Author response letter for bg-2015-325 "Carbon dynamics in highly heterotrophic subarctic thaw ponds"

We would like to thank the two referees for their comments. We are pleased to read that both referees find our manuscript important as it provides detailed knowledge of carbon dynamics from both summer and winter seasons. Below, we answer to the general and specific comments of the referees.

Referee #1

This manuscript is focused on the carbon dynamics and subsequent biological patterns in arctic thaw ponds during both late winter and late summer. The strengths of this paper are the inclusion of winter sampling, as well as the partitioning of carbon fractions and microbial communities. All of these are unusual in the limnological literature, and will advance our understanding of these thaw ponds in new ways.

Some comments that I have made in an effort to improve the clarity of the manuscript:

The second paragraph of the Introduction could use some better organization to clarify main ideas. It opens with a turbidity and nutrient statement about thaw ponds, then expands to detailed and widely ranging comments on carbon dynamics, and ends with light limitation. It is all great information but quite a bit to process- I think some better organization with a clear focus on the major points will help.

Response: We have reorganized this paragraph and divided it into two. Light and related biological aspects (primary productivity and composition of plankton) now have its own paragraph.

Also, please clarify when literature references are broader (e.g., Hudson et al. deals with what is happening in boreal lakes, not these ponds) versus specific to thaw ponds.

Response: The references that refer to boreal studies are better pointed out, e.g. The sentence involving Hudson et al. (2003) now reads:
“The rate of DOM input to aquatic systems has been documented to increase in boreal lakes over the last decades (Hudson et al., 2003), but no such information is available for thaw ponds. However, the recent mobilization of terrestrial carbon stocks stored in permafrost for thousands of years (Vonk et al., 2012) suggests that similar DOM increase could also take place in the North.”

Please clarify how you define the depth of the thermocline. And in the Results, you say the thermocline was situated at 1.6 m- do you mean where it started? This was unclear. One could squint at the figure and decipher it, but that still leaves it unclear overall.

Response: The thermocline has been defined variously but correctly refers to the plane of maximum rate of decrease of temperature with respect to depth, as opposed to the metalimnion that is a layer of water with a steep thermal gradient, and separating the

epilimnion (generally thermally homogeneous) and the hypolimnion. The normal criterion for the presence of a thermocline (a minimum temperature change of 1°C m^{-1}) is not convenient for some studied turbid ponds where the temperature changed linearly by more than 1°C within the first 50 cm (no clear epilimnion). Rather than talking of a thermocline, we now describe how temperature changed from the surface to the bottom in different ponds, and replaced text accordingly (using "thermal structure" or "thermal gradient"):
“Some ponds (KWK 2, 6 and 20) had a thermally homogenous epilimnion until 1 to 1.5 m depth, while in KWK 12 and 23 the temperature declined nearly linearly towards the bottom with a rate of 1-2°C every 50 cm”.

I found the implications for the issue described at the top of p. 11712 a little unclear. Can you clarify from where then the water was sampled (just under ice, so then mixing was likely a bigger issue?). It says the other samples were from 1 m below the ice.

Response: We have indicated in more details the sampling depths in winter:
“…the similar GHG concentrations obtained in surface (**just under the ice**) and bottom waters (**approximately 0.5 m above the sediments**), despite the expected inverse thermal stratification at this period of the year (Laurion et al., 2010). All other samples were collected from about 1 m below the ice and considered as representative of an integrated water column.”

While at times the methods seem a little unconventional to me, the authors do a good job clarifying what they did so that the reader can decide on the quality- I think this is okay.

Thank you for this comment.

The description of the interpretation of the PCA seemed a little oversimplified to me. You say PC1 is more carbon, but PC2 did correlate with a couple of the carbon quality metrics as well as DOC, and PC1 was with TP. I would suggest some further clarification and elaboration on these patterns.

Response: Our original description of the statistical results was a combination of PCA (Fig. 4) and univariate correlation results (Table 4) in the same paragraph. As this seem to have been confusing we have now first explained the PCA in details, and only then go for the correlations between organisms and environmental variables. We believe it is now clear the PC1 is not only correlated with carbon:
“Winter, surface and bottom environmental characteristics formed three distinct groups on the PCA (Fig. 4a), with the first two axes explaining 72.9% of the variation in the environmental variables (PC1 46.0% and PC2 26.9%). Axis PC1 has strong positive correlations with TP (r=0.91), a320 (r=0.80), TSS (r=0.83) and GHG (CO_2 (r=0.92) and CH_4 (r=0.90), and strong negative correlations with pH (r=-0.81) and S289 (r=-0.65), whereas PC2 has positive correlation with temperature (r=0.81), S382 (r=0.69) and SUVA254 (r=0.76), and negative correlation with TN (r=-0.93) and DOC (r=-0.67). Heterotrophs (BB and HNF) were correlated with PC1 while phototrophs (PP, PNF and PPA) were more strongly correlated to PC2 (Fig. 4b). Further, BP and BB were best

correlated with carbon quality indices a320, SUVA254, and S289, while HNF were best correlated with conductivity (Table 4). PP, PNF and PPA were best correlated with a combination of carbon quality indices, DOC and nutrients (Table 4)."

