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), and while no such information is yet available for thaw ponds, the recent mobilization of terrestrial carbon stocks stored for thousands of years in the permafrost (Vonk et al., 2012) suggests a similar DOM increase is taking place also 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 $1°C \cdot m^{-1}$) 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 (CO2 (r=0.92) and CH4 (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 "biomass" 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 carbon **biomass** 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 difference in GHG accumulation, CDOM characteristics, and autotrophic and heterotrophic carbon pools between two seasons 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 of GHG in the water column. We further hypothesized that the thermal structure in summer generates an accumulation of organic carbon in the hypolimnion where heterotrophy dominates. This study follows earlier ones looking at the limnological characteristics and bacterial communities of subarctic thaw ponds in summer (Breton et al., 2009; Negandhi et al., 2013; 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 only capturing ^{14}C fixed in particulate organic matter and not the 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 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 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 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.

Carbon dynamics in highly heterotrophic subarctic thaw ponds

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Abstract

Global warming has accelerated the formation of permafrost thaw ponds in several subarctic and arctic regions. These ponds are net heterotrophic as evidenced by their greenhouse gas (GHG) supersaturation levels (CO_2 and CH_4), and generally receive large terrestrial carbon inputs from the thawing and eroding permafrost. We measured seasonal and vertical variations in the concentration and type of dissolved organic matter (DOM) in five subarctic thaw (thermokarst) ponds in northern Quebec, and explored how environmental gradients influenced heterotrophic and phototrophic biomass and productivity. Late winter DOM had low aromaticity indicating reduced inputs of terrestrial carbon, while the high concentration of dissolved organic carbon (DOC) suggests that some production of non-chromophoric dissolved compounds by the microbial food web took place under the ice cover. Summer DOM had a strong terrestrial signature, but was also characterized with significant inputs of algal-derived carbon, especially at the pond surface. During late winter, bacterial production was low (maximum of 0.8 mg C m^{-3} d^{-1}) and was largely based on free-living bacterioplankton (58%). Bacterial production in summer was high (up to 58 mg C m^{-3} d^{-1}), dominated by particle-attached bacteria (67%), and strongly correlated to the amount of terrestrial carbon. Primary production was restricted to summer surface waters due to strong light limitation deeper in the water column or in winter. The phototrophic biomass was equal to the heterotrophic biomass, but as the algae were mostly composed of mixotrophic species, most probably they used bacteria rather than solar energy in such shaded ponds. ~~According to the δ^{13}C analyses, non-algal carbon supported 51% of winter and 37% of summer biomass of the phantom midge larvae, *Chaoborus* sp., that are at the top of the trophic chain.~~ Our results point to a strong heterotrophic energy pathway in these thaw pond ecosystems, where bacterioplankton dominates the production of new carbon biomass in both summer and winter.

1 Introduction

Traditional view of inland waters as sinks of carbon has changed during the past decades, and at present, lakes and ponds are considered net sources of carbon to the atmosphere (Tranvik et al., 2009). Increasing attention has been given to thaw ponds and lakes after recognizing the cumulative effect of their high abundance and greenhouse gas (GHG) emissions on global warming (Kling et al., 1992; ~~Hamilton et al., 1994; Nakano et al., 2000;~~ Walter et al., 2006; ~~Desyatkin et al., 2009;~~ Laurion et al., 2010), especially when they emit old carbon thus having the potential to act as a positive feedback mechanism on climate (Walter Anthony et al., 2014). Thaw ponds and lakes are dominant in continuous and discontinuous permafrost areas, for example in permafrost regions of Siberia where they represent 90% of all lakes (Walter et al., 2006), but until recently, little has been known on the limnological properties and microbial communities of this important freshwater ecosystem (~~Breton et al., 2009; Tank et al., 2009; Negandhi et al., 2013; Negandhi et al., 2014; Rossi et al., 2013; Sepulveda-Jauregui et al., 2014~~Vonk et al., 2015).

Subarctic thaw ponds can be highly turbid and have relatively high nutrient concentrations (Breton et al., 2009; Rautio et al., 2011a) compared to most high latitude freshwater ecosystems (Pienitz et al., 1997; Hamilton et al., 2001; Medeiros et al., 2012). Moreover, high perimeter/volume ratio, the presence of palsas and bogs and the thawing of organic-rich permafrost in their watershed favor high inputs of dissolved organic matter (DOM) to the ponds. ~~The rate of DOM input to aquatic systems has increased over the last decades as a result of warmer temperatures (Hudson et al., 2003), and the recent mobilization of terrestrial carbon stocks stored for thousands of years in the permafrost (Vonk et al., 2012).~~ Chromophoric DOM (CDOM) can influence water column temperature regimes through its absorption of sunlight (Caplanne and Laurion, 2008), and with suspended particles, they contribute to form a distinct and stable thermocline ~~and strongly regulate the depth of the euphotic zone~~ (Laurion et al., 2010). The rate of DOM input to aquatic systems has been documented to increase~~d~~ in boreal lakes over the last decades (Hudson et al., 2003), and while no such information is yet available for thaw ponds, the recent mobilization of terrestrial carbon stocks stored for thousands of years in the permafrost (Vonk et al., 2012) suggests a similar DOM increase is taking place also in the North.

High DOM concentration also strongly regulates the depth of the euphotic zone. Light is usually limited to the first meter in thaw ponds (Squire and Lesack, 2003; Watanabe et al., 2011), therefore

restricting the water volume where photosynthesis can occur. This type of environment favors heterotrophic bacterial production (BP) that was shown to reach similar rates as in eutrophic lakes (Breton et al., 2009). The limnological characteristics of thaw ponds are likely also beneficial to mixotrophic algae and heterotrophic protozoans, as flagellated species are generally well adapted when lakes are rich in humic compounds (Jones, 2000). In low light conditions they can utilize bacteria as an energy source, and as they are motile they also benefit from the nutrient-rich bottom waters (Arvola et al., 1991). They have been shown to be a key link in the carbon transfer from low to high trophic levels in small boreal forest lakes (Salonen and Rosenberg, 2000).

Bacterial metabolism on allochthonous carbon determines its transfer to mixotrophic algae and heterotrophic protozoans, to zooplankton feeding on bacteria, and via predation to higher trophic levels. Many recent studies consider terrestrially-derived carbon as an important source of energy for aquatic bacteria, especially when algal production is limited, and a subsidy to higher trophic levels despite its lower quality (Jansson et al., 2000; Cole et al., 2006; Berggren et al., 2010). Before entering aquatic ecosystems, DOM has already been degraded and transformed by soil microbes (Wetzel, 1995; Kalbitz et al., 2003), and contains mainly the recalcitrant fraction of this carbon pool and no essential fatty acids required by all organisms (Brett et al., 2009; Brett et al., 2012). However, carbon budgets performed on an increasing number of lakes, taking into account the carbon flow from soils to lakes, and its transformation and transport to the atmosphere and to sediment storage, suggest that an important part of soil carbon is metabolized in lakes (Cole et al., 2007; Tranvik et al., 2009). Part of this metabolization is made possible through the action of sunlight in lake water, which transforms DOM into smaller, more labile molecules (Lindell et al., 1995; Laurion and Mladenov, 2013).

Circumpolar thaw ponds are ice covered for a large fraction of the year, which further affects carbon cycling, and generates large seasonal variations. In the absence of light, autotrophic productivity stops while respiration continues, resulting in high GHG releases in spring (Striegl et al., 2001). The thermal stratification in summer also acts as a barrier and creates another habitat where GHG and organic carbon accumulate, leading to GHG storage flux during the autumnal overturn. The ponds can also be disconnected or poorly connected to a hydrographic network (Bouchard et al., 2011), affecting carbon cycling influenced mainly by evaporation and sedimentation. Hence the dissolved inorganic and organic carbon pools in subarctic thaw ponds may vary widely in time and space. The aim of this study was to demonstrate the difference , and to

98   ~~show how it influences~~in GHG accumulation, CDOM characteristics, and autotrophic and

99   heterotrophic carbon pools <u>between two seasons and depths</u>. Comparisons were made between late

100   winter and summer, and between surface and bottom waters in summer. <u>We hypothesized that in</u>

101   <u>the absence of light in winter or in the aphotic summer hypolimnion, autotrophic productivity stops</u>

102   <u>while respiration continues, resulting in large summer and winter storage of GHG in the water</u>

103   <u>column. We further hypothesized that the thermal structure in summer generates an accumulation of</u>

104   <u>organic carbon in the hypolimnion where heterotrophy dominates</u>~~We also make an attempt to~~

105   ~~estimate the role of terrestrial carbon in the diet of higher organisms in the ponds~~. This study

106   follows earlier ones looking at the limnological characteristics and bacterial communities of

107   subarctic thaw ponds in summer (Breton et al., 2009; Negandhi et al., 2013; ~~Negandhi et al., 2014;~~

108   Rossi et al., 2013<u>; Crevecoeur et al., 2015</u>).

109

2 Methods

2.1 Sample collection and limnological analyses

112   The study was carried out in the discontinuous permafrost region near the village of

113   Whapmagoostui-Kuujjuarapik (55° 20' N 77° 30' W)<u>, Northern Quebec, Canada,</u> along the River

114   Kwakwatanikapistikw (local Cree name; KWK is used to name the ponds hereafter) where a large

115   number of thaw ponds can be found. At this site, ponds are thermokarstic and form over an

116   impermeable clay-silt bed, preventing most hydrological interactions among ponds, on center of

117   thawed lithalsas (or inorganic palsas). They are surrounded by dense shrubs and sparse trees, and

118   occasional areas of mosses and aquatic plants. More details on the study area are provided in

119   Bouchard et al. (2014) and Bouchard et al. (2011).

120   Five ponds were studied during late winter (6-11 April) and summer (8-14 August) in 2009. They

121   are all small (averaged diameter 17 m $\pm$ 1.3 m) and shallow (average depth 2.5 m $\pm$ 0.4 m).

122   Sampling was done with a Kemmerer water sampler (Wildco® USA) through a borehole drilled

123   into the ice in winter, and from an inflatable boat in summer at the middle of the pond. During

124   winter sampling, the water started pouring over the ice through the hole because of the pressure

125   from the weight of snow and ice, and possibly resulting in a mixing of surface with deeper water.

126   This was suggested as the factor explaining the similar GHG concentrations obtained in surface

127   <u>(just under the ice) </u>and bottom waters<u> (approximately 0.5 m above the sediments)</u>, despite the

128   expected inverse thermal stratification at this period of the year (Laurion et al., 2010). All other

129   samples were collected from about 1 m below the ice and considered as representative of an

130   integrated water column. In summer, surface and bottom waters were sampled from just below the

131   surface and approximately 0.5 m above the bottom sediments.

132   Measures of temperature, dissolved oxygen, pH and conductivity were done with a 600R YSI

133   multiparametric probe (Yellow Springs Inc.). Further analyses were performed on water prefiltered

134   through a 50-µm sieve. Total suspended solids (TSS) were obtained by filtering 500-1000 mL of

135   water onto precombusted and preweighted GF/F filters (Advantec MFS Inc.). The 50-µm

136   prefiltration reduced the TSS by an average 5.8% in comparison to bulk water samples, both

137   analyzed in summer (data not shown). Filters were stored at -20°C until they were dried for 24 h at

138   60°C, and weighted to get sestonic dry weight or TSS. They were subsequently combusted at 450°C

139   for 2 h ~~to estimate the organic fraction of TSS and~~ to be able to calculate the organic fraction of

140   TSS. An aliquot of water was filtered through a pre-rinsed cellulose acetate filter (0.2 µm pore size;

141   Advantec MFS Inc.) to analyse soluble reactive phosphorus (SRP; unpreserved samples) and iron

142   (Fe; samples preserved with HNO_3 at 0.15% final concentration). Total phosphorus (TP) and total

143   nitrogen (TN) were analysed from unfiltered water preserved with H_2SO_4 (final concentration of

144   0.15%). All nutrient samples were stored in acid-washed glass bottles in dark and cold (4°C) until

145   further analysis as in Breton et al. (2009).

