AUTHOR'S RESPONSE

Succession within the prokaryotic communities during the VAHINE mesocosms experiment in the New Caledonia lagoon

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POINT-TO-POINT RESPONSES

Reviewer 1 (Danny Ionescu)
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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.
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 figures showing the relative data (Fig. 3 and 4) have been replaced with those showing the absolute data in the main MS, and the relative data has been moved to the Supplement. The Results and Discussion have been amended accordingly.

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 see 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, 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 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 and the figures.

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 ~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 (better 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 as panels b and c. We also calculated the Simpson index and added it to the Shannon index (Fig. S1) for comparison, a difference is not evident, in the MS we thus still refer to Shannon diversity.

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 figures showing the relative data (Fig. 3 and 4) have been replaced with those showing the inferred absolute data in the main MS, and the relative data has been moved to the Supplement as Figures S4 and S5. For calculation of the inferred absolute cell counts, we have now also taken into account different 16S copy numbers per genome and we provide the manually curated 16S copy number table in the Supplement for further use. The Methods, Results and Discussion have been amended accordingly.

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 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 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 skipped 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 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 (new Suppl. Fig. S3). We agree that this paragraph was poorly written and not well focused. In the revision we will streamlined the paragraph on Defluviicoccus accordingly and included the subclade information.

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 confer advantages among other bacteria and facilitate 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 N_2 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 N_2 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 statistical testing to prove that this increase is significant. For this purpose, 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. This was added to Methods and Results.

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: 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
Answer: Done.

23) Figure S1 This figure, as mentioned, should include richness and evenness to fully depict the changes in diversity.
Answer: Done. Supplementary Figure S1 now includes these data.

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.

Reviewer 2 (Pelin Yilmaz)
Received and published: 29 January 2016

1) In this study, Pfreundt et al. are presenting the bacterial community composition analysis results from the VAHINE mesocosm experiment. The VAHINE experiment deals with constructing mesocosms in the oligotrophic South Pacific, and fertilizing them with phosphorus. The manuscript details the changes in bacterial community in the mesocosm and the ambient waters (the lagoon samples) during the duration of this experiment. The results are presented from the perspective of phosphorus starvation, and whether this has an effect on the bacterial community.
The paper is overall well written, although the language could use a bit of polishing here and there. Shortening some sentences for clarity may help readers understand the study better.
Answer: Thank you for the overall positive and very helpful comments. We have addressed all concerns during revision and have made the presentation more concise at several places.

2) My biggest complaint about this paper is its constant referencing to the sister paper from the same authors "Global analysis of gene expression dynamics within the marine microbial community during the VAHINE meso- cosm experiment in the South West Pacific". I understand that the authors may want to publish the results separately. However, since this paper heavily relies on the transcript data for the discussion part, I'm asking myself why not make one big paper, and save the reader from going back and forth between two papers.
Answer: Yes, the companion paper on the metatranscriptome (Pfreundt et al., 2016) is very informative and both are important for understanding the processes within the investigated microbial communities.
Unfortunately, it cannot be joined with the current one due to the extent of information. The companion paper was submitted one full month before this manuscript, but unfortunately it was dealt with quite slowly. However, it is publicly available now accessible as a Discussion Paper in Biogeosciences under doi:10.5194/bg-2015-564.

My specific comments to different sections are listed below.
==Abstract and Introduction==
3) Concise and to the point
Answer: Thank you!

==Materials and Methods==
4) I understand that in total, three mesocosms were deployed, but only one (M1) one was studied for bacterial community composition. Of course I understand the issue of cost, but it seems like a chance missed to have some replicated.
Answer: This is true, and sequencing another 20 samples from a second mesocosm would have indeed been advantageous. The fact that we have a continuous time-series, and 37 samples in total, however, alleviates the lack of replicates to a certain extent,

because it allowed us to do correlation studies and multivariate statistics to infer differences between M1 and the lagoon, as well as revealing dependencies of microbial groups to certain environmental variables.

4) Were there any other negative controls, other than the lagoon sampling? A mesocosm without DIP addition?

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 see 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 N_2 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, 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.

5) Did the authors account for different 16S copies while calculating the pseudo-absolute cell numbers?

Answer: No, as stated on page 20191, line 26 in the MS "*We assumed equal 16S gene copy numbers."* (no absolute assumption was made). While we know that this is not true, and that, for example, copiotrophs mostly have more copies than, for examples Synechococcus, it is impossible to use "true" numbers here, because our abundant OTUs are often not represented by a finished genome.

We have now substantially refined this calculation in the revision by using true 16S copy numbers as given in the Integrated Microbial Genome (IMG) database for the putative close relatives of our abundant OTUs. If no closely related genomes were available, as is often the case, we applied the average of available marine genomes in a given phylogenetic clade. We supply our manually curated table of 16S copy numbers in the Supplement.

6) How were the pearson correlations calculated, and why was pearson correlation selected specifically? Is the data normally distributed? Has the significance of these correlations been tested?

