

Interactive comment on “Optical and molecular-level characterization of fluvial organic matter biodegradation in a highly urbanized river system” by Most Shirina Begum et al.

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Rapid urbanization effects in streams and rivers is a very important topic worldwide, not just for greenhouse gas production via microbial processes, but also in foodwebs of such environments after anthropogenic nutrient introduction. Aquatic and terrestrial organic matter fluorescence and molecular composition analyses by EEMs, PARAFAC, and FT-ICR MS are all in my expertise area, so I have provided these comments on the manuscript, focusing mostly on such areas and their interpretations used to support the main results of the work. I hope they are of use.

Major questions arose when reading this manuscript that need to be addressed prior to publication: 1. How is the priming effect tested? 2. How is biodegradation con-

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firmed/tested? 3. Did the authors measure bacterial abundances as a part of any portion of the incubations? 4. Can the authors report on any lability vs. recalcitrant calculations based on their chemical assessments of the DOM? 5. What did the author's aim to gain when comparing the component ratios of the fluorescence intensities? 6. Can the authors comment/discuss the regions of chemical character on the van Krevelen diagrams that might be indicative of urbanizing watersheds? Meaning – can they comment on any regions that would be more effected by anthropogenic OM or increased nutrients from fertilizers, etc.?

Line 37: What is meant by a positive feedback to climate change? Can the authors be more specific? Increases in greenhouse gases?

Lines 52-69: This section is very generally presented and reads like an overall review of the basic information out there regarding chemical characterizations. Consider a revised section that focuses the material presented and appropriate references on anthropogenic OM studies/urbanizing environments. Some of the statements, (lines 66-69, for example), are not accurate representations of what I think the main idea of this manuscript is. FT-ICR MS has been used to investigate DOM degradation for decades now, but an updated reference list with an urbanizing watershed focus would be appropriate. As of right now, the focus of the introduction is too broad.

Lines 80-86: Primary and secondary goals should be quite clear. Consider a revision that includes stating the methods in further detail. No molecular composition information/FT-ICR MS method is listed here, but seems to be a main focus of the work.

Lines 133-134: The authors should consider microbial inclusions with a filter pore size of $0.7\mu\text{m}$. This should be noted in the text.

Lines 136-137: Were the bottles uncapped within the incubator chamber? Or outside of the chamber while shaking? Did the incubator chamber have a shaker plate, or were they shaken manually?

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Lines 141-145: How does an unfiltered incubation set the grounds to investigate the priming effect? How is the priming effect tested in this work?

Throughout the manuscript, were any glass bottles pre-fired or combusted prior to use? Did the sample collection team triple rinse the bottles with river water to rinse away any residual carbon? Please discuss all precautions taken to reduce bottle-C contamination. More details are required to ensure the control experiments follow the same protocol and/or have minimized contamination procedures.

Lines 178-184: I'm assuming you are describing two separate analyses here. Optical fluorescence information from the post-processed EEMs, and subsequently a statistical PARAFAC analysis, which was not reported. Please separate those reported method details, results, and discussion sections. More information is required regarding how PARAFAC was employed for this work. As of right now, the methods section is severely insufficient.

Line 215: What is your assignment criteria for P if it cannot be confirmed using monoisotopic mass spacing patterns applicable for CHNOS chemical species? Please provide more details.

Lines 246-254: PARAFAC components must be described in the text, and I strenuously suggest component figures added to the manuscript. None are presented, no descriptions of the results are provided, and Figure 3/caption both do not provide appropriate information. Without this information, the results section is insufficient and confusing to read.

Lines 246-254: One can argue that component fluorescence intensities are a direct result of the quantum yield for individual fluorophores. What are the authors trying to gain by comparing intensities? Were they normalized? This needs to be discussed in further detail. Also, in Figure S3, the component fluorescence intensities are barely changing over the 5 day incubation. Can the authors comment on this? Were bacterial abundances also checked for these experiments? Was there any microbial growth

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occurring? How can the authors claim biodegradation without bacterial communities monitored for increases and decreases?

Lines 281-288: Figure 6 is not annotated for PARAFAC components, rather is just a figure of the post-processed EEMs. Therefore, the text in this section does not coincide with the data referenced in the figure. Please adjust all figures/captions and text sections accordingly to be consistent and improve clarity. Consider a revised section that incorporates reporting the results from the EEMs data, followed by a section reporting the PARAFAC results, and then subsequently interpreting both results appropriately in the discussion sections. Currently, this section is too confusing for someone to read and makes little sense.

Lines 295-298: How can the authors make this claim??? FT-ICR MS is not quantitative and the peak intensities are a function of ionization efficiencies. At best, the authors can compare more or less ionizable DOM using the intensity information, but production and consumption cannot be inferred by measuring these changes. Following that comment, how can the authors confirm production versus consumption? Only molecular composition presence and absence of identified peaks can be measured, and that does not insinuate microbial production and consumption only. Abiotic processing (e.g., condensation reactions) can also occur, potentially producing DOM of greater sizes than measurable in a 200-1100 Da analytical window. Using production and consumption inappropriately is a considerable error throughout the manuscript, and the authors are requested to consider clarifying how they are interpreting the FT-ICR MS data.

Lines 300-and on: None of this information can be confirmed by FT-ICR MS molecular composition analyses in this manner. The authors are requested to reinvestigate their data set with accurate definitions (e.g., DOM processing, transformations, abiotic and biotic considerations), and appropriate analyses/interpretations.

Lines 304-307: How can the authors report P results without confirming a method

of molecular assignment? Major molecular series? Aren't those listed the only ones reported? The ionization efficiencies of CHNOSP were above the signal to noise ratio threshold? Can the authors confirm this? What was the percent contribution of CHNOSP molecular species to the entire DOM sample? Less than 1%? 2%? 5%?

The discussion section will require a complete revision based on reinterpreting the results sections as suggested above to improve clarity and strengthen the work.

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