Interactive comment on “New insights on resource stoichiometry: assessing availability of carbon, nitrogen and phosphorus to bacterioplankton” by Ana Soares et al.

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Response to Referee 1

We thank Referee 1 for constructive and relevant comments to the manuscript and for helping us to improve it. We addressed all comments below.

General comment Referee 1:” The manuscript by Soares and others is a novel and important contribution to this topic. In particular, their innovative experimental approach offers an answer to the question: what resource stoichiometry to bacteria actually experience in situ, given that not all measurable forms are bio-available? The work was thoughtfully designed and executed and will be of interest to the readership of Biogeosciences.
Two areas require attention from the authors. First, the conclusion that C is limiting is not adequately supported by the manuscript in its present form (see below). Second, the uncertainties in bioavailable concentrations must be made more clear. Aside from these two areas, the paper is strong and the other comments are minor/clarification.”

Referee comment 1:” Page 1 Line 24. What is the evidence for this in the present study? Although the resource stoichiometry derived from their results suggests that C will likely be limiting before N or P, this does not automatically mean that C is limiting. That extension of resource stoichiometry is applicable only if 1) the bacteria are resource-limited and not under top-down control; 2) the only potentially limiting resources are C, N, or P; and 3) the system is presumed to be at steady state resembling a chemostat.”

Authors’ comment: We agree with the Referee and acknowledge that we do not present direct evidence showing C limitation. We have therefore reformulated all sentences in this regard, clarifying that C was the least bioavailable element (in % bioavailability) out of the three key macronutrients that we work with. We also have now made clear that our bioavailability estimates are informative of maximum potential bioavailability under specific conditions, i.e. when all other macro- and micronutrients of relevance are in excess. Thus, while we can state that access to bioavailable C in our samples tended to be scarce relative to the microbial need and access to N and P, the apparent C limitation is not directly transferrable to natural systems, especially not when considering the dynamic nature of natural ecosystems and the potential presence of top-down controls and/or micronutrient limitation.

Referee comment 2: Page 3 Line 8. While the long incubations have their shortcomings, it is overstated and confusing to say that these are not 'ecologically relevant timescales'. Certainly the majority of the consumption and respiration in fresh DOM happen in a matter of hours to days. However, longer-term degradation rates of more recalcitrant forms are of key importance. Specific to this study, the rapid rates of consumption observed are due to the high concentrations of CNP added and thus, the timescale of the experiment is not ecologically relevant. I suggest that the authors focus this section and justification on the multi-element aspect of their design, which is the important and novel part.

Authors' comment: We agree with the Referee on the ecological importance of long-term degradation of more recalcitrant DOM, particularly in systems with long water residence times. However, resource bioavailability measured with long-term incubations does not reflect readily bioavailable pool sizes that control bacterial metabolism at any given moment. Moreover, during long incubation periods various factors can interfere with the uptake of bioavailable resources. For example, the dynamics of viruses and the development of toxic conditions that can appear from repeated bacterial regeneration of resources can interfere in long-term measurements (Cho et al., 1996). By using our seven-day approach and by maximizing bacterial metabolism, we reduce the incubation length to a minimum and sufficient time period during which bacteria take up most of the readily bioavailable pool (Fig. 1). Our estimates can be used to understand the potential C, N and P bioavailability, as they are performed during "ecologically relevant timescales" in this regard. In our revised manuscript we clarify that the relevance refers to how meaningful the measurements are for understanding the direct controls of bioavailable nutrient pools on short-term metabolism– not the controls the nutrient pools may have months ahead in time.

Referee comment 3: Page 3 Line 30. The third question seems certain to be true, and thus not informative as a question or hypothesis. Yet, quantifying this mismatch is important, so I suggest that the authors rewrite these questions.

Authors' comment: The third question was changed to "By how much do total C:N, C:P and N:P ratios exceed bioavailable C:N, C:P and N:P ratios".

Referee comment 4: Page 4 Line 10. By sampling the rivers at their outlet, much of the bioavailable forms have presumably been consumed in transit. What is the rationale for sampling far downstream from the sources of DOM?

Authors' comment: Our goal was to capture bioavailability patterns across a landscape gradient with different boreal freshwater properties (see first manuscript version page 3 lines 31-32) and not to determine the amount of bioavailable element coming from terrestrial soils. Nonetheless, in the revised manuscript we will clarify that Swedish river systems generally represent substantial water renewal along the watercourse pathways from the Scandes in the west toward the Baltic Sea in the East. In fact it has been suggested that water renewal in running waters offsets the loss of DOC in Swedish

lakes such that rivers in Sweden generally does not have much older DOC than lakes (Muller et al., 2013).

