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Interactive comment on "Geographic and seasonal variation of dissolved methane and aerobic methane oxidation in Alaskan lakes" *by* K. Martinez-Cruz et al.

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Reviewer 2. V. Stepanenko (Referee)

Thank you for providing constructive comments on our manuscript, "Geographic and seasonal variation of dissolved methane and aerobic methane oxidation in Alaskan lakes (bg-2015-49)". We have re-written the manuscript, taking into account your concerns and recommendations.

General comment

This manuscript presents results of estimate of methane oxidation in over 30 Alaskan

lakes. This is the first such study for this region, and, to my knowledge, is the methane oxidation study involving data from the largest lake set so far. The study is significant for the area of greenhouse gas dynamics in lacustrine ecosystems since it presents the new field method for determining methane oxidation potential, and achieves clear conclusions on key factors controlling methane oxidation in Arctic tundra. The manuscript is well written, and the main conclusions are unambiguously stated. These are the strong points.

Our answer: We are grateful for this encouraging comment.

Comment: The weak points are two in my view.

1) The authors sampled 7 yedoma lakes and 23 non-yedoma lakes. So, the reliability of statistics on these two sets is different. Are there any estimates on the sufficient n for the statistical estimates accuracy needed? This is especially relevant for yedoma lakes.

Our answer: Dr. Stepanenko underlined a very important point. In the previous version of our manuscript, we used the Tukey-Kramer test to compare dissolved CH4 and DO concentration, during winter and summer, for yedoma and non-yedoma lakes. This test was performed using the actual sample size (unequal number of yedoma and non yedoma lakes). However, after revision, Dr. Stepanenko is correct and Tukey-Kramer test was not the most appropriate test to make comparisons with different samples size. To avoid confusion, we decided to re-analyze the data using the Kruskal-Wallis multiple comparison test, which is a more appropriate method for comparisons with different sample size and non-normally distributed data. Please note that we obtained the same test results than with Tukey-Kramer test. Thank you for this important observation. We modified the Results section accordingly and we changed the Material and Method section as follows;

“Normality was assessed by the Shapiro-Wilk test. Since most of the data was non-normally distributed and with unequal samples number, significant differences among all parameters were determined using Kruskal-Wallis multiple comparison test (differences were considered significant at $p < 0.05$, $Z > 1.96$). To assess whether CH4 was oxidized during the MO incubation tests, significant differences between CCH4 were determined by an analysis of variance (ANOVA; $p < 0.05$), after normality was assessed by the Shapiro-Wilk test ”.

Comment: 2) The authors admit (p.4228, str.15-30) that measuring methane and oxygen concentrations at 0.5 and 1 m depths they likely underestimate the maximal methane oxidation rate in a lake that is typically located in the thermocline. Thus, the authors should precise that they assess methane oxidation rates in the surface layer, that is not a good proxy for larger depths and lake as a whole. Therefore, I strongly recommend to look through the text and modify it accordingly, replacing "MO in a lake" by "MO in a surface lake layer" etc. The title of the manuscript should be rewritten as well, e.g.: "Geographic and seasonal variation of dissolved methane and aerobic methane oxidation in the surface layer of Alaskan lakes". Otherwise the authors would have to exclude deep lakes from their analysis where the thermocline is well-developed in summer.

Our answer: We partially agree with this comment. As clearly stated in our manuscript, all water samples were taken at a depth of one meter, except in some cases, when the ice layer was thicker than 1 m. We also agree that in several cases, 1-m depth can be considered as surface water. However, and in many cases, lakes were shallow and our sampling regime of near 1-m can reflects mid-column or lake bottom water environments. We clarified that point in the Materials and Methods section, as follows;

“Water samples for MO rates and dissolved CH4 concentration were taken at a depth of within 1 m of the ice-water interface in winter and usually at 0.75 to 1 m water depth

in summer. Due to differences in lake depth and thickness of the ice sheets, samples reflected surface water in deep lakes, but mid water column or even lake bottom water environment in shallow lakes”.

Specific comments

Comment: p. 4222, 5-10, It would be useful to indicate if there was a connection between lakes’ depths and RWCS. E.g., were deeper lakes more stratified in general?

Our answer: As suggested, we checked for a correlation between lake depth and RWCS. We did not find any correlation and we indicated in the manuscript;

“Overall, only one third of the temperature profiles indicated clear stratification. In both seasons, no correlation between RWCS and lake depth was found, probably due to the fact that lakes were shallow and with an uneven depth distribution”.

Comment: p. 4223, 6, Remove one "potential"

Our answer: Changed accordingly.

Comment: p. 4223, 15-20, An interpretation of lag phase is relevant

Our answer: We modified that section to make clearer the interpretation of the lag phase. This section is now;

“In about 60% of the cases during the summer and 80% during the winter, a lag phase was observed; i.e. period of time with no apparent MO (Fig. 4b). This behavior, termed “induction of MO”, has previously been reported for various soils (Bender and Conrad, 1995; Dunfield et al., 1999) and can be interpreted as an adaptation period of the CH4 oxidizers to the culture conditions”.

