

The main objective of this study (I admittedly that it is not completely clear to me) was to study the role of some physicochemical parameters in the greenhouse gas emissions-GHG (CH₄ and CO₂, I think and N₂O was integrated later as complement) in shallow alkaline lakes in the Pantanal of Nhecolândia, Brazil.

The objective is not to test the role of some physicochemical parameters on GHG emissions. The objective is to show the daily and seasonal variability of GHG-emissions from 2 types of lakes with similar chemistry but with distinct biogeochemical functions. The introduction and objectives have been re-written. See below the final part of the introduction:

“Nhecolândia is a sub-region of the Pantanal wetland, where a myriad of shallow saline-alkaline, oligosaline and freshwater lakes and ponds coexist in the landscape, sometimes at short distances from each other (~200 m). Under the influence of cumulative evaporation over the years, the pH of some saline lakes has reached high values, close to or above 10, resulting in an increasing solubility of the organic matter, with dissolved organic carbon (DOC) values up to 750 mg L⁻¹ (Barbiero et al., 2016; Mariot et al., 2007). Martins (2012) noticed that waters of neighboring saline lakes with almost similar chemical composition can have permanent black or green color (Photo 1 Supplement S1), resulting from distinct biogeochemical functioning, but the parameters that control such differences are still poorly understood. Collectively, the size of the region, the number of lakes and diversity of biogeochemical conditions in space and time (day-night and seasonal change) make it difficult to estimate the regional greenhouse gas emissions from Nhecolândia. A prerequisite for such a regional balance and its contribution to the global budget is a better understanding of the diversity of scenarios and of their variability in time and space (Peixoto et al., 2015). The aim of this study is precisely to present this diversity in the specific context of Nhecolândia, and to provide preliminary results of the range of greenhouse gas fluxes (CH₄, CO₂ and N₂O) from the most alkaline black- and green-water lakes.”

And also the contrast between Black and green water lakes mentioned in the introduction:



Photo 1: Aerial picture illustrating the contrast between a lake with black waters and a lake with green waters. Here the bloom is moderate to strong. The two lakes are about a hundred meters apart (source, matuete.com).

The introduction should refer more to literature of the studied type lakes (ponds) in GHG emission topics, and avoid integrate terms that are out the scope of the study and avoid unnecessary statements, for example:

(i) process-based models are mentioned but never used in the manuscript as tool, or even in the discussion of the results,

We agree with this remark, the sentence has been modified and the reference to the process-based models has been deleted.

(ii) please refer properly the ideas and references in the introduction; Saunio et al. (2016) is a neat analysis of the global methane budget, but not for CO₂ and N₂O, then, you need to include literature about the topic (I would remove N₂O, since it is a last graph with few case of the study lakes and only for “green water lakes”). There are incorrect citations for the meaning of the sentences; Bogard et al (2014) is not a good citation for the meaning of the sentence,

We agree. The reference to Saunio et al (2016) is maintained but related to CH₄, not GHGs. Literature about CO₂ and N₂O was included. N₂O was maintained as we are also giving emission/capture from the studied lakes. N₂O emission data from black water lake, initially not shown, was added in the new manuscript. The reference to the work of Bogard et al (2014) was removed from this sentence.

(iii) include more introduction about your type of greenhouse gases studied, there are few information about which gases were measured.

OK, it has been introduce in the “objective”, at the end of the introduction. See below the first part of the Introduction section:

“Wetlands contribute to the creation of large reservoirs of biodiversity, improve the quality of surface water, reduce flood risk associated with extreme rainfall, and supply streams during low water periods (Brinson et al., 1981; Fustec and Lefevre, 2000; Mitsch and Gosselink, 2015; Reddy and DeLaune, 2008; Turner, 1991; Whiting and Chanton, 2001). They are also critically important to global warming because of their role in modulating atmospheric CO₂, CH₄, and N₂O concentrations. (Bastviken et al., 2004, 2011; Lienggaard et al., 2012; Wang et al., 1993). Among the wetlands, tropical wetlands are known to be highly reactive, as permanent high temperatures increase the velocity of the biogeochemical reactions (Fustec and Lefevre, 2000; Reddy and DeLaune, 2008). As an additional restriction, continental alkaline wetlands are characterized by an increase in water pH during evaporation, favoring the solubilization, transfer and accumulation of organic matter in the landscape. Collectively, these conditions can lead to highly reactive portions of landscape; i.e. emission variability in time and space where the greenhouse gas fluxes are poorly constrained (Peixoto et al., 2015).

