

## Overview:

The manuscript presents data on the photo-oxidation of dissolved organic carbon (DOC) using groundwater inflow as the source waters to 4 freshwater lake ecosystems. While it is an interesting study, there are a number of issues with the experimental aspects of the work that need to be clarified at present as there is considerable variability in the DOC concentrations and the DIC yields are surprisingly low with an apparently large pool of missing carbon unaccounted for in the experimental analysis when viewed in terms of a C mass balance.

## General Comments:

### *Carbon mass balance and potential loss of CO<sub>2</sub> to headspace in exetainers:*

The description of the DIC analysis raises a number of questions as to how well the measurements were made in this work. Measuring changes in DIC in the presence of large concentrations of DOC has always been a challenge (Granéli *et al.*, 1998; Granéli *et al.*, 1996) but there are methods available to do this reasonably well (Porcal *et al.*, 2015). However, in the present case the method description seems to indicate that for the DIC and DO measurements there was a headspace in the Exetainer Vials used. Having a headspace when measuring dissolved gases like O<sub>2</sub> and CO<sub>2</sub> is problematic as there will be a considerable amount of gas exchange to the headspace. This is likely the reason why the DIC yield from DOC photo-oxidation are so low in this work and inconsistent with previous studies. At the very least the description has to be improved in a revised manuscript so the analytical issues arising from this type of measurement can at least be understood in a reasonable framework.

In the manuscript there is no real attempt at an overall carbon balance as there were no measurements of POC taken. This is problematic from the point of view of the overall experimental design but it is made more complicated by a misreading of the Cory *et al.* (2014) work by which the authors have confused O<sub>2</sub> and CO<sub>2</sub> (see below for full details) stoichiometries and this unfortunately impacts the interpretation of the results considerably – at best it is a series of typos at worst a serious misunderstanding of the earlier work and of what this paper itself was trying to achieve (i.e. why measure DIC if you can just use a relationship from elsewhere).

### *Role of iron in the photochemical reduction of DOC:*

Iron and pH have been identified previously as playing an important role in the photo-oxidation of DOC in freshwaters (Gu *et al.*, 2017; Molot *et al.*, 2005), it is a pity then that there are apparently no measurements of the iron content of these waters.

## Specific comments:

Line 77: An additional reference of note on the Brownification of fresh waters is the recent review by Kritzberg *et al.* (2020).

Line 179: Where does the data for the residence time of the lakes come from? It would also be useful to include the estimates of sinks/sources and lake inventory that were used in estimating the residence time. In this context it would be useful to know what the catchment sizes were and the average rainfall to each lake. This would help the reader understand more the processes impacting DOC in the lakes.

Line 199: Please indicate if the GF/F filter was pre-combusted before use to remove any DOC on the filter itself.

Line 218: Are these the usual Labco Exetainer vials? If so please provide the part number etc as these are commonly used for dissolved gas samples and so are well known to most researchers.

Line 218: So does this mean there was a 2 mL headspace in the Exetainers? This will impact the measurements of the DO and DIC considerably (Spötl, 2005; Waldron *et al.*, 2014), see also recommendations from the lab at UC Davis:

<https://stableisotopefacility.ucdavis.edu/dictracegassamplepreparation.html>

Line 223: It would be useful to restate here that these are all groundwater samples and not water from the adjacent lake.

Line 233: This is 100  $\mu$ L of groundwater to both the 35 mL Quartz tube and the 12 mL Exetainer? If this is the case how are the data then corrected for the differences in the additions between the DOC and DIC samples?

Line 233: The bacterial community in the groundwater may be significantly different from that found in the lake, most notably in the presumably the abundance of photosynthetic organisms and the response to light. Could photoinhibition of bacterial activity also have been important here?

Line 248: The samples wrapped in Al foil may have been exposed to greater temperatures during the course of the incubations due to solar heating. Some indication of the *in situ* temperatures and the solar irradiation received (e.g. the data from the radiometer) would be helpful then to gauge if this could have been an influence on the experiment.

Line 266: Please report the standards and/or Certified Reference Materials used for the DOC analysis.

Line 268: Were the DIC samples acidified through the Exetainer septum to prevent gas exchange?

