Anonymous Referee #1

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Review of manuscript bg-2019-11

This study address multiple types of CH4 emissions in wetlands (ebullition, diffusion and plant-mediated flux), their temporal variability (diurnal cycles and seasonal differences), the spatial variability among four wetland vegetation communities in both permanent och seasonal wetlands, and links to wetland soil properties. Hence, it standsout as a potentially valuable study for improved understanding of wetland CH4 emissions. However, I have some concerns and questions below that I think should be addressed

We thank reviewer 1 for their constructive comments and suggestions, we have responded to each of these comments in blue font below.

General comments:

It would be good to early on clarify that the word wetland is here used in a broad sense including both wet vegetated environments and open waters/lakes.

We agree, this now reads (lines 52-54):

"Wetlands are considered one of the most valuable ecosystems on Earth (Costanza et al., 2014) and may be classified as both permanently inundated (i.e lakes and shallow waters) and seasonally inundated (i.e. vegetated) biomes."

L 160 and elsewhere: In warm environments, bubbling can sometimes happen rather continuously leading to very high R2 values (I have experience this myself several times in the tropics). Given the short measurement periods and the very high flux rates sometimes found from the floating chambers, I wonder if they did not received considerable bubbling in such a continuous way leading to linear increase in the headspace.

We agree this can occur. However, we are also confident that we were able to detect discrete ebullition. For example, our companion paper now published (*Jeffrey, L. C., Maher, D. T., Johnston, S. G., Kelaher, B. P., Steven, A. and Tait, D. R. (2019), Wetland methane emissions dominated by plant-mediated fluxes: Contrasting emissions pathways and seasons within a shallow freshwater subtropical wetland. Limnol Oceanogr. doi:10.1002/lno.11158*) focuses solely on aquatic emissions and provides examples (Fig. S3 – see below) of disregarded floating chambers featuring ebullition bubbles.