CH₄ released from wetlands accounts for more than 75% of natural CH₄ source, and more than 20% of the global CH₄ source (Schlesinger, 1997), although an important uncertainty on the CH₄ global budget is today attributable to emissions from wetland and other inland waters (Saunio et al., 2016). A data compilation from 196 saline lakes around the world highlighted their role in the global CO₂ emission. Lakes with pH below 9 were identified rather as CO₂ sources, while the most alkaline ones, with higher primary production, were generally weak CO₂ sinks (Duarte et al., 2008). Regarding N₂O, global emission remains largely uncertain, ranging from 6.7 to 36.6 Tg N/yr (IPCC, 2007). About 25% of the global N₂O emission is attributed to uncultivated tropical soils, but exact locations and controlling mechanisms are not clear. Wetland ecosystems contribute considerably to N₂O budgets (XU et al., 2008) and Lienggaard et al. (2012) suggest that the Pantanal wetland in Brazil potentially contributes about 1.7%, a significant single source of N₂O. In this context, to understand the various processes controlling inland water emissions is still regarded as a priority.”

2 The method section is lacking some important information. To mention only a few:

(i) The most important: there is no statistical section, then, there is no idea how you determined significant differences, linear analysis, how many samples per site, time and lake were done.

The method was not fully detailed in the previous version; it has been re-written. In particular, we explain that each value presented hourly is the mean value over duplicate measurements in all floating chamber (3 chambers = 6 measurements or 6 chambers = 12 measurements), and that the error bars denote the standard deviation. The number of chambers is mentioned in the table. See below the table and the modified method section:

Table 1: Date, location, lake characteristics and general conditions during greenhouse gas emission monitoring.

Date	Type of lake (name) Surface km ²	Weather conditions	Phyt. Bloom conditions	EC range $\mu\text{S}\cdot\text{cm}^{-1}$	pH range	DOC $\text{mg}\cdot\text{L}^{-1}$	Procedure Numb of chambers	Water column range meter	Time of gas coll. Minute
Sept. 13, 2012	Black (P) 0.087	Sunny	-	1400-1599	8.81-8.99	51	Fixed 3	0.3 – 0.8	20
Sept. 14, 2012	Green (V) 0.109	Sunny	Moderate	2420-2888	9.48-9.73	236	Fixed 3	0.1 – 0.4	20
Aug. 30, 2013	Black (P) 0.091	Sunny	-	1715-1855	9.21-9.33	37	Fixed 3	0.3 – 1.1	20
Sept. 1, 2013	Green (V) 0.109	Partially cloudy	Strong	2302-2410	9.67-9.78	265	Fixed 3	0.1 – 0.5	20
Dec. 2, 2014	Green (M) 0.053	sunny	No	2014-2204	9.37-9.51	102	Sl. moving 6	0.1 – 0.4	23 to 43
Jul. 7, 2015	Green (M) 0.055	sunny	No	1940-2030	9.28-9.37	82	Sl. moving 3	0.1 – 0.4	21 to 37
Sept. 10, 2015	Green (G) 0.285	Sunny (evening storm)	Strong	34000- 35100	10.3- 10.44	326	Fixed 3	0.1 – 0.2	20
Sept. 12, 2015	Black (P) 0.093	Strongly rainy	-	1382-1450	9.3-9.4	36	Fixed 3	0.4 – 0.7	20

