

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

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Received and published: 27 April 2018

Attending to the general comments:

(1) It is true that the “microbe free” effect of copepod excretion on free-living microorganism’s metabolism is difficult to address and is certain not the scope of our studies. We consider the copepod as a hole considering also its own microbiome and potential microbial epibionts. Have a “microbe free” experiment is also not realistic itself if we try to determine nutrient fluxes and potential role of copepod. Our approach is based on a series of previous studies about the excretion of copepods and its impact on microbial communities (Perez-Aragón et al., 2011; Valdés et al., 2017a; Valdés et al., 2017b).We

C1

have a robust data base regarding the type of compounds excreted by copepods in central-southern Chile. In addition, the excretion of copepods has been studied for several decades and there is an important amount of works and reviews about this topic (Bidigare, 1983; Ikeda et al., 2001; Steinberg and Saba 2008; Hernandez-León et al., 2008; Smith and Whitledge 1977; Pérez-Aragón et al., 2011). Several of these studies have demonstrated that the main compounds excreted by copepods are DON, ammonium. and DOP, although the latter has been much less studied than nitrogen compounds.

(2) We agree with the reviewer that incubation time was an important factor and that the enrichment associated with copepods could be evidenced mainly at the beginning of the incubation, since a later bacterial response could be associated with microbial metabolisms. However, our results indicate that presence of copepods and its metabolisms end products enhance the activity of different microbial communities in very short time response (<2h). We identify examples of active bacteria in our incubations such as LDA, known to avidly react to the different compounds potentially provided by copepods. References associated, for example, to Alteromonadales were included in the discussion of the manuscript. Moreover, the reviewer can check for example in Figure 10 that different active microbial communities were observed in all the stations between the treatment and controls. Therefore, the effect of the copepod metabolism can’t be discarded either. The fact that this “activity enhancement” did not result in significant increase in abundance also indicates that copepods interaction with bacterioplankton could be more complex than expected potentially generating shifts among microbial groups, since some bacteria presented a reduction in the copepod treatments.

(3) We agree in this point, and also this issue was taken in consideration in the Discussion section.

(4) Figure 8 shows the RNA samples not DNA, and thus the active bacterioplankton community. The most abundant communities present in situ and during the experi-

C2

ments are the same, the changes respond to its contribution. Here we study the activity of the microbial communities and as explained in the point (2) has been reported that some opportunistic microorganism as Gammaproteobacteria increases their activity in a very short time in response to enrichment experiments. DNA in situ was used to identify groups present in the study area which could however not usually be the most active ones. But T0 in the graphs were obtained from the activity of bacterioplankton using RNA so is not unexpected to have a different composition particularly in the case of cyanobacteria or photoautotrophs. Moreover, it is common that the incubation experiments the microbial communities differs (in diversity and composition) from the field, since the incubations may favor microorganism rare in nature but featuring opportunistic that allow for a more rapid adaptation to changes in environmental conditions and hence, outcompete other that are originally more abundant. This explanation and references were included in the Discussion section.

(5) The initial samples were taken once all the bottles were incubated both with or without copepods. The time delay between the first bottle put in incubation and the beginning (time T0) of the experiments was around 20 minutes. This time window can explain the differences found in DON concentrations at the initial sampling points and also the variations in the contribution of the most abundant groups. This information has been now added in the Methods section.

Specific Comments:

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

We have now provided the proper reference.

\*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.

C3

We have now added a more comprehensive summary, with new references.

\*P. 3, line 1: "enhance" should be "enhances."

Corrected.

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

Corrected.

\*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.

Latitude and longitudes were now included in the first section of methods along with a map with the transect during the cruise and stations sampled.

\*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.

Temperature and Chl-a values have been included in Table 1 on the new version of the MS.

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

Yes, the incubation seawater was obtained from the DCM. We have now clarified this in section 2.3 of the new version of the MS.

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

Yes, acclimation step is number 1. We have now corrected.

\*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. ..."

C4

Corrected.

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

Thanks for the observation, we have now modified to "temperature controlled room".

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

We have now provided temperature values throughout the MS.

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

We have now clarified this in "preparation of the microcosm and experimental setup" section.

\*P. 4, line 24: should be "to ensure" instead of "of ensuring"

Corrected.

\*P. 4, line 26: suggest specifying "During the excretion phase. . ."

We agree, thanks for the suggestion.

\*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?

The wet oxidation method is used for both particulate and dissolved compounds, the differences are that the oxidation procedure uses seawater samples previously filtered through pre-combusted GF/F filter in Teflon flask for dissolved fraction and for the particulate sample, the filter is used in the Teflon flask for the oxidation procedure. Details are provided in "dissolved inorganic and organic measurements" section.

\*Sections 2.5-2.6: My expertise is in zooplankton physiology, so I cannot assess the

C5

suitability of the genetics and bacterial methods.

\*P. 5, line 22: "using" should be "using"

Corrected.

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.

Done for the entire Results section.

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

We agree we have now modified.

