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).

The authors thank the reviewer for their comments. We have addressed the major issues in individual sections rather than writing a lengthy text at the bottom. Please find our responses below. In addition, each specific comment has been addressed individually.

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

Authors response: We agree that the CH_4 cycle is not simple and does not operate individual of other processes. However, to reduce the complexity of this system, we kept temperature and light levels the same among treatments while the water table was kept stable throughout the experiment (section 2.2). This minimizes the influence of these drivers on spatial variability, which should enhance differences arising from CH_4 cycling functional genes and taxonomy. We believe that using our targeted approach is a strength, rather than a weakness as we can observe many functional genes used across multiple metabolic pathways, which cannot be achieved when using 16s studies. In addition, we were able to make conclusions not observed in more complex whole metagenomic studies.

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.

Authors response: We aim to clarify this by adding additional text to the introductory section. See revisions below.

Original: CH_4 emissions from natural wetlands are known to exhibit both spatial and temporal variability (Crill et al., 1988; Sun et al., 2013). The spatial variability makes wetland CH_4 emissions difficult to model and predict (Wania et al., 2009, 2010), as CH_4 emission within similar environmental conditions (i.e. ecotype) can vary by several orders of magnitude without an apparent explanation (Bridgham et al., 2013). According to current knowledge, both production and consumption of CH_4 within peatland ecotypes is driven by (i) water table depth (WTD), which determines the thickness of oxic and anoxic zones; (ii) plant species composition, which provides substrates and plant mediated transport of CH_4 to the atmosphere; (iii) soil temperature, which affects the rate of microbiological processes; and (iv) substrate availability for biogeochemical processes such as methanogenesis and methanotrophy (Joabsson et al., 1999; Korrensalo et al., 2018; Mastepanov et al., 2013; Strack et al., 2004; Ström et al., 2015).

Authors revision L66-75: CH_4 emissions from natural wetlands are known to exhibit both spatial and temporal variability (Crill et al., 1988; Sun et al., 2013). The spatial variability makes wetland CH_4 emissions difficult to model and predict (Wania et al., 2009, 2010), as CH_4 emissions under similar environmental conditions (i.e. ecotype) can vary by several orders of magnitude without an apparent explanation (Bridgham et al., 2013). According to current knowledge, the magnitude of CH_4 fluxes in peatlands is driven by (i) water table depth (WTD), which determines the thickness of oxic and anoxic zones; (ii) plant species composition, which provides substrates and plant mediated transport of CH_4 to the atmosphere; (iii) soil temperature, which affects the rate of microbiological processes; and (iv) substrate availability for biogeochemical processes such as methanogenesis and methanotrophy (Joabsson et al., 1999; Korrensalo et al., 2018; Mastepanov et al., 2013; Strack et al., 2004; Ström et al., 2015). Interestingly, the microorganisms which produce and consume CH_4 are either not included in models or assume that no spatial variability occurs in the functional potential of these communities (Chadburn et al., 2020). Rather, they picture the below ground microbial community as a uniform black box. Therefore, the need to research whether the functional potential of the microbial community contributes to the spatial variability has become more important in improving model predictions of CH_4 emissions from peatlands.

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.

Authors response: The reviewer is correct in saying that the data indicated that 9 mesocosms contains different number of tillers, a common driver of CH_4 emissions. However, the 9 mesocosms did not exhibit statistically comparable magnitudes of CH_4 fluxes, rather significantly higher or lower CH4 flux depending upon the group (section 3.1.1). This difference

can be observed in figure 1 and was the reason for establishing three flux categories (LFM, MFM and HFM), and the basis for the paper. The mention of gene expression on line 587 is merely a suggestion for future research used in our conclusion.