“Gas fluxes from the lake to the atmosphere were measured using 32-L polyethylene floating chambers, having a base area of 0.195 m². The main conditions during the field campaigns are summarized in table 1. Two procedures were used for these measurements with fixed or slowly moving chambers. The procedure using slowly moving chambers (Photo 2 Supplement S1) was favored when the water level was sufficient and the lake diameter not too large to allow to cross from one bank to another. In this case, depending on the lake diameter, a train of 3 to 6 floating chambers was attached, leaving a gap of 10 meters between two successive floating chambers. Floating chambers were placed in the water every minute at a distance of about 30 m from the lake shore, and then slowly pulled toward the opposite bank at a maximum rate of 5 m min⁻¹. This experimental design allows for scanning the various water column heights, with the least turbulence disruption to the lake surface. To minimize artificial turbulence effects, foam elements were adjusted so that a maximum of 2 cm of the chamber penetrated below the water surface. The collects were carried out once each chamber reached a distance of about 30 m from the opposite bank. The collection times were variable since the first chamber reached the other margin in approximately 20 to 25 minutes, whereas the last chamber took about 35 to 40 minutes. When the water level was too low, or the lake too wide, we opted for a procedure with fixed floating chambers (Photo 3 Supplement S1). In order not to disturb the sediment just below the chamber, they were anchored with a 10-m line to avoid drifting. The line was equipped with a float to the vertical of the anchor. The chambers were located from the center to the border of the lake, and the collects were carried out after 20 min from an inflatable boat with shallow draft. Due to the low water column, it was not possible to place a bubble shield to prevent bubbles from reaching the chamber. Therefore, the results represent the sum of both fluxes by diffusion and ebullition. For each chamber, gas samples were collected in duplicate (about 2 minutes apart) through a 60-mL syringe. Then they were transferred into 30-mL glass bottles, previously capped with gas-tight, 10-mm thick butyl rubber septa and aluminum caps, and evacuated with a hand vacuum pump at 0.75 kPa. Air samples were also collected at the departure of the chamber train for the ambient gas levels. Gas fluxes were calculated by the linear change in the amount of gas in the chambers as a function of sampled time. Thus, for example for a 6-chambers protocol, the mean and standard

deviation on 12 measurements are presented as single gas emission value and error bars, respectively, for a given hour that corresponds to the launching of the first chambers. This operation was repeated every two or three hours or in order to present a complete 24-hour cycle.”

And here is the Photo 2 Supplement mentioned in the text:



Photo 2: Gas collection from a train of 6 slowly moving chambers on green water lake M in the absence of cyanobacteria bloom (December 2014). The first floating chamber has just reached the point of collection. Two samples will be collected in each chamber. The average of these 12 samples will provide 1 flux data for each gas (CH₄, CO₂ and N₂O).

(ii) You need at least a reference to indicate the advantage of the method and/or a comparison between static chamber and your namely "dynamic chamber". What is the purpose of it? Why didn't you set several static chambers and measure it? The way that you collect the gas samples is very difficult to understand. Finally, what is the purpose to use two different methods and no mentioned in the results and discussion sections?

The use of fixed floating chambers provides information on emissions at a fixed point, with a given water column height. It also requires various shifts of the collection equipment. In the case of slowly moving chambers, the chambers scan and involve all the lake water column heights covering the length of a diameter. All the collections are made in series, at the same point, which avoids having to move the equipment. Unfortunately, this procedure was not applicable for larger lakes, or when portions of the lake had a column of water that was too shallow. A photo is provided in "supplement material", allowing to visualize the "slowly moving" procedure. See above.

(iii) There is an important missing information in the gas sample procedure about the manual pressure procedure. This is a critical problem, because if you don't know the % of vacuum made, you didn't know how much dilution contain the sample injected into the vial. Did you have a pressure manometer to measure it?

We agree with this comments. The vacuum pump used was indeed fitted with a manometer. The depression obtained is now mentioned in the text (0.75 KPa). See above.

(iv) The calibration for CH₄ is wrong as you did it, since you used CH₄ standard 10 times over to the atmospheric concentration. So, maybe you will have critical bias in the calibration curve comparing data below to 690ppm from the calibration curve.