Referee comment 5: Page 5 Line 2. This standardized inoculum has important implications for interpreting the results. Elaborate on why this single community was used as opposed to the communities present in the source water.

Authors' comment: **We wanted to ensure that differences in bacterial community composition did not influence our estimates of resource bioavailability (Martinez et al., 1996). This was achieved by using a standard bacterial community in all our assays. We have now explicitly motivated the use of a single bacterial assemblage as inoculum in the manuscript. By using a pooled inoculum representing both headwater inlet and lake water from four different lakes with different properties, we ensured a high diversity of the microbes used to inoculate.**

Referee comment 6: "Page 5 Line 15-30. This experimental approach is rather involved. If space allows, the authors should include a schematic diagram that shows how they forced limitation by CNP and measured the response to addition of the limiting resource. Presumably this method is based on the Wright-Hobbie technique and thus it is important to show how the estimates of ambient concentrations were derived."

Authors' comment: We agree that it is important to include a schematic diagram to help to better visualize our approach. We have added a schematic diagram of the method to the supplementary material in the revised manuscript.

Referee comment 7: "Page 5 Line 30. "The total amount of bioavailable nutrient taken up" is not precise. Especially for C, the nutrient need not be assimilated in order for the bacteria to exhibit a growth response."

Authors' comment: It is true that the leucine incorporation itself reflects growth – not respiration. However, the way our method is designed, the total leucine incorporation is recalculated into absolute units of bioavailable carbon with help from the standard curves. This works as we have the same consistent slope of the standard curves in all lakes and across all C spike levels, implying that our experiment creates conditions with fixed (maximized) growth efficiency. Thus, we think that our sentence is well formulated. We used leucine incorporation as an experimental response variable of all bioavailable element uptake, which in the case of C can be used either for growth or respiration.

Referee comment 8: "Page 6 Line 15. The use of complementary validation methods is an important strength of this paper. Well done."
Author's comment: Thank you for pointing this out.

Referee comment 9: “Page 6 Line 32. This method of calculating cellular N content is strange. What are the assumptions of this method? At the least it assumes that all of the added N is assimilated and that no other N is used.”

Authors’ comment: This method encompasses several assumptions: 1) bacterial growth in the bioassays was effectively limited by N, 2) different N compounds yield similar bacterial biomass increases, 3) all bioavailable N was assimilated when bacterial growth ceased and 4) N bioavailability was independent from the bacterial inocula. The paper from Stepanauskas et al. (1999) describes in detail the experimental setup and the method’s assumptions.

Referee comment 10: “Page 7 Line 5. The validation method used for P availability is more straightforward than for N. Why not use this method for N also? Additionally, were these fitler-P measurements corrected/checked for phosphate binding to the filter?”

Authors’ comment: It is not possible with any standard instrumentation to directly measure changes in absolute concentrations of bioavailable N (and C) with the same analytical precision as routine methods used to determine P (molybdenum blue method, microgram accuracy). In the revised manuscript we add a short section describing limitations and possibilities offered by different bioavailability determination methods based on Berggren et al. (2015).

Estimates of P bioavailability were corrected for potential P filter content, binding of dissolved P species, and abiotic formation of particles (Jansson et al., 2012).

Referee comment 11: “Page 7 Line 30. Needs clarification. No difference between slopes for C, N, and P or among lakes? Also, it is unclear why the regressions were performed individually for each analytical replicate instead of using all of the analytical replicates for a given site/date. From what I can tell, the standard curves were computed individually for each of five analytical replicates and then the standard deviation of their estimates is presented in table 2?”

Authors’ comment: We have changed the sentence on page 7 line 30 from the previous manuscript to: “Since there were no statistical differences between the slopes among lakes for each resource (ANOVA, $p > 0.44$, $n = 20$), slopes were averaged for each resource nutrient across lakes.”

We first performed the regressions individually (Figure 2), because we wanted to test whether there were differences in the bacterial response to nutrient additions between the different lakes. Since we found no statistically significant differences between lake slopes (this is mentioned on page 7 line 29 and page 12 lines 13-14), we combined all data points and performed a new regression for each element based the entire dataset. This rendered the “mean slope” given on Figure 2 (C slope=784 nmol L^{-1} per µg C L^{-1}, N=slope 2667 µg N L^{-1}, P slope=67575 µg P L^{-1}).

In table 2, the mean slope of the standard curves was used to translate amounts five replicate measurements of leucine uptake. The standard deviation of the estimates is given within brackets.