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. Some of the above mentioned 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

Authors response: The research has been conducted using the captured metagenomics approach. We used this approach as we wanted to narrow our research question and try not to overcomplicate our conclusions by including a broader whole metagenomic approach. As this approach uses custom designed probes to target sequences of interest, any off-target sequences (i.e. non methanogen/methanotroph) within our dataset must be ignored and we cannot trust those values to be correct. Therefore, we chose to exclude any other taxa other than methanogens and methanotrophs. During the PCR step, 7 cycles were used for libraries with a genomic DNA input of 150 ng, and 5 cycles where the input was 1 µg to minimise any risk of PCR biases (section 2.5.3). In addition, we did not see any correlation between the samples with low amount of DNA versus those with high. We use the term relative abundance throughout this paper as we cannot call it absolute abundance since that is not what we are measuring; Rather, when using next generation sequencing, we always get relative abundances.

Our results are different from Zhang et al. because we have used a different approach. Zhang et al used absolute abundance, while we focused on the methane producing / consuming community. The main aim of our research was to address whether the composition of both $_{CH4}$ producing and consuming taxa/functional genes shift in dissimilarity in response to variations in CH_4 fluxes, and not whether individual genes such as mcrA correlate to the magnitude of CH_4 flux. This is already well established by Zhang et al. and other studies. Sequence data and calculated abundance were all obtained through the MG-RAST annotation pipeline which is

referenced in section 2.6. We choose not to include all the steps within the MG-RAST pipeline since the pipeline is a well-established method and readers can access more details through the Meyer et al., 2008 reference.

Specific comments:

L 240-241, what data was being transformed? Was standardization or normalization done on the post-QC data?

Authors response: We used a double root transformation on abundance of taxa and functional genes as a form of normalization. We clarified this in the text as follows:

Original: Input data for the PERMANOVA was double root transformed to reduce the influence of highly abundant taxa and genes.

Authors revision: Taxonomic and gene abundances data for the PERMANOVA was double root transformed to reduce the influence of highly abundant taxa and genes.

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

Authors response: This change has been made according to the reviewer's suggestion

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)?

Authors response: As stated in the text mmoX is often targeted in similar experiments, however as we particularly focus upon pmoA in this manuscript and believe the swap to pmoA to be more appropriate. This change has been made according to the reviewer's suggestion

Original: In CH_4 research, key genes such as methyl coenzyme M reductase (mcrA) and methane monooxygenase component A alpha chain (mmoX) are often targeted to determine community composition and functional potential

Authors revision: In CH_4 research, key genes such as methyl coenzyme M reductase (mcrA) and particulate methane monooxygenase subunit A (pmoA) are often targeted to determine community composition and functional potential

L98 “are detected” should be “to be detected”

Authors response: This change has been made according to the reviewer’s suggestion

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.

Authors response: we have added a shot passage of text within the discussion to reflect this. Members of the order Methanosarcinales were included in the calculation of diversity indexes, therefore this will not alter the diversity results.

Original L428: However, the presence of the genera acetoclastic Methanosaeta and Methanosarcina, which possess a more diverse genome allowing them to perform hydrogenotrophic, acetoclastic and methylotrophic methanogenesis, suggests that the community holds a metabolic potential to produce CH_4 under altered environmental conditions.

Authors revisions: However, the presence of the acetoclastic genera Methanosaeta and Methanosarcina, which possess a more diverse genome allowing them to perform hydrogenotrophic, acetoclastic and methylotrophic methanogenesis, suggests that the community holds a metabolic potential to produce CH_4 under altered environmental conditions. Furthermore, members of order Methanosarcinales were also detected that hold the functional potential to perform anaerobic oxidation of CH_4 and is carried out by anaerobic methane-oxidizing archaea, further increasing the functional potential of the methanogenic community.

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.

For clarification, we have rewritten this sentence:

Original: (2) determine whether the β-diversity increases with increasing CH_4 emission

Authors revision: (2) determine whether the combined CH_4 producing and consuming community β-diversity increases with higher CH_4 flux

L142 Is the n=6 per mesocosm?

We have rewritten this sentence to clarify this:

Original: During the experiment, weekly to bi-weekly (final 3 weeks, n = 6) measurements of CO_2 and CH_4 fluxes were conducted.

Authors revision: During the final three weeks of the experiment, bi-weekly measurements of CO_2 and CH_4 fluxes were conducted (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.

