

Thank you for the thorough, thoughtful, and polite reviews of our manuscript. We feel that the reviewers have raised a number of weakness in our original submission, and we have addressed these concerns and incorporated all the recommended changes. In some cases, the suggestions for further analyses did not lead to significant results so these have not been included in the manuscript, but we do detail our approach below. We hope you find that our response to the concerns that were raised has improved the quality of this manuscript. Below, we address the reviewers' comments point-by-point, providing additional detail as needed, and for clarity, each response from us is preceded by an asterisk (*).

Thank you for your time and consideration.

Reviewer(s)' Comments to Author:

Referee: 1

Comments to the Author

This manuscript addresses the role of stream burial for the spatio-temporal patterns of dissolved organic carbon and organic carbon use by benthic microbes in urban streams.

The manuscript is generally well prepared, the applied methods are appropriate and most of the conclusions are supported by the study's results.

General comments:

- The conclusion, that these streams are limited by carbon supply seems insufficiently supported by the results. While the data indicate rather low concentrations of labile carbon, these streams could still be limited by nitrogen or phosphor. This was not assessed with the nutrient diffusing substrata assay since these were supplemented with nitrogen and phosphorus to exclude any inorganic nutrient limitation. While the results comparing different seasons and reaches are of course valid, the conclusion of widespread carbon limitation (abstract, l. 451) and statements based on this conclusion (l. 460, 470; please see specific comments) should be more careful.

*Reviewer number 2 also pointed this out, and we revised the language and tone to be more careful throughout the paper as suggested

- Please indicate the number of samples taken (see also specific comments). Without knowing n, the relevance of the results cannot be assessed by the reader.

*We added n values to the methods

- A number of abbreviations are explained several times in the manuscript and the terms and their abbreviations are used alternately throughout the manuscript (e.g.: Extracellular Enzyme Activity (EEA): explained in l. 55, 126, 128, 240, 353, 354). Please explain them once and then use only the abbreviations.

*these changes were made

Specific comments:

Abstract, l. 5: „other nutrient cycles“ – rather vague statement; which nutrient cycles, other than

what?

*revised for greater specificity

l. 32: „standing stocks exhibit“

*fixed

l. 86-88, 197: How many samples were taken? One per stream, reach and season (18) or were replicates analysed? How many clay tiles per site (here or in l. 197)? Six weeks (l.197) or >6 weeks (l. 88)?

*clarified to explain that we collected one water sample from the downstream and upstream end of each buried and open reach of each stream. Also, clarified that we deployed for a minimum of 6 weeks, and n values added

l. 104: „wavelengths“

*fixed

l. 132-133: It is not clear from these two sentences what was measured: Should this mean that POX was measured using DOPA and peroxidase using DOPA + H₂O₂? Or where both enzymes measured additionally measured using another substrate? „activity of dihydroxyphenylalanine“ – this is the substrate, not the enzyme – peroxidase activity (also figure 4)? The substrate is abbreviated as DOPA, which is not used any more, and the peroxidase is abbreviated as DOPAH₂ or DOPA-H₂O₂, which is explained only in the legend to Figure 4; please be consistent.

*I see the confusion now. Yes, POX was measured using DOPA, and peroxidase was measured using DOPA+H₂O₂. There was not another substrate used to measure the enzymes. Because the measurements derived from DOPA and DOPA+H₂O₂ really measure different aspects of the same thing (recalcitrant carbon acquisition), we decided to simplify the graphic by removing the upper peroxidase panel. We feel that this change simplifies this block of text and the associated results.

l. 134f: Please explain the used indices more detailed: recalcitrant carbon in l. 134 presumably means only POX, not peroxidase or both; please include whole equation in the brackets. LCI: As far as I know, this index is derived directly from lignin and cellulose values, not enzymatic activities; please give reference for the equivalence of these indices (the paper by Sinsabaugh and Follstad Sha referenced in this sentence does not indicate LCI as a measure of enzymatic activity, as far as I can see). Please include equation for CQI (which enzyme for recalcitrant carbon is used?) and explain abbreviation.

*following on the prior comment, we now just describe POX, we have removed references to LCI based on Reviewer 2's comments, and we include the equation for CQI.

l. 155: Resulting in how many samples for buried/open reaches?

*clarified in the text. This resulted in n=8 per carbon treatment in open reaches and n=16 per carbon treatment in buried reaches per season and stream.

l. 157: This is, 8 per site and carbon source?

*clarified in the text. See response above.

l. 162: Should this be „PVC tubes“?

*yes, clarified in the text

l. 189-190 and 193: Periphyton sample from 0.052 versus 0.006 m²: Is this to say that the rock area, from which periphyton was scraped, was within the plastic cylinder?