Referee comment 12: “Page 9 Line 20. Were the total and bioavailable concentrations (or elemental ratios) positively correlated?”

Authors’ comment: No, there was no correlation between the total and bioavailable concentrations. This will be clarified in the revision.

Referee comment 13: “Page 9 Line 23. Again, what is the evidence that C was most limiting, or even limiting at all? The traditional lines of evidence for this (single nutrient bioassays) are not presented, so this is either inferred from the stoichiometry estimated for resources or from the low proportional bioavailability of C compared to N and P. Neither of these shows that C was the strongest limiting factor. Pease elaborate on this and explain 1) the assumptions used for this claim and 2) the specific evidence from this study”

Authors’ comment: We agree with the Referee that we do not have the evidence needed to claim that C is limiting in boreal waters (see answer to Referee comment 1). We have changed the sentence from page 9 line 21 from the previous manuscript version “Surprisingly, in these systems where absolute surface water DOC concentrations are large, C bioavailability was low and was the strongest limiting factor for heterotrophic aquatic production.” to “In these systems where absolute surface water DOC concentrations are large, relative C bioavailability (%) was the lowest, relative to that of N and P.”

Referee comment 14: Page 10 Line 33. There are many other factors related to seasonality that could explain this (light, plant production, hydrology, etc), so how can you conclude that soil microbial activity is the predominant driver? Overall, I found this discussion of seasonality too speculative

Authors’ comment: We agree that important role of other seasonal factors for the amount of bioavailable dissolved organic carbon measured in our study. We have now removed the sentences from the previous manuscript page 10 line 30 to page 11 line 1.

Referee comment 15: Page 11 Line 27. These calculations seem to be the core of the argument that C is limiting and thus require elaboration. Even then, this only shows that C is more likely to be limiting than N or P, but does not show that C was in fact limiting at ambient concentrations.

Moreover, the ranges here are so large that they are not really meaningful. Why not use the ratio of slopes presented in figure 2 to estimate the relative consumption rates of CNP? In your calculations, you already assume that the ratio of leucine:cell is invariant, so the ratio of 1/C-slope to 1/P-slope (=86) is the ratio of C consumption to P consumption when those elements are limiting. No?

In both the lakes and the rivers, the DOM pools have already undergone much degradation by bacteria, light, and reactive oxygen. This needs to be acknowledged, or better yet, discussed in some detail.

Authors' comment: We agree with the Referee. We have thus, reformulated our conclusion and all statements related to C limitation (please see also answers to Referee comment 1 and 13).

We decided to exclude the calculations in question from the manuscript. The reviewer's suggestion of assuming a ratio for the C consumption in relation to P consumption base on Figure 2 is interesting, but it would not work to translate the uptake ratios of the experiment to nature as our methodological approach based on inducing strong limitation of each element is boosting the nutrient use efficiency to max (Jansson et al., 2008). In nature, the nutrient use efficiency (including C use efficiency = BGE) is highly variable and probably much lower than in our experiment. We therefore rather prefer to remove the discussion of C limitation of BP.

We partly agree with the Referee regarding the loss of most of the riverine bioavailable pool, but see our reply to point 4 above. However as we targeted the medium-term bioavailable resource pool, we do not consider this to be a problem. Nonetheless, the discussion of our revised manuscript will expand on the subject of how the river system differ in nature compared to the lakes, and what the implications of that are in the context of macronutrient biovailability.

Referee comment 16: "Page 13, line 1. Avoiding these uncertainties is important, but those are typically on the order of a few percent and can be constrained by experimental validation. Without a robust analysis of the resulting uncertainties from the present approach, it is not possible to discern which method is advantageous. Form Table 2 and Figure 1/2, it appears that the uncertainty in concentration estimated for a single date/site is large. Without such an analysis of the uncertainty in the final estimates, I suggest that the authors focus on the multi-element aspects of their study"

Authors' comment: As suggested we will focus our discussion on the multi-element aspect of our study. Thus, we have removed from the previous manuscript lines 1 to 5 page 13.

Referee comment 17: "Figure 4. What do the diamonds represent in this figure?"

Authors' comment: The diamonds represent average resource ratio values for the lakes for all dates (n=26). We have added to Figure's 4 caption the following sentence: "Data shown as boxplots and includes mean as diamonds.

Referee comment 18: “Figure 5. The vertical axis scale should be fitted to the range of data presented.”

Authors’ comment: Vertical axis scale has been changed from 1 to 100000 to 100 to 100000.

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