Short comment #1

SC1: *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 confirmed/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.?*

Response: Thanks for your comments and questions that are relevant to the key topics addressed in the manuscript. Although we addressed many of the raised questions in the manuscript, here we provide more detailed descriptions and revision plans.

1. Priming effect was tested by comparing the rates of CO_2 production in two separate samples (the mainstem river water enriched in autochthonous DOM and the urban tributary water carrying wastewater treatment plant effluent) and their mixture. We assumed that a higher rate of CO_2 production in the mixture relative to those for the separate samples would indicate an enhanced biodegradation by mixing organic matter of two sources. This will be articulated in the last part of the introduction adding to the study objectives:

"We tested the hypothesis that mixing the river water downstream of a large dam and the tributary water containing WWTP effluent would enhance the biodegradation of existing and/or added organic matter by continuously monitoring and comparing changes in dissolved CO_2 and DOC between the separate and mixed samples."

2. We estimated the rate of biodegradation as the reduction of DOC concentration during a short-term incubation under dark condition (preventing photo degradation) in presence of native microorganisms. The standard method we used has been widely used to examine biodegradation in freshwater systems. Although we did not employ microbiological measurements, our CO_2 measurements in the second incubation experiment confirmed that the changes in CO_2 over the incubation result from organic matter biodegradation.

3. Bacterial abundance was not measured as part of the incubation experiments.

4. Direct report on lability vs. recalcitrance of DOM is not possible from the chemical analyses used in this study; however, intensity and relative abundance of the different components of DOM (C1, C2, C3) can indicate trend and patterns of lability among the samples. This may be articulated in the relevant sections.

5. Though statistically not significant, longitudinal or incubation-induced differences in the mean values can help explore spatial variability of DOM components (Fig. 3) or preferential consumption or production of OM during the incubation (Tables S3, S4). More detailed descriptions of the implications will be provided in the revised manuscript.

6. Figure 7 shows the presence of molecular series indicative of anthropogenic origin. We will cite more papers to articulate which molecular series would indicate organic moieties of anthropogenic sources.

SC1: *Line 37: What is meant by a positive feedback to climate change? Can the authors be more specific? Increases in greenhouse gases?*

Response: Positive feedback to climate change here refers to increase in greenhouse gas emission and resulting warming. We will rewrite the phrase to "inland waters can increase greenhouse gas concentration in the atmosphere and accelerate climate change through warming".

SC1: *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.*

Response: The section was intended to provide an overview of the various OM characterization techniques that are used in this paper. We will rewrite the paragraph to provide a more focused introduction to the OM characterization approaches including some new studies employing FT-ICR-MS analysis in characterizing DOM in urbanized watersheds.

SC1: *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.*

Response: The objectives of the study will be modified to include the methodological details:

"A basin-scale field survey was combined with two laboratory incubation experiments to better understand how downstream changes in riverine OM quality driven by dams and urban water pollution can alter the biodegradation of DOM, with and without POM. Longitudinal and incubation-induced changes in DOM quality were characterized with various optical characterization techniques including UV absorbance at 254 nm (UVA254), fluorescence excitation-emission matrices (EEMs), and fluorescence indices indicative of DOM sources and major chemical components. Fourier transform-ion cyclotron resonance-mass spectrometry (FT-ICR-MS) was used to identify changes in the molecular composition in the second incubation experiment. The specific goal of the second incubation experiment was to investigate whether priming effects of labile OM of autochthonous or anthropogenic origin can enhance the rate of biodegradation of riverine OM and hence CO2 emission from the eutrophic urban river receiving discharges from the upstream reservoir and the polluted tributaries."

SC1: *Lines 133-134: The authors should consider microbial inclusions with a filter pore size of 0.7μm. This should be noted in the text.*

Response: This will be noted in the text and the sentence will be modified as- "A subset of samples were filtered through pre-combusted glass fiber filters (GF/F, Whatman; nominal pore size 0.7 μm) to remove POM assuming no significant difference caused by small microbial inclusion compared to commonly used filters with pore size of 0.2 μm (Servais et al. 1995)".

