the predictability of methane emission from wetlands when subjected to environmental changes. This paper aims to assess the functional potential impact of CH4 producing and consuming microbes on the magnitude of CH4 flux. The authors concluded that the functional potential [of the methane cycling community] plays a minor role in explaining the observed differences in methane flux categories (HFM, MFM, LFM).

Major issues:

The key weakness of this paper is the use of genetic information of the methane cycling community alone in an attempt to address the scientific question the author set out. Firstly, the text does not provide clearly the reasoning of why the authors hypothesized that the differences in the measured methane flux (categories) could be explained by shifts in the composition of the methane cycling taxa in the 9 mesocosms. It would be to lay out the logic. And the data presented in this manuscript indicated that although 9 mesocosms contain different number of tillers (Fig. 2), they exhibited statistically comparable magnitude of CH4 fluxes. Authors also pointed out their understanding that gene expression would be a better proxy. Secondly, only abundance data of these methane cycling taxa relative to each other (i.e. the methane cycling community) was stated (or available). It is uncertain to this reviewer that how the PCR steps in the "captured metagenomics" analysis might have altered such relative abundance. And the use of "captured metagenomics" has, to the disadvantage of the study, prevented one from knowing the abundance of the methane cycling community relative to the total microbial community, as such relative abundance would be helpful to hint the proportion of the whole methane cycling community. These may explain why this study does not find significant correlation between the so-called "functional gene abundance" with the observed CH4 flux categories, when compared to Zhang et al. (2019, cited in this manuscript) that showed a positive correlation of gene abundance (absolute mcrA gene copy number) and methane flux. The use of relative abundance to the specific functional

group is less robust when compared to absolute gene copy numbers. Additionally, it was not obvious that the calculation of the "abundance data" was explained in details or with clarity. It will be helpful for the reads to understand how the sequencing data was process to obtain the abundance – Line 240-241, what data was being transformed? Was standardization or normalization done on the post-QC data? Some of the abovementioned points could be addressed by writing, but sadly, this weakness is a fundamental flaw that transcends through this manuscript, and affects the robustness of the analysis and interpretation

Specific comments:

L45 Missing "the" before "second most important", and please delete "has" in "has in the atmosphere"

L89 Is mmoX a commonly targeted gene in CH4 research? mmoX gene codes for the soluble methane monooxygenase, which is known to use substrates other than CH4. Did the authors mean to say mmoX or particular methane monooxygenase (pmoA)?

L98 "are detected" should be "to be detected"

L100 (there may be a better place to mention the following) mcrA gene is for detecting both methanogenic and methanotrophic archaea. Anaerobic methanotrophs (ANMEs) have been detected, albeit at very low abundance, in wetlands and permafrost-affected areas. Nonetheless, ANMEs have not been mentioned in this manuscript. In this study, *Methanosarcinales* were found among the methanogens, and *Methanosarcinales* contains ANMEs. Authors are suggested to investigate further whether ANMEs have contributed to the taxonomic and functional diversity in their data.

L114-115 Please clarify whether the "beta-diversity" here refers to both of the CH4 producing and consuming microorganisms. And please explain why such increases is thought to increase with increasing CH4 emission.

L142 Is the n=6 per mesocosm?

L166 This reviewer was not able to comprehend the phrase "based of comparison with isotopic mass spectrometer". Please rewrite to clarify.

L179-180 Please provide the access date and/or the version of KEGG database used in this

study.

L189-190 What is "low TE"? Please explain.

Section 2.7 It is not clearly stated that what data is being used to calculate the Bray-Curtis dissimilarity and the various statistical tests. This makes it a bit difficult to interpret the results.

L252 Should it be "between" CH4 fluxes, instead of "within"?

L271 What does "the flux of CH4 held a positive relationship to R_{eco}" actually mean?

L272-273 Authors explained that GPP is calculated from NEE and Reco (GPP = NEE – Reco). What was the reason for the authors to examine such correlation relationship stated in L272-273?

L280 Please add "statistically" before "significant".

L288-289 Is it possible that the less negative value was contributed to higher CH4 oxidation rate in M2 and M4?

L290-291 It is not intuitive as to why a relationship between CH4 flux and the Keeling intercept is investigated, and thus, what it meant if there is a significant relationship. To help readers to follow, please explain.

Figure 3. There are two apparent groups of Keeling intercepts in MFM. Is there any meaning to it? Also, there is a single LFM data point (orange at CH4 flux of ~260 umol m-2 h-1) appearing amidst of the MFM, any explanation why this LFM gave a higher CH4 flux compared to other 5 LFM datapoints? should this datapoint be omitted from the analysis?

L298-299 What unit is it? phyla OR OTU OR genera as in L307?

L308 It would be clearer to say "methanogenic community" (provided that ANMEs are not

detected), instead of “proportion.

L315 It should be “CH4 oxidizing”

L317 *Alphaproteobacteria* is at the class level (!)

L327-329 Such statement is not meaningful in statistics.

L344 the second and third highest “dissimilarity”

Table 1-6 Please explain to the readers how to understand the p-value.

Perhaps missing “in”, in average “in” MFM and HFM?

L369 “CH4 metabolism (PATH: KO00680) made up 17% of the captured genes” … this is confusing because this reviewer learned from the earlier text that “captured metagenomics” data targeted only “the CH4 production and oxidation in pathway map00680” by using the 193,386 individual designed probes.

L382 How should one understand the term “cumulative sum”? Please clarify and provide guidance to readers.

L382-405 It was not easy to follow the comparisons and the results are very similar in the three comparisons.

Discussion When referring to specific results obtained in this study, please cite the corresponding figures/tables. This is helpful for readers to follow and evaluate the arguments.

L431-432 Error: “Proteobacteria” should be before “and”

L435-436 It is not clear what "observed pathways" are being referred to...As stated here,

d13C suggested dominant methane production pathway but d13C does not inform consumption pathways. Genomic information tells only the metabolic potential.

L441-442 Please provide information about "the absence of acetogenesis and fermentation"...then it would be helpful for readers to relate the following statement "the less dominant functional...." at their study site.

L445 The "spatial" info of the highly variable CH4 flux is not given, and it would be good for readers to know the spatial variability represented by M1-M9.

L449-450 R_{eco} was measured for the mesocosm, meaning that the high respiration was a result of the whole community. This reviewer considers that it is inappropriate to use captured metagenomes (targeting methane cycling community) to explain an observation coming from the whole community. Therefore, this statement is considered weak or even misleading.

L492 *Methylocella* is a close relative of high-affinity methanotrophs (upland soil cluster alpha). Would any of the detected *Methylocella* data be coming from high-affinity methanotrophs?

L506 Choice of word. Should not use “were”.

L532 Depending the database used for gene annotation, CODH is a symnonym for carbon monoxide dehydrogenase. CODH/ACS is being used by many if not all methanogens in the reductive acetyl-coA pathway for CO2 fixation, so it is not surprising to see that in the results. And though hdr and CODH genes do not directly involve in methane producing pathway, they are essential for the living of methanogens.

L548 “our” is likely a typo.

L553 The word “indicating” is too strong. And please be more specific to say the microbial group, and not just “microbes”.

L566 The discussion will benefits if authors further elaborate on what they think about the trophic status of methanogenesis in HFM, MFM and LFM.

L576 Is it right that it is over 50% of the methane-cycling community? Please clarify.