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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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1) This study discusses the changes in bacterial community composition in a phosphorus-fertilized mescosom 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 out below.

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Answer: Thank you.

2) 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.

Answer: We agree. The lagoon samples are being counted by flow cytometry at this moment. These data will allow us to calculate the (pseudo)absolute numbers also for the lagoon, which was not possible before. We will exchange the figures showing the relative data with those showing the absolute data in the main MS, and move the relative data to the Supplement.

3) At first I would like to raise three methodological related questions which the authors have not addressed or have done this partially. 3.1) 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 m3 the mesocosm itself has an effect on the microbial community which has to be accounted for.

Answer: The goal of the Vahine project was to study the fate of diazotroph-derived nitrogen (DDN) in a low nutrient, low chlorophyll ecosystem (please se Introductory and Synthesis papers in the SI http://www.biogeosciences.net/special_issue193.html). For this purpose, we decided to deploy 3 replicate mesocosms to isolate a single water mass of several cubic meters from physical dispersion for several weeks, without disturbing temperature and light conditions, and taking into account the biological complexity of the planktonic ecosystem. The DIP fertilization was done only to alleviate any potential limitation in the mesocosms and promote N2 fixation to facilitate our study on the fate of DDN. Consequently, we decided not to perform 3 control mesocosms. We agree that if the main goal of the project would have been to study to effect of a DIP fertilization on planktonic communities, this would have been necessary. Nevertheless,

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we observed some effects likely due to DIP fertilization rather than confinement. With the data we have, we thus tried to tease apart the effects of mesocosm confinement from those of DIP fertilization through multivariate statistical methods, taking advantage of the time series. This is possible because DIP fertilization did not take place until the evening of day 4.

3.2) 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.

Answer: We added a statement to the methods section.

3.3) 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 μm. This especially when the tendency is to switch to pore sizes of 0.1 μm.

Answer: Yes. There is a tradeoff between the amounts of material harvested versus pore size. As we needed substantial amounts of material for metatranscriptomics from the same samples and the time for sampling was another concern, we decided for this pore size.

4) 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.

Answer: In the final Biogeosciences publication, which uses a standard A4 format, figures 3 and 4 will be a full page each. We increased symbol sizes in Figs. 1 and 2.

Abstract 5) UCYN-C is mentioned here and further in the paper but appears in the

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graphs as Cyanothece. 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.

Answer: We added this information wherever one of the two appears in the MS.

Introduction 6) The introduction is concise and to the point.

Answer: Thank you.

Results 7) 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?

Answer: Yes, in M3 the same peak of BP at day 4 was observed. While M1 (investigated here) showed a 4-fold increase, it was 3-fold in M3 and had a higher variation with depth. In M2, a similar peak was observed on day 5 (Van Wambeke et al, this issue, Fig. 4). A methodological error seems very unlikely due to the following reasons: i) This BP peak was seen in all 3 mesocosms, but NOT in the lagoon (outside control). In M1, the same high BP value was observed for all three investigated depths, leading to a very small error. ii) The coinciding 4-fold increase of Rhodobacteraceae 16S genes in mesocosm M1 on day 4, but NOT in the lagoon. Note: BP in M1 increased 4-fold. Water for DNA and RNA extraction was sampled in different containers than water for BP measurements, directly from the mesocosms. iii) The coinciding $\sim$3-fold increase of total Rhodobacteraceae transcripts (Pfreundt et al, submitted to this issue) speaks against a technical bias leading to the observed 16S abundance peak.

8) 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 (bet-

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ter indices like Hill numbers are of course recommended. See Chao et al 2014 DOI: 10.1146/annurevecolsys-120213-091540).

Answer: Thank you for this suggestion. We added richness and evenness to the Suppl. material complementing Fig S1. We also calculated the Simpson index and added it to the Shannon index (Fig. S1) for comparison, the difference is only very slight. The new Figure S1 is attached to this answer as Fig. 1.

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

Answer: We agree. The lagoon samples are being counted by flow cytometry at this moment. These data will allow us to calculate the (pseudo)absolute numbers also for the lagoon, which was not possible before. We will exchange the figures showing the relative data with those showing the absolute data in the main MS, and move the relative data to the Supplement.

10) 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

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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 (R2 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?

