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Interactive comment on "Phototrophic pigment diversity and picophytoplankton abundance in permafrost thaw lakes" *by* A. Przytulska et al.

Anonymous Referee #1

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This manuscript addresses an interesting topic in ecology in relation to phototrophic plankton communities in unique ecosystems at high latitudes. The authors used a multi-level approach combining different techniques to characterize the phytoplankton communities. Although the work represents a valuable effort, I have major concerns about the methodological design and data analysis. I recommend major changes before the manuscript can be accepted for publication in Biogeosciences.

General comments:

1- The authors used a set of techniques (pigments, flow cytometry, epifluorescence microscopy, molecular analyses) to characterize and compare the phytoplankton of thaw lakes in northern Quebec, Canada. Although the effort is very valuable, it is necessary that the results obtained from the different techniques be better integrated

to improve the discussion.

For example, the picophytoplankton fraction was analysed by flow cytometry. What was the relative contribution (%) of each fraction, pico-cyano and pico-eukaryotes, to the total pico fraction?

In Results, page 13, line 25, the authors said the picocyanobacteria abundance in KWK23 was 5.6 *10ˆ5 cel/ml. Then, looking at figure 6 (biovolume), the biovolume for that sample/year was around 5 *10ˆ5 um3/ml. Doing some simple calculations, and assuming 7 ug Chl-a/mm3 of picocyanobacteria biovolume (but please, see Reynolds 1984, The Ecology of Freshwater Phytoplankton), the concentration of chlorophyll a due to picocyanobacteria was in the order of 3.5 ug/L. Looking at the total Chl-a data, line 10, page 11, the relative contribution of picocyanobacteria to the total of the phytoplankton community is important (dominant?). Can the authors discuss this point more in detail?

The molecular data needs to be better linked with the rest of the study. Why did the authors select the eukaryote fraction to do molecular taxonomy (excluding cyanobacteria, 16S RNA)? And, why is it relevant to describe and discuss the non-phototrophic taxa (predators: ciliates, fungi)?

Most of the phytoplanktonic taxa identified by molecular analysis are in the fraction of nano to meso plankton: how does this information match with chlorophyll-a and the contribution of picoplankton to the community?

2- Why did the authors not analyse the nanophytoplantkon fraction with an optical microscope? And why was it necessary to use indirect techniques to infer the phytoplankton composition? Please, justify.

3-The concepts of abundance, concentration and biomass are confused in some paragraphs. In the objectives it is stated: “A secondary objective was to determine the abundance and distribution of picocyanobacteria and picoeukaryotes”. Then, in the

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Results the authors present abundance and biovolume without a clear differentiation of both indicators. For example, in Results, page 13, subsection: “3.3 Picophytoplankton abundance”, it is not clear when the authors describe information about abundance or biovolume. While the text refers to abundance, figure 6 presents biovolume (with no corresponding description in the text). Both variables are complementary but conceptually very different. In page 14, from line 11, is the analysis made with picoplankton abundance or biovolume? This needs to be specified. I would suggest that biovolume be used to explore correlations with environmental and biotic variables.

4- The Material and Methods section has to be improved. The methodological design is complex and should be justified with more detail. Not all the analyses were performed for the same number of samples, lakes and dates. This makes it difficult to follow the results. For example: not all the analyses and sites were sampled on 2011 and 2012 at the two different depths (surface and bottom). It is necessary to explain how many samples, lakes, depths and dates where used for each analysis and why.

5- The statistical analysis section has to be described with more detail. Please, explain why PCA was selected (what was the gradient length of the data?). Using the pigment composition as a proxy of main phylogenetic phytoplankton groups, the authors could explore the % of variance of biological data explained by the environmental data (i.e.: multivariate analysis like CCA or RDA).

Cluster analysis: I would suggest another kind of analysis to compare the sites defined by environmental and biological data (see above). I found the comparison of the two clusters too indirect and poorly supported in terms of statistical significance. In any case, more information about the cluster analysis needs to be presented (which kind of cluster, distance or similarity, which index, which averaging method, which matrix data, etc). The authors compare two clusters built by two different indices “by eye” (distance: is it Euclidean?). Is it possible to identify different groups of lakes based on the environmental data, since the distances are very similar? Regarding the clusters based on biological data, and assuming 40% of similarity as a parsimonious cut point,

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it is possible to find only two groups and one outlier (2012SRB1).

6- Pigment results: Please, analyse pigment ratios to chlorophyll-a based on micro-moles and not micro-grams. Micro-moles/L is not influenced by the molecular weight of each pigment and gives the information about the quantity of molecules of each signal pigment in the total. Since the authors wants to describe the composition of the community, I suggest using micro-moles instead of micro-grams.

It is noteworthy that chlorophyll c (any variety) was not detected when carotenoids such as fucoxanthin, diadinoxanthin and peridinin were found. What is the explanation?

The classification of photoprotective and photosynthetic pigments, as presented in Table 2, is not clearly discussed. And what was the total photoprotective/total photosynthetic pigment ratio? What are the consequences in these differences?

Specific comments: 1- Doing some quick calculations for 2011 data presented in figure 6, the individual size of picocyanobacteria cells in SAS1 was very big ($\sim$ 2.3 um3) in comparison with KWK23 (0.89 um3). It would be interesting to explore and discuss these differences.

2- All the information presented in table 3 (bacterio-chlorophyll) is not well discussed and it does not flow with the rest of the article. I suggest removing this section.

3- Figure 4: I suggest reformatting this figure. It is not easy to follow the differences between carotenoids and sites. The legend of this figure needs to be improved so as to give more information.

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