146

147   2.2 Carbon characterization

148   Dissolved CO_2 and CH_4 were determined as in Laurion et al. (2010). In brief, 2 L of pond water

149   were equilibrated into 20 mL of ambient air for 3 min, with the headspace sampled in duplicated

150   glass vials (Vacutainers©) previously flushed with helium and vacuumed. Gas samples were

151   analyzed by gas chromatography (Varian 3800), and dissolved gas concentrations were calculated

152   according to Henry's Law.

153   The water filtered through pre-rinsed cellulose acetate filters as above was also used to analyze

154   dissolved organic carbon concentration (DOC), and for optical analyses of CDOM. Samples were

155   stored in amber glass bottles in dark and cold (4°C) until the analysis. DOC was quantified using a

156   carbon analyzer (Shimadzu TOC-5000A) calibrated with potassium biphthalate. The absorbance

157   was measured from 200 to 800 nm with a spectrophotometer (Varian Cary 300), using 1-cm quartz

158   cuvettes on dual-beam mode, at 1 nm intervals for winter samples, and 4 nm intervals for summer

159   samples. Null-point adjustment was performed using the mean value from 750-800 nm, and the

160   absorption coefficients (a_λ) were calculated from absorbance measurements (A_λ) at 254 and 320 nm

161   using $a_\lambda = 2.303\ A_\lambda/L$, where L is the length of the cuvette in meters (Mitchell et al., 2002). The

162   absorption coefficient at 320 nm (a_{320}) was used as an index of CDOM concentration. Specific UV

163   absorbance–index ($SUVA_{254}$) was determined from DOC normalized A_{254}, and used as an index of

164   aromaticity (Weishaar et al., 2003).

165   CDOM absorption spectra were further analyzed to calculate the spectral slopes S_λ (Loiselle et al.,

166   2009) between 250 nm and 450 nm (Galgani et al., 2011). Slopes were calculated over 20 nm

167   intervals with a 1 nm step (i.e. 250-269, 251-270,…) for winter samples, and 4 nm steps for

168   summer samples (i.e. 250-269, 254-273,…). The resulting set of spectral slopes was plotted by

169   center wavelengths. Calculations were performed in open source software package SciLab 4.15.

170   The individual spectral slope S_{289} was used to evaluate the amount of fulvic and humic acids likely

171   related to the autochthonous production (Loiselle et al., 2009). Algal-derived carbon has a

172   maximum slope at 289 nm, while terrestrial carbon spectral slopes lack this peak and present a

173   spectrally variable distribution with a steady increase from 260 to 390 nm.

174

175   2.3 Microbial abundance and productivity

176   For the determination of chlorophyll *a* (Chl-*a*) concentrations, 1-2 L of 50 µm-sieved pond water

177   were filtered onto GF/F filters. Samples were collected in duplicates and stored at -80°C until

178   pigment extraction and fluorometric analysis were done in hot ethanol according to Nush (1980).

179   Water samples for the enumeration of nanoflagellates (PNF: phototrophic and mixotrophic cells;

180   HNF: heterotrophic cells) and phototrophic picoplankton (PPA) were preserved with 0.2 µm-

181   filtered glutaraldehyde solution at a final concentration of 1%. Three replicate aliquots (3-5 ml)

182   were filtered through 0.6 µm black polycarbonate filters (Nuclepore). Samples were stained with 4-

183   ,6-diamido-2-phenylindole (DAPI), mounted to slides and stored at -20°C until counting under UV

184   excitation with an epifluorescense microscope (Zeiss Axiovert 200) at 1000× magnification.

185   Discrimination between pigmented and heterotrophic nanoflagellates was done with green

186   excitation light. At least 50 cells or a maximum of 30 fields were counted from each sample.

187   Nanoflagellate cells were further divided into three size categories: < 5 µm, 5-10 µm and > 10 µm.

188 Volumes were converted to carbon biomass using the carbon content coefficient of 0.19 pg C μm^{-3}

189 for PNF and HNF (Putt and Stoecker, 1989). Phototrophic picoplankton carbon content was

190 calculated using the carbon conversion equation (pg C $= 0.433 \times V^{0.866}$, where V is in μm^3) for

191 picoeukaryotic cells (Verity et al., 1992; Campbell et al., 1994).

192 Bacterial abundance (BA) was measured with a flow cytometer (FACSCalibur, Becton-Dickinson)

193 as in Rossi et al. (2013) before and after sonication to obtain estimations of free-living and particle-

194 attached bacterial abundance. Biomass calculation for bacterioplankton was based on biovolumes

195 measured from digital images acquired from DAPI-stained microscope slides using the *Cell C*

196 program (Selinummi et al., 2005)–. Average bacterial carbon content was first estimated with

197 allometric conversion formula recommended for DAPI stained cells (fgC $= 218 \times V^{0.86}$, where V is

198 in μm^3) (Posch et al., 2001). The average cellular carbon content (18.1 fg C $cell^{-1}$) was then

199 multiplied by the bacterial abundance.

200 Bacterial production was measured from unfiltered and filtered (3 μm) water samples using tritiated

201 leucine (^{3}H-leucine) incorporation with centrifugation (Smith and Azam, 1992). A solution of ^{3}H-

202 leucine (specific activity of 164 Ci $mmol^{-1}$) was added to each vial to obtain a final leucine

203 concentration of 30 nM, at which bacteria were saturated (experimentally tested). During the winter,

204 the samples were incubated at ~0°C in the dark for 2 hours. During the summer, samples were

205 incubated at *in situ* temperatures (5-18°C), depending on ponds. BP was stopped by adding

206 trichloroacetic acid (5% final concentration), and samples were then stored at -20°C before the

207 centrifugation step. Winter samples were radioassayed with a Perkin Elmer Tri-Carb 2800, and

208 summer samples with a Beckman LS 6500. Leucine incorporation was converted to carbon biomass

209 using coefficients from Simon and Azam (1989). Bacterial respiration (BR) was estimated from BP

210 according to Del Giorgio and Cole (1998) BR model II.

211 Primary production (PP) was measured from the mixed water column during winter and from the

212 surface in summer. An incubation system generating a gradient of photosynthetically active

213 radiation (PAR), introduced by Rae and Vincent (1998), was used to obtain photosynthesis versus

214 irradiance curves from complete darkness to full sunlight (E_{max}). The value for E_{max} was calculated

215 from *in-situ* PAR measurements available from a weather station located in Whapmagoostui-

216 Kuujjuarapik, and assuming the air-water surface in these humic waters to cause a 50% decrease in

217 radiation levels (Doxaran et al., 2004). A water volume of 20 mL was inoculated with a working

solution of $[^{14}C]$-HCO$_3$ (80 μCi ml^{-1}) to a final concentration of 0.2 μCi ml^{-1}. In winter, the incubations were done outside (~0°C) under natural light conditions in a water bath for 45-60 min, while in summer a circulating water bath was used to keep samples at *in situ* temperature (14 – 18 °C) and incubations were run for 120 min. The incubations were terminated by filtrating samples onto GF/F filters, the method only capturing ^{14}C fixed in particulate organic matter and not the carbon allocated to exudates. The filters were subsequently frozen before they were radioassayed with a Perkin Elmer Tri-Carb 2800 liquid scintillation counter. Chl-a normalized carbon fixation rates were fitted to the equation of Platt et al. (1980) or Jassby and Platt (1976) depending on the presence or absence of photoinhibition, respectively. Data fit to the equations had on average a r^2 value of 0.92, and always higher than 0.81. The iterative non-linear regression of SigmaPlot 11.0 was used to obtain the maximum photosynthetic rate (P$_{max}$). PP at the pond bottom was estimated from the photosynthesis-irradiance curves and PAR estimated from ~~the~~ in situ surface measurements, and diffuse attenuation coefficients calculated from a linear regression using TSS and DOC (Watanabe et al., 2011). Without light under the ice and snow cover ~~(measured)~~, PP was established at zero. Light was measured under the ice with a Li-Cor Li-192 submersible PAR Quantum sensor.

~~2.4 Carbon assimilation in the food web~~

~~Trophic transfer of carbon through the food web was evaluated with $\delta^{13}C$ stable isotopes. The role of different carbon sources for phantom midge larvae (*Chaoborus* sp.) was calculated using $\delta^{13}C$ end-member values of phytoplankton and terrestrial carbon. *Chaoborus* is at the top of the food chain in these fishless ponds. The phytoplankton $\delta^{13}C$ signature was considered a function of the $\delta^{13}CO_2$ signature and the photosynthetic fractionation parameter epsilon (Ɛp):~~

$$\delta^{13}C_{algae} = \delta^{13}CO_{2(aq)} - Ɛp$$

~~$\delta^{13}CO_2$ was obtained from the analyses run for the summer surface waters (G.G. Hatch Stable Isotope Laboratory, University of Ottawa, Canada). Ɛp was calculated from the linear relationship between Ɛp and phytoplankton growth rate divided by the measured CO_2 concentration (μ/CO_2), as described by Laws et al. (1995), where Ɛp = ((μ/CO_2) 0.371)/ 0.015. Phytoplankton growth rate (μ) was estimated as the ratio between algal biomass and PP (Table A1). Algal biomass was calculated from Chl-*a* concentration by using a carbon:Chl-*a* conversion factor of 42.4 of unfed low light-adapted mixotrophic dinoflagellates (Skovgaard et al., 2000). This factor was chosen because~~

mixotrophic species are by far the most abundant group in these ponds, (Dupont, 2008; Forsström, unpublished data). Normally there are large variations in the C:Chl *a* ratios (Moal et al., 1987) and therefore $\delta^{13}C_{algae}$ calculations were tested for C:Chl-*a* ratios from 25 to 100. $\delta^{13}C_{algae}$ was not significantly impacted by ratio change. An average $\delta^{13}C$ signature of -28.21‰ ± 0.15 obtained from the boreal region (Marshall et al., 2007) was used as the terrestrial carbon end member.

Chaoborus population was sampled with 50 μm plankton net, three replicates each approximately 10 individuals were freeze-dried and grinded to a fine powder before stable isotope analysis. POM samples that were considered as food source for *chaoborus* were collected by filtering up to 2 L of water through precombusted GF/F filters. The filters were freeze dried and the organic layer was removed for stable isotope analyses that were performed with a Carlo Erba Flash EA1112 elemental analyzer connected to a Thermo Finnigan DELTA^plus Advantage CF-IRMS. Three replicates were analyzed from each sample. Pike (*Esox lucius L.*) white muscle tissue was used as an internal laboratory working standard, calibrated against International Atomic Energy Agency standards. Internal precision was always better than 0.2‰.

2.45 Data analysis

Differences in environmental and microbial variables among waters were tested using a one-way ANOVA (fixed factor; JMP software, version 11.0.0, SAS Institute Inc. 2013). Conductivity, BP, PPA, SRP, and PNF biomass were log-transformed, while TP, TN, and BB were square-root transformed to achieve normality and homogeneity of variance in residuals (visually checked with predicted values). When differences were detected, a Tukey HSD multiple comparison test was performed. Environmental variables are illustrated with a principal component analysis (PCA) (PAST version 2.17c), where biological variables were entered as vectors which length and direction were determined by Spearman's correlation analysis between biological variables and PCA axis 1 and 2. Correlations between organisms (abundance, biomass and productivity measurements) and environmental variables were tested with non-parametric Spearman's rank correlation coefficient analysis (IBM SPSS statistic 20.0). A significant level of $\alpha = 0.05$ was used for all statistical tests. The contribution of carbon from phytoplankton and terrestrial sources to the *Chaoborus* diet was calculated using the linear IsoError mixing model which partitions two sources with a single isotopic signature (e.g., $\delta^{13}C$).