Comment: p. 4225, 29-30, This requires more quantitative estimates.

Our answer: We agree this is important but the impact of MO on oxygen uptake is difficult to estimate quantitatively, from our results. Therefore, we opted for a suggestion as follows;

“This observation suggests that MO was actively controlling O2 and CH4 concentrations by oxidizing CH4 when O2 was present. To confirm the latter, it would be necessary to measure experimentally the O2 uptake rate by methanotrophs and by the other aerobic processes that compete with MO”.

Comment: p. 4226, Title, May be, "Limiting factors of MO rates" is better?

Our answer: We agree and we changed the title of that section as well as the all text, Tables and Figures, to include “limiting factor” instead of “limiting element”.

Comment: p. 4226, 23-26, "is most likely linked to the higher dissolved CH4 concentration" sounds strangely, since due to eq. (1) it is straightforward to check the contribution of both CH4 and DO into reduction of potential MO.

Our answer: This is correct, we were being too cautious. As we indeed have clear evidences, we modified that section as follows;

“Higher r values for yedoma lakes in summer is explained by the higher dissolved CH4 concentration in presence of a relatively high DO concentration above the oxycline (Fig. 3).”

Comment: p. 4227, Any discussion on maximal MO potential (rmax) for yedoma lakes

is missing

Our answer: We agree and we included a new discussion section, as follows;

“In addition to seasonal variations, permafrost type was also a determining factor of r and rmax. As mentioned before, although no difference in rmax was observed during winter between yedoma and non-yedoma lakes, rmax in yedoma lakes was about twice higher than in non-yedoma lakes during summer. We attribute that difference to a more active MO methanotrophic community in yedoma lakes, as all rmax tests were conducted in aerated vials with a fixed initial standard CH4 concentration in the liquid phase ($\sim$0.6 mg L-1), thus ensuring optimal conditions”.

Comment: p. 4243, Fig.6, a, Horizontal axis should have a label kS$-$O2 p. 4227, 16-25, Δr/ΔKS – is that a ratio of two values or just a notation for Δr for a given ΔKS? In the former case this ratio cannot be expressed in %, and in the latter please denote it as Δr(ΔKS), i.e. Δr as a function of ΔKS.

Our answer: That was certainly unclear. Please check our answer to the last comment, which was on the same point.

Comment: p. 4241, Caption, Replace "3-d" by "3-day"

Our answer: Yes, indeed, changed accordingly.

Comment: p. 4242, Figure 5a consists of two small plots. Please enlarge them

Our answer: We enlarged the plots

Comment: p. 4243, Fig.6, I’m totally confused with this Figure. If Δr is a deviation of r

from its value at a mean K'S−CH4 , Δr must be 0 when K'S−CH4 = 1. Moreover, increasing K'S−CH4 above 1 we must get negative Δr (decrease below r corresponding to mean K'S−CH4). Please clarify what are the values Δr and how they are calculated. Moreover, the authors use Δr' notation, whereas I can't find it in the text.

Our answer: We agree this sensitivity analysis was probably not easy to understand. Based on that comment, we decided to simplify substantially the analysis and we present now a simple error estimation on r, for a given error on KS. With that new presentation, we believe we reached the same conclusion in a simpler way, more understandable for reader unfamiliar with sensitivity analysis. Thanks for this comment. This section has been modified as follows;

"To quantify these potential errors, we conducted a sensitivity analysis. We arbitrarily modified KS-CH4 and KS-O2 and calculated the resulting r (Eq. 1) using the experimental rmax, CCH4, and CO2 measured in the 30 lakes. Fig. 6 shows the error on r caused by a given error on KS-O2 (Fig. 6a) and KS-CH4 (Fig. 6b), for yedoma and non yedoma lakes, in winter and in summer. According to this analysis, an underestimation of KS-O2 or KS-CH4 would lead to an overestimation of the actual MO rate (positive error), while an overestimation of these affinity constants would produce an underestimation of r (negative error). Fig. 6a shows that, an error on KS-O2 ranging from -50% to 200%, would cause from 10% to -6% error on r, for all lakes and all seasons, except in yedoma lakes during winter, where an error from 75% to -50% would be generated. This relatively high sensitivity of r to error in KS-O2 in yedoma lakes during winter is due to DO concentrations close to KS-O2. Likewise, Fig. 6b shows that, from an error on KS-CH4 ranging from -50% to 200%, a resulting error on r from 6% to 4% would be done, for all lakes and all seasons, except in non yedoma lakes during summer, where an error from 50% to -34% would be generated. As above, the latter is due to CH4 concentrations close to KS-CH4 in non yedoma lakes during summer. This sensitivity analysis shows that, other than for KS-O2 in yedoma lakes during winter and KS-CH4 in non-yedoma lakes during summer, errors on KS would have relatively little impact

on determination of methanotrophic rates".

Interactive comment on Biogeosciences Discuss., 12, 4213, 2015.