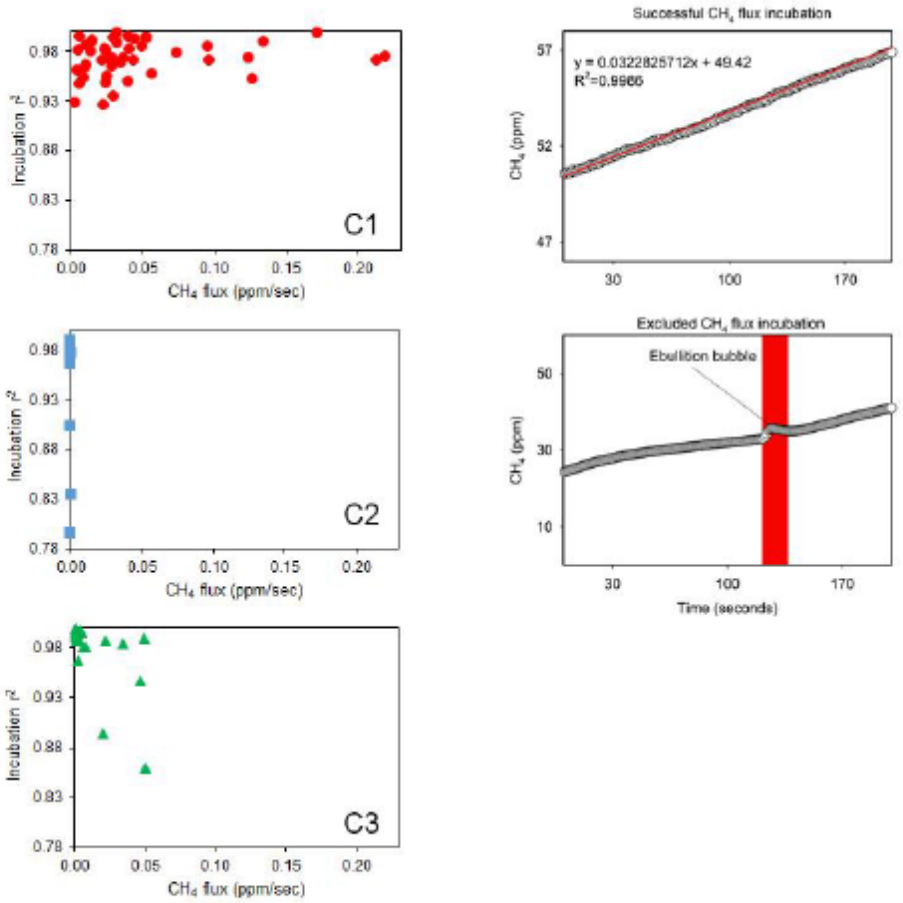

Figure S3. Examples of CH_4 flux linear regression r^2 vs flux rate (ppm/sec) for each campaign (left column) and samples of floating chamber CH_4 fluxes showing increasing CH_4 concentration (ppm) vs time (per second) indicating a successful incubation (top right panel) and an excluded incubation (bottom right panel) due to ebullition bubble release.

To identify this in the manuscript we have added the following:

"One chamber measurement was removed as an outlier (as it was more than three times the standard deviation of the mean) and any chambers capturing ebullition bubbles (determined by a nonlinear increase in concentration) were also disregarded, see example in Jeffrey et al. (2019)."

The high variability in the diffusive flux in Fig 3 also seem to support this guess. Are there any data on surface water concentrations of CH4 that could be used together with modelled piston velocities to estimate diffusive flux, or are there any other independent data to verify the high fluxes found as diffusion fluxes? If not, I would hesitate to report the very high fluxes (up to 10 mmol m-2 d-1) as diffusion and I would instead report values from flux chambers as total open water flux including both diffusion and ebullition. This would be a minor loss for the manuscript, compared to the risk of considerably overestimating diffusive fluxes.

We have recently published a companion paper focused on diffusion, ebullition and plant mediated fluxes from the same site using the same techniques (Jeffrey et al. 2019). In this companion paper we assessed water column concentrations, chamber-derived diffusive fluxes, and calculated convection-driven fluxes. That study highlighted that there was both temporal variability in water column CH4 concentrations (CH_4 ranging from ~60 uM to 250 uM over a diurnal cycle), and also spatial variability with water column CH_4 ranging from 7 to 254 uM throughout the wetland. We also found that convection (occurring only during night) could enhance the piston velocity by up to 17%.

It is likely that this spatial and temporal variability in water column concentrations is the main driver of the observed variability on our current chamber flux estimates. We do not have water column CH4 concentrations from the field campaigns in this present study - however, considering the extremely high water column CH_4 concentrations observed in our companion paper (averaging ~ 80 uM), a diffusive flux rate estimate of 10 mmol/m2/d is not extreme and would only require a piston velocity of ~ 0.5 cm/hr. This is piston velocity is similar to the diffusive transfer velocities in wetland measured by deliberate gas tracer experiments (e.g. Ho et al., 2018).

I think that it is difficult to claim that this study cover seasonal differences for the CH_4 emissions, which are known to have a high day-to-day variability, because there seems to have been on measurement day per season only.

We agree. We accounted for high resolution measurements however these were snapshots in time. We have removed reference to our fluxes representing 'seasonal' differences from the following lines:

Abstract (lines 32-34): "*We account for aquatic CH_4 diffusion and ebullition rates, and plant-mediated CH_4 fluxes from three distinct vegetation communities, thereby examining diurnal and intra-habitat variability"*

Lines 346-351: ***"Figure 5.*** *Fluxes of CH_4 from diel sampling and ebullition over two campaigns from the permanent wetland and adjacent 24 h time series of the seasonal wetland vegetation types. Note: Diffusive fluxes during C2 include chambers featuring lilies, dashed line represents the average, solid line represents the median and dots represent 5^{th} and 95^{th} percentiles. Letters show groups that did not differ significantly ($p>0.05$) using ANOVA on ranks and Dunn's pairwise comparisons within each campaign."*

Lines 369-370: ***"Figure 6.*** *Correlations of CH_4 with temperature (ºC) and photo-synthetically active radiation (PAR) (lum ft^{-2}) for the three wetland vegetation sites of Cattai Wetland during two field campaigns."*

