

## ***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 #2**

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#### 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

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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.

### Specific Comments

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.

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?

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?

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?

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.

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).

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

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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).

#### Technical Corrections

- 1) Page 1, line 14: “. . .purpose of exhausting the pools. . .”
- 2) Page 1, line 16: “base-flow”
- 3) Page 2, line 20: Delete one of these extraneous uses of “on”
- 4) Page 2, line 26: Why “re-growth”? Wouldn’t it be simpler just to call these “growth assays”?
- 5) Page 3, line 11: Delete “single”
- 6) Page 3, line 18: “. . .purpose of rapidly exhausting the pools. . .”
- 7) Page 3, line 23: “N-starved”
- 8) Page 3, line 28: Replace “shares” with “proportions”
- 9) Page 3, line 34: Replace “compromises” with “comprises”
- 10) Page 4, line 16: It says “runoff” here, but the units in the next line suggest that the authors mean discharge.
- 11) Page 4, line 30: “climate-controlled”
- 12) Page 4, line 32: “1000-ml”
- 13) Page 5, line 2: There’s an extra “the” in this sentence. It’s also not very clear (see my comment in Specific Comments above).
- 14) Page 5, line 6: “N-limiting”
- 15) Page 5, line 7: “P-limiting”
- 16) Page 5, line 19: “C-limiting”

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- 17) Page 6, line 10: “seven-day”
- 18) Page 6, line 20: “sensor spots”
- 19) Page 6, line 25: “N-starved”
- 20) Page 7, line 1: “N-amended”
- 21) Page 7, line 23: “pools”
- 22) Page 8, lines 1 and 7: “tests”
- 23) Page 9, line 6: “. . .were validated. . .”
- 24) Page 9, line 16: Replace “neither” with “nor”
- 25) Page 9, line 19: “. . .as a driver of. . .”
- 26) Page 10, line 10: Delete “also”
- 27) Page 10, line 12: “single-element”
- 28) Page 12, line 6: Replace “Whereas” with “However”
- 29) Page 12, line 7: “. . .similar to the lake. . .”
- 30) Page 12, line 19: “. . .method as a proxy. . .”
- 31) Page 12, line 23: Replace “media” with “medium”
- 32) Page 12, line 27: “synthesized”
- 33) Page 12, line 28: “bioavailability”
- 34) Page 13, line 5: Replace “media” with “medium”
- 35) Page 13, line 7: Delete comma
- 36) Fig. 1: Why are the data points from the different treatments not differentiated here?

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