On p. 11727, line 7-9, isn't it also possible that TSS and TP are correlated because particulate organic matter (e.g., bacteria, phytoplankton) contain P?

Response: It is true that particulate organic matter (POC) was positively correlated with TP ($p < 0.001$) but because the biomass of dominant organism (BB, PNF) were not significantly correlated with TP it is unlikely that the biomass of bacteria and phytoplankton explained the correlation between TSS and TP in this inorganic-rich environment.

Referee #2 General comments:

The paper addresses the carbon dynamics of thaw ponds. The study is motivated by the poor knowledge about these ecosystems, not at least when it comes to food web dynamics and biotic processes controlling carbon cycling, i.e. issues that this study addresses. Week parts of the manuscript include quite poor temporal and spatial resolution (5 ponds, 2 sampling occasions, 2 points (shallow and deep) per pond). I suspect that the ability to sample with high resolution may be limited for these sites but it still implies that it is not possible to draw to many conclusions about temporal and spatial variability based on the data. Sometimes the ms do that. I also lack clear aims and hypothesis to test. Without this the study tends to be rather descriptive and unfocused. There are also unclarities in some of the methods and data treatments, implying that some results and conclusions may not be supported by the data. The discussion is long and I suggest the authors try to make it more focused/structured (which would be easier with more clear aims and hypothesis).

Response: Thank you for these comments. We have removed all wording referring to seasonal comparison and now make it clear that we compare only two time periods; one in winter and one in summer. We have also added clear hypotheses to the aims. Please see the detailed responses below. We have made several precisions to the methods and have removed a large section of the discussion, and have made the remaining text more focused.

Specific comments:
11708 L2-3. This is not correct. Super saturation is no evidence of net heterotrophy. Net heterotrophy is defined as when community R exceeds GPP. Ponds could be supersaturated without being net heterotrophic.

Response: We reformulated this sentence to avoid expressing that supersatutation=net heterotrophy as an automatic match. However, when external CO_2 inputs from weathering, runoff or groundwaters are unlikely, such as in case of the studied ponds, these variables can be directly linked, especially when supported by an inverse

relationship between O_2 and CO_2. We have added this information to the results as a support for our statement that CO_2 is coming from O_2 respiration and not from external sources:
"The inverse relationship ($r = -0.9228 = p< 0.0001$) between O_2 and CO_2 suggests that the CO_2 originated from O_2 respiration and not from external sources such as weathering, runoff and groundwater inputs that are unlikely in the studied ponds."

11708 L25-26. It is unclear what you mean with 'production of new carbon'. Please clarify.

Response: We agree that this can be clarified. As this is the last sentence of the abstract and the space is limited for a detailed explanation, we have only added the word "particulate organic" to the sentence to indicate that we mean organic carbon production instead of CO_2 (respiration) or DOM. A more detailed description of the results is given in the manuscript.
"Our results point to a strong heterotrophic energy pathway in these thaw pond ecosystems, where bacterioplankton dominates the production of new **particulate organic** carbon in both summer and winter."

11709 L6-8. Here and at other places. Do you need all these references? I suggest you reduce and just leave key references/examples.

Response: We now only cite three studies here, and we also checked elsewhere to make sure we include a maximum of three references per statement.

11710 L28. 'another habitat' is unspecific. Specify that you mean hypolimnion.

Response: "another habitat" has been replaced by "hypolimnion" as suggested.

11711 L2 Here you can be more specific on how C cycling will be affected.

Response: This sentence has been removed from the introduction, as it did not link with our objectives. The description of a disconnection with the hydrographic network still remains in the methods section as part of the study site description.

11711 L4-6. The aim should be rewritten to better reflect the study. I think it could be more specific and also combined with hypothesis/predictions. It is difficult to demonstrate variability in space and time with only 2 sampling occasions and 2 sampling points per pond so this part should be reformulated.