278

3 Results

3.1 Physicochemical properties of thaw ponds

In late winter, the ponds were covered with 50 to 70 cm of snow and approximately 30 to 60 cm of ice, which absorbed all light. Water temperature was 2.0°C under the ice and the ponds were likely O_2 depleted at the bottom because of the smell of hydrogen sulfide, while O_2 concentration was 4.5 $\pm$ 3.5 mg L^{-1} in the integrated water column sample. In summer, despite their shallow depth, ponds are strongly stratified with nearly 10°C difference in temperature and 10 mg L^{-1} difference in O_2 between surface and bottom (Fig. 1). 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. The pH varied between 6.0 and 7.5, with lowest and highest values respectively measured in summer bottom and surface waters. There was no difference (p = 0.9034) in conductivity between winter (50 $\pm$ 7 µS/cm) and summer at the surface (46 $\pm$ 10 µS/cm), but bottom waters had significantly (p < 0.009) higher conductivity (194 $\pm$ 67 µS/cm) than winter and summer surface waters. Nutrients were relatively high in all samples. TN concentrations were elevated in winter (958 $\pm$ 235 µg N L^{-1}) compared to the summer values (264 $\pm$ 118 µg N L^{-1}) (p = 0.0001). TP was relatively high in winter (97 $\pm$ 43 µg P L^{-1}), and in summer the concentrations were clearly lower at the surface (55 $\pm$ 29 µg P L^{-1}) than at the bottom (3110 $\pm$ 1043µg P L^{-1}) (p = 0.0001). SRP was available during the whole year, with higher concentrations in late winter (26 $\pm$ 15 µg P L^{-1}) and summer bottom waters (31 $\pm$ 36 µg P L^{-1}) than in summer surface where concentrations were relatively low (3.1 $\pm$ 3.0 µg P L^{-1}) but not statistically different from the other values (p = 0.064). All ponds had higher TSS in bottom waters (85 $\pm$ 43 mg L^{-1}), while in winter and summer surface waters the concentrations were generally an order of magnitude lower. In most ponds, organic fraction of TSS followed the same trend as TSS and made on average 24% of TSS. Fe was lower at the surface (0.3 $\pm$ 0.1 mg L^{-1}) than at the bottom of ponds (3.1 $\pm$ 1.6 mg L^{-1}) in summer. No Fe measurements were done during late winter. Limnological properties of the ponds are summarized in Table 1.

305

3.2 Carbon characterization

307   CO_2 and CH_4 concentrations were high in winter (up to 357 and 14.1 µM respectively), and

308   particularly high at the pond bottom in summer (up to 815 and 312 respectively; Table 2). Surface

309   waters, in contact with the atmosphere, had one order of magnitude lower concentrations, but values

310   remained supersaturated. The inverse relationship (r = -0.9228-;= p < 0.0001) between O_2 and CO_2

311   suggests that the CO_2 originated from O_2 respiration and not from external sources such as

312   weathering, runoff and groundwater inputs that are unlikely in the studied ponds. DOC ranged from

313   4.1 to 10.5 mg L^{-1}, with the highest concentrations obtained in winter. Despite the higher DOC

314   observed in winter, CDOM was lowest at this period, as indicated by a_{320} values (7.1 ± 2.5 m^{-1}

315   compared to 27 ± 10 and 70 ± 37 m^{-1} at the surface and bottom in summer, respectively). $SUVA_{254}$

316   followed the same trend as a_{320}, with lower values in winter (0.8 ± 0.2 L mg C^{-1} m^{-1}) compared to

317   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

318   presence of more aromatic compounds, and possibly DOM-Fe complexes in summer. The shape of

319   absorption spectral slopes showed a clear maximum at 382 nm, indicating the presence of terrestrial

320   fulvic and humic acids. Consistent with the $SUVA_{254}$ index, S_{382} values were relatively low in

321   winter (except in pond KWK2) (0.0137 ± 0.0016 nm^{-1}), with similar values in summer surface

322   waters (0.0158 ± 0.0014 mg nm^{-1}), but show significant difference with bottom waters (0.0169 ±

323   0.0007 nm^{-1}) (p = 0.0061). The individual spectral slope S_{289} indicates that higher amounts of algal-

324   derived carbon were present in summer surface waters (0.0148 ± 0.0009 nm^{-1}), especially in the

325   most transparent pond KWK6 (0.0162 nm^{-1}), while their role was limited in the dark habitats under

326   the ice (0.0113 ± 0.0011 nm^{-1}) and in bottom waters (0.0112 ± 0.0007 nm^{-1}). Carbon quantity and

327   CDOM quality properties are summarized in Table 2, and the seasonal differences in absorption

328   spectral slopes are shown in Fig. 2.

329

330   **3.3 Microbial abundance and productivity**

331   The abundance of all organisms examined was highest in the summer bottom waters, but the

332   magnitude and seasonal distribution differed among taxa and ponds (Table 3). Especially, the

333   abundant motile PNF accumulated in the nutrient-rich bottom waters, where their abundance was in

334   most ponds an order of magnitude higher than at the surface (110 ± 89 vs. 27 ± 8 × 10^6 L^{-1}). The

335   less abundant HNF also favored the bottom waters, with an order of magnitude difference in the

336   abundance between bottom and surface waters (9.2 ± 11.6 vs. 0.8 ± 0.7 × 10^6 L^{-1}). The smaller

phototrophic picoplankton (PPA) and bacteria were more uniformly distributed, although the abundances were significantly higher (p < 0.05) at the bottom. Abundance was also quantified for free-living and particle-attached bacteria. During late winter, 74 ± 9% of bacterioplankton was attached to particles, and 46 ± 21% and 70 ± 17% in summer surface and bottom waters (Table ~~B2~~A1).

When converted to biomass, the seasonal and vertical distribution pattern was even more accentuated (Table 3, Fig. 3). Overall, lower biomasses were encountered in winter, and higher at the bottom of the water column in summer. Total phototrophic biomass as Chl-*a* concentration (5.6 ± 2.5 µg L^{-1}) as well as the PNF biomass (152 ± 68 µg C L^{-1}) under the ice were relatively high. Summer surface phototrophic biomass was similar to winter waters (p = 0.1213), and it was different (p < 0.05) and highest in the dark bottom waters, likely reflecting cell sedimentation. The biomass of strictly phototrophic PPA was very low in winter (5.8 ± 4.4 µg C L^{-1}) and an order of magnitude higher in summer. The bacterial biomass was always high when compared to literature, with the lowest biomass occurring in winter (109 ± 67 µg C L^{-1}), two times higher biomass in summer at the surface (223 ± 36 µg C L^{-1}), and five times higher at the bottom (520 ± 146 µg C L^{-1}). The HNF contributed little to the total microbial biomass, especially in the illuminated summer surface waters where they averaged 3.2 ± 2.9 µg C L^{-1}. Their biomass was higher at the bottom (39.2 ± 49.4 µg C L^{-1}) and in winter samples (8.9 ± 4.7 µg C L^{-1}).

Snow and ice cover prevented light penetration under the ice, and therefore we assumed that no PP occurred *in situ* in late winter. However, the photosynthetic capacity of late winter phytoplankton community was not null, and represents the potential at the ice-out, which took place 2-3 weeks later. The maximum photosynthetic rate (P$_{max}$) of late winter phytoplankton community was for most ponds an order of magnitude lower than P$_{max}$ of the summer community (2.0 ± 2.1 vs. 30.6 ± 26.1 mg C m^{-3} d^{-1}). BP rates were very low in winter (0.4 ± 0.2 mg C m^{-3} d^{-1}) and different (p = 0.0001) compared to the summer when surface (31 ± 14 mg C m^{-3} d^{-1}) and bottom (42 ± 19 mg C m^{-3} d^{-1}) BP rates. BR rates calculated from BP were low in winter (2.0 ± 0.6 mg C m^{-3} d^{-1}) and equally high during summer in surface (28 ± 6 mg C m^{-3} d^{-1}) and in bottom (33 ± 6 mg C m^{-3} d^{-1}). Particle-attached bacterioplankton played an important role in pond production at both sampling seasons (late winter: 42 ± 20%; summer surface: 66 ± 20%; bottom: 68 ± 44%; Table ~~B2~~A1).

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), a_{320} (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 S_{289} (r=-0.65), whereas PC2 has positive correlation with temperature (r=0.81), S_{382} (r=0.69) and $SUVA_{254}$ (r=0.76), and negative correlation with TN (r=-0.93) and DOC (r=-0.67). Heterotrophs (~~bacteria biomass;~~ BB and HNF) were correlated with PC1 ~~and its associated carbon quality indices,~~ while phototrophs (PP, PNF and PPA) were more strongly correlated to PC2 ~~and its associated nutrients and DOC~~ (Fig. 4b~~, Table 4~~). Further, BP and BB were best correlated with carbon quality indices a_{320}, $SUVA_{254}$, and S_{289}, 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).

~~**3.4 Carbon assimilation**~~

~~The variability in mean *Chaoborus* $\delta^{13}C$ values between winter (-34.1‰) and summer (-34.3‰) was small (Fig. 5a). Phytoplankton $\delta^{13}C$ values ranged between -34.3‰ and -41.4‰ in different ponds (Table B1). The values of POM fell between the terrestrial and algal end member values reflecting the presence of both types of carbon in the water column (Fig. 5a). According to the mixing model, on average 42% of *Chaoborus* carbon diet came from terrestrial sources in winter, while in summer the terrestrial share was 10% smaller for most ponds, thus *Chaoborus* diet became clearly dominated by algal sources (Fig. 5b). Only in one pond (KWK6) terrestrial carbon contributed substantially (70%) to the *Chaoborus* diet.~~

4 Discussion

The large variations in physicochemical characteristics of thaw ponds between late winter and summer and between surface and bottom waters ~~over the seasons and down the water column~~ were reflected in their ecological properties. The cold and nutrient-rich winter waters had the lowest microbial abundance and productivity. The warm and illuminated summer surface waters favored primary production, while the colder and darker bottom waters accumulated nutrients, carbon

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compounds and microorganisms. 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.

4.1 Spatial and seasonal variations in GHG

Greenhouse gas storage in pond waters occurred in winter under the ice cover and in summer under the steep thermocline, due to the isolation of these water masses from atmospheric venting. Partial pressures measured were especially large in summer hypolimnetic waters (in average 13 674 µatm of CO_2 and 4783 µatm of CH_4) compared to values at the surface (in average 1206 µatm of CO_2 and 9 µatm of CH_4). Such heterogeneity in the vertical distribution of GHG is typical for seasonally stratified lakes where bottom values are often many folds higher than at the surface (Eller et al., 2005; Guérin et al., 2006; Bastviken et al., 2008). Partial pressures of CO_2 at the surface of thaw ponds (598-1545 µatm) were comparable to values obtained in other regions, for example in European boreal lakes (990 µatm; Kortelainen et al. 2006), Canadian boreal streams and rivers (1850 µatm; Teodoru et al. 2009), Western Siberian lakes and ponds (1935 µatm; Shirokova et al. 2012), or Canadian subarctic and arctic ponds (1896 µatm; Breton et al. 2009). Surface water CH_4 partial pressures (6-13 µatm) were also in the same range as values reported in lakes and ponds from North-America (11-59 µatm; Bastviken et al. 2008) Finland (23 µatm; Juutinen et al. 2009), Canadian Subarctic and Arctic (22 µatm; Breton et al. 2009), and Western Siberia (30 µatm; Shirokova et al. 2012).

The dominance of BR over PP, combined with the isolation of water masses during the long winter, followed by a very short spring mixing period (Laurion et al., 2010) and the steep summer stratification of these ecosystems controlled the accumulation of GHG in the hypolimnion. 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. This may be partly due to the more abundant microbial community in summer, although most GHG produced likely came from benthic respiration, especially in such shallow systems

(Kortelainen et al., 2006). Summer also provides fresh organic matter that may stimulate GHG producers that indeed presented higher respiration rates. The smaller CH_4 accumulation rates in winter might also result from more optimal conditions for methanotrophy under the ice cover where bubbling CH_4 is trapped and dissolves in water. The amount of CH_4 in a lake was shown to depend on the size of the anoxic layer (Fendinger et al., 1992; Bastviken et al., 2004), and in the present study, this layer represents a particularly large proportion of the water volume in summer. During the autumnal turnover period (lasting about 2 months), the whole water column becomes oxygenated, and in the first part of the winter surface layer of the pond may contain sufficient oxygen (Deshpande et al., 2015) for efficient CH_4 consumption. Anoxic methanotrophy has also been reported (Sivan et al., 2011) and may occur at our study sites. Nevertheless, these GHG storage periods indicate that subarctic thaw ponds will release large amounts of CH_4 during the autumnal mixing period, and also during spring turnover if it lasts long enough (Walter et al., 2006; Laurion et al., 2010).