Answer: The referee probably refers to Suppl. Fig S2. We calculated Pearson correlations using the cor() function in R on the different taxonomic groups in M1 and the lagoon. This was added to the Methods section. We applied the Shapiro-Wilks normality test to the distributions of all taxonomic groups separately and found that 22 out of 60

distributions significantly deviated from normality. The trends tend to be the same whether Pearson or Spearman rank correlation is used.
We did not compute significance values for the correlations initially, because it is visible from the heatmap plots in Fig. S2 that the strong correlations (showing strong relationships between taxonomic groups in M1 and the lagoon) are rare and thus likely significant. Nevertheless, we now calculated all p-values for these correlations. In the revision, we now changed the method for correlation calculation to the rcorr() function of the Hmisc package for R, which includes the significance calculation. We now show Spearman rank correlations in Suppl. Fig. S2 instead of Pearson, and we indicate whether a value is significant or not.

7) The authors make plenty use of the correlation values in the results and discussion.
Another interesting analysis that the authors might consider adding here would be tests of significant associations between the taxa and groups of sites (lagoon vs. mesocosm, or periods in the mesocosm). These tests are implemented in R package indicspecies.
Answer: Thank you for this suggestion, we did a similar analysis, but not with this R package. The multivariate statistical analyses including significance tests (PERMANOVA) of the effects of the site, time, and depth on OTU distribution is presented in **section 3.2.4 / Fig. 5** of the Discussion paper. Additionally, we did a constrained ordination to infer associations with environmental variables (**section 3.2.5 / Fig.6**), and believe that together, these analyses are sufficient for this paper.

==Results and Discussion==
8) Figures are really hard to read, I think this is also partly because of the pdf format from BGD, but it would greatly help if the authors could increase symbol sizes in figures.
Answer: Sure, we increased symbol and text sizes.

9) If I understand correctly, PP, APA, Chla and BP were measured for all mesocosms. If so, it would be good to see the data from M2 and M3 as well.
Answer: This data is provided in several companion (and cited) manuscripts (Van Wambeke et al., 2015, Berthelot et al, 2015). In this MS, we only included M1 and lagoon data to make our data (which is also M1 and lagoon) directly comparable.

10) The comparison and usage of both pseudo-abundance and relative abundance is confusing, especially since the figures display rather different trends.
Answer: We constrained results and discussion to the absolute data in the revised MS, but still supply the relative figures in the Supplement (Fig. S4 and S5), because the relative data is the original data and the absolute numbers are derived.

11) I suspect that the Deferribacteres is just mostly SAR406 clade, please indicate this in the text and figure captions
Answer: Indeed, we checked this and it is almost entirely SAR406. As suggested, we added this information to the MS.

11) In figures 4 and 6, it would be good to mark DIP addition as well.
Answer: We do not understand, why the referee suggests this for Fig. 4 (relative abundances of taxa in the lagoon) and Fig. 6 (canonical correspondence analysis). We now indicated the DIP fertilization in the old Fig. 3 (which is now Fig. S4), and in the new Fig. 3, showing the absolute taxon abundances in M1.

12) UCYN-C and A should also marked in the figure captions, the manuscript text and figure captions do not match
Answer: Done.

13) I fail to see this suggested correlation between SAR11 and SAR86 in the data presented by the authors. I also fail to understand why they should be correlated – they occupy a similar niche, but they have different nutrient preferences, but I don't really see how that would lead to a correlation between the two groups.
Answer: Please refer to our comprehensive **answer to point 14 by referee Danny Ionescu**. Briefly, we do indeed see very strong correlation (r=0.8-0.9) between the 2 groups when comparing total transcript abundance (Pfreundt et al, 2016) over the full three weeks. It is not known why, but this has also been observed over a diel cycle (Aylward et al, 2015). We did not specifically test 16S abundance correlation between the two groups.

14) There is a big increase in relative abundance of SAR11 clade in the lagoon sample on day16, do the authors have a suggestion as to why that might be?
Answer: No, there is no indication in the transcriptional data that might suggest this strong increase in cell number. A similar increase, but with lower amplitude, is seen in M1 on day 8.

15) Figure 6 is an incredibly busy figure, and it is really hard to find anything in it. The authors should consider splitting the CCA biplot into two complementary figures showing sites and species separately.
Answer: This is a good suggestion. Thank you. We now created panels A and B, that show sites and OTUs separately, and increased the text size.

16) Defluviicoccus issue is interesting, although it's not surprising a bacterium associated with biological phosphorus removal systems would respond to DIP input. The authors are right that the "genus" is rather broadly defined. Looking at the sequences of this genus, I noticed that there is "marine" and "wastewater" of Defluviicoccus group. It would be interesting if the authors could show that their 16S amplicons associated with Defluviicoccus are indeed falling into this marine branch.
Answer: This is a good suggestion. We revised our statement accordingly and shortened this section, also in in agreement with the comment from reviewer 1. We used the SINA aligner and built a phylogenetic tree with Mr. Bayes for placement of our *Defluviicoccus*

OTUs. Indeed, as seen in new **Supplementary Fig. S3**, our OTUs fall into a tight cluster with 16S sequences from marine samples.

17) I searched the Bioproject database with the given accession number, which returned no results. I persisted and searched for the manuscript title, authors and other things, but still got no results. Please make sure your sequences are available to public.
Answer: This issue has been resolved with NCBI, and the data is now accessible under the given Bioproject ID PRJNA304389.

LIST OF CHANGES MADE TO THE MANUSCRIPT

- New author Mathieu Caffin was added, who performed the Flow Cytometry analyses

Abstract

- A sentence was added stating the inference of absolute counts from 16S data and Flow Cytometry.

Introduction

- A sentence was added introducing the calculation of absolute counts from 16S data and Flow Cytometry.