Response: We have rewritten the last paragraph of introduction with a clear statement on the objective, and also have added hypotheses.
"Circumpolar thaw ponds are ice covered for a large fraction of the year, which further affects carbon cycling, and generates large seasonal variations. The aim of this study was to demonstrate the variability in GHG accumulation, CDOM characteristics, and autotrophic and heterotrophic carbon pools between two opposing time periods and

depths. Comparisons were made between late winter and summer, and between surface and bottom waters in summer. We hypothesized that in the absence of light in winter or in the aphotic summer hypolimnion, autotrophic productivity stops while respiration continues, resulting in large summer and winter storage GHG fluxes. We further hypothesized that the thermal structure in summer generates an accumulation of organic carbon in the hypolimnion where heterotrophic processes and organisms dominate. This study follows earlier ones looking at the limnological characteristics and bacterial communities of these subarctic thaw ponds in summer (Breton et al., 2009; Negandhi et al., 2013; 2014; Rossi et al., 2013; Crevecoeur et al., 2015)."

11711 L4-6. L8-9. 'make an attempt' does not sound very scientific. Reword.

Response: This sentence no longer exists in the revised manuscript.

11711 L14. I suggest you write that the study region is in N. Quebec, Canada.

Response: We have added this information as suggested.

11715 L25. I believe this technique only capture 14C fixed in particulate OM and not C allocated to exudates. Would be good to explain what PP (net vs gross, part vs. tot) the data represent.

Response: The referee is correct and we have clarified the method:
"The incubations were terminated by filtrating samples onto GF/F filters; the method captured only ^{14}C that was fixed in particulate organic matter and did not include carbon allocated to exudates. "

L11716 L3-4. It is not clear how you derived PAR. Please explain in more detail.

Response: As suggested, we now specify the use of in situ surface measurements:
"…and PAR estimated from **in situ surface measurements** and diffuse attenuation coefficients calculated from a linear regression using TSS and DOC (Watanabe et al., 2011)."

L11716 L5. How do you know that there were no light if it was not measured?

Response: The light was indeed measured. We have revised the sentence to clarify this, and now include information on the photometer that was used to measure PAR:
"Light was measured under the ice with a Li-Cor Li-192 submersible PAR Quantum sensor."

L11716 L12-The lack of algae d13C data is a weakness. Although these data are very hard to obtain it would be good with complementing the modelling approach with some sort of uncertainty analysis to show how the results could vary with variation in d13C algae. This could be accomplished by using literature data on the fractionation factor.

Response: After careful considerations we have decided to completely remove the 13C results from the manuscript. Addition of complementary modelling and uncertainty analyses would lengthen an already long manuscript, while the referees asked to shorten it. We are convinced that our algae d13C estimations are correct, but agree with the referee that more details would be needed. We will present these results in another manuscript that focuses on the diet top consumers in thaw ponds, and will keep the focus on the microbial food web in the current manuscript.

L11716 L15. Provide details on method for d13C CO2.
L11716 L26. The test with variable C:chla ratios is good. Please show the results from this test (now you just mention that there were no change).
11717. L20-22. Please provide info if/how you accounted for trophic fractionation for 13C in consumers?

Response: These three comments refer to the algae d13C values in the previous comment, and no longer apply as the 13C results have be removed from the revised manuscript.

Results. There is a general lack of statistical support when stating that there are differences between seasons or depths.

Response: We have added correlations and ANOVA-outputs throughout the results section.

11718. L4. Yes, it is likely that ice and snow absorbed all light but since light was not measured this is an assumption. Reformulate.

Response: Please see the comment above about the PAR measurements under the ice. They were indeed measured.

11719 L11-12. As written it is unclear if this is a general presence or if it applies for summer or winter only. Rewrite and clarify.

Response: We have specified that the season in question is summer:
"$SUVA_{254}$ followed the same trend as a_{320}, with lower values in winter (0.8 ± 0.2 L mg C^{-1} m^{-1}) compared to summer surface (4.8 ± 1.0 L mg C^{-1} m^{-1}) and bottom waters (7.7 ± 2.4 L mg C^{-1} m^{-1}), indicating the presence of more aromatic compounds, and possibly DOM-Fe complexes **in summer**."

11720 L18. Give stats when you state something is significant different

Response: We have added correlations and ANOVA-outputs throughout the results section.

11720L25. ':::cover most likely prevented:::'

Response: We remain with the original sentence because light was measured (see above responses).

11721 L27. Add results from uncertainty analysis. This may show that there are some uncertainty around these numbers from mixing model. If so I suggest you express the resource use by the consumers less strongly than is now the case ('...clearly dominated by ::: ').

Response: The mixing model results have been removed from the manuscript (see above responses).

11722. L4-5. You do not have the detailed temporal and spatial resolution to be able to make these general statements about variability. I suggest you specifically write surface vs. deep and late winter vs. summer (or mid-summer) instead of trends over seasons and down the water column.