Lines 408-409: *"This was associated with the lowest fluxes of CH_4 for both sampling periods (Fig. 5, Table 1)."*

Lines 467-469: *"These were similar to our findings with highest CH_4 fluxes of each campaign time series occurring near midday (10:50 am during C1; 4.88 mmol m^{-2} d^{-2} and 12:15 pm during C2; 2.06 mmol m^{-2} d^{-2}) (Fig. 3)."*

Lines 554-556: *"Our CH_4 emissions rates were at the low end of the scale of measurements made in southern hemisphere subtropical systems but within range of northern hemisphere subtropical systems of similar latitudes (Fig. 9)."*

Lines 593-597: Conclusion: *"Results reveal distinct differences between the areal CH_4 fluxes of four different eco-types located within a remediated subtropical Australian wetland and indicate high*

variability between campaigns. By combining novel and well established techniques we delineated several CH_4 pathways of both seasonal and permanent wetland sources (ebullition, diffusion and plant-mediated pathways) and linked these to hydrological drivers."

Specific comments:

Abstract: Please define "AVS".

Amended.

L84-86: Tiny language thing: Two "now" in same sentence.

Amended.

P156-158. How many replicate floating chamber measurements were performed during each measurement time at each location, and how many measurements times during each campaign?

We have added details as follows (Lines 175-177):

"A total of 39 CH_4 floating chamber incubations averaging ~8 minutes in duration were recorded over the two campaigns, with 19 during C1 (nine at night) and 30 during C2 (12 at night)."

L185: 10 minute intervals in the daytime sampling would return in the order of 4-6 measurements per hour, but Figure 4 does not show that many points. Were fluxes really measured at 10 min intervals as said here?

We agree this was potentially confusing, this was the approximate intervals between incubation start times. The manuscript stated incubation times '*Vegetation incubation times ranged from 6 to 15 minutes*'. To clarify the number of vegetation incubations measured each day and night, per campaign, we have re-worded this paragraph as follows (lines 205-212):

"*During the first time-series (C1), an average of 16.7 ± 2.9 daytime flux measurements (i.e. after sunrise) and 7.3 ± 1.6 night time (i.e. after sunset) were recorded within each habitat. During the second campaign (C2) an average of 27.7 ± 2.9 (day time) and 10.3 ± 1.5 (night time) flux measurements were recorded within each habitat. In addition, CH_4 fluxes from the adjacent exposed sediments or shallow overlying water at each site were also measured at ~4 hourly intervals to determine the influence and role of plant-mediated CH_4 fluxes compared to non-vegetated CH_4 fluxes…."*

L226-230: Please show unit and value of R, as there are several versions to choose from. Should there not be a conversion from ppm to partial pressure in the equation, e.g. s*(1/1000000)*Total_Pressure?

R is in the units of $m^3.atm.K^{-1}.mol^{-1}$, which has a value of $8.205*10^{-5}$ in this case. Text has been added to clarify this point. We assume atmospheric pressure is 1 atm in our calculations, this has been added to the methods section (Lines 251-256):

" $F = (s(V/RT_{air}A))t$ *(1)*

where s is the regression slope for each chamber incubation deployments (ppm sec^{-1}), V is the chamber volume (m^3), R is the universal gas constant (8.205×10^{-5} $m^3.atm.K^{-1}.mol^{-1}$), T_{air} is the air temperature inside the chamber (K), A is the surface area of the chamber (m^2) and t is the conversion factor from seconds to day, and to mmol. We assume that atmospheric pressure is 1 atm."

Given the variability, was there really a significant difference between day and night?

We have performed statistical analysis to assess differences between day and night fluxes and have added the following to the manuscript methods (Lines 262-266):

"2.6 Statistical analysis

As the CH_4 flux data was non-parametric we used a Kruskal-Wallis one way analysis of variance (ANOVA) on ranks to test for significant differences between each campaign, between flux pathways and between diel variability, where $p<0.001$. Dunn's multiple pairwise comparisons were then used to analyse specific sample pairs ($p<0.05$)."