Line 268: What was the volume of sulfuric acid added to each vial?

Line 271: What does well mixed mean in this case? That the headspace in the exetainers was shaken with the water layer. Where the samples acidified prior to this mixing? – see the comment also above regarding the acidification steps.

Line 298: Unfortunately this statement is incorrect as the original citation (Cory *et al.*, 2014) assumes a 0.5 mol O<sub>2</sub> consumed to 1 mol DOC oxidized for partial photooxidation, not CO<sub>2</sub> as stated in the present manuscript. The Cory *et al.* (2014) value is also not valid as other work has shown this value can vary depending on the river water itself (Xie *et al.*, 2004).

Line 299: What does [DIC\*2] signify here? Are you suggesting that half the photo-oxidized carbon turns into some other form of carbon? As the previous sentence in the manuscript linking CO<sub>2</sub> production from DOC was erroneous, this sentence is also incorrect. It begs the question as to how the C balance is achieved if only 50% of the DOC photo-oxidized forms CO<sub>2</sub> what happens to the other 50% of the C as normally CO production is only a small pathway.

Line 301: What is this pool of carbon then, if it is converted from DOC but it is not DIC it has to then be POC by default, unless the authors are arguing for a 3<sup>rd</sup> form of dissolved carbon? See the work Porcal *et al.* (2015) for more details on the carbon balance in these types of experiments.

Line 366: Table 1 - I found some of the statistical relationships to be not credible here; given the data provided so it would be extremely useful to include more details on how the statistics were generated here and for the values to be rechecked. For example when the P/B column indicates a  $p < 0.001$  values for DIC results but the data clearly overlap at the 1 or  $2\sigma$  level then something is not right with regard to the p value reported:  $49.1 \pm 11.4$  compared to  $25.3 \pm 7.2$  and  $20.4 \pm 1.9$  compared to  $17.7 \pm 3.0$

Line 366: Table 1: The DIC yields from photo-oxidation are very low, for example only at Lacawac there is approximately a  $30 \mu\text{mol L}^{-1}$  increase in DIC for a  $1500 \mu\text{mol L}^{-1}$  decrease in DOC so either there is very large POC production or there is something very wrong with the DIC or DOC values. Given the issues noted above for the DIC measurements it is most likely those values that are questionable.

Line 490: The red precipitate is likely iron oxide but was this found in the controls as well?

Line 491: If there is a precipitate then the iron is not being released 'back into the water', it is now precipitating out into the solid phase as the complexing agents responsible for solubilizing it have been destroyed.

Line 495: Sloppy feeding is a term normally applied to zooplankton grazing on bacteria and not bacteria themselves. Why mention this in the context of  $\text{O}_2$ ? As producing DOC does nothing to the  $\text{O}_2$  content necessarily – the  $\text{O}_2$  is used up in respiration.

#### References:

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- Granéli, W., Lindell, M., de Faria, B.M., de Assis Esteves, F., 1998. Photoproduction of dissolved inorganic carbon in temperate and tropical lakes – dependence on wavelength band and dissolved organic carbon concentration. *Biogeochemistry* 43 (2), 175-195.
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- Gu, Y., Lensu, A., Perämäki, S., Ojala, A., Vähätalo, A.V., 2017. Iron and pH Regulating the Photochemical Mineralization of Dissolved Organic Carbon. *ACS Omega* 2 (5), 1905-1914.
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- Porcal, P., Dillon, P.J., Molot, L.A., 2015. Temperature Dependence of Photodegradation of Dissolved Organic Matter to Dissolved Inorganic Carbon and Particulate Organic Carbon. *PLoS ONE* 10 (6), e0128884.
- Spötl, C., 2005. A robust and fast method of sampling and analysis of  $\delta^{13}\text{C}$  of dissolved inorganic carbon in ground waters. *Isotopes in Environmental and Health Studies* 41 (3), 217-221.

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- Xie, H., Zafiriou, O.C., Cai, W.-J., Zepp, R.G., Wang, Y., 2004. Photooxidation and Its Effects on the Carboxyl Content of Dissolved Organic Matter in Two Coastal Rivers in the Southeastern United States. *Environmental Science & Technology* 38 (15), 4113-4119.