We understand that this phrase may be confusing. We have adjusted this section as follows:

Original: The CH_4 emission and its $\delta_{13}C$ signature were determined using a cavity ring-down laser absorption spectrometer (CRDLAS) with the closed chamber technique described above (G2201i, Picarro, Santa Clara, USA). The surface of each peat mesocosm was covered with a transparent cylindrical chamber for 25-30 minutes while the CH_4 mixing ratio and $\delta_{13}C$-CH_4 was recorded with 1 second intervals. Data was averaged into one minute averages. CH_4 emission were calculated using linear fitting, and the $\delta_{13}C$ signature of emitted CH_4 was determined with a Keeling plot intercept approach (Keeling, 1958; Thom et al., 1993). The resulting $\delta_{13}C$-CH_4 values were corrected by adding a constant value of 3.4 ‰, based of comparison with isotopic mass spectrometer.

Authors revision: The CH_4 emission and its $\delta_{13}C$ signature were determined using a cavity ring-down laser absorption spectrometer (CRDLAS) with the closed chamber technique described above (G2201i, Picarro, Santa Clara, USA). The surface of each peat mesocosm was covered with a transparent cylindrical chamber for 25-30 minutes while the CH_4 mixing ratio and $\delta_{13}C$-CH_4 was recorded with 1 second intervals. Data was averaged over one minute and the $\delta_{13}C$ signature of emitted CH_4 was determined with a Keeling plot intercept approach (Keeling, 1958; Thom et al., 1993). We compared values from the CRDLAS instrument with an isotope ratio mass spectrometer (IRMS) by taking air samples from the flux chamber during measurements from the CDRLAS and analyzing these with the IRMS (Rinne et al., 2022). The values from the IRMS indicated a bias of -3.4 ‰ on the CRDLAS, thus we have corrected the values of the $\delta_{13}C$ signature by adding 3.4 ‰.

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

We have changed the text accordingly:

Original: Genes encoding enzymes closely related to the CH_4 production and oxidation in pathway map00680 were identified from the Kyoto Encyclopedia of Genes and Genomes (KEGG).

Authors revision: Genes encoding enzymes closely related to the CH_4 production and oxidation in pathway map00680 were identified from the Kyoto Encyclopedia of Genes and Genomes (KEGG), database version 88.

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

We have rewritten this sentence to better explain this:

Original: Depending on the extracted DNA concentration, 150 ng or 1 μg of genomic DNA in a total volume of 100 μl low TE

Authors revision: Depending on the extracted DNA concentration, 150 ng or 1 μg of genomic DNA in a total volume of 100 μl low Tris-Ethylenediaminetetraacetic acid buffer (TE buffer).

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.

We have rewritten this sentence to clarify the data used for these tests:

Original: Further statistical tests for use on genomic data, including the Permutational multivariate analysis of variance (PERMANOVA), α-diversity and β-diversity, and Nonmetric Multidimensional Scaling (NMDS)

Authors revision: absolute abundances for taxonomic and functional sequences from the KEGG ko:00680 metabolism pathway were used as input for the statistical tests including the Permutational multivariate analysis of variance (PERMANOVA), α-diversity and β-diversity, and Nonmetric Multidimensional Scaling (NMDS).

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

Changed to "among"

Original: After observing such large variability within CH_4 fluxes

Authors revision: After observing such large variability among CH_4 fluxes

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

We mean "correlated positively" and have changed the text to clarify this:

Original: In an attempt to investigate the relationships between carbon fluxes we conducted a correlation test and found that the flux of CH_4 held a positive relationship to R_{eco}

Authors revision: In an attempt to investigate the relationships between carbon fluxes we conducted a correlation test and found that the flux of CH_4 correlated positively to R_{eco}

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?

We examined this due to the influence of GPP and Reco on available substrate. We have changed the text to make this point more clearly:

Original: In an attempt to investigate the relationships between carbon fluxes we conducted a correlation test and found that the flux of CH_4 held a positive relationship to R_{eco} ($R^2 = 0.60$, $p \leq 0.04$), but not to GPP or NEE (fig 2). When analysing CO_2 fluxes, GPP held a strong negative relationship to R_{eco} ($R^2 = 0.70$, $p \leq 0.002$), while NEE held a strong positive relationship to GPP ($R^2 = 0.82$, $p \leq 0.001$) (fig 2).