And abstract (lines 39-39):

…"Significantly higher CH_4 emissions ($p<0.001$) of the seasonal wetland were measured during flooded conditions…"

And to our results (lines 334-351):

"CH_4 fluxes from the three vegetation types were significantly higher during C1 than during C2 ($p<0.001$). During C1, the CH_4 fluxes from the Juncus and Phragmites were not significantly different from each other but were both significantly higher ($p<0.001$) than Juncus/Forest however, during C2 the CH_4 fluxes of each seasonal wetland habitat were significantly different between all habitats ($p<0.05$) (Fig. 5). The highest average CH_4 fluxes in each of the vegetation types always occurred during the daytime but were not significantly different to night time fluxes (Fig. 5, Table 1). Phragmites consistently emitted the highest CH_4 fluxes (2.27 ± 1.42 mmol m^{-2} d^{-1} during C1 and 0.77 ± 0.46 mmol m^{-2} d^{-1} during C2). The Juncus/ Forest ecotype within the seasonal wetland consistently produced the lowest CH_4 fluxes of all sites, with a negligible flux that was not significantly different from zero occurring during C2 (-0.01 ± 0.08 mmol m^{-2} d^{-1})."

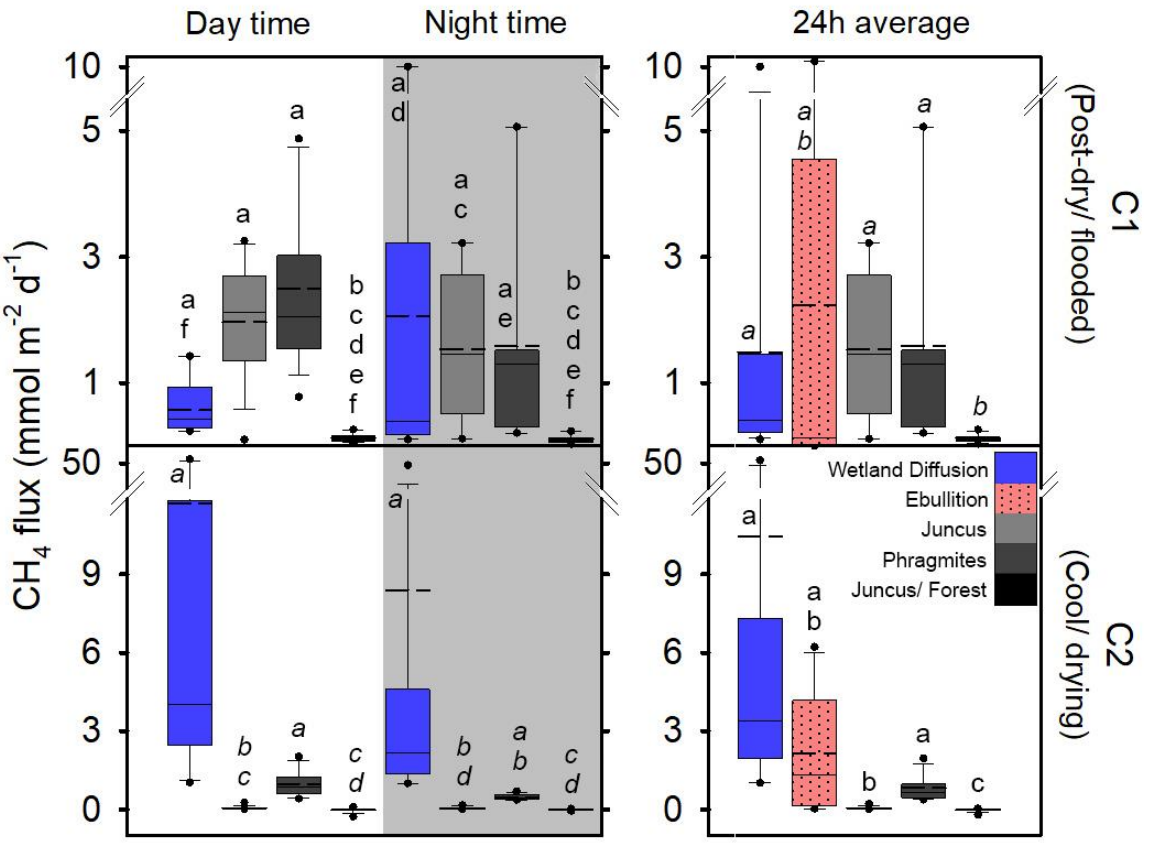

Figure 5. *Fluxes of CH_4 from diel sampling and ebullition over two campaigns from the permanent wetland and adjacent 24 h time series of the seasonal wetland vegetation types. Note: Diffusive fluxes during C2 include chambers featuring lilies, dashed line represents the average, solid line represents the median and dots represent 5th and 95th percentiles. Letters show groups that did not differ significantly ($p>0.05$) using ANOVA on ranks and Dunn's pairwise comparisons within each campaign.*

L264-265: This statement does not seem to hold for Veg C right?

After statistical analysis, this now reads (Lines 338-340):

"The highest average CH_4 fluxes in each of the vegetation types always occurred during the daytime but were not significantly different to night time fluxes (Fig. 5, Table 1)."

L265-266: See above: Was there a significant diel variability?

As address above this now reads (lines 338-341):

"The highest average CH_4 fluxes in each of the vegetation types always occurred during the daytime but were not significantly different to night time fluxes (Fig. 5, Table 1). Phragmites consistently emitted the highest CH_4 fluxes (2.27 ± 1.42 mmol m^{-2} d^{-1} during C1 and 0.77 ± 0.46 mmol m^{-2} d^{-1} during C2)…."

Line 267-268: Is the Veg C flux really negative or rather not significantly different from zero, ie Veg C flux is to be seen as zero?

As the flux is nominal we have re-worded as (lines 341-344):

"…The Juncus/ Forest ecotype within the seasonal wetland consistently produced the lowest CH_4 fluxes of all sites, with a negligible flux that was not significantly different from zero occurring during C2 (-0.01 ± 0.08 mmol m^{-2} d^{-1})."