*clarified in the text. The plastic cylinder was used for BOM sampling, and rocks ranging from 0.006-0.04 m²) were collected separately.

l. 198: Using molecular methods (qPCR) indicates that samples should be taken under sterile conditions. Was this removal using a toothbrush sterile?

*this entire paragraph was removed because we report no relationships between algal abundance or bacterial counts and our EEA, EEM, and NDS findings

l. 259: „response was“

*fixed

l. 261: LME – abbreviation not explained

*clarified in the statistical analysis section of the methods

l. 267: „seasons“

*This sentence was deleted based on a comment by Reviewer 2

l. 277-278: Please clarify whether the presented results stem from the present study, in which case this sentence should be moved to the results section, or from an earlier study, in which case the sentence should be rephrased as something like „An earlier study showed that these streams etc. ... (Beaulieu 2014)“.

*fixed

l. 282, 368, 369, 418 and elsewhere: Past tense should be used when reporting results from the present study.

*Revised in the instances mentioned and in other instances we found

l. 296: Primary production was not presented in this manuscript; please clarify that this refers to an earlier study.

*fixed

l. 369: Please check sentence structure; better without „that“?

*deleted “that”

l. 376 and 388: These sentences contradict each other („EEA patterns do not match etc.“, „Although some EEA patterns did not conform ... others did“). Please clarify, which EEA metrics did not match DOM patterns (e.g., „Although EEA metrics related to carbon uptake ...“ or similar).

*We specified the measurements that matched and the ones that did not

l. 397: Same comment as to l. 277 and l. 296.

*fixed

l. 403-405: Since spatial DOM properties and EEAs did not match, a more cautious statement would be appropriate.

*Revised to be more cautious: “The combined approach of using EEA and EEMs provided distinct and complementary information about the characteristics of, and microbial use of, the DOM pool. While some patterns in the EEMs were not confirmed by EEA, the combined approach showed that stream burial and seasonality influence the DOM pool, and although we did not see corresponding seasonal differences in EEA, we did find spatial differences in how microbes use carbon sources in buried versus open reaches of the urban stream network.”

l. 417: C-limitation

*fixed

l. 424: P/H actually indicated higher quality DOM in buried reaches. Please clarify.

*this was a complete oversight on our part. These sentences were revised to indicate evidence that open reaches export labile DOM into buried reaches

l. 460f, 470f: This is only true if carbon is indeed the limiting nutrient, which was not assessed in this study.

*In addressing the comments we deleted the reference on 460, and added additional supporting material to the statement in line 470

l. 476: „context of“?

*fixed

Figure legends:

Figure 5: „an index“

*fixed

Figures: It would be more informative to include both reaches and seasons into the plots, even when the differences are not significant. Also, I suggest to use either boxplots or column plots.

*Reviewer #2 made similar recommendations, and we revised figures accordingly

Referee: 2

Comments to the Author

General comments:

The authors present a well-written paper exploring how different urban infrastructures and seasonality affect the pattern in DOM quality and bacterial metabolism in urban stream networks. They used fluorescence indices to assess the relative contribution of autochthonous/allochthonous sources to stream DOM, and its likely recalcitrance or lability. In addition, they used a combination of enzymatic and nutrient diffusing substrata assays to further

explore the microbial strategies of resources utilization in these urban systems. Overall, they observed that DOM in buried reaches had a higher humic character compared to their open counterparts, which was reflected by bacterial strategies of recalcitrant matter uptake. The findings further our understanding of how human impact the functioning of stream ecosystems, in this case by directly structuring the fluvial network, and thus believe that the work is worthy of publication. I have, however, a few main issues with the paper at the moment that would need to be addressed:

The paper is mainly oriented towards qualitative aspects of the DOM pool, either by measuring its chemical properties using fluorescence, or indirectly using microbial indicators. Yet, there is no explicit indication of how much carbon is potentially consumed in these streams, such that it is hard for me to appreciate the biogeochemical importance of the patterns reported here. It is my understanding that the DOC concentration was measured at the different sites along with metabolic measurements, and thus one could estimate the fraction of DOC that can be consumed over a given timeframe (e.g., % labile DOC per day) or during transit in these streams. At the moment, I am left wondering if bacterial activity is really causing the export of more recalcitrant DOM downstream in buried streams for instance (457-459).

