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Interactive comment on "Filtering artefacts in bacterial community composition can affect the outcome of dissolved organic matter biolability assays" *by* Joshua F. Dean et al.

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We thank the reviewer for their constructive comments on our manuscript. Here we give our initial response to these comments, and will provide a modified manuscript after the discussion is closed and under the guidance of the editor. We also include a response to both reviewers at the end of this comment as they both raised the same point regarding expanding the discussion about the impact of filtration on DOC structure and concentrations.

Methodology:

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- Line 33: The samples were refrigerated at 4 degC. The samples were not refrigerated during transport, but the travel time was less than an hour and were refrigerated immediately upon arrival at the university. These notes will be added to the methodology in our modified manuscript.

- We chose 0.2 and 0.7 μm pore sizes as these are the most commonly used filter sizes for DOM incubations and provide a reasonable spread in pore size. The reviewer is correct in that 0.45 μm is common as a size definition or filtration choice for normal DOC concentration analyses (as mentioned in our introduction, pg 2 line 9). But 0.45 μm is not commonly used for incubations unless an inoculum is added, in which case we would consider a 0.45 μm treatment to be analogous to our 0.2 μm treatment; we did not have the budget for a fourth treatment in this experiment.

- It took 6 months to analyse the samples because our own lab for running DOC concentrations had technical problems, so we had to find another lab to run these samples. This took some time and there was a wait to finally run the samples. 6 months frozen storage should not have had any significant impact on the concentration and isotopic composition of the DOC samples (Gulliver et al., 2010; Peacock et al., 2015).

Results:

- Figure 1. We will correct the missing labels.

- Figure 2. We will shrink the marker points as requested.

- Figure 3c. We will include the P2, P7 and UF labels under Day 0 and Day 14.

Discussion:

Reviewer 2 states: “It would be good to see more of a discussion concerning the impact of pore size on DOC concentration, and particularly how this may change over time. For example, would any pore size be fine if you are going to analyse the sample quickly?”

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This comment is echoed by Reviewer 1, who states: “It would be good to include some discussion/references on how the filter pore size might (or might not) affect the DOC concentrations and characteristics. As a starter, both papers below demonstrate no significant differences in DOC and d13C-DOC be- tween 0.2 and 0.7 μm filtrations: Denis et al. (2017) A comparative study on the pore- size and filter type effect on the molecular composition of soil and stream dissolved organic matter. Organic Geochemistry 110: 36–44 Bouillon et al. (2014) Contrast- ing biogeochemical characteristics of the Oubangui River and tributaries (Congo River basin). Scientific Reports 4: 5402 | DOI: 10.1038/srep05402”

- We will add a short discussion as requested by both reviewers in Section 3.1 “DOM carbon dynamics” and Section 4.3 which will be renamed “Limitations, implications and future work”; this is outlined below:

To be added to Section 3.1: Based on the lack of a significant difference between DOC concentrations in the treatments at the initial time point (Figure 1), there is very little DOC contained between the 0.2 and 0.7 μm size range; this is supported by previous work (Zsolnay, 2003; Bouillon et al., 2014; Denis et al., 2017). Most DOM molecules are very small, less than $\sim$0.1 μm in size (Gustafsson and Gschwend, 1997), with very little in the 0.2 to 0.7 μm size range contributing to the overall DOC concentration. This is also reflected in the DOM quality indices in this study, although the P2 treatment shows some differences initially (Figure 2). The lower E2:E3 ratio and higher SR initial values for P2 compared to P7 suggests that the P2 samples are of lower molecular weight in general, while the higher SUVA254 values suggest greater aromaticity in the P2 samples (Helms et al., 2008). This difference is most clearly seen in the absorbance at 440 nm (Figure 2e). However, these differences appear relatively minor in magnitude, with the structural indices converging by the first time point (day 5) across all treatments during incubation (Figure 2). This suggests that what small initial structural differences existed between the P2 and P7 treatments appeared unimportant to the overall dynamics of the DOM pool, which is supported by the lack of difference in

initial DOC concentrations (Figure 1), and previous work (Zsolnay, 2003; Bouillon et al., 2014; Denis et al., 2017).

To be added to Section 4.3: This study suggests that filter size has relatively little impact on DOC concentration and structure, supporting previous studies (Zsolnay, 2003; Bouillon et al., 2014; Denis et al., 2017). The choice of filter size for DOM sample storage, therefore, is relatively unimportant. What is more important for sample storage, and the subsequent degradation of the DOM pool by latent microbes, is the immediate treatment and storage conditions. For example, acidification, storage in dark conditions, refrigeration and freezing can all reduce microbial activity and maintain the integrity of DOM samples, but the best method is to analyse the samples as soon as possible after collection (Gulliver et al., 2010; Peacock et al., 2015).

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