

**Dear Dr Battin,**

Thank you very much for your work with the manuscript (bg-2013-195). We are also very grateful for the two reviewers that provided thorough, insightful and constructive comments that helped us substantially improve this manuscript. We have addressed all comments in a detailed manner and feel that the manuscript has substantially improved. Our responses are highlighted in bold and the cursive written sentences of our response were added to the manuscript.

## **Response to Reviewer 1:**

Comments on the manuscript:

Pg8951, L26 – Authors state that “CDOM typically dominates the DOM pool in these lakes”. Consider revising as CDOM levels are reported only in absorbance units and not as concentrations. The true concentration of the colored components of the DOM pool are unknown and their absorbance is also often increased by high levels of iron. A more precise statement might be that these systems have high carbon normalized absorbance or SUVA, or that they are simply CDOM rich.

**Author's response: corrected to: "...and that these lakes are chromophoric DOM (CDOM) rich,..."**

8952, L1 – “CDOM concentrations” – rephrase to CDOM levels, absorbance, or something other than concentration.

**Author's response: changed to levels.**

L11 – When talking about the loss of photolabile DOM in photoreactions cite the literature on this, such as: Zhang, Y., H. Xie, et al. (2006). "Factors affecting the efficiency of carbon monoxide photoproduction in the St. Lawrence estuarine system (Canada)." *Environmental Science & Technology* 40(24): 7771-7777. Moran, M. A., W. M. Sheldon, et al. (2000). "Carbon loss and optical property changes during long-term photochemical and biological degradation of estuarine dissolved organic matter." *Limnology and Oceanography* 45(6): 1254-1264. Stubbins, A., J. Niggemann, et al. (2012). "Photo-lability of deep ocean dissolved black carbon." *Biogeosciences* 9(5): 1661-1670.

**Author's response: references added.**

L14-18: These points about anoxia, redox, re-dissolution etc. should also be referenced. Methods or early in results – detail DOC recoveries and how the optical properties of the CDOM compare to either the initial water before acidification if measured and literature values for similar environments.

**Author's response: references were added. The DOC recoveries were mentioned already: "...adsorption efficiencies ranged between 58 and 64 %,...". We added another sentence to the method section: "The shape of the absorbance as well as the fluorescence signals of the SPE extract did not change noticeably, when compared to the original water sample."**

8958, L5 – Photochemical experiments do not seem the best way to test the hypothesis that “DOM is released from the sediments” – this would be done by determining fluxes from sediments or comparing sediment leachate DOM molecular signatures with those of overlying waters. The photochemical experiments test whether summer surface DOM is photodegraded. Restate or remove this.

**Author's response: Sentence was removed**

8959, L20 – “Microbial decarboxylation” is a overly specific term that suggests a mechanism whereby carboxyl groups are cleaved from DOM by microbes. I believe the authors mean respiration, whereby dissolved inorganic carbon is formed through the oxidation of DOC.

**Author's response: changed to "Microbial respiration, whereby DOC is oxidized and dissolved inorganic carbon is formed, seemed to be an effective pathway..."**

L24. The 1.7 mg L<sup>-1</sup> is described as a rate, but has no temporal unit. Why? Is it per day, per second, per year?

**Author's response: It is the total loss of DOC over 147 days (almost 5 months) during a previous experiment with water from the same lake as studied here (Bastviken et al. 2004).**

**We have now clarified this.**L26 – how is the rate of DOC loss between April and June calculated?

**Author's response: In this estimate we assumed that the DOC loss for these two months was the same as the observed experimental loss during 5 months using water from the same lake (i.e. 1.7 mg L<sup>-1</sup>). We combined this assumption with the observation that surface water lost 2.8 mg DOC L<sup>-1</sup> relative to bottom waters after circulation and estimate that this relative difference between summer surface and bottom waters could be due to in then order of 1.7 mg L<sup>-1</sup> (60%) enhanced microbial respiration because of higher surface water temperatures and 1.1 mg L<sup>-1</sup> (40 %) photorespiration. This is of course a crude estimate which on one hand may overestimate the microbial respiration part by using total respiration obtained from 5 months for a 2 month period, and on the other hand does not account for photochemical enhancement of microbial respiration. Nevertheless we think the outcome that microbial and photochemical contributions are of similar orders of magnitude convincing and of interest for readers. We have tried to clarify this in the revised text. The paragraph was revised to: *“To provide a crude estimate of relative contribution of microbial versus light-dependent removal of DOC in surface water between April and June in our study we assumed that the total respiration found in this previous study over 5 months (1.7 mg C L<sup>-1</sup>) represented the contribution of the light independent microbial DOC mineralization from April to June (2 months). We also assumed negligible input of water and DOC from land given the very dry conditions this spring. Then, given a total difference of 2.8 mg DOC L<sup>-1</sup> between surface and bottom water in June (Table 1), at least 1.1 mg L<sup>-1</sup> or 40% of this relative DOC loss in the surface water should be directly or indirectly related to photochemical DOC degradation. This calculation likely overestimates the light independent microbial contribution by using a 5 month value for 2 months, thereby underestimating the influence of photochemistry, and should not be taken literally. Rather it illustrates similar magnitudes of light independent and photochemically induced DOC mineralization processes in lake surface.”***