Methods

- 2.1. A statement was added that only one mesocosm was sampled.
- 2.2. A more detailed section on the “Inference of absolute cell numbers from 16S data and flow cytometry” was added.
- 2.3. A paragraph on significance testing and correlation was added.

Results

- 3.2.1. A paragraph about the use of absolute instead of relative abundances was added. Information on Deferribacteres being mostly SAR406 was added. Absolute abundance results instead on relative values are presented now.
- 3.2.2. Section on Cyanobacteria has been moved up before “3.2.3 Heterotrophic Bacteria”. Absolute abundance results instead on relative values are presented now. Significance value for increase of UCYN-C has been added.
- 3.2.3. Absolute abundance results instead on relative values are presented now. This changed slightly the numerical dominance relations within alphaproteobacteria with SAR116 being most numerous most of the experiment. The NS7 marine group within Flavobacteriia does not appear anymore in the dominant flavobacterial groups (now defined as >5000 cells ml^{-1}). This section now ends with a section on total heterotrophs increase in P2, which before was the end of the “Cyanobacteria” section.

Discussion

- A new section 4.1. has been introduced that discusses chances and error sources for the inference of absolute counts from 16S and Flow Cytometry data.
- 4.2. Two sentences on Synechococcus fluorescence have been removed.
- 4.3. A sentence on temperature dependency of Sufflavibacter has been added. A new reference (Garren et al., 2015) has been added in this context.
  Line 654: Sentence on SAR11 and SAR86 correlation has been formulated more clearly.
  Line 670: Defluviicoccus paragraph has been streamlined and new supplementary figure S3 introduced.
- 4.5. Line 731: Interpretation added, that Flavobacteria increase towards the end of the experiment is not due to M1 specific effects.
- 4.6. Line 785: Possibility that Trichodesmium may reside in deeper water before the bloom has been added. Line 798-801: Sentence has been shortened.

Figures

- Fig.1. New panels e and f were added, that display BP and N2 fixation, which were before Fig. 2c and 2d.
- Fig. 2. Flow cytometry data for the lagoon was added, now Fig 2c and 2d.
- Fig. 3. is exactly the same scheme as before, but now shows absolute abundances inferred from 16S data. A line marking the time of DIP addition has been added. The abundance cutoff for families shown in panels c, d, e has been changed to >5000 cells/ml and in f to 250 cells/ml for cyanobacterial genera.
- Fig. 4. Changes analogous to Fig. 3
- The old Figures 3 and 4 are now supplementary Figures S4 and S5.
- Fig. 6. This plot has been split into two panels for better visualization. Panel a now shows only the OTUs (objects), and panel b only the samples (sites).
- Fig. S1. Simpson index has been added to panel a. New panels b and c now show Shannon evenness and Chao1 richness estimates, respectively.
- Fig. S2. Has been changed to Spearman instead of Pearson correlation values, and now also indicates significance of correlation values.
- Fig. S3 is new and shows phylogenetic placement of Defluviicoccus OTUs.

Generally, grammar, spelling, or style was corrected in some places and is not mentioned in detail in this list. Other small changes may not be listed, but are visible in the tracked version of the MS.

Succession within the prokaryotic communities during the VAHINE mesocosms experiment in the New Caledonia lagoon

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Abstract

N_2 fixation fuels ~50 % of new primary production in the oligotrophic South Pacific Ocean. The VAHINE experiment has been designed to track the fate of diazotroph derived nitrogen (DDN) and carbon within a coastal lagoon ecosystem in a comprehensive way. For this, large-volume (~50 m^3) mesocosms were deployed in the New Caledonia lagoon and were intentionally fertilized with dissolved inorganic phosphorus (DIP) to stimulate N_2 fixation. This study examined the temporal dynamics of the prokaryotic community together with the evolution of biogeochemical parameters for 23 consecutive days in one of these mesocosms (M1) and in the Nouméa lagoon using MiSeq 16S rRNA gene sequencing and flow cytometry. Combining these methods allowed for inference of absolute cell numbers from 16S data. We observed clear successions within M1, some of which were not mirrored in the lagoon. The dominating classes in M1 were alpha- and gammaproteobacteria, cyanobacteria ~~(mainly *Synechococcus*)~~, eukaryotic microalgae, ~~on days 10 and 14~~ Marine Group II euryarchaea, ~~on days 12–23 also~~ *Flavobacteriia*, and *Acidimicrobia*. Enclosure led to significant changes in the M1 microbial community, probably initiated by the early decay of *Synechococcus* and diatoms. However, we did not detect a pronounced bottle effect with a copiotroph-dominated community. The fertilization with ~0.8 µM DIP on day 4 did not have directly observable effects on the overall community within M1, as the data samples obtained from before and four days after fertilization clustered together, but likely influenced the development of individual populations later on, like *Defluviicoccus*-related bacteria and UCYN-C type diazotrophic cyanobacteria (Cyanothece). Growth of UCYN-C led to among the highest N_2 fixation rates ever measured in this region and enhanced growth of nearly all abundant heterotrophic groups in M1. We further show that different *Rhodobacteraceae* were the most efficient heterotrophs in the investigated system and we observed niche partitioning within the SAR86 clade. Whereas the location in- or outside the mesocosm had a significant effect on community composition, the temporal effect was significantly stronger and similar in both locations, suggesting that overarching abiotic factors were more influential than the enclosure. While temporal community changes were evident, prokaryotic diversity (Shannon Index) only declined slightly from ~6.5 to 5.7 or 6.05 in the lagoon and M1, respectively, throughout the experiment, highlighting the importance of multiple and varying sources of organic matter maintaining competition.