*Given the structure of our original data, we approached this question using nutrient spiraling theory. We first took our respiration rate in the buried reaches that was calculated from diel oxygen deployments (details in Beaulieu et al. 2014) and converted it into a per m loss rate (k). Then we multiplied this value by the length of the buried reach to estimate the fractional loss of DOC in transit through the buried reaches (we focused just on the buried reaches due to no autotrophic component to confound this estimation). This estimation yielded values between 2 and 53% loss in the buried reaches, or an average of about 20% with no pattern in space or time. This amount of loss would be reasonably significant, yet we find no other confirmation with our other data. For example, DOC concentration did not vary between the top and bottom of buried reaches, and neither did DOM quality metrics from EEMs. Given the preponderance of the direct evidence that does not support our speculation that buried reaches export more recalcitrant export, we share your skepticism, and we have omitted this idea from the text.

I have a hard time agreeing with the idea of a carbon limitation of bacterial respiration in natural systems as it is not clear to me when one can conclude that carbon is not limiting. In other words, bacterial will always respire the available substrate in all systems (but their growth may be more limited), and thus in my opinion the discussion should be oriented towards if bacterial respiration is lower or higher at any given place or time or if a given system is dominated by more labile or less available molecules. I think the tone could be easily adjust throughout the manuscript to reflect this (for instance, L74, L266, L308, L451).

*We agree that adjusting the tone would be appropriate, and we revised this through the manuscript

The lack of inner-filter correction here might be problematic here if the water sampled contained any color. The problem is that most of the indices derived from the EEMs rely on the region most affected by the IFE, that is the region typical of the protein-like at low excitation and emission wavelength. In addition to underestimating the contribution of the proteinaceous material overall, you may end up being in a situation where for a similar concentration of these compounds in a sample, your concentration estimate may be quite variable depending solely of

much fluorescence is absorbed by the sample itself. Could the authors at least provide absorbance values and what would be the impact of the IFE for the samples they have measurements for (L107)?

*We have provided a range of absorbance values for the samples we measured in spring and we have responded below the impact of IFE for samples that have absorbance measurements.

Finally, the management recommendation made towards the end of the manuscript does not make a lot of sense to me: how is increasing autochthony in the system something desirable (L42-43)? I can see the point of directing efforts to restore streams towards more natural conditions, but from a microbial and C cycling point of view, in what way is this beneficial?

*we revised aspects of the conclusion to emphasize a greater need to understand if altering the sources of DOM in daylighted streams might alter the bacterial community (after Attermeyer et al. 2014)

More specific comments:

Title: I think the main focus here is on how the urban infrastructure influence DOM quality, which ultimately shape bacterial metabolism in these streams. Also, related to one of my main point above, I would remove the “carbon limitation” from the title. Thus, I think a more appropriate title could be: “Urban infrastructure influence dissolved organic matter quality and bacterial metabolism in an urban stream network”.

*changed as suggested

Introduction:

L41: I would be careful with the statement of allochthonous DOC being recalcitrant. I think a great deal has been learnt in the last decade showing that yes, as a whole terrestrial DOC can be considered relacitrant. Yet a small fraction of this pool has been shown to be composed of low molecular weight, labile compounds rapidly replenished from soils, which may in fact support most the metabolism of aquatic bacterial communities (see references below).

*we added information from the findings of Guillemette et al. 2013, and revised the language to de-emphasize “labile v recalcitrant” and to instead emphasize allochthonous v autochthonous.

L75-L77: I don’t think this hypothesis is really needed here as it is more of a tautology than a testable hypothesis i.e. one can already anticipate that adding glucose for instance will increase bacterial activity.

*We deleted the final hypothesis

Methods:

L107-109: Without the absorbance, it is hard for me to judge if the IFE is a problem here. The authors should follow the paper of Kothawala et al. 2013 L&O:Methods to calculate the percent fluorescence lost due to the IFE, and provide the reader of by how much their results are likely to vary.

*Absorbance was measured for spring samples only and we have updated the methods section with the range of absorbance values for these samples. We also added information on the percent fluorescence lost due to the IFE, which we calculated based on Kothawala et al. 2013.

Additionally, we added that when comparing metrics calculated from EEMs that were corrected with and without the IFC, we found <5% difference in EEM metrics.

L110: Given the authors can fix the IFE issue mentioned above, I would suggest performing a Parallel Factor (PARAFAC) analysis of their fluorescence data as it is a more powerful tool to deal with fluorescence data.

* We performed a PARAFAC analysis as suggested. We had to run autumn in a separate PARAFAC model because the model with all seasons could not be validated (following Stedmon and Bro 2008 L&O Methods). So even though the original EEM fluorescence intensities were similar in magnitude for all seasons, the PARAFAC results for the two different models were not comparable. We analyzed differences between spring and summer (which had 4 components) and buried and open, but we found none, and we analyzed reach scale differences in autumn (which had 3 components), and again found none. Since these results were not informative and because we couldn't directly compare spring and autumn given the lack of model validation, we do not include the PARAFAC results. Given the <5% difference in the EEM metrics with and without IFC, we must focus on the relative differences they show across seasons and between reaches.

