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Sent: Monday, April 24, 2017 5:04 PM

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Subject: Freshwater Biology - Decision on Manuscript ID FWB-P-Feb-17-0064

24-Apr-2017

Dear Dr. Arango

Your manuscript ID FWB-P-Feb-17-0064 entitled "Carbon limitation of heterotrophic respiration is linked to dissolved organic matter quality in urban streams" has now been assessed by the reviewers, whose reports are included at the bottom of this letter.

While the reviewers see strengths in your paper, they made substantial criticisms and suggestions for improvement. Both reviewers call into question your conclusions about widespread carbon limitation in the studied system, but also raise a number of concerns aboiut methodology and interpretation of data. However, I do think it should be possible for you to address this and therefore invite you to revise your paper in light of the comments made. I will then return your paper to the referees for a second review before making a final decision.

Please return your revised paper within the next 8 weeks. Instructions for using Manuscript Central are given below. When submitting your revised manuscript, you will be provided with a space to respond to the reviewers' comments. Please use this space to document the changes you have made to your original manuscript.

To revise your manuscript, log into <https://mc.manuscriptcentral.com/fwb> and enter your Author Centre, where you will find your manuscript title listed under "Manuscripts with Decisions." Under "Actions," click on "Create a Revision." Your manuscript number has been appended to denote a revision.

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IMPORTANT: Your original files are available to you when you upload your revised manuscript. Please delete any redundant files before completing the submission.

I look forward to receiving the revised paper soon.

Yours sincerely,

Dr. Stefan Bertilsson

Associate Editor, Freshwater Biology

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.

- 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.

- 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.

Specific comments:

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

l. 32: „standing stocks exhibit“

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)?

l. 104: „wavelengths“

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 dihydroxyphenlyanaline“ –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.

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.

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

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

l. 162: Should this be „PVC tubes“?

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?

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

l. 259: „response was“

l. 261: LME – abbreviation not explained

l. 267: „seasons“

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)“.

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

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

l. 369: Please check sentence structure; better without „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).

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

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

l. 417: C-limitation

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

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

l. 476: „context of“?

Figure legends:

Figure 5: „an index“

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.

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 it 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).

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).

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)?

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?

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”.

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).

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.

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.

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.

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.

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

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.

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.

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?

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

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

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

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).

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?

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

L476-477: Please revised synthaxe

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.

Figures need significant improvement:

Please reorder seasons as spring, summer, and fall.

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).

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).

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.

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