1 Introduction

The South West Pacific ocean is recognized as an area with one of the highest dinitrogen (N_2) fixation rates in the global ocean (Garcia et al., 2007; Luo et al., 2012). In this region, plankton taxa capable of N_2 fixation (N_2-fixing or diazotrophic organisms) are very diverse (Moisander et al., 2010) and fuel up to 60 % of ~~the~~ primary production (Bonnet et al., 2015a)~~(Bonnet et al., 2015a)~~, yet their interactions with surrounding planktonic communities are rarely studied. Within this vast oceanic region, most of the studies ~~regarding the~~investigating coupling between phytoplankton and heterotrophic bacteria have targeted the Nouméa lagoon, New Caledonia. Phytoplankton and bacterial production shows seasonal patterns, with maxima in December and January and an annual heterotrophic bacterial production representing 21 to 34 % of primary production (Torréton et al., 2010). High N_2 fixation rates are a recurrent feature in the Nouméa lagoon (Biegala and Raimbault, 2008; Garcia et al., 2007), and diazotroph-derived N (DDN) was shown to be significantly channeled through the heterotrophic bacterial compartment in this environment (Berthelot et al., 2015; Bonnet et al., 2015b)~~(Berthelot et al., 2015; Bonnet et al., 2015a)~~. Yet, no information is available on the potential effects of this N_2 fixation on bacterial successions.

The production and quality of varying sources of organic matter is expected to vary as blooms of diazotrophic organisms are transient events. Such variation is expected to influence not only biogeochemical fluxes, but also the succession of different heterotrophic bacteria, according to their metabolic capabilities. Indeed, the importance of quality and quantity of dissolved organic matter (DOM) in structuring bacterioplankton communities has been well established (Alonso-Sáez and Gasol, 2007; Beier and de Albuquerque, 2015), either by comparing the continuum of trophic conditions *in situ* (West et al., 2008), or following phytoplankton blooms under natural (Teeling et al., 2012) or experimental (Lebaron et al., 2001) conditions. Since the 1970s, heterotrophic bacterial succession during phytoplankton blooms has been observed with culture-dependent techniques (Fukami et al., 1981a) and linked to the possible origin of DOM sources (release, lysis, sloppy feeding, degradation of phytoplankton detritus), DOM size (low or high-molecular weight molecules), or quality (Biddanda and Pomeroy, 1988; Murray et al., 2007; Nagata, 2000; Riemann et al., 2000).

The VAHINE experiment (Bonnet et al., 2016)~~(Bonnet et al., 2015b)~~ provided a unique opportunity to study the fate of DDN in the marine planktonic food web and the interactions between phytoplankton and heterotrophic bacteria by simultaneously addressing biogeochemical parameters, stocks and fluxes, as well as biodiversity based on 16S rRNA gene sequencing and flow cytometry cell counts. Taking into account different 16S copy numbers,

we used the absolute cytometry cell counts for *Synechococcus* and *Prochlorococcus* to calculate a sample-specific cells count to 16S ratio and infer cells mL^{-1} for all operational taxonomic units (OTUs) obtained from MiSeq sequencing.

After fertilization with dissolved inorganic phosphorus (DIP), ~0.8 µmol L^{-1}) on the evening of day 4, to alleviate ~~any~~ potential limitation often observed in the region (Moutin et al., 2007), two periods of about 10 days each (P1 and P2, see description in section 2.1) were ~~clearly~~ identified in terms of diazotroph succession. During the first period, diatom-diazotroph associations (DDAs) were dominating (Turk-Kubo et al., 2015) the diazotrophic community with N_2 fixation rates decreasing from 17.9 ± 2.5 to 10.1 ± 1.3 nmol N L^{-1} d^{-1} (Bonnet et al., 2015b)~~(Bonnet et al., 2015a)~~. Unicellular N_2-fixing cyanobacteria of the UCYN-C type (*Cyanothece*-like) dominated the diazotroph community in the mesocosms during the second period, but did not appear in Nouméa lagoon waters (Turk-Kubo et al., 2015). N_2-fixation rates increased during days 15-23 and reached > 60 nmol N L^{-1} d^{-1}, which are among the highest rates measured in marine waters (Bonnet et al., 2015b; Luo et al., 2012)~~(Bonnet et al., 2015a; Luo et al., 2012)~~. These two periods corresponded to characteristic successions in phytoplankton taxa, chlorophyll stocks, primary and heterotrophic bacterial production, discussed in detail in companion studies in this issue (Berthelot et al., 2015; Leblanc et al., 2016; Van Wambeke et al., 2015)~~(Berthelot et al., 2015; Leblanc et al., 2015; Van Wambeke et al., 2015)~~.

In this manuscript we focus on prokaryotic community dynamics and possible parallels between or successions among different groups of phytoplankton, heterotrophic bacteria and N_2-fixing organisms.