SC1: *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?*

Response: The bottles were taken out of the chamber, uncapped and placed on a shaker plate for aeration for ~30 minutes. More details will be provided, such as "the bottles were taken out of the chamber, uncapped, and gently shaken for 30 min on a shaker plate at ~100 rpm every two days".

SC1: *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?*

Response: Incubation with unfiltered samples was supposed to create more realistic conditions for testing the priming effect on all organic matter components including POM.

SC1: *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.*

Response: All the reported values were checked by strict QA/QC procedures that our lab maintains. More details will be provided in the revised manuscript, as follows:

"The glass bottles and other glassware used in the experiment were cleaned by placing in 10% HCL acid for >24 hours and rinsing carefully with DI water. The clean dry bottles were always rinsed at least once before collecting the sample. Similarly, any glassware used for filtering and storing the sample was rinsed before using. Gloves and other protective wears were always used to prevent contamination."

SC1: *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.*

Response: We did not conduct PARAFAC with the relative small data set. Instead, we used peak wavelengths of three components identified for a large data set we had collected in the same study site (Han River watershed). We are now testing and comparing PARAFAC runs with the reported values and therefore we may be able to report the PARAFAC results if the modeling results can conform to PARAFAC validation criteria despite the relatively small number of samples (<100).

SC1: *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.*

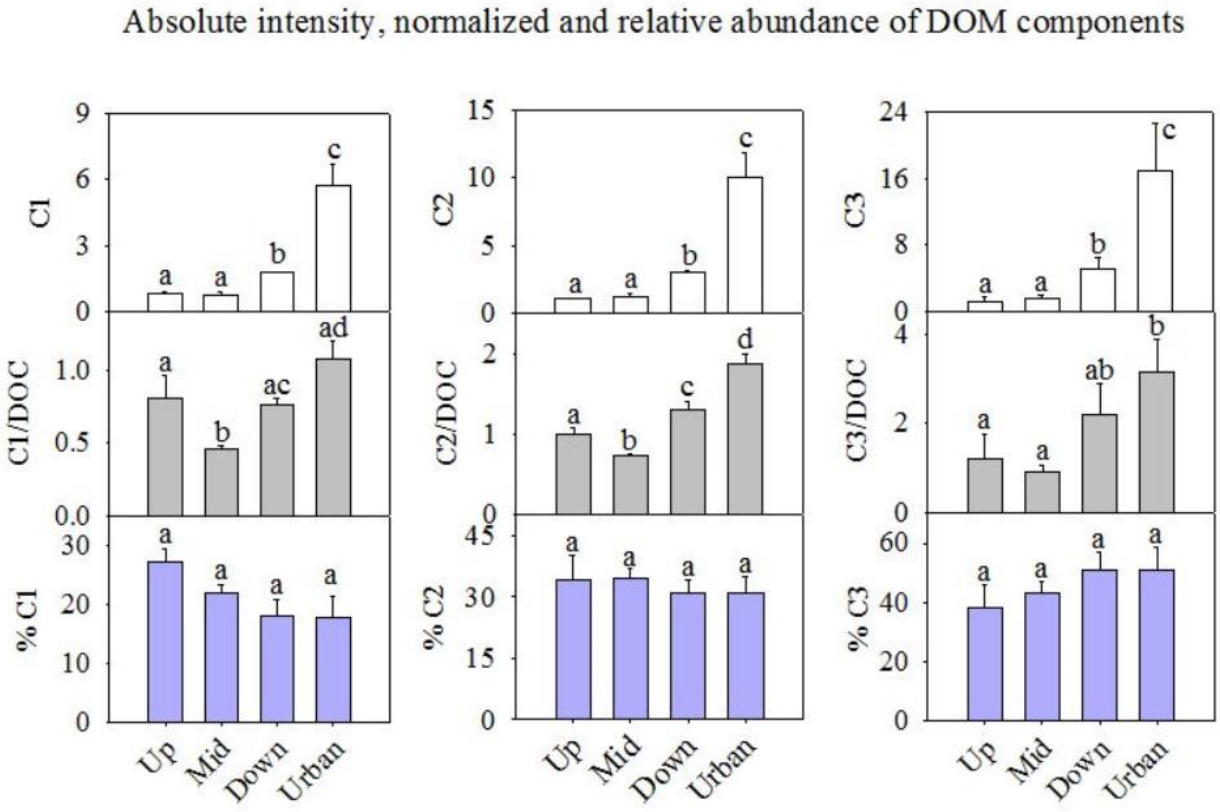
Response: In the present study, no criteria for the P-containing compounds (natural organic phosphorus) were used. Our home-coded analysis software was tested using the data set published in supporting information of Anal. Chem. 2007, 79, 1758-1763 by by Boris P. Koch, Thorsten Dittmar, Matthias Witt, and Gerhard Kattner. Our analysis results were almost identical to those of Anal. Chem. (2007) paper. Although we did not use any criteria for the natural organic phosphorus was used, the monoisotopic mass spacing patterns will be used in the revised manuscript to confirm the presence of natural organic phosphorus. The criteria we are planning to use is based on the fact that masses for P (30.974), $^{14}N^{16}O^{1}H^{+}$ (31.0581), and $^{15}N^{16}O^{+}$ (30.9950) are a little different from each other (Talanta, 2005, 66, 348-358).

