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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We thank referee 2 for constructive and relevant comments and suggestions of technical corrections, which helped us to improve the manuscript. Please find our response below.

General Comments
This manuscript presents the results from a test of a new method of determining the relative bioavailability of carbon, nitrogen and phosphorus for lake and riverine bacterioplankton.The technique, which combines radiolabeled leucine incubations with reciprocal nutrient amendments, is a novel approach to backing out the proportion of total dissolved C, N and P that bacteria can rapidly take up if other factors are not limiting. The authors test the approach with seasonal samples from four Swedish lakes and single-date samples from seven rivers. Overall, the authors provide a very interesting analysis and the paper is in good shape. Please see below for my specific and technical comments. The only general comment that I would make is that the approach explicitly considers bioavailability in the absence of any co-limitation. In other words, the method cannot incorporate any interactions between limiting factors. While this may be a necessary shortfall of the approach, its significance perhaps deserves some thought and maybe some treatment in the discussion.

Authors’ comment to the general comment: The Referee is correct. Our method determines the maximum pool sizes of readily bioavailable macronutrient fractions that can be used given that all other nutrients are provided in access. In the revised manuscript version we clarify that our bioavailability estimates are defined under these specific operational conditions. We also explain that, in order to translate the implications of the results to natural systems, factors like nutrient co-limitation and potential limitation by micronutrients or even top-down controls (e.g., grazing as pointed out by Reviewer 1) need to be taken into account.

Specific Comments

Referee comment: 1) “Page 1, line 17-18: Make sure the readers know that these percentages are based on the initial concentrations. I know that this might sound obvious, but I was initially confused about whether these were percentages of final (post-incubation) or initial (pre-incubation) amounts.”

Authors’ comment: We thank the Reviewer for pointing this out. This has been clarified in the new manuscript version.

Referee comment: 2) “Page 5, line 2: Where exactly was the inoculum sampled? And how could it have been sampled only once, given that the lake and river samples were collected over a lengthy period and the incubations run soon after each sample collection? Was it maintained in the laboratory?”

Authors’ comment: The inoculum consisted of a mixture of water from both the epilimnion and inlet of the lakes. By including communities from several different sampling sites, we ensured a large microbial diversity on the inoculum. The inoculum was maintained in the fridge at approximately 4 ˚C. Because our experiment strongly maximized bacterial metabolism (selecting for fast-growing opportunistic bacteria), we do not think that the inoculum played a large role on the outcome of our experiment. Previous studies have further demonstrated bacterial growth to be independent of bacteria inocula (Tranvik and Hofle, 1987).

Referee comment: 3) Page 5, line 8: Could there be an effect of incubating bacterioplankton in such a small volume of water? Could biofilms on the walls of these small vials start to have a disproportionate impact on the results?

Authors’ comment: We did not test or control for the potential development of biofilms in the tubes walls. However, based on the results for phosphorus presented in Figure 5, we can compare our measurement of the amount of leucine incorporation (normalized per unit of bioavailable P; filled square) with corresponding data extracted from Jansson et al. (2012; the box plot). In the latter case, Jansson et al. did not involve incubations in Eppendorf tubes but in much larger (700+ ml) volumes. There was an overlap in magnitude of leucine incorporation when comparing these two data sources, but it can be noted that our measurements are in the upper range compared to those from Jansson et al. (2012). Biofilm accumulation could have potentially contributed to this difference in our incubation tubes. However, when looking at the time series of our incubations (Figure 1), it is clear that most of the leucine incorporation in our case happened already within 3 days, which should be a time-frame too short for substantial biofilm formation. Thus, we do not consider that biofilms strongly influenced our results.

Referee comment: 4) Page 5, line 16: Maybe I’m missing something, but why didn’t the controls consist of lake water without any added C, N or P?

Authors’ comment: Since our design is based on the idea of inducing strong limitation of the nutrient to be evaluated for maximum potential bioavailability, we did not consider relevant to incubate lake water without any nutrient additions. On the lines that the Reviewer refers to, we tested whether the inoculum or L16 added any bioavailable C, N and P to our assays. By using Mili-Q water instead of lake water, we made sure the inoculum and L16 were the only possible sources of limiting resource in our bioassays. At the same time, this also tested that leucine incorporation (or bacterial growth) was in fact controlled by the induced limiting resource and that no bacterial growth occurred in the absence of the bioavailable limiting resource (see previous manuscript version page 12 lines 16-18).

Referee comment: 5) Page 5, line 24: Presumably these standard curves would be system-specific? Or at least limited to similar environments within a region? Some discussion of should perhaps be added to the discussion.

Authors' comment: We did not find significant differences among standard curves for the different lakes (page 12 line 13 previous manuscript version), which is interesting since the lakes represent gradients in DOC and catchment features representative to a range of boreal conditions. Possibly, corresponding standard curves from the rivers could have been different from those in the lakes, but it would have been a major time-consuming effort to determine those curves for all of the rivers. Therefore, results from the rivers should be interpreted with caution, even if the rivers do not represent fundamentally different chemical conditions. We added a short section on the subject to the discussion part in the new manuscript.

Referee comment: 6) Page 6, line 24 to page 7, line 8: It sounds like these methods assume no changes in cellular stoichiometry with nutrient availability (i.e. elemental homeostasis).

Authors' comment: Yes the Reviewer is correct; an invariant cellular stoichiometry was assumed in the validation method used to calculate N bioavailability. This, as well as other method assumptions, has been scrutinized in Stepanauskas et al. (1999).

However, there were no assumptions regarding cellular stoichiometry for the method used to calculate P bioavailability since this was based on direct measurements of the content of P in bacterial growth cultures harvested from filters.

Referee comment: "7) Page 11, line 5: Is this consistent with turnover rates of these elements in these ecosystems?"

Authors' comment: We do not know of studies looking at the turnover rates of these elements in the soils in the study area. We have deleted the sentence in question and replaced it with a clearer sentence that brings into attention the main mechanism suggested by Jansson et al. (2012), i.e., the apparent temperature-dependence of mobilization of bioavailable P from soils.

Referee comment: 8) Page 11, line 23: Perhaps mention threshold elemental ratios here, as well as the work that has focused on them in bacteria (Sinsabaugh, Chrzanowski, etc).

Authors' comment: We have accepted the suggestions from Referee one (see Referee comment 1, 13, 15) and removed the discussion on threshold element ratios and inferences on C limitation. As the section in question has been deleted, we did not include these references.

Referee technical corrections: 9 – 35)

Authors' comment: We thank the Referee for technical corrections. All have been addressed in the new manuscript version, apart from the technical corrections in sentences that have been removed from the manuscript.

36) Fig. 1: Why are the data points from the different treatments not differentiated here?

Authors' comment: The majority of the data points overlap, differentiating these points would make the figure more complex and not necessarily more informative.

References:

Jansson, M., Berggren, M., Laudon, H., and Jonsson, A.: Bioavailable phosphorus in humic headwater streams in boreal Sweden, Limnology and Oceanography, 57, 1161-1170, 2012.

Stepanauskas, R., Leonardson, L., and Tranvik, L. J.: Bioavailability of wetland-derived DON to freshwater and marine bacterioplankton, Limnology and Oceanography, 44, 1477-1485, 1999.

Tranvik, L. J., and Hofle, M. G.: Bacterial growth in mixed cultures on dissolved organic carbon from humic and clear waters, Applied and Environmental Microbiology, 53, 482-488, 1987.