Original Submission

Ms. Ref. No.: bg-2018-508

Title: Composition and cycling of dissolved organic matter from tropical peatlands of coastal Sarawak, Borneo, revealed by fluorescence spectroscopy and PARAFAC analysis

Authors: Yongli Zhou, Patrick Martin, Moritz Müller

Overview and general recommendation

1. Recommendation

Major revision

2. Comments to Author

Note: I also read the companion paper 'Distribution and cycling of terrigenous dissolved organic carbon in peatland-draining rivers and coastal waters of Sarawak, Borneo' and the relevant review comments and the authors' answers to the comments.

This study (bg-2018-508) aimed to distinguish different fractions of dissolved organic matter (DOM) in peat-draining rivers, estuaries, and coastal waters of Sarawak, Borneo, using fluorescence spectroscopy and parallel factor (PARAFAC) analysis. The authors observed that the terrigenous fractions showed high concentrations at freshwater stations within the rivers, and conservative mixing with seawater across the estuaries, while the autochthonous DOM fraction showed low concentrations at all salinities. The authors claim that, based on the fluorescence data and little changes in optical properties of DOM, at least 20%–25% of the DOC at even the most marine stations (salinity >31) was terrestrial in origin. Although not all of the data provided is new to the relevant field, the content of this paper fulfills the requirements for the submission to *Biogeosciences* of which aims and scopes are to publish studies *on all aspects of the interactions between the biological, chemical, and physical processes in terrestrial or extraterrestrial life with the geosphere, hydrosphere, and atmosphere*. The title is representative of the article contents and the abstract summarize the contents clearly. Therefore, I recommend accepting this paper after the authors revise all the necessary points.

I have serious concerns about the use of sodium azide (NaN_3) as a preservative for samples analyzed by UV absorption and fluorescence spectroscopy. Also, the emission wavelengths used to calculate fluorescence index (FI) seem inadequate. Finally, estimation of %tDOM by fluorescence is questionable.

2.1 Major comments

2.1.1. Estimate of terrestrial contribution

FDOM is only a small portion of the bulk DOM, and thus estimation of %tDOM by fluorescence is troublesome. PARAFAC components can be used to better understand biogeochemical processes that occur during the estuarine mixing, but PARAFAC components alone are not sufficient to estimate the tDOM contribution at given salinity. To make it possible, you must assume that all the rest of components in riverine DOM other than FDOM (PARAFAC component C1 in this case) behaves in the same way as C1 does during the estuarine mixing and that marine end-member has no C1. Please explicitly state your assumptions. It's not enough in the current form. Also, how do you explain %tDOC of >100% in Samunsam and Western Region (in March) at salinity >10 under your assumption?

In addition, Fmax/DOC is known to be susceptible to errors caused by the fluorescence intensity and DOC measurements (Korak et al. 2014), and the authors should include an evaluation of such an uncertainty (error propagation analysis), since %tDOM estimation is I believe the most important part of this study.

Generally, in estuarine environments, contribution from estuarine vegetation (mangrove and marsh) is done by an end-member mixing model (0.1 salinity increment) using DOC concentrations of the fresh and marine end-member (Cawley et al. 2014). Because the main subject of this study is tropical peatlands, I feel that what the authors want to investigate is not riverine (derived from upper regions) inputs but inputs from the peatlands located in the estuary. The authors may reassess contributions from the peatlands using the method reported in, for example, Cawley et al. (2014).

2.1.2. NaN_3

Although you said 'NaN_3 did not contribute any blank fluorescence', it did contribute to sample absorbance, as you mentioned in the companion paper. Indeed sample preservation is still a major challenge, and I do use NaN_3 to preserve samples for DOC analysis. However, I never use NaN_3 to preserve samples for optical analysis because of the strong UV absorbance by NaN_3 even at a low concentration (0.005% (w/v) in this study). I agree that if your samples have high absorbance, you could correct for the NaN_3 absorbance accurately. However, when measuring EEM for samples containing NaN_3, it seems that you failed to correct for the inner-filter effects (IFEs) caused by NaN_3, because for the IFEs correction you used the absorbance of CDOM that were obtained by subtracting the absorbance of NaN_3 from that of samples containing NaN_3. In that way, you underestimated fluorescence in the EEM regions where NaN_3 absorbed light (Ex 250–280). This is very serious because you mentioned the protein-like component 'showed consistently low values across the study region', and this could be due to underestimation of the protein-like component. The relative degree of the underestimation will be larger with decreasing sample absorbance relative to that of NaN_3.

If you will correct (or may have corrected) for IFEs including NaN_3 absorbance, please

explain the degree of uncertainty of the correction. Because, although you said all samples had the same NaN_3 concentration, there should be some variation in the concentration caused by, for example, repetitive volumetric measurements of samples (30 mL) and NaN_3 solution (150 µL).

2.1.3. FI

Did you apply instrument-specific correction for EEM? If so, the emission wavelength for FI must be 470/520 nm instead of 450/500 nm (Cory et al. 2010; Kida et al. 2018), because the emission peak often lies between 450 and 500 nm when the correction applied, which makes FI meaningless (FI must be calculated on the right side of the emission peak). If not, please write so in M&M section, because in that case your results are not directly comparable with other studies. It is often observed that if not corrected for the instrument-specific bias, the variability of FI between instruments is large for a given sample.

2.2 Minor comments

Table 2. Was the distribution of the PARAFAC components and chlorophyll-a normally distributed? If not, Spearman's rank correlation should be used instead. Note that strong parametric linear relationships between PARAFAC components are unlikely considering the theory of PARAFAC. If components have a strong linear correlation, PARAFAC cannot resolve these components and they appear as a single combined component. Correlations between PARAFAC components are generally expressed by a log-log plot or Fmax/DOC plot (Stedmon and Markager 2005).