We agree. The value mentioned in the first ms was wrong. This part of the method has been actualized as follow: “Gas concentrations (CH₄, CO₂ and N₂O) were measured by gas chromatography model Shimadzu GC-2014 (Shimadzu Co., Columbia, MD, 5 USA). The chromatographer was equipped with a packed column, an electron capture detector (ECD) to analyze N₂O, and a flame ionization detector (FID) to quantify CO₂ and CH₄. Prior to detection, CO₂ was reduced to CH₄ using a methanizer. The gas analyzer was calibrated with NOAA CMDL certified standars CO₂ (357.5 and 1531 ppm), CH₄ (1.016 and 9.639 ppm) and N₂O (313 and 11,240 ppb) gas standards (minimum and maximum, respectively). Analytical accuracy was better than 0.02 ppm CH₄ and precision was better than 0.005 ppm expressed as the standard error of the mean for multiple measurements of standards. The analyses were performed in the Environmental Science Laboratory (UFSCar, Sorocaba, Brazil).”

(v) Section 2.2.2. title should be "physicochemical analysis" (or similar), since using the title “Biogeochemical field indicators” is very vague, according to the measurements made in the study.

Of course, it has been changed to “Field physico-chemical measurements”.

3 Results sometimes are discussed (in the result section) in speculative way for example:

(i) I cannot see Fig 3. Opposite trend mentioned in page 35 Lines 26-29.

“Opposite” has been changed to “distinct” and “opposition” to “difference”.

(ii) Carefully in the temperature results, you didn’t measure at the same time (even you measured different years I think), then, temperature fluctuations is due that environmental conditions during a day, or I am wrong and you measured all lakes at the same day, did you do that?

We agree. The date corresponding to the measurements given as an example in Fig. 3 is mentioned. For Lakes P and G, measurements were taken on the same day, the two lakes being only a hundred meters apart. For Lake V, the data corresponds to the next day, and we specify that the meteorological conditions were similar. See below:

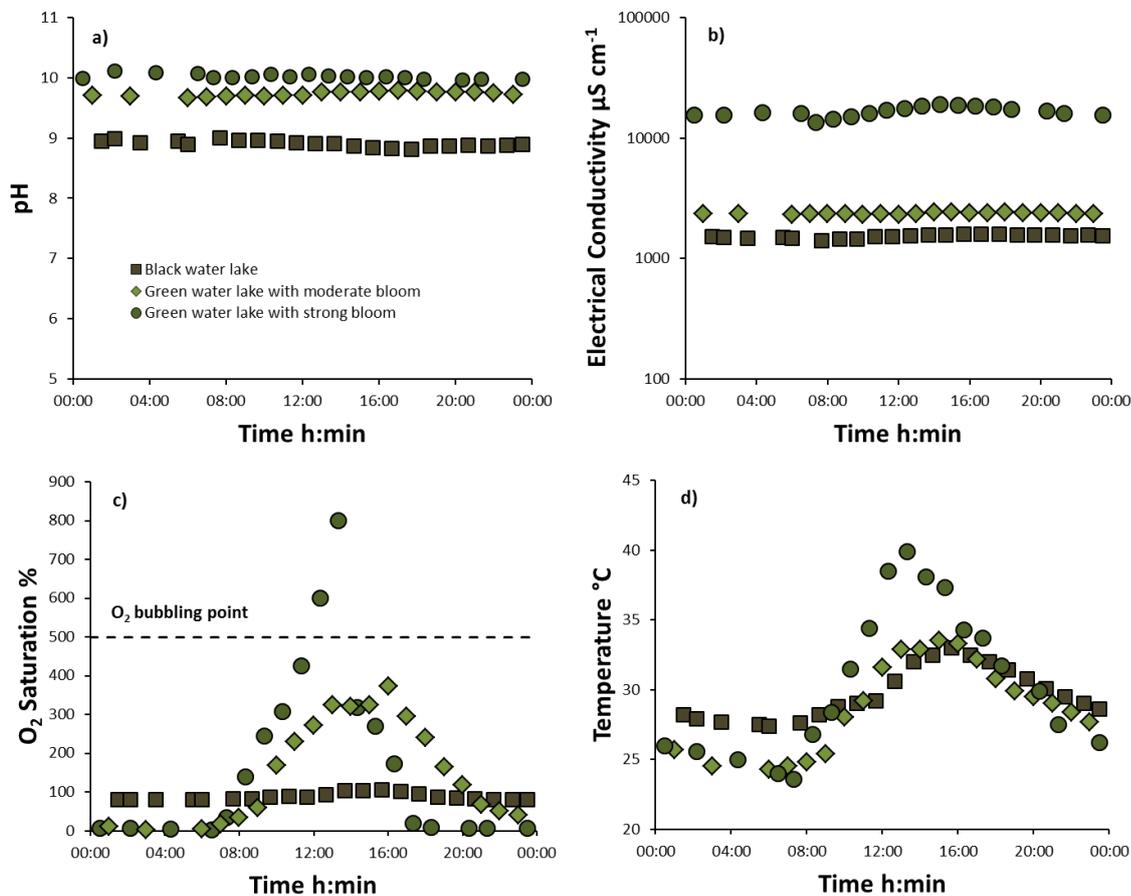


Figure 2: Changes in (a) pH, (b) E.C., (c) dissolved O₂ and (d) temperature at 5 cm below the lake surface, over 24-hours monitoring. The measurement were carried out with similar climate conditions on September 13th, 2012 for black water lake P and green water lake G with strong bloom, and on September 14th, 2012 for lake V with moderate bloom. The dashed line in Fig. 2c represents the O₂ bubbling point for a solution at the equilibrium with atmospheric O₂.

(iii) There is no term of variation (e.g. standard deviation, standard error, variance, among others) and number of samples in the gas emission section, then I don't believe that statements as in Page 8, line 8 "The differences in the emission values between the floating chambers were moderate".

This part has been modified and detailed. In particular, we detailed the achievement of results at each hour of collection. "Gas fluxes were calculated by the linear change in the amount of gas in the chambers as a function of sampled time. Thus, for example for a 6-chambers protocol, the mean and standard deviation on 12 measurements is presented as single gas emission value and error bars, respectively, for a given hour that corresponds to the launching of the first chambers. This operation was repeated every two or three hours, in order to present a complete 24-hour cycle."

(iv) Which peaks are in Figure 7, the figure is very confusing, would be better to represent in another way (all about gas data), because error bars (also indicate what is the term of variation) are mixing and it is impossible to understand.

We agree with this comment. As was also suggested by other referees, we have grouped all the data of a given gas on the same graph. To reduce the confusion in the error bars, we opted for different colors (dark brown for black water lakes, and different shades of green for green water lakes). See

below, for example, the figure for methane emission (dissolved methane and fluxes are grouped on a single Fig):