L269-271: See above comment. I think data and its variability indicate the floating chambers received lots of ebullition in spite of the gas accumulation being linear. Please provide independent evidence supporting that numbers represent diffusive flux only, or consider reporting fluxes as total flux.

In addition to our earlier response addressing this point and our evidence supporting that the reported data represent diffusive flux only (see response to General comment no. 2), we note that the rates are within those reported in previously published studies of diffusive fluxes from similar latitudes, and thus are representative of both open water and lilies.

The higher fluxes during C2 are also likely due to the re-emergence of lily species (Nymphaea sp.) during C2 which are included in some chamber measurements (but were not present not C1). These were mentioned in the manuscript, but we have now added further details to the following areas to clarify for the reader (lines 346-351):

"Figure 5. *Fluxes of CH_4 from diel sampling and ebullition over two campaigns from the permanent wetland and adjacent 24 h time series of the seasonal wetland vegetation types. Note: Diffusive fluxes during C2 include chambers featuring lilies, dashed line represents the average, solid line represents the median and dots represent 5^{th} and 95^{th} percentiles. Letters show groups that did not differ significantly (p>0.05) using ANOVA on ranks and Dunn's pairwise comparisons within each campaign."*

lines 353-357:

…" The permanent wetland showed an inverse trend with seven-fold and significantly higher (p<0.001) diffusive fluxes during the cool/drying C2 when lilies were present (10.46 ± 15.81 mmol m^{-2} d^{-1}) compared to the post-dry/flooded C1 when no lilies were present (1.49 ± 2.75 mmol m^{-2} d^{-1}), while the ebullition rates were similar during both campaigns (Fig. 5, Table 1)….."

lines 326-335:

"…A lag time (ranging from weeks to months) for recovery of the CH_4 pool post-drought has been observed in other systems (Boon et al., 1997) and also during lab-based experiments (Knorr et al., 2008; Freeman et al., 1992). Further, during C2 the return of macrophyte species Nymphaea caspensis most likely enhanced CH_4 gas transport from the rhizosphere to the floating chambers, as discussed in detail in Jeffrey et al. (2019). Therefore this combination of drivers most likely explain the higher CH_4 fluxes during C2 when the system (and lilies) had sufficient time to recover, despite lower water column temperatures that would normally reduce microbial metabolism rates. This hypothesis is also supported by the shift of net positive redox potential…"

Lines 171-175:

"To account for spatial and temporal variability, measurements were conducted during both day-time and night-time, and sampling within vegetated areas featuring lilies (Nymphaea capensis); that

were only present during the second campaign, forested areas (Melaleuca sp.) and in areas where no aquatic vegetation was present (i.e. open water).”