2 Material and methods

2.1 Mesocosm description, sampling strategy and stocks/fluxes analyses

Three large mesocosms (~50 m^3) were deployed in the nutrient-poor waters of the Nouméa lagoon close to the Boulari passage (22°29.073 S - 166°26.205 E), 28 km off the coast from January 13 to February 4, 2013. Details of the location, deployment and sampling strategy are described in (Bonnet et al., 2016)~~(Bonnet et al., 2015b)~~. The three triplicate mesocosms were fertilized with ~0.8 μM KH_2PO_4 between day 4 and day 5 of the experiment to promote a diazotroph bloom. Samples were collected every morning at 07:00 AM over a period of 23 days from 3 selected depths (1 m, 6 m, 12 m) in each mesocosm (M1, M2 and M3) and in surrounding

waters (hereafter referred to as Nouméea lagoon waters). For sequencing of the microbial community and metatranscriptomics, only one of the mesocosms and the Nouméa lagoon as a control was sampled. Further details regarding the sampling are given separately (Pfreundt et al., 2016; Van Wambeke et al., 2015)~~(Pfreundt et al., 2015; Van Wambeke et al., 2015)~~.

Subsampling and analysis procedures for stocks and fluxes are not given in detail here as they were mainly used for statistical analyses (see section 2.3) but they are described in full in companion papers from this issue: Inorganic nutrients and chlorophyll *a* (Chl) concentrations, DIP turnover time, N_2 fixation rates and primary production (PP) were analyzed according to procedures detailed in (Berthelot et al., 2015). *Synechococcus* and *Prochlorococcus* cell numbers were counted by flow cytometry (Leblanc et al., 2016)~~(Leblanc et al., 2015)~~. Heterotrophic bacterioplankton abundances were also determined by flow cytometry and corresponded to the sum of cells with high and low nucleic acid content, discarding autotrophic cell counts (Van Wambeke et al., 2015). Heterotrophic bacterial production (BP) was determined by the ^3H leucine technique, and alkaline phosphatase activity (APA) was determined on the total fraction (i.e., unfiltered samples) using MUF-P substrate (Van Wambeke et al., 2015). Three periods of the experiment were defined. P0 was defined as days 2-4, before DIP fertilization of the mesocosms. P1 (days 5-14) was characterized by DIP availability and correspondingly lower APA inside the mesocosms (Van Wambeke et al., 2015). P2 (days 15-23) was characterized by increasing Chl *a* concentrations, N_2 fixation rates, PP and BP inside the mesocosms and also in the Nouméa lagoon, albeit to a lower extent. Further, APA increased steeply corresponding to DIP becoming limiting in the mesocosms during P2 (Berthelot et al., 2015; Van Wambeke et al., 2015).~~, N_2 fixation rates;~~

2.2 16S rRNA gene amplicon sequencing and OTU clustering

DNA was extracted from seawater samples collected ~~as described in section 2.1~~ every two days at 1 m and 12 m depth in M1 and in Nouméa lagoon waters. A volume of 10 L was pre-filtered through a 1 mm mesh to keep out large eukaryotes and filtered on 0.45 µm pore size, 47 mm diameter polyethersulfone filters (Pall Supor), immersed in RNA resuspension buffer (10 mM NaAc pH 5.2, 200 mM D(+)-sucrose, 100 mM NaCl, 5 mM EDTA) and snap frozen in liquid N_2. Tubes with filters were vortexed, then agitated in a Precellys bead beater (Peqlab, Erlangen, Germany) 2x (2 x 15s) at 6500 rpm after adding 0.25 mL glass beads (0.10-0.25mm, Retsch, Frimley, U.K.) and 1 mL PGTX (39.6g phenol, 6.9 mL glycerol, 0.1 g 8-hydroxyquinoline, 0.58 g EDTA, 0.8 g NaAc, 9.5 g guanidine thiocyanate, 4.6 g guanidine hydrochloride, H_2O to 100 mL (Pinto et al., 2009). RNA and DNA was extracted simultaneously by adding 0.7 mL

chloroform, vigorous shaking, incubation at 24 °C for 10 min and subsequent phase separation by centrifugation. RNA and DNA was retained in the aqueous phase, precipitated together and stored at -80 °C for further use for metatranscriptomics analysis (Pfreundt et al., 2016)~~(Pfreundt et al., 2015)~~ and 16S tag sequencing. For the latter, ~100 ng of RNase treated total DNA was sent to a commercial provider (LGC Genomics, Berlin, Germany) for amplicon sequencing with primer pair S-D-Bact-0341-b-S-17/S-D-Bact-0785-a-A-21 (Klindworth et al., 2013), that targets bacteria and archaea. Briefly, 16S hypervariable regions 3-4 were amplified and the amplicons sequenced on a MiSeq (Illumina) sequencer, generating between 31,466 and 749,629 paired-end reads (2x300) per sample.

All bioinformatics steps were done using the USEARCH package and following the UPARSE pipeline (Edgar, 2013) unless mentioned otherwise. Briefly, lowest quality tails were truncated from all reads and the paired reads merged using the -fastq_mergepairs command and yielding between 25,221 and 604,457 reads of average length > 400 nt per sample. Merged reads were then quality filtered, reads shorter than 350 nt discarded, and converted to fasta. All fasta files were concatenated for OTU clustering, with the following steps: dereplication at 100% identity and keeping information on the weight of each unique sequence, sorting of unique sequences by decreasing weight and discarding of singletons (weight=1), clustering into OTUs with the -cluster_otus command using a maximum dissimilarity of 2 %. The last steps includes chimera filtering (Edgar, 2013). Finally, the merged reads from the different samples were mapped back onto the generated OTUs using vsearch v1.1.3 (Torbjørn Rognes et al., 2015) with at least 98 % identity to create the final OTU table. For taxonomic classification of the OTUs, they were submitted to https://www.arb-silva.de/ngs/ and classified using the SILVA SSU taxonomy and database release SSU 119.1 (Quast et al., 2013). Cyanobacterial OTUs that SILVA had only classified to family level were manually curated by BLASTN searches of these OTUs against NCBI nt. If a hit with > 95% sequence identity was found, the genus of this classification was added to the OTU table. For all further use, relative OTU abundances in each sample were calculated.