Answer: Unfortunately, only total heterotrophs with the distinction between high- and low nucleic acid (HNA and LNA) were counted, from which it is not possible to tease out SAR11. We believe that the data from our metatranscriptome study (Pfreundt et al, published in BGD, 2016), which shows very strong correlation between SAR86 and SAR11 total transcript abundances, is sufficient to state that loss of SAR11 was uniform across samples. The manuscript dealing with the metatranscriptomic data is now accessible under doi:10.5194/bg-2015-564. Please see Figure S6 in that MS. Strong correlations like this would be very unlikely if there was random loss of SAR11 in the different samples. We thus conclude that loss was uniform.

The correlation (or not) of SAR11 and SAR86 in terms of 16S abundance is discussed further down in our response to point 14.

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

Answer: Done.

Discussion 12) 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

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phycourobilin: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.

Answer: The two mentioned sentences were indeed not central for the flow and the central statements of this analysis. Therefore, we skip them in the revision.

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

Answer: Done.

14) 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.

Answer: We apologize for the unclear writing here. First of all, we did not expect SAR11 and SAR86 to correlate in abundance. Rather, constrained ordination (Fig. 6 in the MS) showed that abundances of individual OTUs of the two clades could be explained by the same combinations of environmental variables. We wrote in the conclusion: “Linked with the observation of tight correlation between SAR11 and SAR86 transcription during a diel cycle (Aylward et al., 2015) and over three weeks (Pfreundt et al., 2016), we show that the temporal dynamics of individual OTUs of these clades over three weeks can be explained by very similar combinations of environmental variables.” We did not intend to say that the clade abundances were generally correlated, and the referee indeed correctly calculated that they are not. We always referred to single abundant OTUs. We wrote in Discussion section 4.2: “It appears striking that for both, SAR11 and SAR86, 16S abundance was not well correlated with the respective total transcript accumulation (Pfreundt et al., 2015), but that the two clades were correlated among each other both in transcript abundance and abundance of dominant

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OTUs" We apologize for mistakenly referring to the 16S data as correlated in the Discussion section 4.2. Indeed, correlation was only tested for the transcriptome data. We rewrote the sentence to be more clear and amend this error, so that it now says: ""It appears striking that for both, SAR11 and SAR86, 16S abundance was decoupled from the respective total transcript accumulation, but that the transcript abundance of both clades was highly correlated (Pfreundt et al., 2016) and that some specific abundant SAR11 and SAR86 OTUs seem to share a similar niche (Fig. 6)."

15) 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 Defluviicoccus 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.

Answer: It might not appear surprising that a bacterium associated with biological phosphorus removal systems would respond to DIP input. However, the Defluviicoccus found in high phosphorus systems are adapted to several orders of magnitude higher phosphorus loads then those applied to M1 and are not exactly known from marine environments. Therefore, we were indeed surprised to see this taxon coming up. However, also in agreement with a comment from the 2nd reviewer that there is "marine" and "wastewater" subclade within the Defluviicoccus group, we checked during revision whether our 16S amplicons associated with Defluviicoccus are indeed falling into this marine branch. We agree that this paragraph was poorly written and not well focused. In the revision we will streamline the paragraph on Defluviicoccus accordingly and include the subclade information.

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16) Page 20201 Line 7: Information....Shows

Answer: Changed to “Information retrieved from analysis of cultured representatives showed . . .”

17) 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.

Answer: Changed to “...metabolic pathways that give them an advantage among other bacteria and facilitates interaction with, and attachment to phytoplankton detritus.”

18) Page 20202 Just out of curiosity – in the accompanying transcriptome study were Cyanobacteria the sole N2 fixers?

Answer: Data about the presence of N2 fixers can be found in Turk-Kubo et al, where the authors employed qPCR and nifH amplicon sequencing to generate absolute nifH gene copy numbers. This showed that heterotrophic diazotrophs were present, but at much lower abundances. In our metatranscriptome paper, we did find transcription of nifHDK genes from one group of heterotrophs, the Chromatiaceae. We have to mention that nifH transcripts were very rare generally, consistent with generally low relative abundances (<1%) of diazotrophs in this community, thus imposing a large error on nifH transcript abundances seen in this study. Further, as sampling took place in the late morning, only those diazotrophs with daytime N2 fixation can be seen.

19) 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.