4.2 Seasonality in CDOM properties

We used a_{320} as an indicator of the quantity of CDOM, and $SUVA_{254}$, S_{289} and S_{382} as indicators of the aromatic content of CDOM and the relative proportions in allochthonous (terrestrial) versus autochthonous (algal) carbon sources. The high a_{320} and $SUVA_{254}$ values indicate a large contribution by terrestrial humic substances in DOM at the surface of thaw ponds in summer, with values similar to a few years earlier in the same ponds (Breton et al., 2009; Watanabe et al., 2011). CDOM values were clearly ~~decreased~~ smaller ~~along the~~in winter (late winter a_{320}; Table 2) and ~~became~~ less aromatic ($SUVA_{254}$) than in summer~~,~~. ~~The high DOC values in winter (average of 8.3 mg L^{-1} compared to 5.8 mg L^{-1} in summer surface) but its low aromaticity~~ suggest~~ings~~ that carbohydrates or protein-like compounds from bacteria-induced CDOM-degradation made up a larger fraction of DOM in winter~~,~~. Laurion et al. (unpublished data) tested the role of bacterial CDOM degradation in arctic thaw ponds and found that its importance can almost match that of photodegradation in certain cases, without significant losses in DOC, potentially indicating a bacterial transformation into less colored compounds. 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} in) thus likely resulted from a combination of a generation of dissolved compounds from the uninterrupted but slower microbial

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activity, and out-freezing inputs associated to the formation of ice~~an interruption of allochthonous inputs, and a larger contribution by bacteria induced CDOM degradation under the ice~~. Although BP in late winter was low, the long subarctic winters allow accumulation effects that could lead to a decrease in CDOM. The labile part of DOC is known to be consumed within days by bacterioplankton, leaving the recalcitrant fraction of DOC to dominate in environments where fresh inputs are limited (Del Giorgio and Davis, 2002; Roehm et al., 2009; Guillemette and del Giorgio, 2011). However this is not clearly observed in the present study thaw ponds, where less aromatic and non-chromophoric fractions increased along winter, likely as the result of DOM recycling by a rich microbial food web, together with a lack of new carbon inputs from the frozen catchment.

The high a_{320} and $SUVA_{254}$ values found in bottom waters are very likely caused by the elevated dissolved iron concentrations (up to 5.1 mg L^{-1}; $r^2 = 0.947$ between $SUVA_{254}$ and Fe in summer samples, n=10). High concentrations of iron (>2 mg L^{-1}) were shown to form complexes with humic substances and increase DOM absorbance (Maloney et al., 2005; Xiao et al., 2013). During the autumnal turnover, CDOM-Fe complexes from bottom waters are exposed to photochemical reactions at the surface, potentially generating hydroxyl radicals that dissociate carboxyl groups from humic substances. These reactions can lead to lower molecular weight carbon components at the same time as increasing the water clarity (Brinkmann et al., 2003). The spectral slope values indicate that algal-derived proteins (S_{289}; Galgani et al. 2011) were more abundant in summer surface waters when primary production and Chl-*a* were highest, and at the same time fulvic and humic acids (S_{382}) also seemed more abundant than in winter. Algal proteins are generally highly available to heterotrophic organisms and were likely supporting the higher summer BP (Kritzberg et al., 2004). Nevertheless, Xiao et al. (2013) showed how high Fe concentrations, such as found in bottom waters, can significantly lower DOM absorption slopes, thus interpretations of bottom water S_{λ} should be done cautiously.

4.3 Thaw pond microbial food web

Primary production rates in summer at the surface of thaw ponds were in the same range as in other arctic and subarctic lakes (Lizotte, 2008), while in late winter snow and ice cover prevented *in situ* PP. However, when exposed to light, the production of carbon by the winter phytoplankton community was in the same range as the heterotrophic production, suggesting that the high

phytoplankton biomass encountered during late winter were made of mixotrophic species that switched from hetero- to autotrophic production at ice-out. Similarly, the high Chl-*a* and PNF biomass in the light-limited bottom waters in summer were most likely composed of mixotrophs. Many species of Chrysophycaea, Cryptophyceae and Dinophyceae are known to be 99% mixotrophic (Olrik 1998 and references therein). Chrysophyceae were dominating the phytoplankton community in KWK2 and KWK20 (Dupont 2008, L. Forsström, unpublished data). Many Chrysophyceae species have flagella, and thus can thrive in sheltered humic ponds that have been shown to favor diurnal migrations of phytoplankton (Jones, 1991). This is especially advantageous in steeply stratified and shallow ponds where phytoplankton can migrate within a few hours (Arvola et al., 1991), and are able to use nutrient-rich hypolimnetic waters (Salonen et al., 1984). The high nutrient concentrations in bottom waters likely explain the high biomass of PNF observed in these waters. Anoxic bottom waters were also devoid of *Daphnia* sp. (M. Wauthy et al., unpublished data) providing to phytoplankton a refuge from grazing. Absence of light, hypoxia and low temperatures further prevent photo-oxidation of algal pigments, preserving them for extended periods (Vallentyne, 1960). Thus some of the Chl-*a* and PNF encountered at the bottom may have been from dead yet still not degraded cells. Another factor that could have caused bias in very high bottom water Chl-*a* values is the occurrence of chlorophyll-*b* containing green-sulphur bacteria that are known to colonize the anoxic layer of the studied ponds (Rossi et al., 2013), and that may interfere with the spectrophotometric analyses of Chl-*a*. However, green-sulphur bacteria were not found in all ponds with high values of Chl-*a*. In winter, the lack of PP and allocthonous inputs of carbon, and a DOM pool composed by a large fraction of molecules with low aromaticity (low $SUVA_{254}$ and S_{289}) suggest that intensively recycled DOM formed the basal organic carbon pool in thaw ponds.

BP and BB were strongly linked to CDOM originating from a terrestrial source (high $SUVA_{254}$ and a_{320}), indicating a positive link between bacterioplankton and terrestrial carbon (Tranvik, 1988; Crump et al., 2003). These results are in accordance with the experiment from Breton et al. (2009) which showed that bacterioplankton in thaw ponds was more carbon than nutrient-limited (glucose was used as a labile carbon source). On the contrary to several previous studies (Granéli et al., 2004; Säwström et al., 2007; Roiha et al., 2012), the amount of DOC did not seem to control the BP in our study. During the long ice-covered winter period, bacterioplankton was likely degrading the particulate carbon accumulated during the previous open water season, and producing dissolved

516 ~~compounds, explaining the DOC maximum in late winter.~~ The studies that reported a positive

517 correlation between DOC and BP have been conducted in summer, when BP and DOC

518 concentrations were highest, but without necessarily taking into account the variability in DOC

519 type, which had a dominant role in our study.

520 The presence of a mixture of autochthonous and allochthonous carbon may have helped in making

521 the terrestrial carbon available to bacteria. Phytoplankton and macrophytes apparently produced

522 significant amounts of dissolved carbon in surface waters during summer as indicated by the larger

523 S_{289} peak. This carbon is considered more labile (lower molecular weight) and with a higher

524 nutritional value to heterotrophs (Kritzberg et al., 2004; Brett et al., 2009). The availability of high

525 quality phytoplankton carbon during summer possibly enhanced the bacterial use of more

526 recalcitrant terrestrial carbon though the priming effect (Bianchi, 2011), that is a small supplement

527 of nutritionally rich algal cells increasing bacterioplankton terrestrial carbon assimilation.

528 Correlation between TSS and TP (r=0.903 p<0.001, n=15) indicates that phosphorus was binding to

529 particles, which may be more accessible to attached bacterioplankton than to phytoplankton in

530 summer surface waters where SRP concentrations were low. The high proportion of particle-

531 attached bacterial abundance (62 ± 30 %) and productivity (59 ± 30 %) in thaw ponds suggests that

532 particle attachment is beneficial. Although studies have shown that BP is often nutrient-limited

533 (Kritzberg et al., 2004; Vrede, 2005), there were no correlation between BP and nutrients in thaw

534 ponds.

535 One possible additional explanation for the difference between winter and summer BP is the

536 difference in temperature that is known to have a large impact on production (Adams et al., 2010).

537 On the other hand, bacterial communities are known to adapt at low temperatures, and some

538 communities can have multiple temperature maxima (Adams et al., 2010). In our study, the highest

539 BP was found in relatively cool bottom waters. Moreover, bacteria biomass (BB) was not correlated

540 to temperature, although several studies found temperature as a main driver of bacterial biomass

541 (Ochs et al., 1995; Rae and Vincent, 1998). Trophic interactions certainly contributed to the

542 observed patterns in microbial abundance. Rotifers are the dominant zooplankton group in the

543 studied thaw ponds, while the cladoceran community is composed of a few *Daphnia* sp. observed

544 only at the surface (Bégin, 2014). These two zooplankton groups were only found in summer, when

545 they could exert a grazing pressure on bacteria and phytoplankton, but experiments would be

needed to confirm the extent of grazing. We tentatively estimated the top-down control of nanoflagellates on bacteria, using the average nanoflagellate ingestion rate of 0.66 pg C cell^{-1} day^{-1} measured for *Dinobryon* sp. in Svalbard (Laybourn-Parry and Marshall, 2003). From these calculations, it seems that nanoflagellate grazing on bacteria could have been efficient enough in late winter when the grazing potential exceeded 100% of the BP, while summer removal estimations corresponded to only 1.7% at the surface and 9.3% at the bottom of the BP, possibly contributing to explaining the accumulation of BB in summer

In winter, the lack of PP and allocthonous sources of carbon, and a DOM pool composed by a large fraction of molecules with low aromaticity (low SUVA$_{254}$ and S$_{289}$) suggest that intensively recycled DOM formed the basal organic carbon pool in thaw ponds. This carbon was channeled to the top of the food chain by bacterial grazers (nanoflagellates) and to their predators (*Chaoborus* sp.), as suggested by the mixing model results indicating that *Chaoborus* was more dependent on terrestrial carbon in winter. Similar results showing the increasing importance of terrestrial carbon to zooplankton in winter have also been reported in the subarctic lake Saanajärvi in Finland, where *Daphnia umbra* was almost entirely dependent on terrestrial carbon under the ice (Rautio et al., 2011b). During summer, a lower dependence on terrestrial carbon was likely because algal-derived carbon was more available (high S$_{289}$ and PP), when nearly 70% of *Chaoborus* carbon had an algal origin in the majority of the ponds.

We also considered whether methane-oxidizing bacteria were included in the zooplankton diet as inferred from the mixing model results. These bacteria are known to occur in environments where both oxygen and CH$_4$ are available (Hanson and Hanson, 1996), and they were suggested to contribute to zooplankton diet (Kankaala et al., 2006). The metalimnion of thaw ponds, where there is more CH$_4$ and still some O$_2$, is a potential living habitat for methanotrophs, relatively abundant in the studied ponds (Rossi et al., 2013). However, methanotrophs are known to have highly negative δ^{13}C values (< -60‰), while in our study the *Chaoborus* δ^{13}C values always fell between the algal (median -40‰) and terrestrial (-27‰) end-members. Therefore, although methanotrophs were present, they did not seem to have a significant role in *Chaoborus* diet.

5 Conclusions

Low light intensity, strong stratification and high concentrations of CDOM in subarctic thaw ponds are the main drivers creating an environment highly beneficial to heterotrophs. We have also shown some important differences in their ecosystem dynamics between winter and summer. Although a high biomass of bacterioplankton is found under the ice, the ponds are producing relatively small amounts of carbon in late winter, likely caused by the lack of fresh carbon inputs and its associated priming effect. Nevertheless, the ponds are accumulating GHG during winter. The relatively high phototrophic biomass and primary production potential encountered under the ice are at the same level as the heterotrophic biomass and production, indicating the importance of mixotrophy in winter. In summer, fresh primary production and terrestrial carbon inputs enhance the heterotrophic production. Although a higher phototrophic (mixotrophic) biomass is encountered in summer, primary production is restricted by light and space and is significant only at the pond surface, thus the heterotrophic carbon production remains significantly higher. Consequently, GHG continue to accumulate in hypolimnetic waters during this strongly stratified summer period, and are most likely released during the autumnal turnover period, in addition to the constant emissions of GHG throughout the summer from the highly supersaturated surface waters.

