

## ***Interactive comment on “Succession within the prokaryotic communities during the VAHINE mesocosms experiment in the New Caledonia lagoon” by U. Pfreundt et al.***

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This study discusses the changes in bacterial community composition in a phosphorus-fertilized mesocosm deployed in an oligotrophic marine environment. The paper concludes that the fertilization effects were not immediate but rather delayed. The different bacterial succession events in the mesocosm may be due to the mesocosm itself rather than the DIP fertilization. Overall it appears that there are stronger environmental parameters than P starvation that govern the bacterial community composition and succession.

The paper is overall well written with the exception of several places which are pointed

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out below. I believe the presentation of the data and the following discussion can be simplified and clarified by the authors using the (pseudo)absolute numbers they so carefully generated rather than the relative abundance. By doing so there is no need to switch back and forth between the two methods of data presentation.

At first I would like to raise three methodological related questions which the authors have not addressed or have done this partially.

1) The authors compared community and activity in 3 fertilized mesocosms to parallels in the lagoon waters. I am surprised that the experimental design did not contain any control non-fertilized mesocosm. Personally having worked with mesocosms with volumes around 300 m<sup>3</sup> the mesocosm itself has an affect on the microbial community which has to be accounted for.

2) Second, the authors chose to sample only one of the mesocosms. Therefore, no biological duplicates are available. While the financial / man-power reasons behind such a decision can be understood, this drawback of the experimental design must be clearly stated.

3) The authors claim very clearly in the last sentence of the introduction that they focus on the prokaryotic community. Hence, I am a bit confused about the decision to use a filter with a pore size of 0.45  $\mu\text{m}$ . This especially when the tendency is to switch to pore sizes of 0.1  $\mu\text{m}$ .

As general comment with respect to the figures, the page formatting of this journal splits the standard page into two halves, thus long figures designed for a full page become illegible. In this case figures 3 and 4 and 6 are useless unless zoomed in on a computer screen. With respect to figures 1 and 2 the size of the symbols should be increased.

Abstract

UCYN-C is mentioned here and further in the paper but appears in the graphs as Cyan-

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othece. It would be good to add to the legend of the figures UCYN-C in parentheses, especially since *Atelocyanobacterium thalassa* (UCYN-A) may be confusing.

#### Introduction

The introduction is concise and to the point.

#### Results

The increase in BP on day 4 at the surface is very high compared to the day before and after. That on day 21 seems to be part of some trend. Were similar values obtained from the other mesocosms around these time periods? How do the authors exclude a methodological error?

The Shannon index is affected by the community evenness. To better describe the diversity in the sample I suggest adding (to the supplementary material) the richness and evenness values for the same samples. Generally the Simpson index is less dependent on the evenness and it should be used instead of the Shannon (better indices like Hill numbers are of course recommended. See Chao et al 2014 DOI: 10.1146/annurev-ecolsys-120213-091540).

The authors discuss (and show in Fig 3 and 4) the change in abundance of different groups. In Fig 2 a and b they show absolute numbers for cyanobacteria and prokaryotes. Very often diversity studies lack the absolute numbers and therefore are “forced” to use only relative abundance. However, in this study the authors clearly have the data to convert these relative abundances to absolute numbers and they have also invested the time to come up with a reliable method to do so. Nevertheless they chose to present these data as supplementary. Comparing the two figures some trends are very different both at the class level and within the shown classes. In my opinion the absolute abundance should be the main (and only) figure in this case. This will also simplify the discussion which alternates between absolute and relative abundance. There is no such figure for the lagoon data – one should be added to replace Fig. 4.

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For example the Rhodobacteraceae are said to have the most extreme dynamics. While this is true in relative abundance, the Flavobacteriaceae have a 9 fold steady increase in cell numbers between day 10 and 18 while Rhodobacteraceae “merely” increase < 4 fold.

The authors discuss the abundance of SAR11 clade. Later in the manuscript they argue that while a loss is possible due to the large pore size of the filter, the loss should be uniform throughout the samples. The reasoning is the correlation between the SAR11 and SAR86 transcript abundance which should be interpreted as a uniform loss of SAR11 across the samples. I have not been able to read the cited manuscript by the same leading author but the cell abundance of SAR11 and SAR86 as reported in Fig S3 is not highly correlated ( $R^2$  of 0.5 if I remove day 8 (the increase in SAR11)). This does not mean that the SAR11 data is incorrect! Can the authors perhaps bring evidence from the flow cytometry with respect to the abundance of the size class that would match SAR11?

Page 20193 Line 26: use “in contrast to” and not “in opposition to”

#### Discussion

Page 20195 Lines 22-26: This sentence regarding the pigments of *Synechococcus* and results from another paper appear “out of the blue” and are not in context. If the authors insist of having this here to explain results from an accompanying paper they should start with the results obtained in the flow cytometry and then continue with their explanation. It may be clearer if the authors state that some cultured isolates from these particular clades have orange autofluorescence due to a high phycoerythrin:phycoerythrobilin ratio.

There is no direct connection between the above paragraph (discussing the pigmentation) to the *Synechococcus* ecotypes discussion that follows. Therefore, these two should be somehow contextually separated.

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Page 20196 Line 18: erase “the” in “the both communities”

Page 20198 Line 18: I am not sure I understand the statements here: 1) SAR 11 16S abundance and SAR11 transcripts are not correlated (and the same for SAR86); 2) SAR11 and SAR86 are correlated with each other in transcript abundance and 16S abundance. At least the latter is not correct when absolute cell counts are considered, as pointed out earlier. It is not clear to me why would one expect SAR11 and SAR86 to be correlated in abundance.

Page 20199 Lines 3 onwards: This paragraph is poorly written and therefore hard to follow. The words “enhanced biological phosphorus removal systems” appear twice consecutively. There is no need in one sentence to say “in such systems” and again “enhanced. . .” at the end of the sentence.

Looking at the figures (absolute cell numbers S3) and trying to understand the why is it surprising to find *Defluviococcus* responding to the DIP fertilization when it seems to be related to high phosphorus systems. I can only assume that the surprise is the discrepancy between the CCA and the change in abundance. The latter shows no increase in Rhodospirillaceae to the fertilization in the first days. This entire paragraph should be rewritten to clearly state the authors’ intentions.

Page 20201 Line 7: Information. . . Shows

Page 20201 Line 24-25: Change to . . . metabolic pathways that gives them an advantage among other bacteria and facilitates interaction with, and attachment to phytoplankton detritus.

Page 20202 Just out of curiosity – in the accompanying transcriptome study were Cyanobacteria the sole N<sub>2</sub> fixers?

Page 20204. Do the UCYN-C really increase? – this is not clear from the graphs and it appears to me that it would be within the error margins of the method.

Page 20204 Line 21: The correlation between SAR11 and SAR86 has been mentioned  
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a lot but no data to support this has been shown. If there are specific OTUs that are correlated, this cannot be seen at the family level and therefore this should be clearly shown in a figure.

Figure 3, 4 and S3 why is the Y axis of these graphs differently scaled than the other panels?

Figure 6: in the legend: Objects and not Objets

Figure S1 This figure, as mentioned, should include richness and evenness to fully depict the changes in diversity.

Figure S2 – I think the extra note in the caption is not needed. The figure shows correlation between the groups in and out of M1. Pity this figure does not show the famous SAR11/SAR86 correlation.

#### References

Submitted papers (to the same issue) should be changed to the final citation once this is known.

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Interactive comment on Biogeosciences Discuss., 12, 20179, 2015.