Authors revision: Previous research has shown that CH_4 flux holds a strong correlation to both GPP and R_{eco}, which can influence the availability of CH_4 substrates (Ström et al., 2005). In an attempt to investigate whether the relationships between carbon fluxes matched previous research, we conducted a correlation test and found that the flux of CH_4 held a positive relationship to R_{eco} ($R^2 = 0.60$, $p \leq 0.04$), but not to GPP or NEE (fig 2). When analysing CO_2 fluxes, GPP held a strong negative relationship to R_{eco} ($R^2 = 0.70$, $p \leq 0.002$), while NEE held a strong positive relationship to GPP ($R^2 = 0.82$, $p \leq 0.001$) (fig 2).

L280 Please add "statistically" before "significant".

Authors response: This change has been made according to the reviewer's suggestion

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

Authors response: This is of course possible, as the d13C of the emitted methane reflects both processes involved in methanogenesis and methanotrophy. However, combining the d13C value with the fact that this mesocosm had high methane emission makes it likely that the variation is caused by the methanogenesis, not methanotrophy (e.g. Hornibrook 2009; Rinne et al., 2022). We will include a paragraph on the interpretation of d13C in relation to methane emission in discussion section (see next response).

Original: L564-566: Furthermore, the positive correlation between $\delta_{13}C$-CH_4 to CH_4 emission rate indicates the CH_4 emission to be mostly controlled by the trophic status for methanogenesis, rather than methanotrophy (Hornibrook, 2009).

Authors revision: Furthermore, the positive correlation between $\delta_{13}C$-CH_4 to high CH_4 emission rates, especially observed in HFM, indicates that the CH_4 emission is mostly controlled by the trophic status for methanogenesis, rather than methanotrophy (Hornibrook, 2009).

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. Explain the keeling method more clearly, why we use keeling to investigate ch4 fluxes

We have revised the text to explain this further:

Original: Distinct isotopic signatures of individual mesocosms are shown in fig 3. All mesocosms fell within the range of hydrogenotrophic methanogenesis ($\delta_{13}C = -110‰$ to $-60‰$) (Chanton, 2005; Whiticar, 1999). However, M2 (MFM) and M4 (HFM) indicated a slight tendency towards acetoclastic methanogenesis with less negative isotopic signature ($\delta_{13}C$ = -60‰ to -50‰), both yielding mid -60‰ $\delta_{13}C$ Keeling intercepts. A significant positive correlation ($R_2 = 0.5$, $p \leq 0.001$) and significant relationship also existed between CH_4 flux and the Keeling intercept shown in fig 3.

Authors revision: Distinct isotopic signatures of individual mesocosms are shown in fig 3. The relationship between d13C and CH_4 fluxes can be indicative of the processes controlling the spatial variability of the CH_4 emissions (Hornibrook 2009; Rinne et al., 2022). A positive correlation between d13C and CH4 fluxes indicates that the variation is due to the substrate availability for methanogenesis, while a negative correlation is indicative for methanotrophy to

be the dominant cause for the variability of CH_4 flux. All mesocosms fell within the range of hydrogenotrophic methanogenesis ($\delta 13C = -110‰$ to $-60‰$) (Chanton, 2005; Whiticar, 1999) and held a significant positive correlation ($R2 = 0.5$, $p \leq 0.001$), indicating the dominant methanogenesis pathway to be hydrogenotrophic. However, M2 (MFM) and M4 (HFM) indicated a slight tendency towards acetoclastic methanogenesis with less negative isotopic signatures ($\delta 13C = -60‰$ to $-50‰$), both yielding mid -60‰ $\delta 13C$ Keeling intercepts.

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?