L275: I do not follow the end of this sentence and do not see how Figure 4 can support this statement.

We agree. We have amended this sentence, incorporated new results from the ANOVA as follows (lines 357-360):

“Overall, the diffusive fluxes of the permanent wetland were within range of CH_4 fluxes from the three seasonal wetland habitats but were significantly higher than Juncus/Forest during both campaigns, and Juncus during C2 (Fig. 5). Diel diffusive flux variability was not significant between day time and night time (Table. 1, Fig. 5).”

L330 and elsewhere: Is re-flooding the only possible explanation of the differences found in the redox between the seasonal and the permanent wetland? Could not the difference also represent a difference between areas with emergent aquatic plants having O2 leaking out from the roots and maintaining oxidized conditions, and on the other hand areas without this type of root zone aeration in the permanent wetland? This root zone aeration is mentioned below in another context. Should it not also be highlighted here when discussion the sediment redox depth profiles?

Although we agree this is another plausible explanation, especially for the seasonal wetland sites, it is unlikely to apply for the permanent wetland, as the *opposite trend* occurred due to the absence of lilies during C1; where the positive redox potentials were observed. During C2 when the lilies returned, lower redox was observed. To clarify this point, in the permanent wetland discussion we have added: (lines 537-540)

“…Further, although aquatic vegetation can facilitate root zone aeration therefore increasing sedimentary redox potential, as no aquatic vegetation was present in the permanent wetland during C1, this further suggests water level drawdown of the was the main driver of redox conditions.”

And to the seasonal wetland discussion we have added the following text (lines 510-514):

“The differences are therefore likely explained by the higher positive redox potentials (Table 1) that may be partially attributable to rhizome aeration by the nearby trees, and more abundant thermodynamically favourable terminal electron acceptors (i.e. Fe(III) and SO_4^{2-}) (Fig. 5) all of which can inhibit methane production within the sediments (Burdige, 2012).”

L 387-389 and elsewhere: Some studies have highlighted different patterns. See e.g. Milberg et al. 2017 AoB Plants. doi.org/10.1093/aobpla/plx029

We have added this paper to the discussion as follows (lines 463-467):

“…Milberg et al. (2017) found no apparent diel patterns of CH_4 fluxes from Phragmites australis during seven campaigns within the Swedish growing season. Kim et al. (1998) showed that CH_4 emissions peaked around midday and that daytime emissions were about 3-fold higher than night time emissions, positively correlating with temperature and PAR…”

L410-411 and elsewhere: Is the difference between passive and pressurised gas transfer the only possibility? The sediment redox potentials reported correlate with CH_4 fluxes. Could the sediment conditions not also be influenced also by root depth or root density varying between plant species? If there are no clear explanations, and speculations are necessary, it would be good to highlight not

only one alternative (that are frequently discussed in the literature) but also other possible alternatives.

We agree on the need to canvas a wider range of possible explanations and have now discussed potential alternatives as follows (lines 490-499):

"In comparison, in Phragmites these day:night ratios were almost triple this (67% and 94% higher) during the same periods. This may potentially be due to the more efficient daytime conductive gas transfer pathway of CH_4 through Phragmites australis compared to the more passive diffusive CH_4 gas transfer pathway of Juncus kraussii and/or the effectiveness of these different species to alter sedimentary redox conditions. This suggests that non-pressurized pathways may result in lower net rhizosphere-atmosphere gas exchange of CH_4 from seasonal wetland vegetation. Alternatively, root depth and root density differ between these two species (Moore et al., 2012, De La Cruz et al., 1977), therefore further influencing redox dynamics in the rhizosphere, and the potential extent of net gas exchange."