8960, L3: Authors need to describe at least in modest detail the photochemical changes to optical properties and how these compare to previous work, how they are generally interpreted, and why in this case they indicate “limited photoreactivity”.

**Author's response: The following paragraphs were added: *“The absolute decrease in absorption coefficients (Fig. 3) after 24h simulated sunlight exposure was significant in all samples including the late winter and summer surface and deep samples. The photobleaching under winter conditions between surface and deep water CDOM was very similar with a detectable differences only below 240 nm. This stands in contrast to the comparison between***

*surface and deep CDOM in summer, where the surface CDOM is much less photobleached in the whole ultraviolet (UV) range (200 - 400 nm) (Fig. 3).*

*The measurements of EEM spectra before and after the same irradiation time showed that the photochemically-induced changes were reflected in areas in the EEM spectra that are centered around two maxima with excitation, emission couples of 280/380 nm and 340/375 nm (Fig. 4). The summer surface sample was the only sample that showed a clear differences in photochemically-induced degradation of fluorescent DOM (FDOM) between surface and bottom water, confirming the same trend shown in the absorbance data (Fig. 3). These results imply that water that had not been exposed to light for extended periods (under ice or near the bottom during summer stratification) was much more photoreactive.*

L13-14: A shift to LMW has been shown during photochem in many previous studies using ultrafiltration and size exclusion (e.g. Thomson et al., 2004; Lou and Xie, 2006; Helms et al., 2008) and for whole waters using FT-ICR MS (e.g. Stubbins et al 2010). Helms, J. R., A. Stubbins, et al. (2008). "Absorption spectral slopes and slope ratios as indicators of molecular weight, source, and photobleaching of chromophoric dissolved organic matter." *Limnology and Oceanography* 53(3): 955.

Lou, T. and H. Xie (2006). "Photochemical alteration of the molecular weight of dissolved organic matter." *Chemosphere* 65(11): 2333-2342. Already cited: Thomson, J., A. Parkinson, et al. (2004). "Depolymerization of chromophoric natural organic matter." *Environmental Science & Technology* 38(12): 3360-3369.

Stubbins, A., R. G. Spencer, et al. (2010). "Illuminated darkness: Molecular signatures of Congo River dissolved organic matter and its photochemical alteration as revealed by ultrahigh precision mass spectrometry." *Limnology and Oceanography* 55(4): 1467. Last paragraph about photo-bleached compounds needs to cite and place the current findings in the context of Kujawinski et al. 2006; Gonsior et al 2009; Stubbins et al. 2010; and Rossel, Vahatalo, Dittmar et al. 2013, all of which report somewhat similar findings. The author should know all of these FTMS refs.

**Author's response:** All the mentioned references were added. *"...This trend is in agreement with previous studies where ultrafiltered DOM was photo-degraded and analyzed by size exclusion chromatography (Lou and Xie, 2006; Thomson et al., 2004). Whole water samples were also previously analyzed using optical properties (Helms et al., 2008) and ultrahigh resolution mass spectrometry of Congo River water (Stubbins et al., 2010) with again similar findings."*

*"A set of photosensitive aromatic compounds in the Cape Fear River estuary has been previously shown using FT-ICR-MS (Gonsior et al, 2009) and long term exposure to simulated sunlight (57 days) of Congo River water also confirmed the preferential photo-degradation of the aromatic DOM content (Stubbins et al, 2010)."*

8961, L5-20. Again cite literature showing that aromatics are lost and also connect this FTMS data to the optical data in the paper which also indicates that aromatics were lost.

**Author's response:** see references above and: *"The loss of aromatics is also in agreement with the observed photochemically-induced changes in the optical properties and the decrease*

*in conjugated aromatic compounds indicated by the changes in absorbance and fluorescence (Fig.4).”*

8962 – When discussing the photolability of surface summer versus other times of year and deep samples, the authors should again turn to the literature which indicates photoreactivity decreases with irradiation time:

Zhang, Y., H. Xie, et al. (2006). "Factors affecting the efficiency of carbon monoxide photoproduction in the St. Lawrence estuarine system (Canada)." *Environmental Science & Technology* 40(24): 7771-7777.