Authors response: The division of mesocosms to LFM, MFM and HFM groups was based on their average methane emission rates while Figure 3 shows the individual measurements of methane emissions. At one time, the emission from LFM and MFM mesocosms was high, leading to the data point mentioned moving away from the other data points. As this study is focused on a replicated peak growing season, and not a temporal scale, we prefer not to remove the data point just because it is an outlier.

L298-299 What unit is it? phyla OR OTU OR genera as in L307? (Add and genus level)

We have changed this sentence to clarify this:

Original: In total, 20 methanogenic Archaea and 5 methanotrophic Bacteria were detected.

Authors response: In total, 20 genera of methanogenic Archaea and 5 methanotrophic Bacteria were detected.

L308 It would be clearer to say "methanogenic community" (provided that ANMEs are not detected), instead of "proportion.

Authors response: This change has been made according to the reviewer's suggestion

L315 It should be "CH4 oxidizing"

Authors response: This change has been made according to the reviewer's suggestion

L317 Alphaproteobacteria is at the class level (!)

Added this information:

Original: 5 genera of CH_4 reducing Bacteria were detected including methanotrophs from Alphaproteobacteria, Gammaproteobacteria and Verrucomicrobia class.

Authors response: 5 genera of CH_4 reducing Bacteria were detected including methanotrophs from class level Alphaproteobacteria, Gammaproteobacteria and Verrucomicrobia.

L327-329 Such statement is not meaningful in statistics.

Authors response: This sentence has been removed according to the reviewer's suggestion

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?

Author response: The explanation of the p-value is described in the table text "p-value of the permutation test (Probability of getting a larger or equal average contribution in random permutation of the group factor)"

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. (explain about off target hybridization)

Authors response: The method of captured metagenomics allows the user to target high number of genes sequences, but as with any method it is not perfect. Off target sequences are known to hybridize to the custom designed probes if they hold a high enough similarity to the binding site. Therefore, we filter out the off target sequences using bioinformatics. One such way is by using the MG-RAST pathway filter, i.e. path 00680 for the CH4 cycle.

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

We have changed the text to explain this:

Original: In total, 21 genes of the 109 contributed to 70% of the cumulative sum (table 4, 5 and 6).

Authors response: In total, 21 genes of the 109 contributed to 70% of the cumulative sum, i.e. the contributions for each gene in descending order (table 4, 5 and 6).

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

Authors response: Yes, the results for the three comparisons are very similar and thus difficult to interpret for a clear take home message. Throughout the passage of text, we have referred to tables, figures and statistics to clarify the reader. We believe this further reinforces our conclusion that the functional potential of the methane producing and consuming community displays minor importance in explaining spatial variability of CH_4 fluxes.

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.

Authors response: This change has been made according to the reviewer's suggestion, please find references to tables and figures now within the discussion.

L431-432 Error: "Proteobacteria" should be before "and"

Authors response: This change has been made according to the reviewer's suggestion

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.

We understand that this sentence may be misinterpreted, which is why we have rewritten it as follows:

Original: we can expect CH_4 production and consumption to still occur, but possibly using alternative metabolic pathways than currently observed

Authors revision: our results indicate that we can expect CH_4 production and consumption to still occur, as the community holds the functional potential to continue producing or reducing CH_4, possibly using alternative metabolic pathways such as acetoclastic or methylotrophic methanogenesis.

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.

Information added.

Original: In the absence of acetogenesis and fermentation, the less dominant functional groups (i.e. acetoclastic and methylotrophic methanogens) may still remain dormant, due to the absence of necessary substrates to metabolize.

Authors revision: In the absence of acetogenesis and fermentation, that produce the necessary products for acetoclastic and methylotrophic methanogenesis, the less dominant acetoclastic and methylotrophic methanogens may still remain dormant due to the absence of necessary substrates to metabolize.

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.

Authors response: We have added a reference to figure 1 in this sentence. The variability within and across different mesocosms is apparent from the boxplots.

Original: We observed a high spatial variability in CH_4 flux, which is consistent with research conducted in other temperate peatlands

Authors revision: We observed a high spatial variability in CH_4 fluxes (fig 1), which is consistent with research conducted in other temperate peatlands

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.