L412-413 and elsewhere: See above. Another perspective could be that that no significant CH4 fluxes were found from the Veg C site. I suggest letting the statistics decide the perspective.

As now addressed in the results, we have added 'significant' to this as follows (lines 500-501):

"The Juncus/ Forest habitat emitted significantly lower fluxes of CH_4 during both time series campaigns and was a net sink for CH_4 during C2 (Table 1, Fig. 8)..."

L419-425: Why is not possibly more extensive root zone aeration by the additional tree roots mentioned as one hypothesis?

As mentioned above, we have added to this hypothesis as follows (lines 507-514):

"Shading by the overhanging trees may inhibit the daytime diffusive CH_4 gas transport through Juncus/ Forest habitat assumable to lower rates of photosynthesis, however PAR was only lower during C2 (Fig. 7) and so does not appear to explain the CH_4 flux differences observed during C1. The differences are therefore likely explained by the higher positive redox potentials (Table 1) that may be partially attributable to rhizome aeration by the nearby trees, and more abundant thermodynamically favourable terminal electron acceptors (i.e. Fe(III) and SO_4^{2-}) (Fig. 5) all of which can inhibit methane production within the sediments (Burdige, 2012)."

L428-429: See above. (a) Consider the possibility that the floating chambers reflect total flux and not diffusion only. (b) I am not convinced this study can make claims about seasonal differences based on one measurement day per season only as day-to-day fluxes can be highly variable. Therefore, parts of the discussion about reasons for the seasonal difference seem obsolete.

(a) As per previous comments, we are confident that reported diffusion values are accurate and likely due to the presence of lilies enhancing the flux as mentioned in detail above. (b) As per previous suggestions and reviewer #2 comments also, we have removed all claims to quantifying 'seasonal fluxes' from the manuscript and stick to the changes in drivers in our discussion.

L451: I suggest removing "Permanent" here, because many large non-permanent wetland areas are also important (most tropical wetlands vary greatly in size over a year).

Removed and this now reads (lines 546-548):

"Within the global wetland CH_4 budget both subtropical systems and southern hemisphere systems are poorly represented (Bartlett and Harriss, 1993;Bastviken et al., 2011) (Fig. 9)."

Fig 1 and elsewhere: Why were not all measurements and core collections taking place nearby each other? How comparable are the results if data were collected far apart?

At the seasonal wetland sites (Veg A, B and C) cores were taken nearby, but not directly at the site of the flux measurements to ensure minimal disturbance of the site. As the permanent wetland was fairly homogenous (as found during previous study of the wetland i.e. Jeffrey et al., 2019), the cores were extracted from a location to avoid trampling disturbance to fragile sediments and lily habitat, and to avoid artificial ebullition release prior to deployment. We have added the following to our methods explaining this (lines 222-224):

"The cores were sampled in close proximity to the time series habitats (5 to 15 m) in December 2016, but within the permanent wetland the cores were taken from elsewhere to avoid disturbance of the shallow water column and sediments."

Figure 4 and elsewhere: (a) Does Fig 4 really show seasonal fluxes? Can at all seasonal fluxes be claimed from two measurement days as shown here? How to know that these two days were representative of whole seasons? (b) Please inform readers how many replicate measurements were made at each time point for the fluxes?

We have removed all terms referring our study to 'seasonal fluxes' and replaced with 'campaigns' and as above have referenced our companion study and included the number of chamber measurements featured in this study in our methods (lines 171-178):

"To account for spatial and temporal variability, measurements were conducted during both day-time and night-time, and sampling within vegetated areas featuring lilies (Nymphaea capensis); that were only present during the second campaign, forested areas (Melaleuca sp.) and in areas where no aquatic vegetation was present (i.e. open water). A total of 39 CH_4 floating chamber incubations averaging ~8 minutes in duration were recorded over the two campaigns, with 19 during C1 (nine at night) and 30 during C2 (12 at night). The average r^2 value of linear regressions of CH_4 concentrations versus time during chamber incubations was 0.97 ± 0.05."

End of Referee #1 response file