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

Yes, there is a gap between the moment we started the experiments and the moment we took the initial sample. The initial sample (T0) was taken after all the bottles were conditioned (when all the bottles had the copepods and/or the seawater). The gap of the time was around of 15-20 minutes. We have now added this information in the Methods section.

\*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?

We have now corrected. Thanks for the observation.

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

C6

We have now clarified this sentence and all the statistical tests were in parentheses.

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

Corrected.

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

Corrected.

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

Thanks for the observation, now it was modified accordingly.

\*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.

We modified the nomenclature to DIN: DIP and DON: DOP for inorganic and organic ratios, respectively.

(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.

We did not measure urea, so it is not the molar ratio that we are using. DON: DOP ratio is much less commonly measured than the inorganic ratio and only we have a couple of references with the measure of this ratio to compare our interpretation.

(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.

We agree and now it was modified.

C7

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

We agree and now it was modified.

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

Corrected.

\*P. 8, line 23: should be "shown" not "showed"

Corrected.

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

Done.

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

Yes, we have now deleted 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.

Agree. Now it was modified.

\*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?

This issue was discussed in the general comment (2).

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

Yes. We have now modified.

C8

\*P. 10, line 12: "show" instead of "showed"

Corrected.

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?

Section 4.0 has been now modified, avoiding a general analysis of both regions.

\*P. 10, line 22: "region" should be "regions"

Corrected.

\*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.

The increase in DON at different time or in only one-time point during the experiments has been reported by other authors (Gardner and Paffenhöfer, 1982; Steinberg et al., 2002). DON excretion is release in a non-continuous way in highly concentrated spurts, while ammonium is release continuously, which is in agreement with our results.

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

Yes. Thanks for the observation.

\*P. 10, line 31: is  $22,8 \times 10^3$  correct? Should it be 228 or 22.8? The formatting is a bit odd and makes me think it is a typo.

The formatting is correct.  $22.8 \times 10^3 = 22,800$ .

\*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.

In average, bacteria increase their abundance but between 1 and at the end of incubation. We have now modified this sentence.

\*P. 11, line 10: But in LD B, you said no significant differences were observed over time

C9

(although your reported stats results don't match that - p 7, line 30)

We agree. We have now deleted this sentence.

\*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.

The initial sample was taken after all the bottles set for incubation (T0), that is when all the bottles had the copepods and/or the seawater. The gap of the time was around of 15-20 minutes. We have now added this information in the Methods section.

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

Corrected.

\*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.

Thanks for the observation, now it has been corrected

\*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.

(1)(2)We agree and we have now rewritten this section.

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

Ok, corrected.

\*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

We have now deleted "substantial amounts" to avoid misunderstanding. However, our results showed an increase in DOP concentrations at different sampling points during

C10

the experiments.

\*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.

We agree and we have now modified this sentence.

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

We have now clarified in Table 1, Figure 4 and through the whole manuscript.

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

We have now provided temperature values at DCM in Table 1.

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

"\*" corresponds to the additional sampling time that we took at LD B and LD C stations. We have now clarified in the figure caption.

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

Thanks for the observation. Now it was clarified.

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

Done.

\*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?

We try to add all the information in one table, but is difficult to follow the results and is necessary extend the table losing clarity. Thus, we decided not added the information in the Table S2.

\*Figures 2-5: The data are really complicated and results are hard to grasp between

C11

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.

We tried but it's hard to put all these information in the graphs without losing clarity in the figure since we should add this information between sampling time and treatments. Sorry for this thus do not agree with adding the statistical information in the figures.

\*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.

We agree. Now it was modified in the figure.

Please also note the supplement to this comment:

<https://www.biogeosciences-discuss.net/bg-2017-563/bg-2017-563-AC2-supplement.pdf>

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Interactive comment on Biogeosciences Discuss., <https://doi.org/10.5194/bg-2017-563>, 2018.

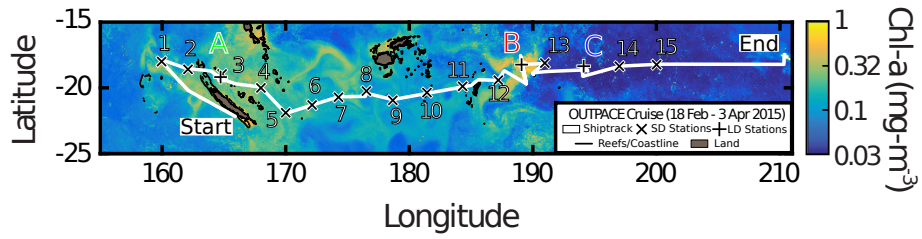


Fig. 1.

