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Interactive comment on "Nitrogen and phosphorus recycling mediated by copepods in Western Tropical South Pacific" *by* Valentina Valdés et al.

Anonymous Referee #2

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The authors present a study that simultaneously examines the role of zooplankton and bacteria on nitrogen and phosphorus cycling in low-nutrient systems. Few studies have tried to assess these factors simultaneously to link the two processes. Although the authors provide a lot of interesting results, I am not convinced that their data back up the larger assertions of the role of copepod excretory products play in spurring growth of microbes. Also, I am not convinced that in situ conditions were accurately enough recreated to directly apply these results to the in situ setting.

More specifically:

1) It is challenging to know how much N and P is being utilized by the bacteria (and in

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what forms) when you don't have baseline data for N and P excretion rates for copepods in the absence of microbes. Without this information, you are unable to say the actual impact either party (copepods or microbes) is having on buildup or drawdown of different N or P compounds.

2) In many cases, there were not significant or clear differences between the controls and copepod treatments. For instance, the bacterial community structure for LD A and LD B stations grouped more by time point than by treatment. That implies an effect of the incubation itself, and not due to extra nutrients brought on zooplankton excretion. Also, in 2 out of the 3 experiments, it appears that bacterial abundance was higher in the controls at the end of the experiment. If they were using the nutrients to grow, we would expect to see significantly higher abundances in the copepod bottles.

3) Copepod densities in the bottles were much higher than in situ conditions. While high densities of zooplankton are definitely necessary for detectable changes in excretory products, this also meant that the change in N and P facilitated by zooplankton is likely to be much larger within this experiment than what bacteria experience in the field.

4) When looking at the dendrogram results in Figure 8, it appears that the DNA samples taken from the in situ environment show a very different community structure when compared to the communities that were present in the incubation bottles at time T0. If the community structure in the bottles at the beginning of the experiment isn't representative of the local community, the changes observed might not reflect what would occur in situ.

5) I am concerned that there are significant differences between copepod and control treatments at T0. The methods say that the organisms were placed in new incubation water at the beginning of the excretion step. I assume initial samples were taken immediately. While the authors attempt to explain the differences seen in DON (p. 11, line 26), there is no information addressing the differences in bacterial community structure at T0 for several of the experiments.

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Additionally, the entire document needs to be reviewed for use of proper English grammar. Most of the issues are minor, but do make the paper a bit harder to read and understand. I will point out several below in my comments, but there are certainly more within the document.

Specific Comments:

Introduction:

*P. 2, line 7-8: Citation needed for sentence about thermal stratification and global warming decreasing the nutrient supply

*P. 2, lines 19-23. There has been a lot of work on zooplankton excretion and the various products. A more comprehensive summary should be provided about N cycling of body nitrogen and support for phytoplankton needs in oceanic environments.

*P. 3, line 1: “enhance” should be “enhances.”

Methods:

*P. 3. Second sentence of section 2.1: Should read “The transect began west of New Caledonia and ended near Tahiti.”

*P. 3, section 2.1: You should include latitude/longitudes for your LD stations. Also, you should include a map of the transect and your specific sites used for the LD experiments.

*P. 3, line 26: it says that you used a CTD rosette to collect water. Further information on hydrographic parameters should be included. Especially on chlorophyll concentrations within the DCM.

*P. 4, line 7: was the incubation seawater also collected from the DCM? I assume so, but that is not clearly stated.

*P. 4, line 8: isn’t the acclimatizing step number 1?

*P. 4, line 8-9: If you were using the filtered seawater, it should read “seawater (22.5 L) was immediately filtered through a 0.7 um filter. . ..”

*P. 4, line 10: 25C doesn’t really seem cold. Perhaps “temperature controlled room” is a better term than “cold room?”

*P. 4, line 18: by controlled temperature, is that also in the 25 degrees C room? You should specifically say the temperature.

*P. 4, line 21: what 6 bottles were added? Were they all controls? You need to explain this furtherâĂŤit is confusing as currently written.

*P. 4, line 24: should be “to ensure” instead of “of ensuring”

*P. 4, line 26: suggest specifying “During the excretion phase. . .”

*P. 5, line 12: I am not familiar with the wet oxidation method, so I looked up the paper you cited. It looks that paper assesses PON and POP. What modifications need to be made to look at dissolved materials?

*Sections 2.5-2.6: My expertise is in zooplankton physiology, so I cannot assess the suitability of the genetics and bacterial methods.

*P. 5, line 22: “u sing” should be “using”

Results:

*Generally speaking, the authors did a good job displaying a very complicated data set. However, there are some places where the clarity of the results presentation can be improved with some minor modifications. I provide some of those suggestions below for Table S2 and Figures 2-5 and 8.

*P. 7, line 21: If these are ANOVA results, you should say so.

*P. 7, line 24: “notorious” is not a correct term to use. “Notable” instead?