Moran, M. A., W. M. Sheldon, et al. (2000). "Carbon loss and optical property changes during long-term photochemical and biological degradation of estuarine dissolved organic matter." *Limnology and Oceanography* 45(6): 1254-1264.

Stubbins, A., J. Niggemann, et al. (2012). "Photo-lability of deep ocean dissolved black carbon." *Biogeosciences* 9(5): 1661-1670.

AND suggestions that samples from deep, aphotic waters are also more photoreactive than surface waters in other environments e.g. the ocean: Mopper, K., X. Zhou, et al. (1991).

"Photochemical degradation of dissolved organic carbon and its impact on the oceanic carbon cycle." *Nature* 353(6339): 60-62.

**Author's response: We included all references in the revised manuscript.**

## Response to Reviewer 2

Specific comments:

Abstract:

L8: If photobleaching is not the sole factor explaining the higher DOC concentrations above the sediments, it seems logical to state here what other factor(s) might be involved. I suggest moving the later sentence about the release of DOC from sediments here.

**Author's response: A good suggestion. Has been done in the revised manuscript. Thanks.**

Intro:

P8951-L16-18: While that may be true for humic or highly coloured lakes, it has been shown that light may in fact inhibit microbial degradation in more productive or algal dominated systems e.g. Tranvik & Bertilsson 2001. As a side note, since this study was conducted in a humic lake, I would be more specific in the conclusions that the patterns observed and the proposed mechanisms underpinning these patterns may be particularly relevant in the case of humic lakes, but that they may differ with lake type.

**Author's response: agreed and now mentioned in the discussion.**

P8951-L16: This paragraph starting is quite long and carries two distinct ideas: DOM photodegradation and release from the sediments. I suggest splitting for clarity.

**Author's response: paragraphs were separated**

P8952-L1: I would avoid using the word “concentration” when referring to CDOM through the manuscript as we don’t really know how much of the DOM pool is actually chromophoric.

**Author's response: agreed and “concentrations” were changed to “levels”.**

P8952-L17: Please provide proper referencing supporting the importance of redissolution.

**Author's response: references were added:**

*Skoog, A. C., and Arias-Esquivel, V. A.: The effect of induced anoxia and reoxygenation on benthic fluxes of organic carbon, phosphate, iron, and manganese, Sci Total Environ, 407, 6085-6092, 10.1016/j.scitotenv.2009.08.030, 2009.*

*Cottrell, B. A., Timko, S. A., Devera, L., Robinson, A. K., Gonsior, M., Vizenor, A. E., Simpson, A. J., and Cooper, W. J.: Photochemistry of excited-state species in natural waters: A role for particulate organic matter, Water Research, 47, 5189-5199, <http://dx.doi.org/10.1016/j.watres.2013.05.059>, 2013.*

Methods:

P8954-L14: How were the controls used in regard to the light exposed samples in the data treatment?

**Author's response: We added a sentence to explain how dark controls were used: “The differences between the dark controls and the irradiated samples were used to evaluate the photo-degradation at the molecular level.”**

P8954-L23: Were the samples diluted prior to fluorescence measurements to minimize the inner filter effect as in Zepp et al. 2004? If not, how was that taken into account here as this effect may be quite important in highly coloured waters, and differ between the before and after the light treatment?

**Author's response: “All samples were diluted 1:10 prior to all optical property analyses to avoid inner filtering effects and to be able to apply the linear range of the quinine sulfate calibration of the EEM spectra.”**

Results:

P8958-L14: Did the authors observed similar patterns in CDOM or fluorescence intensity? Some information on this should be presented in Table 1.

**Authors response:** We added a discussion of spectral slope ratios according to Helm et al 2008 and a table with the spectral slope data (Table 2): *“The spectral slope ratios ( $S_r$ ), obtained by dividing the slope calculated between 275-295 nm and 350-400 nm (Helms et al., 2008), showed also significant changes towards higher values after irradiation time, but was relatively similar between depths (18 % increase in the April surface and 20 % in the April bottom water sample) (Tab. 2). In general, these results are in agreement with the Helms et al. study and would suggest a decrease in molecular weight after irradiation.”*

P8957-L26: I am a bit confused here regarding the inputs and “sinks” of DOC. There was a decrease in DOC concentrations of  $\sim 4$  mg L<sup>-1</sup> between April and June, both at the surface and at the bottom. Yet the authors stated in the previous paragraph that a dilution or depletion was unlikely. Then what is the likely cause? Also, the difference in DOC between the surface and the bottom is quite small between April and June  $\sim 0.5$  mg L<sup>-1</sup> ( $25.0-22.7 = 2.3$  mg L<sup>-1</sup> for April and  $21.5-18.7=2.8$  mg L<sup>-1</sup> in June; Table 1), especially in regards to the change in total DOC. Was this difference significant given the measurement errors, and can it be that there was in fact inputs of DOC in the epilimnion compensating for the loss of DOC by photomineralization?