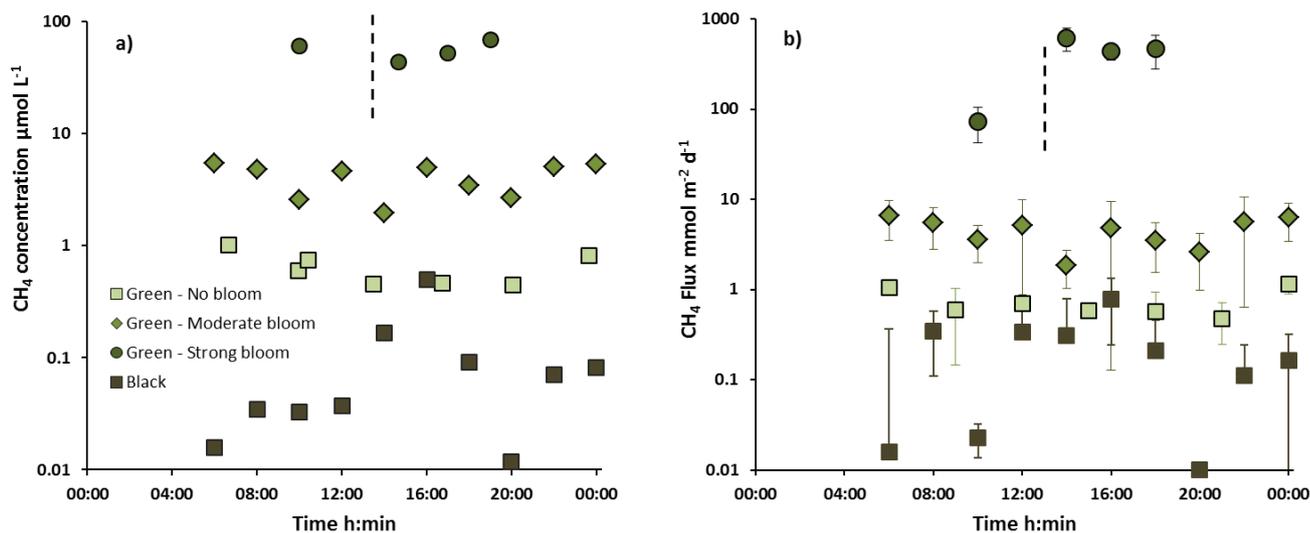


Figure 5: (a) Dissolved methane concentrations at the top of the water column, (b) and methane fluxes over 24 hours monitoring in black water lake (lake P) and green water lakes for no- (lake M), moderate- (lake V) and strong- (lake G) bloom conditions. Due to the logarithmic scale used, some negative values of the error bars (denoting standard deviations) are not drawn. The dashed line represents the beginning of the ebullition in lake G (13:20).

(v) How did you know that CH₄ bubbling was moderated (Page 8 Line 12)? the heterogeneity could be as result of moving the chambers, even it is confused why you measure with different methods (static and dynamic).

In fact, as has been pointed out by other referees, nothing allows to discuss on the role of methane ebullition from the sediment. This part has been deleted from the manuscript.

(vi) Most of the time I need to assume your term of bloom and I believe you, however I cannot see the trends in the Figure 9 and why black water is not shown. What is the meaning of trend for this study? In some figures, some parameters didn't change along the time, so is it a trend?, if I am right you need to include in Figure 9 the trend for "Black water lakes".

A picture showing the contrast between two lakes with black and green waters is proposed as supplement material. In this picture, the intensity of the bloom is obvious, and here we have only a moderate to strong bloom, not enough for the O₂ bubble point to be exceeded in the afternoon. "Trend" was removed from the sentence. However we maintained it for black water lakes: "For the black water lake, no clear trend towards emission or consumption of N₂O was observed." These N₂O data on black water lake have been incorporated in the figure. See above and below:

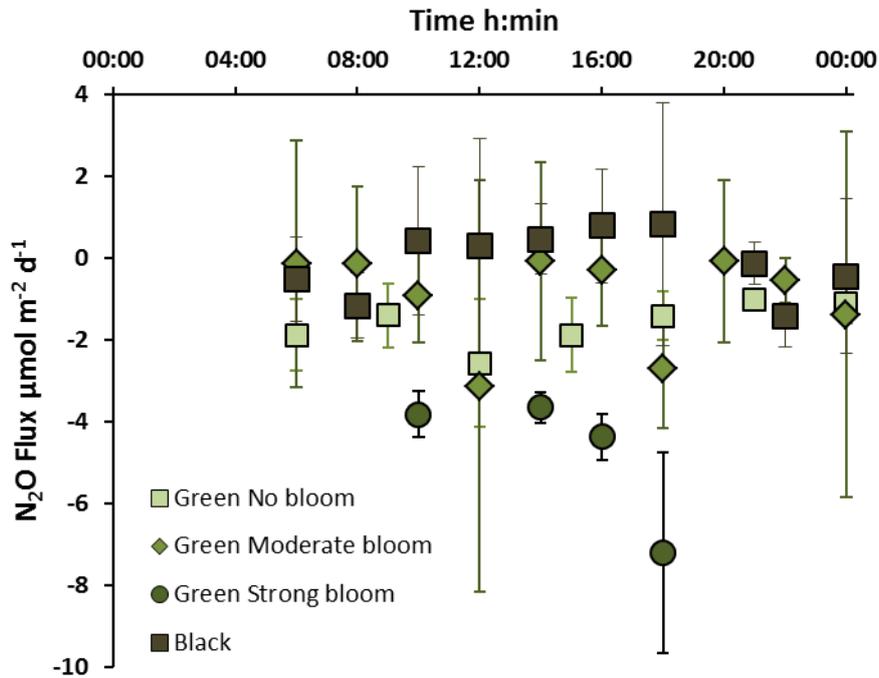


Figure 8: Nitrous oxide fluxes from black water lake and green water lakes for no-, moderate- and strong bloom conditions.