L220-221: Cory et al. 2010 revised the original endmember values of McKnight et al. 2001, which are now around 1.5 and 1.2 for the microbial and terrestrial endmembers, respectively.

*we revised this accordingly

L138: Please define CQI and which enzymes are involved in this calculation.

*reviewer number 1 agreed, and this is now defined more clearly

Results:

L223-224: Note that this is the case regardless of IFE correction or not i.e., fluorescence only allows for relative comparison, as it is not a quantitative method. I would revise or delete the statement.

*This sentence was deleted

L244: As far as I can tell, the same results are used in the calculation of LCI and CQI, and thus the relationship found here does not provide any useful additional information.

*this result was deleted

L256-257: I guess the reason you did not find a difference is probably due to the fact that respiration and biomass are correlated here. But since you have made both measurements, why not exploring patterns in bacterial growth efficiency that could be used as another index of substrate quality?

*this comment is addressed in detail above

L266-267: This conclusion should be moved to the discussion.

*Deleted here, and revised to take into account other comments about bacterial C limitation versus bacterial response to carbon molecules of differing lability

L270-273: This data should be shown as a new figure.

*We added this figure

Discussion:

L277-278: Figure 8 should be removed from the paper as it does not bring anything new. Please cite the Beaulieu et al. 2014 paper instead here.

*removed

L279: The McKnight et al. paper does not tell anything about substrate quality, it is a method paper developing the fluorescence index.

*we revised this sentence to instead emphasize the changing seasonal nature of the DOM pool

L323-346: I do not think low HIX in the spring and fall and in the open reaches is that counter-intuitive. The HIX index was developed in the soil literature and is often an indicator of soil-derived OM inputs in aquatic systems, and to me it is likely that soil OM, not only leaf inputs, may drive the pattern in HIX. I do not have the hydrological data to judge this, but to me spring freshet and fall storms may well bring soil OM with high HIX values (see Marin-Spiotta et al. 2014. Biogeochemistry. 117:279–297).

*we revised this section to include the possibility that autumn rains flushed soil OM, which is consistent with increases in the buried and open reaches during that season.

L329-331: Not clear how Fig. 8 shows that leaf can generate recalcitrant DOM. Also, this statement is in contradiction with a recent showing the opposite (Attermeyer et al. 2013. Ecology. 94:2754-2766), and actually with the authors' own statement that CBOM leaches labile DOM (L371). So which one is it?

*Figure 8 has been removed. This sentence was revised with evidence from more recent studies that support storm flow as a potential driver of higher HIX as well as a laboratory study that showed microbial degradation of leaf leachate increases humic compounds

L430-431: As I pointed above, calculating bacterial growth efficiency could support this idea.

*this comment is addressed in detail above

L476-477: Please revised synthaxe

*revised

References:

I noted that besides the papers authored by the investigators of this study, most of the cited references are prior to 2010 and I would encourage the authors to search for more recent literature. For instance, see papers from the groups of M. Xenopoulos and D. Butman, and the fluorescence reviews of Hudson et al. 2007. River Research and Applications and Fellman et al. 2010. Limnology and Oceanography. Also, A. Attermeyer, M. Berggren and F. Guillemette also did quite some work regarding the bacterial processing of DOM from different sources, which could help supporting some of the claims made by the authors in the Introduction and Discussion.

*Based on this suggestion, we searched for some more recent work and the following new references were most applicable to our work: Attermeyer et al. 2013, Attermeyer et al. 2014, Guillemette et al. 2013, Hur et al. 2009, Marin-Spiotta et al. 2014, and Singh et al. 2014.

Figures need significant improvement:

Please reorder seasons as spring, summer, and fall.

*our original order was in the order we sampled, but we revised as suggested

Please show the open vs. buried data even if no significant differences are found so the reader can appreciate when a difference exists (which should be marked by an asterix).

*these figures have been updated

Figure 1, 2, and 3 should be merged together as a 4-panel figure and displayed according to Fig. 1 (displayed as Fig. 8 could also be a good option).

*We revised figures as suggested and converted all to box plot

Similarly, it would be easier to see how the raw enzymatic data shown in Fig. 4 and 6 vary across seasons and between infrastructure types if they were presented in the same figure.

*Revised as suggested

Fig. 8 can be removed from the paper as this data as already been presented in another paper.

*This figure was removed and we cited the prior paper in the text