**Author's response:** It is correct that the differences between 2.3 and 2.8 mg L<sup>-1</sup> is close to our standard deviations (Table 1) and therefore uncertain. We thank the reviewer for pointing this out. We have clarified this part of the paragraph as follows: *“In June the bottom water DOC levels were close to (slightly lower than) surface levels in April surface. This is logical given the combination of (1) lake circulation mixing the larger surface water volume with a smaller bottom water volume, (2) increased temperatures and thereby increased DOC mineralization throughout the water column, and (3) some photomineralization during the circulation period affecting the whole water column. After lake circulation the surface water DOC levels was likely reduced further by additional photo-mineralization and enhanced microbial degradation as temperatures increased further. Additional DOM sources that may have influenced the DOC concentrations between April and June cannot be ruled out but the months of April, May and June 2011 were particularly dry in this region and large amounts of leaching from forest soils after spring mixing was unlikely.”*

P8958-L5: I am still not convinced that the authors provided strong evidence of the importance of DOM release from the sediments to explain the patterns they observed. I would have expected to see some release rate measurements, or some comparison between the optical or molecular properties of the DOM standing just above the sediments and from the sediments themselves. Did the authors perform such measurements? Also, did the authors observe a similar pattern (i.e. higher DOC concentration near the sediments) in March as well, or was it constrained in April only when the sediments were anoxic?

**Author's response:** The strongest support of DOM release from the sediments are derived from DOC distribution under ice when the temperature profile (and the microbial degradation) and the light influence throughout the water column is more uniform than during summer. This is now clarified. Unfortunately we did not measure fluxes of DOM directly. However, we have more detailed DOC profiles from August 1999 and March 2000 (under ice) which clearly support our findings and indicate considerable DOC release from sediments under ice. This data has now been added (new Figure 2).

P8958-L14: Here the authors should provide sufficient details on the results presented in the different figures (figure 6 should not be presented in the text before Fig. 4 and 5). Also, I would have expected to hear a bit about which fluorescent peaks were preferentially photodegraded in the experiments, and some discussion in regards to previous study e.g. Helms et al. 2013 and others.

**Author's response:** We used only figure 4 (now 5) and 5 (now 6) here and that means that the order of figures is now correct. A discussion about the fluorescent peak was also added: *“The measurements of EEM spectra before and after the same irradiation time showed that the photochemically-induced changes were reflected in areas in the EEM spectra that are centered around two maxima with excitation, emission couples of 280/380 nm and 340/375 nm (Fig. 4).” This preferential photo-degradation of FDOM at longer wavelengths indicated a shift towards lower wavelengths of the remaining fluorescent peaks after irradiation similar to findings reported previously (Helms et al., 2013)*

P8958-L19-21: An alternative explanation for this result could be that CDOM or fluorescent DOM might also have been significantly degraded by microbes prior to the photobleaching experiments as recently shown for lakes e.g. Guillemette & del Giorgio 2012; Koehler et al. 2012; Kothawala et al. 2012.

**Author's response:** We agree and added a discussion about microbial degradation of CDOM: *“An alternative explanation would be a microbial degradation of CDOM during spring and the much warmer surface water. However, this was shown to be a rather slow process and a decrease in fluorescent intensity of up to 40 % in 3.5 years has been demonstrated (Kothawala et al., 2012). Another study suggested the involvement of lake bacterial communities to produce and degrade CDOM, but this observation was associated with increased protein-like fluorescence associated with increasing bacterioplankton biomass (Guillemette and del Giorgio, 2012). In this study we did not see any change in the protein-like fluorescence between winter and summer. Therefore, the involvement of bacteria in the degradation of CDOM in the June surface water sample was probably not significant when compared to photochemical degradation.”*

P8959-L7-8: The statement about the solubility of oxidized DOM should be supported by the literature.