Inference of absolute cell numbers from 16S data and flow cytometry.

~~To calculate pseudo-~~Aabsolute cell numbers were inferred from these relative OTU abundances in three steps. First, raw read counts per OTU were normalized to equal 16S copy numbers ("16S-norm reads") using a manually curated table of 16S copies per genome for all OTUs (Suppl. Dataset). For OTUs with no available genome, an average copy number of all genomes from marine representatives of the next higher phylogenetic clade was used (for the detailed procedure see Suppl. Dataset). Second,~~,~~ ~~for each sample~~ the cumulated ~~relative~~

~~abundances of~~raw reads for *Synechococcus* (Syn) and *Prochlorococcus* (Pro) were divided by the respective sum of *Synechococcus* and *Prochlorococcus* cytometric cell counts for each sample, producing a sample-specific ratio (~~%~~16S-norm reads/cells mL^{-1}). We used ~~these two groups~~Syn and Pro, because flow cytometry is well-established for them and we could expect them to be reasonably precise. This ratio was then used to calculate cells mL^{-1} ~~numbers~~ for all ~~investigated phylogenetic groups~~OTUs by dividing their 16S-norm reads ~~relative abundance~~ in each sample by the sample-specific ratio. We are aware that missing genomic information may lead to errors in this calculation. OTUs for which no 16S copy number could be estimated, were set to 1 copy/genome.

Calculation of diversity measures.

The Shannon and Simpson indices~~ex~~ as ~~a~~ measures of diversity were~~as~~ calculated separately for M1 and Nouméa lagoon waters using the *diversity* function in the R package vegan (Oksanen et al., 2015).

2.3 Multivariate statistics of 16S community data, samples and environmental data

Statistical tools were all used with relative 16S abundance data. A canonical correspondence analysis (CCA) (XLSTATS ~~on~~ Excel software) was used to test for significant effects of biogeochemical parameters on variations in the bacterial 16S-community structure. These parameters were PP, BP, Chl, APA, DIP, N_2 fixation rates, particulate organic carbon concentrations (POC) and temperature (T). CCA generates ordination axes which are linear combinations of the environmental variables that best explain microbial diversity composition data (Braak, 1986)~~(ter Braak, 1986)~~. The variables were the log-transformed biogeochemical stocks and fluxes, the objects were the 34 dominating OTUs in terms of abundance (expressed in relative OTU abundance), and the sites were sampling time and origin of the samples.

Non Metric Multidimensional Scaling (NMDS) was used to find the best display of differences in 16S community structures. The full OTU table containing relative abundances of each OTU per sample, with samples as rows and species as columns, was used for unconstrained ordination with the R package vegan (Oksanen et al., 2015). Additionally, the same procedure was run with the subset tables containing only "heterotroph" OTUs (i.e. all that are not classified as *Cyanobacteria* or chloroplasts) or only "autotroph" OTUs (i.e. all classified as chloroplasts or *Cyanobacteria* without *Melainabacteria*). First, the abundances were standardized using Wisconsin double standardization, which divides abundances by the species maximum and then standardizes samples to equal totals. This converts abundances per species

into relative values and therefore reduces the weight of dominant species and increases the weight of rare species. Then, Bray-Curtis distance matrices were computed and an NMDS ordination calculated using metaMDS. MetaMDS did not use any further transformations or standardizations. Assuming that samples of day 2-8, day 10-16, and day 18-21 cluster together, ellipses were drawn using the standard error for each cluster in the ordination plot with a confidence limit of 0.95. Like this, we could consider clusters with non-overlapping ellipses as significantly different from each other at a significance level alpha = 0.05. Permutational Multivariate Analysis of Variance (PERMANOVA) was conducted using the adonis function in the R package vegan on the Bray-Curtis distance matrices described above. We tested the dependence of sample distances on the factors *location* (M1 or lagoon waters), *depth* (1 m or 12 m) and *time* (separation of samples into three phases: day 2-8, 10-16, 18-21).

To test significance of higher *Cyanothece* 16S abundances in M1 during P2, 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 were significantly different. We found that P2 in M1 was significantly different from the other 3 groups (Tukey's HSD p-values 0.00002, 0.00009, 0.00003) and that in the lagoon P2 was not significantly different from P0/P1.

Pearson and Spearman rank correlations were performed in R using the rcorr() function, which also calculates the significance of the derived correlation coefficients. A coefficient was defined as significant, if $p \leq 0.05$.