Answer: Yes, we admit that this is hard to see from the graphs due to generally low abundance of N2 fixing cyanobacteria compared to Synechococcus and Prochlorococcus. The relative abundance of Cyanothece (UCYN-C) in M1 is in fact one order of magnitude higher in samples from days 16, 19, and 21 than before or in the lagoon. We did some statistical testing to prove that this increase is significant. For this pur-

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pose, we defined two groups, days 2-14 (P0/P1), and days 16-21 (P2), both in M1 and the lagoon, leading to 4 groups. We applied ANOVA and the Tukey's Honestly Significant Difference (Tukey's HSD) test to see whether the means of these groups are significantly different. We found that P2 in M1 is significantly different from the other 3 groups (Tukey's HSD p-values 0.00002, 0.00009, 0.00003) and that in the lagoon P2 is not significantly different from P0/P1. This analysis shows that UCYN-C significantly increased in M1 during phase P2, as stated in the MS.

20) Page 20204 Line 21: The correlation between SAR11 and SAR86 has been mentioned 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.

Answer: Whereas there is very strong support for the correlation on transcript level (see Figure S6 from the referenced MS, Pfreundt et al., 2016), which we mostly discussed, the "correlation" of 16S-based abundance was solely taken from Fig. 6, the constrained ordination, which showed that abundances of SAR11 and two out of four dominant SAR86 OTUs could be explained by the same variables, thus these OTUs might share a similar niche. We mistakenly denoted this as "correlation", and have changed this now (see our answer to point 14).

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

Answer: We are sorry, but we don't understand the question, as no specific panel is mentioned. Scaled differently to which other panels? Maybe the referee is referring to the panel f in each of these figures, which has a sqrt-scale instead of a linear scale. We chose this scaling because the abundance difference between Synechococcus and especially the diazotrophic cyanobacteria is so large.

22) Figure 6: in the legend: Objects and not Objets

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Answer: Done.

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

Answer: Done. The figure is attached to this answer as Fig. 1.

24) 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.

Answer: We think that all information given in the caption is helpful and thus did not shorten it. Of course this figures does not show SAR11/SAR86 correlation, because the intention of this analysis was a different one, namely testing which phylogenetic groups behaved differently between the lagoon and M1, and which ones were not influenced by the confinement in M1.

References 25) Submitted papers (to the same issue) should be changed to the final citation once this is known.

Answer: The bibliography was updated to the current status.

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

Response Letter Figure 1. a) Development of the Shannon (red) and Simpsons (black) diversity indices in the Nouméa lagoon and mesocosm M1 shows that these are very similar. Generalized linear models were fitted with glm() in R and the predicted values plotted as straight lines. b) Shannon evenness calculated using the RAM package for R and the function evenness(index="shannon"). c) OTU richness displayed as rarefaction curves calculated on the raw count data.
Phases are labelled: early - blue (T2-T8), middle - orange (T10-T14), late - green (T16-T21).

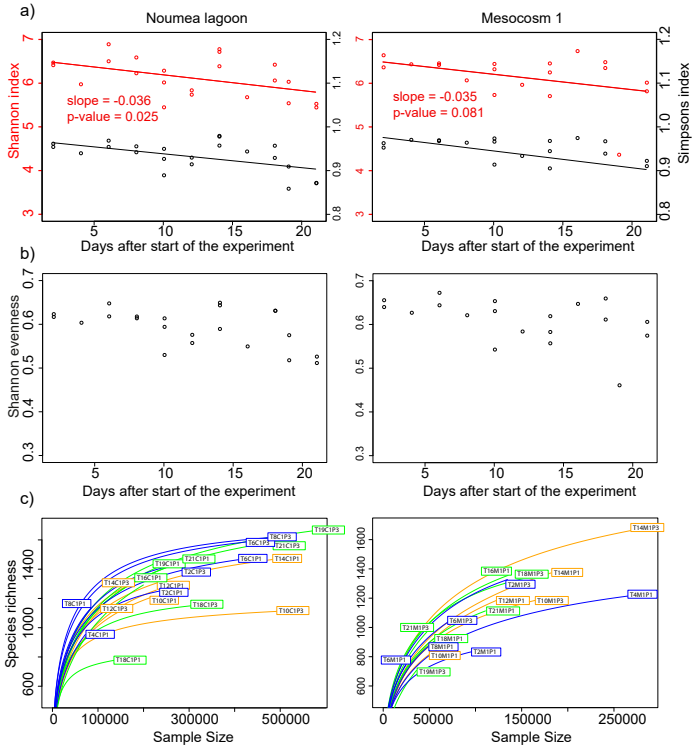

Fig. 1.

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