SC1: *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.*

Response: More description will be added in the revision to clarify that the PARAFAC was not conducted as part of this study. Aforementioned, PARAFAC results may be presented if test runs conform to model validation criteria.

SC1: *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 occurring? How can the authors claim biodegradation without bacterial communities monitored for increases and decreases?*

Response: Fluorescence intensities were compared among the watershed compartments to examine anthropogenic impacts along the Han River from the up- to downstream reach as well as how the organic matter composition changes during the short term incubation. The fluorescence intensities were not normalized by DOC concentration, but we may use the specific fluorescence normalized by DOC in the revised manuscript or SI, as shown in the figure below. Specific fluorescence intensities clearly show impoundment effects in the middle reach and longitudinal increases from the mid- to the downstream reach. As answered before, we did not monitor any bacterial abundance and community composition parameters. However, we followed standard procedures used in many biogeochemical studies of biodegradaion focusing on BDOC and optical characterization. The limitation associated with the lack of microbiological measurements will be discussed in the revision.



SC1: *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.*

Response: Text and figures will be changed accordingly for consistency of the terms. To refer to the altered areas on the EEMs, we will use the terms such as "areas near the humic-like/ microbial humic-like/ protein-like OM", whereas to refer to the DOM components- C1, C2, and C3, humic-like/ microbial humic-like/ protein-like component/fluorophore will be used.

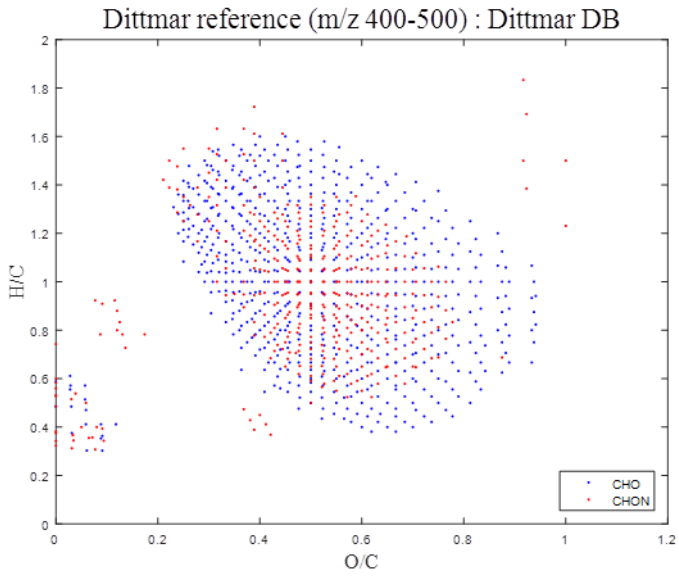
SC1: *Lines 295-298: How can the authors make this claim??? FT-ICR MS is not quantitative and the peak intensities are a function of ionization efficiencies. At best, the authors can compare more or less ionizable DOM using the intensity information, but production and consumption cannot be inferred 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.