We agree with the reviewer that Reco is the result of a much larger community than just the methane cycling community, and it also includes autotrophic respiration from the plants. We have weakened this statement as follows:

Original: One potential reason for the high respiration from HFM could be the significantly higher relative abundance of pmoA. The pmoA gene codes for the first step in methanotrophy, where CH4 is reduced to methanol, and finally CO2, which is often used as a proxy for methanotrophy (Franchini et al., 2015; Freitag et al., 2010). The higher abundance of pmoA may indicate a higher rate of methanotrophy, which may help to explain the higher CO2 flux respired by the methanotrophs in HFM. In addition, higher plant productivity causes higher autotrophic respiration, which generally makes up ~50% of Reco. However, the vegetation may also be supplying more substrates to the microbial community, which in turn is consumed and respired in the form of CO2.

Authors revision: The high respiration from HFM coincides with a significantly higher relative abundance of pmoA. The pmoA gene codes for the first step in methanotrophy, where CH_4 is reduced to methanol, and finally CO_2, which is often used as a proxy for methanotrophy (Franchini et al., 2015; Freitag et al., 2010). The higher abundance of pmoA may indicate a higher rate of methanotrophy, which would contribute to higher respiration. However, as we have used a targeted approach, we cannot conclude that the pmoA gene is significantly higher than other carbon reducing genes outside of methanotrophy. Moreover, autotrophic respiration from plants can be as large as heterotrophic respiration in peatlands, which further complicates this picture (see e.g. Järveoja et al. 2020). Thus, we can only conclude that methanotrophy can contribute to higher Reco but it is not the only contributor.

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?

Author response: We can of course not guarantee that all taxonomic assignments are 100% correct all the time, however, there appears there are enough high-affinity methanogen sequences that the classifications would hold. Also, do we know what the ppm levels of methane would be in our samples? High-affinity methanotrophs may become a factor if the methane has very low. Regardless, since we do not see any significant change in the abundance between the groups, I suggest that the taxonomic assignments are trustworthy.

L506 Choice of word. Should not use "were".

Changed accordingly.

Original: contrary to results found by Zhang et al. (2019) were the authors observed significant correlation between mcrA and CH_4 flux.

Authors revision: contrary to results found by Zhang et al. (2019) where the authors observed significant correlation between mcrA and CH_4 flux.

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 involved in methane producing pathway, they are essential for the living of methanogens.

Authors response: I believe you have written it very clearly and we agree with your statement. We have added this information to the text.

Original: These genes code for CO dehydrogenase and are involved in the Acetyl-CoA pathway, which is not directly included in methanogenesis.

Authors revision: These genes code for CO dehydrogenase and are involved in the Acetyl-CoA pathway, which is not directly involved in methane producing pathway, but are essential for the living of methanogens, therefore it is expected to observe CO dehydrogenase genes in high abundance.

L548 "our" is likely a typo.

The typo has been removed.

L553 The word "indicating" is too strong. And please be more specific to say the microbial group, and not just "microbes". (Suggesting)

Changed to 'suggesting'

Original: HFM held the highest dissimilarity indicating that as the CH_4 flux increases, the abundance and variability of microbe's increase.

Authors revision: HFM held the highest dissimilarity, suggesting that as the CH_4 flux increases, the abundance and variability of microbial group.

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

Authors response: we have added additional discussion material following L566.

Authors revision: Covariation of d13C-CH4 with methane emission rates suggests that the spatial variation in methane emissions are determined largely by the variations in the precursor availability and thus trophic status within a mire (Rinne et al., 2022). Similarly, within the mesocosms studied here, the d13C-CH4 correlates positively with methane emission rates between the mesocosms and flux categories, indicating the trophic status to exert major control on this variation.

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

Authors response: Yes, this statement is correct. For clarity we have added that it is 50% of the methane producing and consuming community.

Original: The dominant methanogen, Methanoregula, made up over 50% of the community composition.

Authors revision: The dominant methanogen, Methanoregula, made up over 50% of the methane producing and consuming community composition.