Response: We have modified the opening sentence of the discussion as requested by the referee:
"The large variations in physicochemical characteristics of thaw ponds between late winter and summer and between the surface and bottom waters were reflected in their ecological properties."

11722 L9. Super saturation does not imply a dominance of energy flows through heterotrophs.

Response: We have clarified the sentence, and please also see the earlier comment about supersaturation and net heterotrophy:
"The overall supersaturation in GHG and the clear dominance of heterotrophy over phototrophy **as expressed in the biomass and activity of the microbial food web components** indicate that the majority of the energy was flowing through the heterotrophic food web."

11723 L9-10. How was the C accumulation calculated? There are only 2 time points, one in winter and one in summer so it is hard to understand how accumulation rates could be calculated. Also, winter concentrations are affected by freezing per see.

Response: In response to this comment, we now specify that the length of the isolation periods estimated from a previous study was used to calculate the GHG accumulation rates, and some assumptions that needed to be made:
"When normalized by the duration of the **known** isolation period **(Laurion et al., 2010**), GHG accumulated more rapidly in summer at the pond bottom (6.8 ± 2.2 µM CO_2 d^{-1} and 1.9 ± 0.9 µM CH_4 d^{-1}) than in winter under the ice cover (1.5 ± 0.3 µM CO_2 d^{-1} and 0.030 ± 0.027 µM CH_4 d^{-1}), assuming a constant accumulation rate during the two sampling periods and excluding freezing exclusion effects."

11724 L7. It is likely that SDOM decreased during winter but you actually do not have any time series data to show this.

Response: We agree and have rephrased the sentence instead of describing tendencies that are difficult to make with only two time points:
"CDOM values were smaller in winter (late winter a_{320}; Table 2) and less aromatic ($SUVA_{254}$) than in summer,….."

11724 L9-13. This part is unclear. It is not obvious why high DOC suggest dominance of carbohydrates or protein like compounds. Also, interruption of allochthonous input plus degradation of CDOM would decrease DOC conc. Part of the increase in DOC conc. should also be explained by outfreezing during ice formation.

Response: We have separated the DOC and CDOM into two different sentences to avoid confusion between the two variables. We have also introduced the effect of out-freezing, as suggested by the referee:
"CDOM values were smaller in winter (late winter a_{320}; Table 2) and less aromatic ($SUVA_{254}$) than in summer, suggesting that carbohydrates or protein-like compounds from bacteria-induced CDOM-degradation made up a larger fraction of DOM in winter… The higher DOC values observed in winter (average of 8.3 mg L^{-1}) compared to summer at the surface (5.8 mg L^{-1}) thus likely resulted from a combination of a generation of dissolved compounds from the uninterrupted but slower microbial activity, and out-freezing inputs associated to the formation of ice."

11725 L17-18. You should discuss the correlations between biological variables and environmental variables.

Response: We are unfortunately unable to understand what the referee means with this comment. It refers to the second opening sentence in the "thaw pond microbial food web" section in the discussion: "When exposed to light, the production of carbon by the winter phytoplankton community was in the same range as the heterotrophic production…"
If the request is to discuss the correlations between primary producers and environmental variables, or heterotrophic producers and environmental variables, these were discussed thoroughly in the following paragraphs.

11726 L14, L20. This is somewhat confusing. You first write that BP were strongly linked to CDOM but then that DOC did not control BP.

Response: This is right; BP was strongly linked to CDOM variables SUVA and a_{320} but not to DOC. CDOM variables and DOC are different indices characterizing DOM. DOC quantifies bulk carbon concentrations of DOM, while SUVA and a_{320} are optical indices of the DOM quality. They do not always correlate.

11726 L21-23. This has already been mentioned in the discussion.

Response: We have removed the sentence to avoid redundancy.

11728 L5. I assume you mean 'input' rather than 'sources' of carbon.

Response: We have replaced the word source by the word input as suggested.

Table 1 and 2. Could these be combined to save space?

Response: These tables as well as Table 3 are in the same format. They could form one giant table, but we feel that separating the variables into the same categories that were used in the sections of the manuscript (3.1. physiochemical properties, 3.2. carbon characterization and 3.3. microbial abundance) makes the reading easier.

Table 2. Why is KWK23 summer surface data included twice?

Response: Thank you for noticing this typo; the "Summer surface" title was on the wrong line.

Table 4. Are the statistics for summer or winter data? Give n values.

Response: It is now specified that the correlations were done for all data grouped. We have also added n in the table.

Figure 2. Do you need to show this figure?

Response: Although Table 2 gives the accurate values for S_{289} and S_{382}, we thought that the graphical presentation of the spectral slope signatures is visually powerful and presents in more details the differences between seasons and depths.

Fig 5. What does the error bars represent?

Response: This figure has been removed along with d13C results.