P2L26 'extremely high DOC concentrations' Please specify the DOC range, as it depends on person when a DOC concentration is 'extremely' high.

P3L11 Sampling

How was the weather on the sampling days? In addition to seasonal changes, daily changes in rainfall and water flow conditions would affect DOM concentrations and compositions. If you discuss seasonal changes, at least the weather should be the same.

P3L30 Was the condition of the photodegradation experiment sterile (biodegradation-free)? If not, how about the effect of biodegradation? Please add some more details about the photodegradation experiment. For example, water inside the bottles was repetitively sub-sampled or you prepared many bottles and each bottle was collected as a sub-sample?

P4L3 'To minimize self-quenching of fluorescence intensity' Please add information on the maximum absorbance value of the measured samples, since IFE correction becomes invalid if sample absorbance

is too high. Also, how you measured absorbance data is completely lacking. Please explain it in this section, and reference to the companion paper alone is not sufficient.

P4L27 ‘chemical compound classes’ The authors need to be careful here. What PARAFAC can do is to statistically deconvolute EEMs into underlying building blocks, termed ‘components’, and these components are rarely related to specific chemical compounds. I think the authors understood that, but for those who are not familiar with PARAFAC, the author’s statement may be misleading.

P4L28 Specify how many samples were removed.

P4L29 Please add in Fig. 3 the excitation and emission loadings of the validated split dataset.

P5L1 Fmax is not just a score value. “Fmax is calculated by multiplying the maximum excitation loading and maximum emission loading for each component by its score, producing intensities in the same measurement scale as the original EEMs” (Murphy et al. 2013).

Also, Fmax cannot be a major of the concentration of each component *in a sample*, “because different fluorophores can have very different efficiencies at absorbing and converting incident radiation to fluorescence (Murphy et al. 2013).” Rather, “Quantitative and qualitative information may however be obtained from changes in the intensity of a given component, or in the ratios of any two components, *between samples in the dataset* (Murphy et al. 2013).”

P5L8&L15 a_{350}, $S_{275–295}$, S_R, and $SUVA_{254}$ appeared for the first time here without explanations what they are. This is not kind for those who are not familiar with the optical indices. This is relevant to my comment on P4L3. Now I think that you need to make another section in M&M that explains the absorbance measurement and absorbance-based indices. However, personally I think that you can completely cut the sentences with respect to S_R, a_{350} and $SUVA_{254}$ since you mentioned about S_R and a_{350} only once or twice and did not discuss SUVA results (just correlation with HIX). As for $S_{275–295}$, you may want to use it to support your idea that an FDOM-based estimate of tDOM is OK. However, I am not totally convinced that being correlated with $S_{275–295}$ supports the correctness of your fluorescence-based %tDOM (P11L24), because estimations of %tDOM based on $S_{275–295}$ is non-linear (Fichot and Benner 2012).

P5L16 “$SUVA_{254}$, 3.08–6.89” SUVA value of 6.89 is too high. Even the highest aromaticity sample (Ar >40%) in Weishaar et al. (2003) had the SUVA value of 5.3, and the possible maximum SUVA value (~5) has been recently suggested from a molecular analysis (Kellerman et al. 2018). Iron(III) is most probably interfering with SUVA determination in your sample dataset (Poulin et al. 2014; Kida

et al. 2018). If the authors did not measure Fe(III) and also have no stored sample for Fe(III) measurement, please state in the manuscript that some of SUVA values in this study was overestimated by interferences from Fe(III) to an unknown degree. Note that, if Fe(III) contributes to $SUVA_{254}$ to a similar degree for all the samples, $SUVA_{254}$ and $SUVA_{280}$ would still have a high correlation.

Another possibility is the interference by NaN_3 even after the blank correction. This is possible when the sample CDOM absorbance was low. Please add the information on the NaN_3 absorbance contribution to sample absorbance at 254 nm. According to Fig S1&2 of the companion paper, decadic absorption coefficient of the NaN_3 solution was about 4 m^{-1}, which was about 10%–30% of that of Rajang, Sematan, and Lundu samples and 50%–200% of marine samples. These values are not trivial.

P6L3 Please add seasonal climatic information (dry? rainy?) after months so that readers can easily understand climatic conditions, not only in the M&M section.

P9L31 "correlating strongly with DOC-normalized amino acid yields" This is not a correct citation. The correlation coefficient was r = 0.62 (Fig. 8b in Yamashita et al., 2015), at best moderate correlation.

2.3 Technical corrections

P2L5 & L7 '0.2-0.25 Pg C yr^{-1}' and '40% - 50%' should be 0.2–0.25 Pg C yr^{-1} and 40–50% (or 40%–50%). Please check the usage for minus (−), hyphen (-), en dash (–), and em dash (—). I did not correct for the rest of the manuscript.

In Fig 2&4, it would be better to set the x axis to the same scale (maximum salinity of 35) except for the Simunjan River results so that comparisons between rivers become easier and more straightforward.

The caption of Fig. 4 says 'while they distinguish samples from different regions in the panel (z)', but I can't find the panel (z).

In Table 1, pleased add Tucker congruence coefficient (TCC) values so that readers can evaluate how much the comparisons are quantitative. Add the relevant explanations in M&M section as well.

In reference list, please add a space between references to improve visibility. It's OK not to do it this time but I'm suggesting this for future reviewers.

References

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