**Author's response:** This is inferred from fundamental chemistry, because the electronegativity of oxygen will certainly introduce a polarization of covalent bonds and increase the overall solubility. We have explained this in the revised manuscript.

P8959-L14: Is this result consistent with what other studies have previously found (e.g. Stubbins et al. 2010)?

**Author's response:** yes. We added the following: *“Such highly oxidized formulas produced after solar simulated irradiation have been previously shown and confirmed our observation (Gonsior et al., 2009).”*

P8959-L20: I don't remember seeing rates of microbial decarboxylation specifically measured in Bastviken et al. 2004. Is it oxic respiration that is meant here? Also, I don't really follow the calculation that follows. How was the rate of 1.7 mg L<sup>-1</sup> calculated, and what are the temporal units here? Or is it representing the total amount of DOC consumed over 2 months? If I get it right, the authors are trying to apportion how much of the DOC loss between April and June was due to microbial vs. photodegradation in the lake surface, and they come up with a 40% of total DOC loss potentially induced by solar irradiation. How exactly was that calculated, and where the 22 mg L<sup>-1</sup> comes from? When looking at Table 1, I calculated a similar difference in DOC

concentrations at the surface or at the bottom of the lake between the two sampling dates (4 mg L<sup>-1</sup>), which contradicts a bit the importance of photomineralization in the surface. Could it be that the microbial degradation was simply higher than assumed based on Bastviken et al. 2004, and could this explain most of the differences observed between April and June at the surface and bottom?

**Author's response: It is correct that we mean oxic respiration. The explanation to the questions are given above in the response to comments by Reviewer 1 and this is now clarified in the text. From this explanation it is clarified that the calculation probably overestimates light independent microbial respiration and thereby underestimates the direct or indirect contribution from photochemistry.**

P8960: The discussion in the two paragraphs about how light induced changes in the optical and chemical properties, and in the size distribution of the DOM molecules is interesting, but should be put into context with previous work e.g. Helms et al. 2008; Stubbins et al. 2010; Helms et al. 2013 and others.

**Author's response: references were added. (see details under comments by Reviewer 1)**

Technical comments:

P8951-L26: Remove space after CDOM

**Author's response: corrected**

P8955-L3: Remove space SPE- DOM

**Author's response: corrected**

P8956-L16: Give the full acronym for IUPAC

**Author's response: corrected**

Legend Fig. 5: greater than 10%

**Author's response: corrected**

Legend Fig. 6: Please indicate what the circles are referring to.

**Author's response: We assume Figure 7 (now Figure 8) was meant by the reviewer. We added an explanation to the caption: “; the red circle highlights the completely photo-degraded more saturated DOM component during spring time sunlight exposure, whereas the black circle indicates slow photo-degrading unsaturated and presumably aromatic DOM.”**

Relevant literature:

Guillemette F. & del Giorgio P.A. (2012). Simultaneous consumption and production of fluorescent dissolved organic matter by lake bacterioplankton. *Environ. Microbiol.*, 14, 1432-1443.

Helms J.R., Aron S., Jason D.R., Minor E.C., Kieber D.J. & Mopper K. (2008). Absorption Spectral Slopes and Slope Ratios as Indicators of Molecular Weight, Source, and Photobleaching of Chromophoric Dissolved Organic Matter. *Limnology and Oceanography*, 53, 955-969.

Helms J.R., Stubbins A., Perdue E.M., Green N.W., Chen H. & Mopper K. (2013).

Photochemical bleaching of oceanic dissolved organic matter and its effect on absorption spectral slope and fluorescence. *Marine Chemistry*, 155, 81-91.

Koehler B., von Wachenfeldt E., Kothawala D. & Tranvik L.J. (2012). Reactivity continuum of dissolved organic carbon decomposition in lake water. *J. Geophys. Res.*, 117, G01024.

Kothawala D.N., von Wachenfeldt E., Koehler B. & Tranvik L.J. (2012). Selective loss and preservation of lake water dissolved organic matter fluorescence during long-term dark incubations. *Science of The Total Environment*, 433, 238-246.



Stubbins A., Spencer R.G., Chen H., Hatcher P.G., Mopper K., Hernes P.J., Mwamba V.L., Mangangu A.M. & Wabakanghanzi J.N. (2010). Illuminated darkness: Molecular signatures of Congo River dissolved organic matter and its photochemical alteration as revealed by ultrahigh precision mass spectrometry. *Limnology and Oceanography*, 55, 1467.

Tranvik L.J. & Bertilsson S. (2001). Contrasting effects of solar UV radiation on dissolved organic sources for bacterial growth. *Ecology Letters*, 4, 458-463.