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7 References

Adams, H. E., Crump, B. C., and Kling, G. W.: Temperature controls on aquatic bacterial production and community dynamics in arctic lakes and streams, Environ. Microbiol., 12, 1319-1333, doi: 10.1111/j.1462-2920.2010.02176.x, 2010.

Arvola, L., Ojala, A., Barbosa, F., and Heaney, S.: Migration behaviour of three cryptophytes in relation to environmental gradients: an experimental approach, Brit. Phyco. J., 26, 361-373, doi: 10.1080/00071619100650331, 1991.

Bastviken, D., Cole, J., Pace, M., and Tranvik, L.: Methane emissions from lakes: Dependence of lake characteristics, two regional assessments, and a global estimate, Global biogeochemical cycles, 18, doi: 10.1029/2004GB002238, 2004.

Bastviken, D., Cole, J. J., Pace, M. L., and Van de Bogert, M. C.: Fates of methane from different lake habitats: connecting whole-lake budgets and CH_4 emissions, J. Geophys. Res.-Biogeo. 2005-2012, 113, doi: 10.1029/2007JG000608, 2008.

Bégin, P. N.: Rotifer abundance, biodiversity and controlling variables in subarctic thermokarst lakes and ponds, M.Sc thesis, Université Laval, Québec, 2014.

Bianchi, T. S.: The role of terrestrially derived organic carbon in the coastal ocean: a changing paradigm and the priming effect, Proc. Natl. Acad. Sci. U. S. A., 108, 19473-19481, doi: 10.1073/pnas.1017982108, 2011.

Bouchard, F., Francus, P., Pienitz, R., and Laurion, I.: Sedimentology and geochemistry of thermokarst ponds in discontinuous permafrost, subarctic Québec, Canada, J. Geophys. Res., 116, doi: 10.1029/2011jg001675, 2011.

Bouchard, F., Francus, P., Pienitz, R., Laurion, I., and Feyte, S.: Subarctic thermokarst ponds: Investigating recent landscape evolution and sediment dynamics in thawed permafrost of northern Québec (Canada), Arct. Antarct. Alp. Res., 46, 251-271, doi: 10.1657/1938-4246-46.1.251, 2014.

Breton, J., Vallières, C., and Laurion, I.: Limnological properties of permafrost thaw ponds in northeastern Canada, Can. J. Fish. Aquat. Sci., 66, 1635-1648, doi:10.1139/F09-108, 2009.

Brett, M. T., Kainz, M. J., Taipale, S. J., and Seshan, H.: Phytoplankton, not allochthonous carbon, sustains herbivorous zooplankton production, P. Natl. Acad. Sci. U.S.A., 106, 21197-21201, doi: 10.1073/pnas.0904129106, 2009.

Brinkmann, T., Sartorius, D., and Frimmel, F.: Photobleaching of humic rich dissolved organic matter, Aquat. Sci., 65, 415-424, doi: 10.1007/s00027-003-0670-9, 2003.

Campbell, L., Nolla, H. A., and Vaulot, D.: The importance of *Prochlorococcus* to community structure in the central north pacific ocean, Limnol. Oceanogr., 39, 954-961, doi: 10.4319/lo.1994.39.4.0954, 1994.

Caplanne, S., and Laurion, I.: Effect of chromophoric dissolved organic matter on epilimnetic stratification in lakes, Aquat. Sci., 70, 123-133, doi: 10.1007/s00027-007-7006-0, 2008.

Crevecoeur, S., Vincent, W. F., Comte, J., and Lovejoy, C. Bacterial community structure across environmental gradients in permafrost thaw ponds: methanotroph-rich ecosystems, Front. Microbiol., 6, 192. doi: 10.3389/fmicb.2015.00192.

Crump, B. C., Kling, G. W., Bahr, M., and Hobbie, J. E.: Bacterioplankton community shifts in an arctic lake correlate with seasonal changes in organic matter source, Appl. Environ. Microbiol., 69, 2253-2268, doi: 10.1128/aem.69.4.2253-2268.2003, 2003.

Del Giorgio, P., and Davis, J.: Patterns in dissolved organic matter lability and consumption across aquatic ecosystems, in: Aquatic ecosystems: Interactivity of dissolved organic matter, edited by : Findlay, S. E. G., and Sinsabaugh, R. E., Academic press, San Diego, ca, 399-424, 2002.

Del Giorgio, P. A., and Cole, J. J.: Bacterial growth efficiency in natural aquatic systems, Annu. Rev. Ecol. Syst., 503-541, 1998.

Deshpande, B. N., MacIntyre, S., Matveev, A., and Vincent, W. F.: Oxygen dynamics in permafrost thaw lakes: anaerobic bioreactors in the Canadian subarctic, Limnol. Oceanogr., 2015.

Doxaran, D., Cherukuru, R. C. N., and Lavender, S. J.: Estimation of surface reflection effects on upwelling radiance field measurements in turbid waters, J. Opt. A-Pure Appl. Op., 6, 690-697, Pii S1464/4258(04)75616-0, doi: 10.1088/1464-4258/6/7/006, 2004.

Dupont, C.: Microbial diversity of thermokarst pond and their greenhouse gas production, M.Sc thesis, Institut national de la recherche scientifique, Université du Québec, Québec, 2008.

Eller, G., Kanel, L., and Kruger, M.: Cooccurrence of aerobic and anaerobic methane oxidation in the water column of lake Plußsee, Appl. Environ. Microbiol., 71, 8925-8928, doi: 10.1128/AEM.71.12.8925-8928.2005, 2005.

Fendinger, N. J., Adams, D. D., and Glotfelty, D. E.: The role of gas ebullition in the transport of organic contaminants from sediments, Sci. Total. Environ., 112, 189-201, doi 10.1016/0048-9697(92)90187-W, 1992.

Galgani, L., Tognazzi, A., Rossi, C., Ricci, M., Galvez, J. A., Dattilo, A. M., Cozar, A., Bracchini, L., and Loiselle, S. A.: Assessing the optical changes in dissolved organic matter in humic lakes by spectral slope distributions, J. Photoch. Photobio. B, 102, 132-139, doi: 10.1016/j.jphotobiol.2010.10.001, 2011.

Granéli, W., Bertilsson, S., and Philibert, A.: Phosphorus limitation of bacterial growth in high arctic lakes and ponds, Aquat. Sci., 66, 430-439, doi: 10.1007/s00027-004-0732-7, 2004.

Guérin, F., Abril, G., Richard, S., Burban, B., Reynouard, C., Seyler, P., and Delmas, R.: Methane and carbon dioxide emissions from tropical reservoirs: significance of downstream rivers, Geophys. Res. Lett., 33, doi: 10.1029/2006gl027929, 2006.

Guillemette, F., and del Giorgio, P. A.: Reconstructing the various facets of dissolved organic carbon bioavailability in freshwater ecosystems, Limnol. Oceanogr., 56, 734-748, doi: 10.4319/lo, 2011.

Hamilton, P. B., Gajewski, K., Atkinson, D. E., and Lean, D. R. S.: Physical and chemical limnology of 204 lakes from the Canadian arctic archipelago, Hydrobiologia, 457, 133-148, doi 10.1023/A:1012275316543, 2001.

Hudson, J. J., Dillon, P. J., and Somers, K. M.: Long-term patterns in dissolved organic carbon in boreal lakes: the role of incident radiation, precipitation, air temperature, southern oscillation and acid deposition, Hydrol. Earth. Syst. Sc., 7, 390-398, doi: 10.5194/hess-7-390-2003, 2003.

Jassby, A. D., and Platt, T.: Mathematical formulation of the relationship between photosynthesis and light for phytoplankton, Limnol. Oceanogr., 21, 540-547, 1976.

Jones, R. I.: Advantages of diurnal vertical migrations to phytoplankton in sharply stratified, humic forest lakes, Arch. Hydrobiol., 120, 257-266, 1991.

Jones, R. I.: Mixotrophy in planktonic protists: an overview, Freshwat. Biol., 45, 219-226, doi: 10.1046/j.1365-2427.2000.00672.x, 2000.

Juutinen, S., Rantakari, M., Kortelainen, P., Huttunen, J. T., Larmola, T., Alm, J., Silvola, J., and Martikainen, P. J.: Methane dynamics in different boreal lake types, Biogeosciences, 6, 209-223, doi: 10.5194/bg-6-209-2009, 2009.

Kling, G. W., Kipphut, G. W., and Miller, M. C.: The flux of CO2 and CH_4 from lakes and rivers in arctic Alaska, Hydrobiologia, 240, 23-36, doi 10.1007/Bf00013449, 1992.

Kortelainen, P., Rantakari, M., Huttunen, J. T., Mattsson, T., Alm, J., Juutinen, S., Larmola, T., Silvola, J., and Martikainen, P. J.: Sediment respiration and lake trophic state are important predictors of large CO_2 evasion from small boreal lakes, Global Change Biol., 12, 1554-1567, doi 10.1111/j.1365-2486.2006.01167.x, 2006.

Kritzberg, E. S., Cole, J. J., Pace, M. L., Granéli, W., and Bade, D. L.: Autochthonous versus allochthonous carbon sources of bacteria: results from whole-lake ^{13}C addition experiments, Limnol. Oceanogr., 49, 588-596, doi: 10.4319/lo.2004.49.2.0588, 2004.

Laurion, I., Vincent, W. F., MacIntyre, S., Retamal, L., Dupont, C., Francus, P., and Pienitz, R.: Variability in greenhouse gas emissions from permafrost thaw ponds, Limnol. Oceanogr., 55, doi: 115-133, doi: 10.4319/lo.2010.55.1.0115, 2010.

Laurion, I., and Mladenov, N.: Dissolved organic matter photolysis in Canadian arctic thaw ponds, Environ. Res. Lett., 8, 035026, Artn 035026, doi 10.1088/1748-9326/8/3/035026, 2013.

Laws, E. A., Popp, B. N., Bidigare, R. R., Kennicutt, M. C., and Macko, S. A.: Dependence of phytoplankton carbon isotopic composition on growth rate and $[CO_2]aq$: theoretical considerations and experimental results, Geochim. Cosmochim. Acta, 59, 1131-1138, doi: 10.1016/0016-7037(95)00030-4, 1995.

Laybourn-Parry, J., and Marshall, W. A.: Photosynthesis, mixotrophy and microbial plankton dynamics in two high arctic lakes during summer, Polar Biol., 26, 517-524, doi: 10.1007/s00300-003-0514-z, 2003.

Lindell, M. J., Graneli, W., and Tranvik, L. J.: Enhanced bacterial-growth in response to photochemical transformation of dissolved organic-matter, Limnol. Oceanogr., 40, 195-199, doi: 10.4319/lo.1995.40.1.0195, 1995.

Lizotte, M. P.: Phytoplankton and primary production, in: Polar lakes and rivers: limnology of arctic and antarctic aquatic ecosystems, edited by: Vincent, W. F. and Laybourn-Parry, J., Oxford University Press, Oxford, UK, pp. 197–212, 2008.

Loiselle, S. A., Bracchini, L., Cozar, A., Dattilo, A. M., Tognazzi, A., and Rossi, C.: Variability in photobleaching yields and their related impacts on optical conditions in subtropical lakes, J. Photoch. Photobio. B, 95, 129-137, doi: 10.1016/j.jphotobiol.2009.02.002, 2009.

Maloney, K. O., Morris, D. P., Moses, C. O., and Osburn, C. L.: The role of iron and dissolved organic carbon in the absorption of ultraviolet radiation in humic lake water, Biogeochemistry, 75, 393-407, doi: 10.1007/s10533-005-1675-3, 2005.