3 Results

3.1 Biogeochemical context

Here, we present data on the prokaryotic community in M1 and the Nouméa lagoon from depths of 1 m and 12 m, which was close to the bottom of the mesocosm. As a foundation for understanding and discussing these data, selected biogeochemical parameters are shown in Fig. 1 ~~and Fig. 2c, 2d~~ for the corresponding sampling locations. On the evening of day 4, M1 was fertilized with ~0.8 µM DIP, leading to lower APA than in the lagoon until day 19, when APA in M1 increased steeply (Fig. 1c~~, 2e~~ 1d) and DIP turnover times dropped below initial levels (Van Wambeke et al., 2015).~~,~~ Of the three mesocosms, the here investigated M1 was the one with the lowest increase of Chl *a* during P2, still reaching higher levels than in Nouméa lagoon waters, whereas PP in M1 did not exceed the values measured in the lagoon (Fig. 1a, 1b). BP exhibited two prominent peaks in M1 with a 4-fold and 2-fold increase, on day 4 and on day

21, and was otherwise close to values measured for Nouméa lagoon waters, only slightly exceeding them on most days after day 11 (Fig. 1ed).

3.2 Bacterial community based on 16S tag MiSeq sequencing

3.2.1 An overview

We identified a total of 3,600 OTUs belonging to bacteria, archaea, and chloroplasts using 16S rRNA gene sequencing data from all samples combined (17 samples from M1, 20 samples from the Nouméa lagoon, Supplementary Suppl. Dataset). In the following, the term "16S community" will be used for the entirety of all 16S reads belonging to these OTUs. On this basis, the Shannon and Simpson indicesex as a measures for overall diversity wereas calculated. With values between 5.6 and 7 (Shannon) at both investigated depths over the 23-days experiment, diversityit was relatively high., although vValues above 5 have been reported for the edge of the South Pacific gyre (Yin et al., 2013), but based on clone libraries, which might underestimate diversity. Over the course of the experiment we observed a slight decrease in diversity both in M1 and in the lagoon (Suppl. Fig. S1).

Throughout Results and Discussion, we will use absolute abundances inferred from 16S sequencing data and flow cytometry counts for *Synechococcus* and *Prochlorococcus* (see Methods section 2.2 and Suppl. Dataset). The temporal evolution of relative (16S) and absolute (flow cytometry) *Synechococcus* abundances in M1 diverged substantially after day 4 (compare Suppl. Fig. S4f and 2a). As *Synechococcus* and *Prochlorococcus* together constituted up to 30 % of all 16S reads, changes in absolute cell counts for these groups can highly influence the temporal evolution of relative 16S data. To elude possible ambiguous interpretation of these data, we used the combined *Synechococcus* and *Prochlorococcus* cytometric counts to calculate a `16S/cells ml^{-1}´ ratio for each sample (see section 2.2). This ratio was then used to calculate absolute cell numbers from relative 16S abundance in M1 for all other groups investigated here (Figs. 3, 4). Different 16S copy numbers per genome for different OTUs/phylogenetic groups were taken into account (Suppl. Dataset). We hope that this combinatorial approach using 16S sequencing, flow cytometry and 16S copies/genome may serve as a blueprint for future 16S based studies. In the following, the term "16S community" will be used for the entirety of all 16S reads belonging to these OTUstotal inferred cell count per sample..

The dominating classes in M1 were alpha- and gammaproteobacteria, cyanobacteria, chloroplasts, Marine Group II (MGII) euryarchaea (*Thermoplasmata*, mainly days 10 and 14), Acidimicrobiia, and and on days 12-23 also *Flavobacteriia* (Fig. 3a). Of these, alpha- and gammaproteobacteria exhibited clearly different dynamics between M1 and the lagoon with

completely different peaking times, whereas cyanobacteria, chloroplasts, ~~and~~ MGII euryarchaea, and Flavobacteriia showed similar trends (Fig. 3~~a~~, 4~~a~~, correlations in Suppl. Fig. S2a).

Amongst the classes contributing less than 5 % to the total 16S community, dynamics were generally different between M1 and the lagoon (Suppl. Fig. S2b). Inside M1, *Deferribacteres* (almost entirely the SAR406 clade) and *Deltaproteobacteria* abundances ~~decreased from > 1.2 % to < 0.7 % of the 16S community~~halved after the DIP fertilization and increased again when DIP concentrations fell below 0.1 nM on day 12 (Fig. 3c). ~~*Cytophagia* and *Flavobacteriia* also~~ All other groups increased at ~~the same~~similar times, ~~while~~ except for *Actinobacteria*, ~~and *Phycisphaerae* increased on day 20~~which responded later (Fig. 3b). Except for the increase in *Flavobacteriia* and cyanobacteria (predominantly *Synechococcus*), none of these late-phase increases were observed in the lagoon (Fig. 4), although several of the less abundant classes (*Cytophagia*, *Betaproteobacteria*, *Actinobacteria*) more than doubled from day 16 to 18 in the lagoon, but ~~also~~ decreased just as quickly after that (Fig. 4b).