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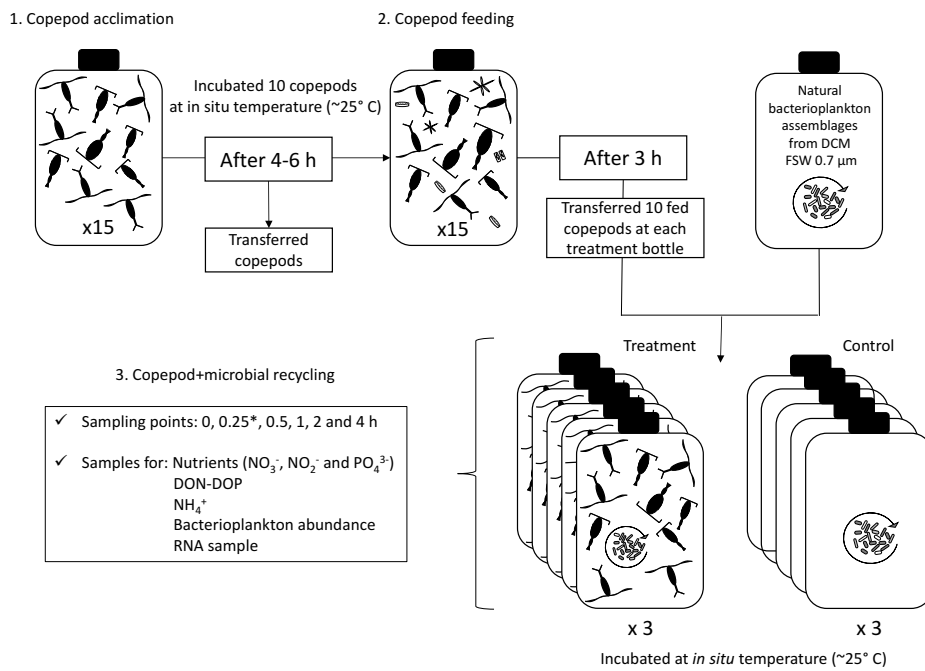


Fig. 2.

C14

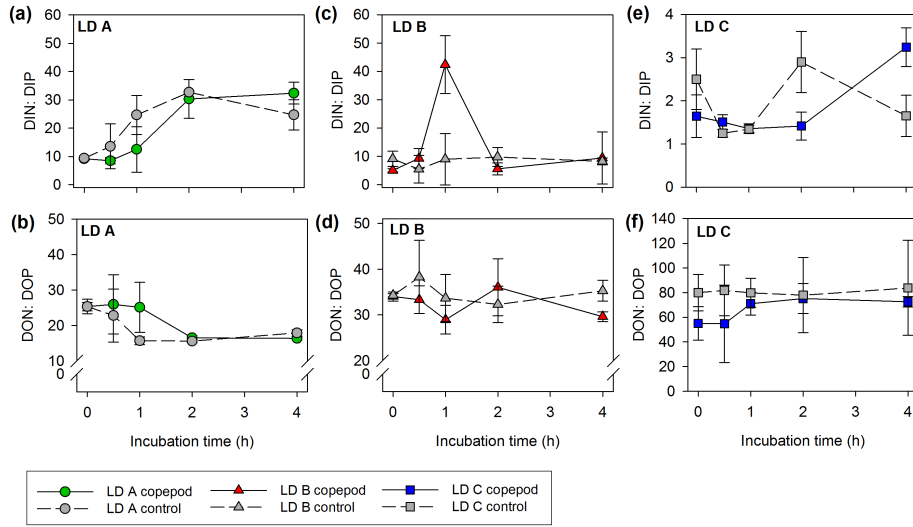


Fig. 3.

C15

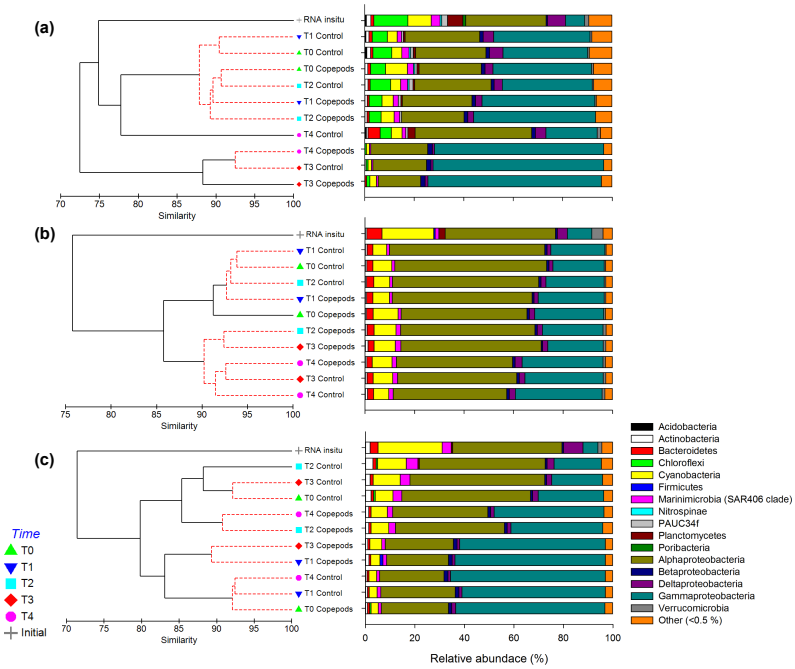


Fig. 4.

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