Marshall, J. D., Brooks, J. R., and Lajtha, K.: Sources of variation in the stable isotopic composition of plants, Stable isotopes in ecology and environmental science, 22-60, doi: 10.1002/9780470691854.ch2, 2007.

Medeiros, A. S., Biastoch, R. G., Luszczek, C. E., Wang, X. A., Muir, D. C. G., and Quinlan, R.: Patterns in the limnology of lakes and ponds across multiple local and regional environmental gradients in the eastern Canadian arctic, Inland Waters, 2, 59-76, doi 10.5268/Iw-2.2.427, 2012.

Mitchell, B. G., Kahru, M., Wieland, J., and Stramska, M.: Determination of spectral absorption coefficients of particles, dissolved material and phytoplankton for discrete water samples, Ocean optics protocols for satellite ocean color sensor validation, Revision, 3, 231-257, 2002.

Moal, J., Martinjezequel, V., Harris, R. P., Samain, J. F., and Poulet, S. A.: Interspecific and intraspecific variability of the chemical-composition of marine-phytoplankton, Oceanol. Acta, doi: 10, 339-346, 1987.

Negandhi, K., Laurion, I., Whiticar, M. J., Galand, P. E., Xu, X., and Lovejoy, C.: Small thaw ponds: an unaccounted source of methane in the Canadian high arctic, PLoS One, 8, doi: e78204, 10.1371/journal.pone.0078204, 2013.

Nush, E.: Comparison of different methods for chlorophyll and phaeopigment determination, Arch. Hydrobiol. Beih, 14, 14-36, 1980.

Ochs, C. A., Cole, J. J., and Likens, G. E.: Population-dynamics of bacterioplankton in an oligotrophic lake, J. Plankton. Res., 17, 365-391, doi: 10.1093/plankt/17.2.365, 1995.

Olrik, K.: Ecology of mixotrophic flagellates with special reference to chrysophyceae in Danish lakes, Hydrobiologia, 370, 329-338, doi: 10.1023/A:1017045809572, 1998.

Pienitz, R., Smol, J. P., and Lean, D. R. S.: Physical and chemical limnology of 59 lakes located between the southern Yukon and the Tuktoyaktuk peninsula, Northwest Territories (Canada), Can. J. Fish. Aquat. Sci., 54, 330-346, doi: 10.1139/cjfas-54-2-330, 1997.

Platt, T., Gallegos, C. L., and Harrison, W. G.: Photoinhibition of photosynthesis in natural assemblages of marine-phytoplankton, J. Mar. Res., 38, 687-701, 1980.

Posch, T., Loferer-Krossbacher, M., Gao, G., Alfreider, A., Pernthaler, J., and Psenner, R.: Precision of bacterioplankton biomass determination: a comparison of two fluorescent dyes, and of allometric and linear volume-to-carbon conversion factors, Aquat. Microb. Ecol., 25, 55-63, doi: 10.3354/ame025055, 2001.

Putt, M., and Stoecker, D. K.: An experimentally determined carbon: volume ratio for marine "oligotrichous" ciliates from estuarine and coastal waters, Limnol. Oceanogr., 34, 1097-1103, 1989.

Rae, R., and Vincent, W. F.: Phytoplankton production in subarctic lake and river ecosystems: development of a photosynthesis-temperature-irradiance model, J. Plankton Res., 20, 1293-1312, doi: 10.1093/plankt/20.7.1293, 1998.

Rautio, M., Dufresne, F., Laurion, I., Bonilla, S., Vincent, W. F., and Christoffersen, K. S.: Shallow freshwater ecosystems of the circumpolar arctic, Ecoscience, 18, 204-222, doi 10.2980/18-3-3463, 2011a.

Rautio, M., Mariash, H., and Forsström, L.: Seasonal shifts between autochthonous and allochthonous carbon contributions to zooplankton diets in a subarctic lake, Limnol. Oceanogr., 56, 1513-1524, doi: 10.4319/lo.2011.56.4.1513, 2011b.

Roehm, C. L., Giesler, R., and Karlsson, J.: Bioavailability of terrestrial organic carbon to lake bacteria: the case of a degrading subarctic permafrost mire complex, J. Geophys. Res.-Biogeo., 114, Artn G03006, doi: 10.1029/2008jg000863, 2009.

Roiha, T., Tiirola, M., Cazzanelli, M., and Rautio, M.: Carbon quantity defines productivity while its quality defines community composition of bacterioplankton in subarctic ponds, Aquat. Sci., 74, 513-525, doi: 10.1007/s00027-011-0244-1, 2012.

Rossi, P. G., Laurion, I., and Lovejoy, C.: Distribution and identity of bacteria in subarctic permafrost thaw ponds, Aquat. Microb. Ecol., 69, 231-245, doi: 10.3354/ame01634, 2013.

Salonen, K., Jones, R. I., and Arvola, L.: Hypolimnetic phosphorus retrieval by diel vertical migrations of lake phytoplankton, Freshwat. Biol., 14, 431-438, doi: 10.1111/j.1365-2427.1984.tb00165.x, 1984.

Salonen, K., and Rosenberg, M.: Advantages from diel vertical migration can explain the dominance of *Gonyostomum semen* (Raphidophyceae) in a small, steeply-stratified humic lake, J. Plankton Res., 22, 1841-1853, doi: 10.1093/plankt/22.10.1841, 2000.

Säwström, C., Laybourn-Parry, J., Granéli, W., and Anesio, A. M.: Heterotrophic bacterial and viral dynamics in arctic freshwaters: results from a field study and nutrient-temperature manipulation experiments, Polar Biol., 30, 1407-1415, doi: 10.1007/s00300-007-0301-3, 2007.

Squires, M. M., and Lesack, L. F. W.: Spatial and temporal patterns of light attenuation among lakes of the Mackenzie Delta, Freshwater Biol., 48, 1–20, doi: 10.1046/j.1365-2427.2003.00960.x, 2003.

Selinummi, J., Seppälä, J., Yli-Harja, O., and Puhakka, J. A.: Software for quantification of labeled bacteria from digital microscope images by automated image analysis, BioTechniques, 39, 859-863, 2005.

Shirokova, L. S., Pokrovsky, O. S., Kirpotin, S. N., Desmukh, C., Pokrovsky, B. G., Audry, S., and Viers, J.: Biogeochemistry of organic carbon, CO_2, CH_4, and trace elements in thermokarst water bodies in discontinuous permafrost zones of western Siberia, Biogeochemistry, 113, 573-593, doi: 10.1007/s10533-012-9790-4, 2013.

Simon, M., and Azam, F.: Protein-content and protein-synthesis rates of planktonic marine-bacteria, Mar. Ecol. Prog. Ser., 51, 201-213, doi: 10.3354/meps051201, 1989.

Sivan, O., Adler, M., Pearson, A., Gelman, F., Bar-Or, I., John, S. G., and Eckert, W.: Geochemical evidence for iron-mediated anaerobic oxidation of methane, Limnol. Oceanogr., 56, 1536-1544, doi: 10.4319/lo.2011.56.4.1536, 2011.

Skovgaard, A., Hansen, P. J., and Stoecker, D. K.: Physiology of the mixotrophic dinoflagellate *Fragilidium subglobosum*. I. Effects of phagotrophy and irradiance on photosynthesis and carbon content, Mar. Ecol. Prog. Ser., 201, 129-136, doi: 10.3354/meps201129, 2000.

Smith, D. C., and Azam, F.: A simple, economical method for measuring bacterial protein synthesis rates in seawater using ^{3}H-leucine, Mar. Microb. Food Webs, 6, 107-114, 1992.

Teodoru, C. R., del Giorgio, P. A., Prairie, Y. T., and Camire, M.: Patterns in pCO_2 in boreal streams and rivers of northern Quebec, Canada, Global Biogeochemical Cycles, 23, doi: 10.1029/2008gb003404, 2009.

Tranvik, L. J.: Availability of dissolved organic carbon for planktonic bacteria in oligotrophic lakes of differing humic content, Microb. Ecol., 16, 311-322, doi: 10.1007/BF02011702, 1988.

Tranvik, L. J., Downing, J. A., Cotner, J. B., Loiselle, S. A., Striegl, R. G., Ballatore, T. J., Dillon, P., Finlay, K., Fortino, K., Knoll, L. B., Kortelainen, P. L., Kutser, T., Larsen, S., Laurion, I., Leech, D. M., McCallister, S. L., McKnight, D. M., Melack, J. M., Overholt, E., Porter, J. A., Prairie, Y., Renwick, W. H., Roland, F., Sherman, B. S., Schindler, D. W., Sobek, S., Tremblay, A., Vanni, M. J., Verschoor, A. M., von Wachenfeldt, E., and Weyhenmeyer, G.

A.: Lakes and reservoirs as regulators of carbon cycling and climate, Limnol. Oceanogr., 54, 2298-2314, doi: 10.4319/lo.2009.54.6_part_2.2298, 2009.

Vallentyne, J.: Fossil pigments, Comparative biochemistry of photoreactive systems, 83-105, 1960.

Walter Anthony, K. M., Zimov, S. A., Grosse, G., Jones, M. C., Anthony, P. M., Chapin, F. S., 3rd, Finlay, J. C., Mack, M. C., Davydov, S., Frenzel, P., and Frolking, S.: A shift of thermokarst lakes from carbon sources to sinks during the Holocene epoch, Nature, 511, 452-456, doi: 10.1038/nature13560, 2014.

Verity, P. G., Robertson, C. Y., Tronzo, C. R., Andrews, M. G., Nelson, J. R., and Sieracki, M. E.: Relationships between cell-volume and the carbon and nitrogen-content of marine photosynthetic nanoplankton, Limnol. Oceanogr., 37, 1434-1446, 1992.

Vonk, J. E., Sanchez-Garcia, L., van Dongen, B. E., Alling, V., Kosmach, D., Charkin, A., Semiletov, I. P., Dudarev, O. V., Shakhova, N., Roos, P., Eglinton, T. I., Andersson, A., and Gustafsson, O.: Activation of old carbon by erosion of coastal and subsea permafrost in arctic Siberia, Nature, 489, 137-140, doi: 10.1038/nature11392, 2012.

Vonk, J. E., Tank, S. E., Bowden, W. B., Laurion, I., Vincent, W. F., Alekseychik, P., Amyot, M., Billet, M. F., Canário, J., Cory, R. M., Deshpande, B. N., Helbig, M., Jammet, M., Karlsson, J., Larouche, J., MacMillan, G., Rautio, M., Walter Anthony, K. M., and Wickland, K. P. Reviews and Syntheses: Effects of permafrost thaw on arctic aquatic ecosystems, Biogeosciences Discuss., 12, 10719-10815, 2015. doi:10.5194/bgd-12-10719-2015.

Vrede, K.: Nutrient and temperature limitation of bacterioplankton growth in temperate lakes, Microb. Ecol., 49, 245-256, doi: 10.1007/s00248-004-0259-4, 2005.

Walter, K. M., Zimov, S. A., Chanton, J. P., Verbyla, D., and Chapin, F. S.: Methane bubbling from Siberian thaw lakes as a positive feedback to climate warming, Nature, 443, 71-75, doi: 10.1038/nature05040, 2006.

Watanabe, S., Laurion, I., Chokmani, K., Pienitz, R., and Vincent, W. F.: Optical diversity of thaw ponds in discontinuous permafrost: a model system for water color analysis, J. Geophys. Res.-Biogeo., 116, Artn G02003, doi: 10.1029/2010jg001380, 2011.

Weishaar, J. L., Aiken, G. R., Bergamaschi, B. A., Fram, M. S., Fujii, R., and Mopper, K.: Evaluation of specific ultraviolet absorbance as an indicator of the chemical composition and reactivity of dissolved organic carbon, Environ. Sci. Technol., 37, 4702-4708, doi: 10.1021/es030360x, 2003.

Xiao, Y. H., Sara-Aho, T., Hartikainen, H., and Vähätalo, A. V.: Contribution of ferric iron to light absorption by chromophoric dissolved organic matter, Limnol. Oceanogr., 58, 653-662, doi 10.4319/lo.2013.58.2.0653, 2013.

Figure captions

Figure 1. Summer temperature and oxygen profiles from sampled ponds.