4 Discussion of the results remains mainly speculative, and the statements are sometimes questionable for example:

(i) Page 9 Lines 16-20 is a very vague discussion,

This is actually not a discussion, but a link to discussion furtherly developed in the ms.

(ii) Please read the manuscript mentioned in Page 9 Line 29; you are working with ponds and it is out of the scope to mention methane paradox. Grossart and Tang are working with a oligotrophic lake with a very particular conditions, and Bogard et al. (2014) is an enclosure experiment to confirm methane paradox in water lakes. I think your results are more correlated to the microbial activity in sediments but no as production of methane in the water column (in oxic conditions), and your experiment doesn't allows to speculate it.

We agree, this speculative discussion was removed from the manuscript.

(iii) I am sorry but section 4.3 is a very speculative supposition, you didn't test any experiment to validate your supposition about microbubbling CH₄. Additionally, you can't mentioned methanotrophy, since you didn't measure methanotrophy activity. Because you showed large gas emissions, it doesn't mean that methanotrophy is suppressed. You are measuring only the total emission; that is the result of the CH₄ produced minus CH₄ oxidized by methanotrophs. Then you don't know the rate of methanotrophy activity, which probably is large or small, but you need to have proof to mention it.

As suggested by other referees this section has been re-written. We focused on the O₂ microbubbling, showing that the onset of this phenomenon enhanced methane emissions. A figure

was added comparing the calculated K600 coefficient for lakes V and G. “The consistent change in the calculated K_{600} values (Fig. 6), which coincided with the occurrence of the abrupt generalized ebullition of lake G, emphasize that CH_4 behave quite differently in these 2 lakes.” With regard to methanotrophy, we agree with the comment. Of course, nothing in our dataset allows us to discuss methanotrophy. The error originally comes from a confusion methanotrophic / methanogenic. This point has been changed.

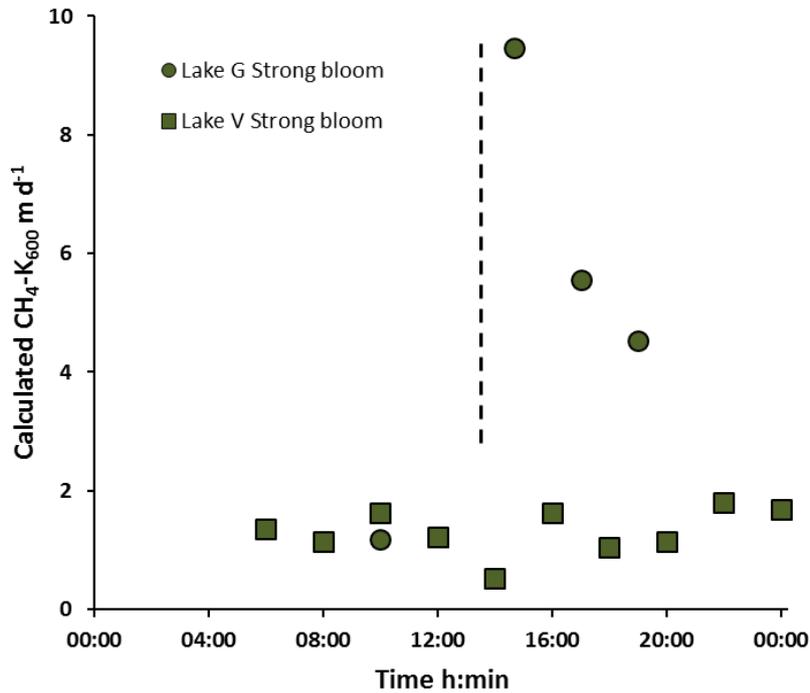


Figure 6: Calculated exchange gas coefficient for Methane in lakes V and G in strong bloom condition. The dashed line represents the beginning of the ebullition in lake G (13:20).

(iv) Section 4.4 is very speculative since you didn't measure during rainfall conditions, please remove it.

This part was removed from the discussion and shifted to the section “future directions”