*P. 7, line 30: I don’t understand why there is a difference at time T0. Is there a gap

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between when you started the experiment and took your initial sample? If that is the case, you need to specifically mention that in the methods.

*P.7 , line 30-31: Your final sentence appears to contradict itself. If there were non-significant differences, wouldn't the p-value need to be >0.05?

*P. 8, line 1-2: Consider re-wording for clarity. Specifically, all of the statistical test results should be together within parentheses.

*P. 8, line 6: missing a "(" before the "LD B. . ."

*P. 8, line 7: "trough" should be "through"

*P. 8, line 8: "estimated" does not make sense here. Perhaps "significant differences were only observed between. . ."

*P 8, second paragraph:

(1) You should be very specific here about which N:P ratio you are discussing at any given time. It is confusing at the beginning when you define it only using nitrate/nitrite and phosphate, but then discuss inorganic and organic ratios.

(2) Would you expect DON and DOP to be comparable to Redfield? Urea (one of the more common forms of DON), actually has 2 N per molecule, so if it is molar ratios that you are using, then it is not a 1:1 comparison that could be made between DON and DOP. Are there other papers that have using the organic N:P ratio? If so, it would be really helpful to bring those in to support your use and interpretation of this ratio.

(3) Line 18: you say that the DON:DOP is close to Redfield. But, the numbers that you mention in the prior sentences (and those in figure 4) are much higher than Redfield.

(4) Line 20-21: The deviation from Redfield appears to be that LD C is much higher, not lower.

(5) Line 21: should be "ratio" not "ration"

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*P. 8, line 23: should be “shown” not “showed”

*P. 8, line 24: “plankton” is a plural term, so it should be “bacterioplankton were more abundant. . .”

*P. 8, line 30: is “and between time” referring to a different time than the sampling times you mention earlier in the sentence?

*P. 9 , line 14: On the other hand makes it sound like this is contradictory result. The two genetic markers seem to be showing similar trends. "Additionally" might be a better way to begin this thought.

*P. 9, line 27: if LD A and LD B show variability associated with time points, can you draw conclusions then based on presence of copepods? Or is this an artifact of the culturing conditions?

*P. 10, line 2: do you mean “larger” instead of “longer?”

*P. 10, line 12: “show” instead of “showed”

Discussion:

*Section 4.0: Can you say the MA region had higher nutrient concentrations when LD B had the lowest of all of your sites?

*P. 10, line 22: “region” should be “regions”

*P. 10, line 27: You said there was significant increase in DON. But this was only during one time point. The other one is during timepoint T0, which seem suspicious.

*P. 10, line 31: do you mean “increase” instead of “increment?”

*P. 10, line 31: is 22,8x10ˆ3 correct? Should it be 228 or 22.8? The formatting is a bit odd and makes me think it is a typo.

*P. 10, line 32: You suggest DON assimilated by growing bacteria. But it is mostly in the control of LD A that the bacterial abundances increase between 1-2 hours.

*P. 11, line 10: But in LD B, you said no significant differences were observed over time (although your reported stats results don't match that - p 7, line 30)

*P. 11, line 26: How long after adding the copepods did you take your initial measurements? Could it really happen that fast? You need some more specific information to back up this as a reasonable assertion.

*P. 11, line 27: "crustaceans" instead of "crustacean"

*P. 12, line 10: Is this supposed to be phosphate or DOP? All the rest of the P references in this paragraph are to DOP, so I wanted to double-check.

*Section 4.2, first paragraph:

(1) But it didn't seem like bacterial abundance was increased within the copepod bottles for most of the timepoints.

(2) You said in your bacterial community results that LD B experiments grouped by time primarily, not by treatment.

*P. 13, line 18: "diminished" instead of "diminish"

*P. 13, line 28: Define "substantial amounts." You need to be able to better quantify this. I don't really think this statement is firmly backed up by the data presented

*P. 13, lines 32-34: It didn't really seem like you saw bacterial growth overall. This statement is not directly supported by your results.

Tables and Figures:

*Table 1: Redefine what this N:P ratio includes in the table caption.

*Figure 1:

(1) You mention "in situ temperature" a few times. What is that temperature?

(2) What does "*" mean after the 0.25 sampling point?

(3) In the caption, it says the feeding phase lasts for 3 hours. But other places in the document it states 4 hours.

(4) Specify in the caption that each of the 15 bottles contains 10 fed copepods.

*Table S2: You should include the overall ANOVA results as part of Table S2. It would be much easier for the reader if we could find all of those statistical results in one place. Perhaps on the gray lines?

*Figures 2-5: The data are really complicated and results are hard to grasp between the text (where ANOVA are reported), table S2 (where post-hoc data are reported), and graphs (where visual trends are reported). Can you find a way to mark statistically significant differences on these graphs? Then the reader can go to these for a summary.

*Figure 8: Can you use the prefixes in the descriptions of each line? "T1 copepod" or "T2 Control." It makes it very difficult to see the trends you mentioned when you have to look back at the legend each time you read a line to figure out the color-coded timepoint.

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