Figure 2. Absorption spectral slope curve (S_λ) of dissolved organic matter as an indicator of the presence of algal-derived carbon (peak at S_{289}) and terrestrial carbon (steady increase in the slopes from 260-390 nm).

Figure 3. Seasonal changes in the biomass of phototrophic picoplankton (PPA), pigmented nanoflagellates (PNF), bacteria (BB), and heterotrophic nanoflagellates (HNF). Error bars represent standard errors.

Figure 4. a) Seasonality in pond environmental variables illustrated with principal component analysis during late winter (triangles), summer surface (diamonds) and summer bottom (crosses). b) Spearman correlation coefficients between biological variables and principal components 1 (x-axis) and 2 (y-axis). BP=bacterial production, BB=bacterial biomass, HNF=heterotrophic nanoflagellate biomass, PP=primary production, PNF=pigmented nanoflagellate biomass, and PPA=phototrophic picoplankton biomass.

Figure 5. a) The δ^{13}C isotopic signature of algal carbon (Phyto), *Chaoborus*, particulate organic matter (POM) and allochthonous carbon (t POM) averaged for samples collected in winter and summer. b) The relative contribution of allochthonous carbon to *Chaoborus* diet in winter and summer for the 5 sampled ponds.

Table 1. Limnological properties of the subarctic ponds sampled in late winter (W) and during summer at the surface (S) and bottom (B) of the water column, including pH, conductivity (Cond.), total phosphorus (TP), total nitrogen (TN), soluble reactive phosphorus (SRP), total suspended solids (TSS), particulate organic carbon (POC) and iron (Fe).

Site	pH	Cond. ($\mu S\ cm^{-1}$)	TP ($\mu g\ L^{-1}$)	TN ($\mu g\ L^{-1}$)	SRP ($\mu g\ L^{-1}$)	TSS ($mg\ L^{-1}$)	POC ($mg\ L^{-1}$)	Fe ($mg\ L^{-1}$)
Winter								
KWK 2	6.6	60	61.9	957.4	20.8	2.7	0.8	na
KWK 6	6.8	49	50.6	687.8	7.7	3.7	1.1	na
KWK 12	6.6	46	73.2	1038.4	18.4	4.1	1.2	na
KWK 20	6.7	52	180.7	1301.6	47.1	53.9	16.6	na
KWK 23	6.4	42	119.3	802.7	33,9	23.5	6.8	na
Summer surface								
KWK 2	7.4	38	34.9	289.3	1.2	3.4	0.9	0.3
KWK 6	6.9	61	43.9	227.9	0.9	7.2	2.3	0.2
KWK 12	7.3	36	23.9	311.9	0.7	2.6	0.9	0.3
KWK 20	7.5	47	92.6	263.4	7.1	14.2	1.8	0.5
KWK 23	7.1	48	78.0	227.9	5.7	16.1	2.1	0.4
Summer bottom								
KWK 2	6.2	200	341.5	496.1	1.5	27.7	4.4	2.5
KWK 6	6.2	265	197.7	389.4	na	13.6	2.3	1.1
KWK 12	6.1	247	207.1	447.6	1.0	37.0	8.8	2.9
KWK 20	6.0	155	377.0	289.3	73.8	85.8	5.7	5.1
KWK 23	6.2	102	431.8	266.7	45.7	126.8	7.8	4.1

Table 2. Concentrations of carbon dioxide (CO_2), methane (CH_4), and dissolved organic carbon (DOC), and dissolved organic matter (DOM) optical properties, including absorption coefficient of DOM at 320 nm (a_{320}), specific UV-absorbance index ($SUVA_{254}$), and absorption spectral slopes at 289 nm (S_{289}) and 382 nm (S_{382}), of subarctic pond water sampled in late winter and during summer at the surface and bottom of the water column.

Site	CO_2 (µM)	CH_4 (µM)	DOC (mg L^{-1})	a_{320} (m^{-1})	$SUVA_{254}$ (L mg C^{-1} m^{-1})	S_{289} (nm^{-1})	S_{382} (nm^{-1})
Winter							
KWK 2	316	14.1	7.7	8.5	0.9	0.0099	0.0155
KWK 6	354	5.1	5.2	3.0	0.5	0.0112	0.0122
KWK 12	215	2.4	10.5	9.5	0.8	0.0105	0.0154
KWK 20	na	na	10.3	8.1	0.8	0.0125	0.0130
KWK 23	357	2.5	7.9	6.6	0.8	0.0124	0.0123
Summer surface							
KWK 23	357	2.5	7.9	6.6	0.8	0.0124	0.0123
KWK 2	72	0.6	5.6	26.4	4.7	0.0141	0.0169
KWK 6	27	0.5	4.1	11.8	3.4	0.0162	0.0135
KWK 12	55	0.3	6.0	26.5	4.5	0.0142	0.0170
KWK 20	71	0.3	7.1	38.9	6.1	0.0145	0.0156
KWK 23	53	0.3	6.3	30.7	5.4	0.0150	0.0161
Summer bottom							
KWK 2	na	na	6.1	51.5	6.7	0.0109	0.0156
KWK 6	422	145.2	4.2	21.4	4.3	0.0125	0.0173
KWK 12	761	259.0	7.4	67.9	7.4	0.0111	0.0173
KWK 20	815	311.9	9.3	115.6	9.8	0.0107	0.0172
KWK 23	570	131.6	7.5	94.5	10.2	0.0109	0.0171

Table 3. Phototrophic and heterotrophic properties of subarctic pond water sampled in late winter and during summer at the surface and bottom of the water column, including chlorophyll-*a* concentration (Chl-*a*), maximum photosynthesis parameter (P_{max}), *in situ* primary production (PP), pigmented nanoflagellate abundance (PNF), phototrophic picoplankton abundance (PPA), bacterial production (BP), bacterial respiration (BR), bacterial abundance (BA) and heterotrophic nanoflagellate abundance (HNF).

Site	Chl-*a* (μg L^{-1})	P_{max} (mg C m^{-3} d^{-1})	PP (mg C m^{-3} d^{-1})	PNF ($\times10^6$ L^{-1})	PPA ($\times10^5$ mL^{-1})	BP (mg C m^{-3} d^{-1})	BR (mg C m-3 d^{-1})	BA ($\times10^6$ mL^{-1})	HNF ($\times10^6$ L^{-1})
Winter									
KWK 2	2.8	1.2	0	33.0	0.2	0.2	1.3	4.2	2.0
KWK 6	9.9	0.8	0	51.0	0.9	0.5	2.2	11.2	3.3
KWK 12	11.5	5.8	0	29.1	0.7	0.3	1.6	2.5	2.4
KWK 20	2.8	0.8	0	6.3	0.2	0.8	3.0	8.7[a]	2.4
KWK 23	0.8	1.5	0	10.0	0.1	0.4	2.0	3.7	0.3
Summer surface									
KWK 2	5.4	4.5	2.6	16.3	3.3	27.2	25.7	13.3	0.0
KWK 6	13.4	27.9	15.3	20.0	4.2	37.4	31.2	11.3	0.8
KWK 12	2.2	5.9	3.2	28.8	0.9	31.2	27.9	9.6	0.7
KWK 20	14.3	59.0	26.4	33.1	17.3	15.2	18.0	14.8	1.9
KWK 23	12.3	55.6	15.6	35.0	3.8	45.9	35.3	12.7	0.4
Summer bottom									
KWK 2	180.2	na	0.8	149.6	9.4	57.6	40.5	35.7	26.2
KWK 6	87.2	na	4.2	201.6	3.0	39.0	32.0	14.3	6.3
KWK 12	158.9	na	0.3	72.5	26.8	48.8	36.6	38.0	3.7
KWK 20	203.4	na	0.3	na	4.0	39.1	32.0	27.2	na
KWK 23	37.1	na	0.0	4.5	6.2	23.0	23.2	24.3	0.6

[a] BA was calculated from DAPI-stained microscope slide

Table 4. Spearman correlations between biological and environmental variables. Only statistically significant correlations are given.

Biological variable	Environmental variable	n	r	p
Bacteria production (BP)	Temperature	15	0.600	0.018
	TN	15	-0.524	0.045
	a_{320}	15	0.704	0.003
	$SUVA_{254}$	15	0.699	0.004
	S382	15	0.633	0.011
Bacteria biomass (BB)	O_2	15	0.555	0.049
	Conductivity	15	0.668	0.007
	TP	15	0.600	0.018
	TSS	15	0.521	0.046
	Fe	10	0.879	0.001
	CH_4	13	0.588	0.035
	a_{320}	15	0.825	<0.001
	$SUVA_{254}$	15	0.857	<0.001
	S382	15	0.716	0.003
HNF	Conductivity	15	0.657	0.011
	CH_4	15	0.636	0.026
Primary production (PP)	Temperature	10	0.896	<0.001
	SRP	10	-0.662	0.007
	TN	10	-0.827	<0.001
	DOC	10	-0.610	0.016
	$SUVA_{254}$	10	0.533	0.041
	S289	10	0.732	0.002
PNF	SRP	14	-0.732	0.003
	DOC	14	-0.647	0.012
PPA	TN	15	-0.665	0.007
	a_{320}	15	0.825	<0.001
	$SUVA_{254}$	15	0.717	0.003
	S382	15	0.564	0.029

