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Biogeosciences Discussions

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

Anonymous Referee #1

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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.

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.

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.

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.

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?

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.

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.

Page 5 Line 30. This approach requires high confidence in the regressions used. Uncertainty in the slope and intercept for the standard curves can be propagated to estimate the uncertainty in the ambient concentrations that are estimated. The authors should include such calculations of uncertainty for at least a few representative samples (perhaps as a supplement if space is limiting).

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.

Page 6 Line 15. The use of complementary validation methods is an important strength of this paper. Well done.

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.

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?

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?

Page 9 Line 20. Were the total and bioavailable concentrations (or elemental ratios) positively correlated?

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

Page 10 Line 33. There are many other factors related to seasonality that could eplain 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

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 limitng. 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.

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

Figure 4. What do the diamonds represent in this figure?

Figure 5. The vertical axis scale should be fitted to the range of data presented.

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