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***Interactive comment on* “Effect of permafrost thawing on the organic carbon and trace element colloidal speciation and microbial activity in thermokarst lakes of Western Siberia” by O. S. Pokrovsky et al.**

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Reviewer No 3 (anonymous) 1. The reviewer correctly points out that some of our interpretations/conclusions are a little over-rated with regard to biological processes. We agree and moderated our conclusions on the role of bacterio- and phyto-plankton. Unfortunately, we do not have in this study the total number of bacteria and Chl a. The reviewer suggests including CDOM optical characteristics and we added the values of UV absorbance at 280 nm together with a pertinent discussion. We agree with the reviewer that including ‘microbial activity’ in the title does not reflect what the study mostly covers

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(OM-TE interactions) and we modified the title as following: “Effect of permafrost thawing on the organic carbon and trace element colloidal speciation in thermokarst lakes of Western Siberia”. The reviewer correctly points out that culturable heterotrophic bacteria may only represent a small proportion of the species present (the ones that respond well to culture conditions), not necessarily the ones that had an important function in situ, with a possible bias towards rare species. We added necessary discussion in the text as requested. We would like to point out that heterotrophic, aerobic bacteria growing on nutrient-rich agar yield a closest culturable approximation for natural consortia of bacteria present in organic-rich, well oxygenated waters of the thermokarst lakes. It is also important to note that all three types of nutrient media, organic-rich (eutrophic), 10% diluted and minimal nutrient Bacto oligotrophic agar yielded similar trends of bacterial number evolution along the chronosequence of lake development. We added necessary discussion in the revised version. Because the method for culturing bacteria under 3 trophic conditions and main results are presented in Shirokova et al. (2009), we modified the text accordingly. 2. The reviewer inquires, are there any evidences in the literature that microbes use OM and leave out the associated minerals? This is a really interesting point. To our knowledge, there is no such information available. We believe that heterotrophic aerobic microbes do not need high concentration of Fe and Al (mineral part of colloids) present in the lake water. In fact, at high concentration of colloidal-bound nutrient metals, there is also significant proportion of easier-available LMW complexes of those metals. As a result, it is unlikely that bacteria will be specially “looking for” inorganic part of colloids. 3. The reviewer correctly points out that photolysis may be another important factor affecting DOM molecular size via coagulation/flocculation, especially in such exposed ecosystems and suggests discussing this issue. Following this and other reviewers’ remark, we added a big deal of discussion in the revised version of the manuscript (sections 3.1 and 3.2). 4. The reviewer is concerned by a delay between sampling and measurements and suggests discussing these aspects. Following this important remark, we added this discussion in the revised version: Filtered and dialysated samples were kept at 4-5°C in the darkness

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before the analysis. Temperature was reduced to decrease the coagulation and bacterial production in the experimental samples during storage (Chen and Buffle, 1996; Wilkinson et al., 1997). 5. The reviewer points out that, although the key words such as GHG fluxes, heterotrophic lake status, allochthonous C (peat) bacterial utilization and hypothesis are presented in the introduction, but afterwards the implications of OM-TE interactions along the chronosequence is not sufficiently discussed nor the hypothesis tested. We partially agree with this comment and added some discussion on the consequences of permafrost thawing in section 4. We would like to notice that bacterial aspect of lake development and processes of CO₂ emission to the atmosphere are presented in previous work (Shirokova et al., 2009) and briefly summarized in revised manuscript. 6. The reviewer suggests that the sentence about microbial transformation of organic colloids in the course of thermokarst lake development (p. 8045, line 25) needs to be better supported. We would like to point out that the last sentence of the introduction is our hypothesis and as such we reformulated this sentence in the revised version. It is known for long that the boreal lakes and rivers yield net annual CO₂ evasion to the atmosphere (so-called net heterotrophy) and between 30% and 80% of the total OC (in the form of organic and organo-mineral colloids) that entered the freshwater ecosystems is lost in lakes via mineralization and subsequent CO₂ emission to the atmosphere (Cole et al., 1994, 2007; Hope et al., 1996; Kelly et al., 2001; Sobek et al., 2003; Teodoru et al., 2009; Tranvik et al., 2009) as we stated in the introduction. 7. The reviewer inquires whether we have data on the presence of phytoplankton and zooplankton in old khasyres, because it would corroborate the observed organic ligands and the putative phytoplankton exometabolites. Unfortunately, we have only qualitative data on the presence of phytoplankton and zooplankton in lakes at their mature stage of development. We added explicatory sentence in section 2.1 of the revised manuscript. 8. The reviewer noticed a contradiction in the results: all OM fractions decrease along the chronosequence, but the relative proportion of small-size DOC increases from 15 to 20-23%. We believe this contradiction is apparent because in the first case, we are dealing with mass concentration and in the second, with relative frac-

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tion (percentage). The reviewer correctly pointed out on an error on p. 8049 should be small-size autochthonous, not allochthonous carbon. In the revised version, description of processes of transformation of OC along the lake chronosequence were clarified and better supported by numerous literature references thanks to insightful comments of all reviewers. 9. The reviewer is right that we cannot refer to < 1 kDa fraction as 'potentially bioavailable' since heterotrophic bacteria consume both colloidal and truly dissolved OC. Following this important remark, we corrected the term to LMW (low molecular weight fraction) in the text and in figure captions. We would like to underline that, in contrast to organic carbon, the bioavailable metal fraction should be certainly < 1 kDa molecular size to be able to pass through the cell membranes. 10. The question on facultatively oligotrophic (FO) and oligotrophic (O) bacteria is resolved in the revised manuscript because these bacteria are presented in details in our previous work (Shirokova et al., 2009). In revised manuscript, only eutrophic bacteria are discussed. 11. The reviewer correctly pointed out that, after presentation of results in Fig. 7, there is a discussion on CO₂ supersaturation that seems out of context. We corrected the text as following: "In addition to pelagic respiration of DOC, benthic respiration and dissolved organic matter (DOM) photolysis are known to be important factors controlling the OM degradation and CO₂ release to the atmosphere in boreal lakes (e.g., Jonsson et al., 2001, 2008) with even much larger contribution of benthic respiration occurring in shallow ponds (Kortelainen et al., 2006)." 12. The reviewer requires provide the comparison with other lake types in terms of Fe and Al concentration and explain what makes thermokarst lakes unique. As it is stated in the text, specific and unique feature of thermokarst lakes developed on frozen peat of Western Siberia is that typical concentrations of Al and Fe metals range from 0.1 to 1 mg/L which is even higher than the concentration of other major elements such as Mg, K, Si. The most likely cause of such high concentrations are elevated DOC content and low pH of the lake water. We also added great deal of discussion on the geochemistry of studied lakes and specific weathering reactions responsible for observed chemical composition of the lake water. Note that, to our knowledge, these are the first data of metal

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concentration in thermokast lakes developed on permafrost terrain. 13. The reviewer correctly pointed out that it will be useful to include some discussion on microgels and nanogels and large colloids transformation and we discussed this issue and provided references on Verdugo's group papers. We would like to point out that nanogel coagulation via Ca bridging of exopolysaccharides, certainly possible in seawater or in some polluted highly mineralized European river, are rather unlikely in extremely low mineralized lakes of Western Siberia formed via soil ice thawing and atmospheric precipitation interaction with peat deposits.

We corrected all technical issues noted by this reviewer and significantly revised the English of the manuscript.

We appreciate very useful comments of all three reviewers that allowed significant improvement of the manuscript

Sincerely Oleg Pokrovsky

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