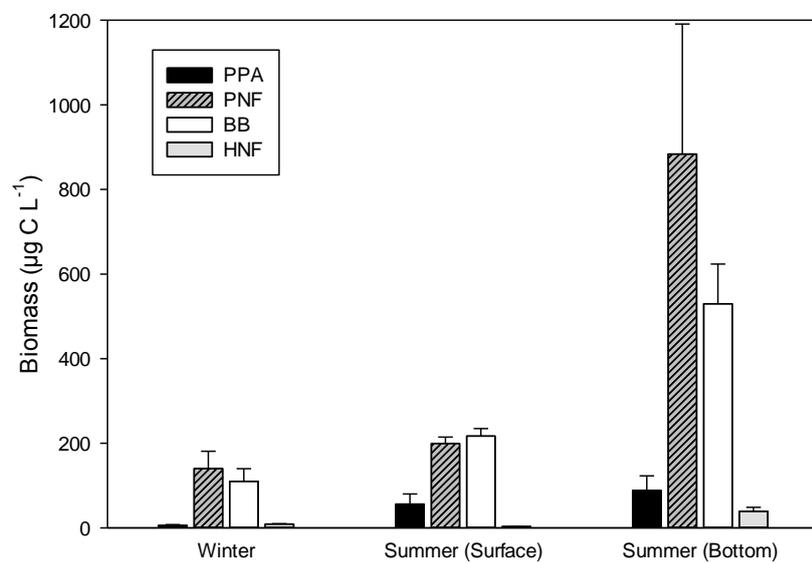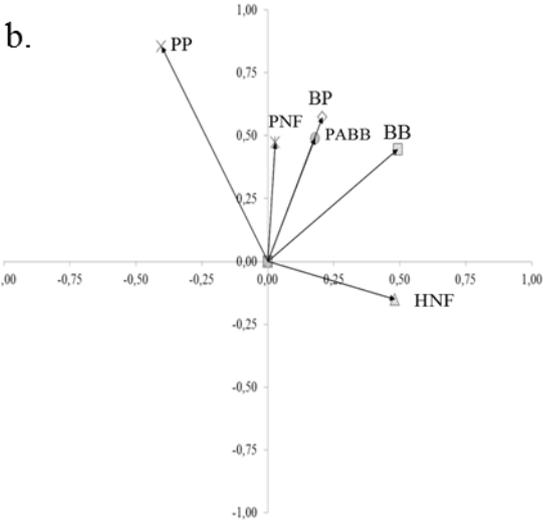**Figure 1.**
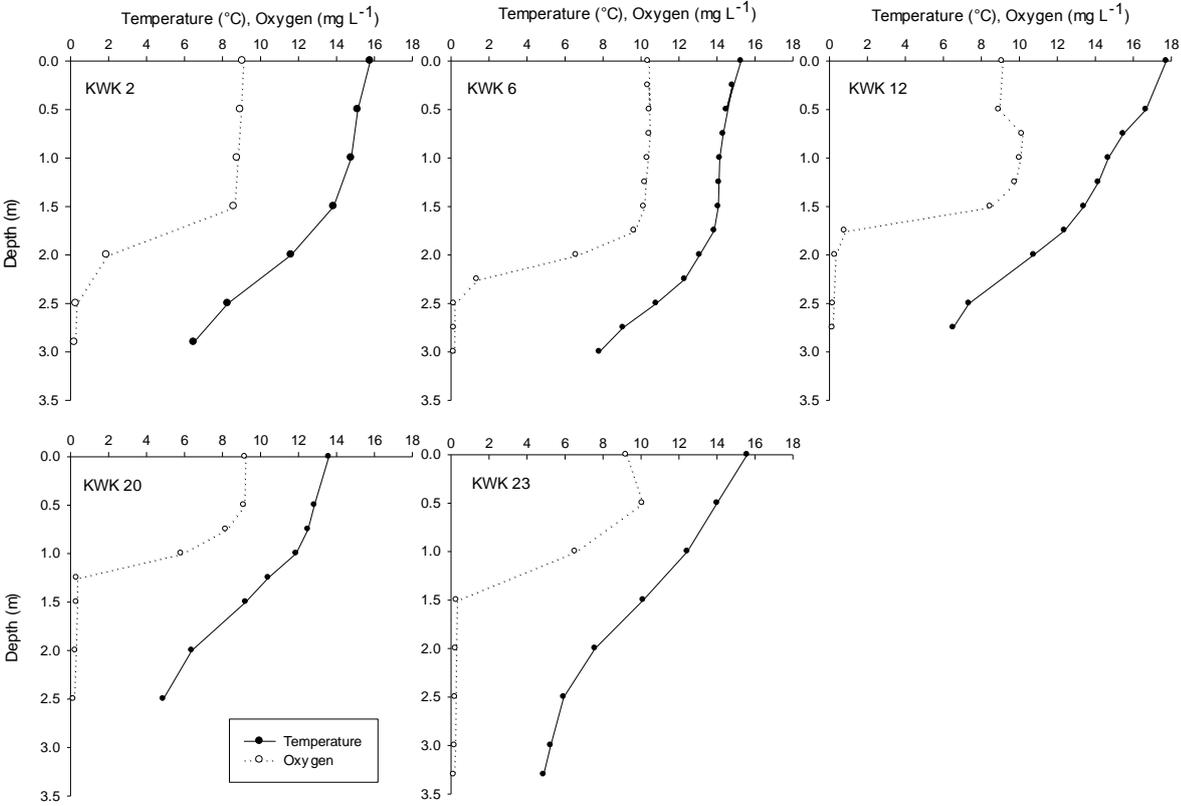
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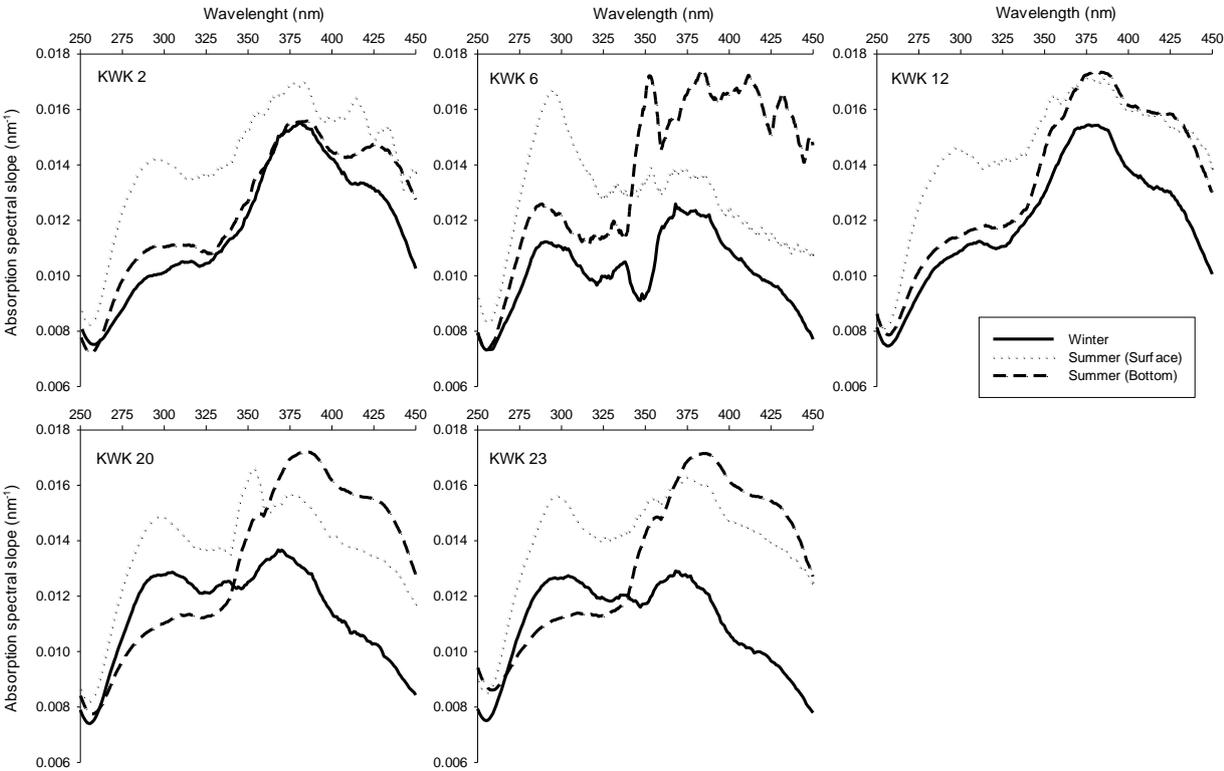
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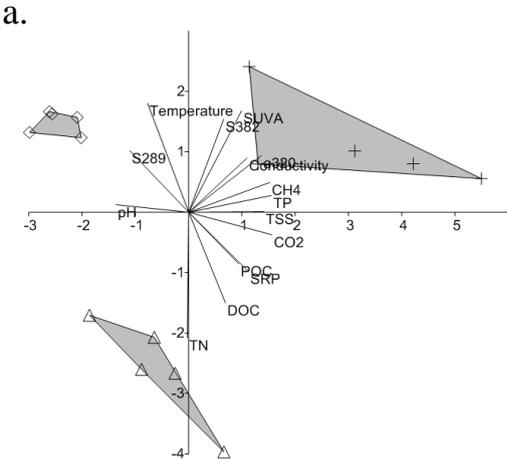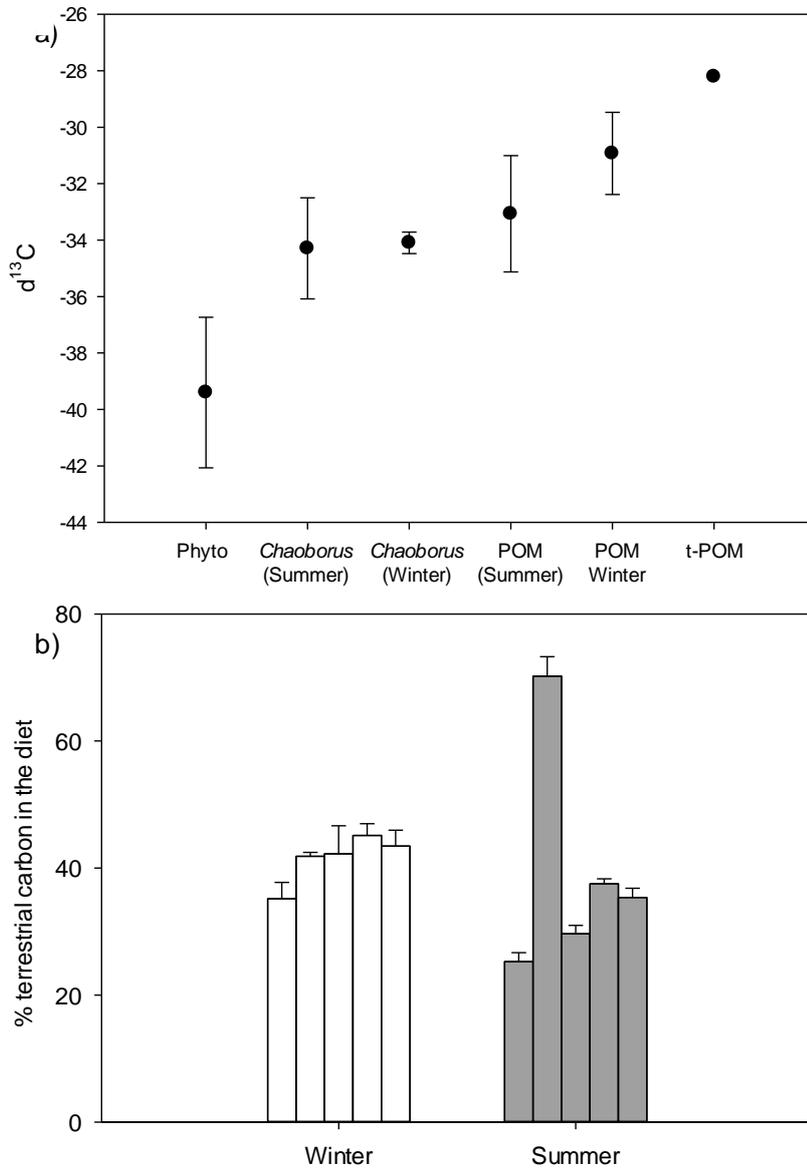
Figure 5.

Appendix A:

Table A1. Carbon isotopic signatures ($\delta^{13}C$) of the algae, and the variables that were used to calculate $\delta^{13}C_{algae}$, including the phytoplankton growth rate (μ), the photosynthetic fractionation parameter (ϵ_p) and the isotopic signature of CO_2.

Site	$\delta^{13}C_{algae}$	μ	CO_2 (μM)	ϵ_p	$\delta^{13}CO_{2(aq)}$
KWK 2	-40.3	1.8	71.7	23.1	-17.2
KWK 6	-34.3	1.2	27.3	21.8	-12.5
KWK 12	-41.4	1.5	55.3	23.0	-18.4
KWK 20	-38.7	2.3	71.5	22.6	-16.1
KWK 23	-36.8	2.6	52.8	21.5	-15.3

Appendix ~~B~~<ins>A</ins>:

Table ~~BA~~<ins>A</ins>1. Particle attachment of bacterioplankton in winter, during summer at the surface and bottom of ponds, including sonicated bacterial abundance (BA_T) representing the total abundance, bacterial abundance before sonication (BA_{FL}) representing free-living bacteria, proportion of particle-attached bacteria (BA_P), total bacterial production (BP; as in Table 3), filtered (3µm) bacterial production (BP_{FL}) and proportion of particle attached bacterial production (BP_P).

Site	BA_T ($\times 10^6$ mL^{-1})	BA_{FL} ($\times 10^6$ mL^{-1})	BA_P (%)	BP (mg C m^{-3} d^{-1})	BP_{FL} (mg C m^{-3} d^{-1})	BP_P (%)
Winter						
KWK 2	4.2	0.8	81	0.2	0.1	54
KWK 6	11.2	3.2	72	0.5	0.2	54
KWK 12	2.5	0.5	79	0.3	0.2	48
KWK 20	na	na	na	0.8	0.4	47
KWK 23	3.7	1.4	62	0.4	0.4	7
Summer surface						
KWK 2	13.3	5.8	57	27.2	4.7	83
KWK 6	11.3	8.5	24	37.4	3.3	91
KWK 12	9.6	6.0	37	31.2	17.4	56
KWK 20	14.8	4.1	73	15.2	7.0	54
KWK 23	12.7	7.7	40	45.9	18.6	60
Summer bottom						
KWK 2	35.7	11.3	68	57.6	1.7	97
KWK 6	14.3	8.3	42	39.0	1.5	96
KWK 12	38.0	11.2	70	48.8	26.0	47
KWK 20	27.2	3.7	86	39.1	1.0	97
KWK 23	24.3	4.2	83	23.0	26.6	0