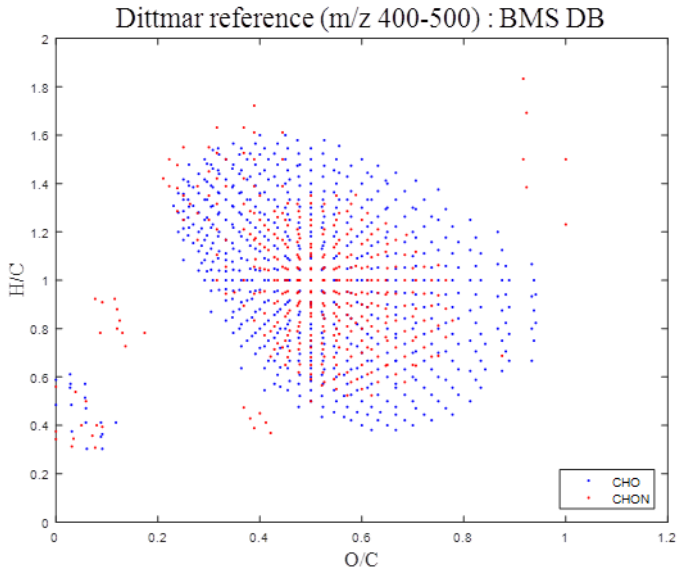
Response: Our approach is based on a previous study that considered changes in peak intensity as the production or consumption of organic moieties during a short incubation (Ward et al. 2013). We are also well aware of the fact that FT-ICR-MS data cannot be used to provide direct quantitative information, but believe that the used visual presentation can provide at least "qualitative" information on the potential compositional changes caused by microbial processing. We will make clear on which assumptions and limitations our qualitative approach is based in the revised manuscript. We are also aware that absolute abundances of the peaks appearing ESI-FT-ICR MS spectra is not quantitative. However, the normalized relative abundances of the peaks in the ESI-FT-ICR MS spectra can be informative for indicating, *at least*, the fold-increase or decrease of the compounds under examination, we think. Furthermore, this semi-quantitative approach was also used in the previous paper by Ward et al. 2013.

SC1: *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.*

Response: As addressed above, our home-coded analysis software was tested using the data set published in the supporting information of Anal. Chem. 2007, 79, 1758-1763 by Boris P. Koch, Thorsten Dittmar, Matthias Witt, and Gerhard Kattner. Our analysis results were almost identical to those of Anal. Chem. (2007) paper, indicating that our analysis ability is acceptable. Below, two van Krevelen plots are shown. For clear demonstration, these are plotted for the data only in the *m/z* region between 400 and 500. The first one is plotted using the data analysis results from Anal. Chem. 2007. In the case of the second plot, data analysis was made using our home-coded analysis software for the mass spectrometry peak list from Anal. Chem. 2007 and the analysis results are simply plotted in the van Krevelen diagram format. The two plots are almost identical to each other, clearly demonstrating that our home-coded analysis software are very accurate in analyzing the DOM FT-ICR MS data. This point will be clarified in the revised manuscript. Further, a full list of natural organic compounds will be provided in the supporting information.



A van Krevelen plot using the data analysis results from Anal. Chem. 2007 paper



A van Krevelen plot made using the analysis results by our home-coded analysis software for the FT-ICR MS data from Anal. Chem. 2007 paper

SC1: *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%?*

Response: As stated above, in the present study, no criteria for the P-containing compounds (natural organic phosphorus) is used. In the revision, the criteria based on the fact that masses for P (30.974),

$^{14}N^{16}O^{1}H^{+}$ (31.0581), and $^{15}N^{16}O^{+}$ (30.9950) that are a little different from each other (Talanta, 2005, 66, 348-358) will be used, and the comments and questions raised above can be addressed based on the future analysis results.

SC1: *The discussion section will require a complete revision based on reinterpreting the results sections as suggested above to improve clarity and strengthen the work.*

Response: Discussion section will be revised based on the comments and suggestions provided here as well as by the reviewers.