3.2.2 Cyanobacteria

Synechococcus sp. (OTU-9) was the dominant OTU throughout the experiment in M1 and the lagoon, being almost an order of magnitude more abundant than the second ranked OTU at some time points. The steep drop of *Synechococcus* abundance on day 4 in M1, two days after the mesocosm closure, was equally seen in absolute counts by flow cytometry (from ~ 78000 to ~ 44000 cells mL^{-1}, Fig. 2a) and in the relative 16S data (Suppl. Fig. S4f). Please note that the inferred absolute abundances shown in Figs. 3f and 4f are not the same as the flow cytometry counts for Syn and Pro. We used the sum of Syn and Pro to infer the sample-specific ratios for the calculation to reduce putative counting biases in one of the two groups (see Discussion). Inferred cell counts for *Synechococcus* were only 61000 and 25000 cells mL^{-1} for these two days. At the same time in M1, *Prochlorococcus* inferred abundance reached a maximum of 37000 cells mL^{-1} on day 6, corresponding to ~16000 cells mL^{-1} counted with flow cytometry (Fig. 2a and 3f). Variations in *Synechococcus* abundance correlated with changes in Chl concentrations, except for days 2-5 with high *Synechococcus* cell counts but low Chl concentrations (Fig. 1b, 2a). N_2-fixing cyanobacteria were generally one to two orders of magnitude less abundant than *Prochlorococcus* and *Synechococcus* and were in M1 most prevalent on days 2 - 4, owing mainly to *Trichodesmium* with ~5000 cells mL^{-1} (Fig. 3f). After the DIP fertilization, *Trichodesmium* 16S tags dropped quickly by a factor of ten, leaving *Calothrix* as the most abundant diazotroph until day 12. Important to mention, the SILVA

database used for 16S classification lists all *Richelia intracellularis* 16S sequences as descendants of the *Calothrix* node. This is discussed in section 4. Candidatus *Atelocyanobacterium thalassa* (UCYN-A) constituted the third most abundant diazotroph until day 14. UCYN-A may be seen as a heterotrophic diazotroph, as it misses photosystem II, cannot fix CO_2 into biomass (Tripp et al., 2010), and lives in symbiosis with unicellular algae (Thompson et al., 2012). Between day 14 and 16, *Cyanothece* (UCYN-C) 16S tags increased by an order of magnitude, exhibiting significantly higher levels in M1 during P2 than during P0/P1 and in the lagoon (ANOVA followed by Tukey's Honestly Significant Difference test, all p-values <0.0001). UCYN-C was the most abundant diazotroph on day 16 in M1 with ~400 cells mL^{-1} (Fig. 3f). Heterotrophic diazotrophs like *Bradyrhizobium* and *Mesorhizobium* were, with a maximum of 0.00013 % of the community for the former, not present in numbers anywhere close to N_2-fixing cyanobacteria (Suppl. Dataset). In the lagoon samples, the two dominant diazotrophs (*Trichodesmium* and *Calothrix*/*Richelia*) were the same, but with different dynamics (Fig. 4f). Notably, *Cyanothece* OTUs did not increase in abundance in the lagoon.

3.2.32 Heterotrophic bacteria

As alpha- and gammaproteobacterial 16S abundances showed close to zero correlation between M1 and the lagoon (Suppl. Fig. S2a), we examined the dynamics within these classes at family level. We observed clear successions within M1 that were not mirrored in the lagoon (Figs. 3c, 3d and 4c, 4d). ~~Already o~~On day 4 (two days after the mesocosms had been closed around the water column), ~~the relative share of~~ *Rhodobacteraceae* ~~16S tags~~ increased from <10000~~3.5~~ to ~~12.5 %~~almost 35000 cells mL^{-1} in M1, and dropped~~, exhibiting the most extreme dynamics of all families~~ (Fig. 3c). One particular OTU (297), classified as *Thalassobium* sp., contributed 79 % of *Rhodobacteraceae* sequences on that day, but only 2-20 % in other samples. This peak corresponded to increased transcript accumulation from *Rhodobacteraceae* seen in the metatranscriptome analysis (Pfreundt et al., 2016)~~(Pfreundt et al., 2015)~~ and a 4-fold increase in bacterial production on that day (Fig. 1e~~d~~). This situation occurred the morning before the DIP fertilization and was accompanied by a ~~relative~~ 30-50% increase in SAR86, SAR11, and *Rhodospirillaceae* ~~by 35 and 45 % to >13 % and 9.7 % of the 16S community, respectively~~. Notably, a similar increase of SAR11 was observed in the lagoon (Fig. 4c), speaking against a M1-specific trigger for this group. In addition, a M1-specific ~~60 % drop in *Alteromonadaceae* down to 2 %, and a~~ severe drop of *Synechococcus* from >60000 to 25000 cells mL^{-1} ~~20 % to < 8 % of the 16S community were also~~was observed (Fig. ~~3c, 3d,~~ 3f). While *Rhodobacteraceae*

dropped to ~ 5000 cells mL^{-1} on day 6~~to initial values~~ - similar to lagoon ~~those in the~~values ~~lagoon - on day 6~~ and ~~contributed around 3 %~~stayed fairly stable until day 1~~4~~6, ~~relative abundance of~~ SAR11, dominated by the Surface 1 group, increased from 30000 to >100000 cells mL^{-1}~~8 to 25 % of the 16S community~~ on day 8 and outcompeted all other heterotrophic bacteria in M1 (Fig. 3c~~, 3d~~). SAR11 was replaced by SAR116~~, *Rhodospirillaceae* (mainly AEGEAN-169), and SAR86~~ two days later, ~~on day 10, which constituted~~ the ~~three almost equally dominant~~most numerous alphaproteobacteria ~~groups until day 18 with 7 to 12 % of the 16S community each.~~ for most of the experiment, and surpassed in numbers only by SAR86 at the end of the experiment (Fig. 3d). ~~On day 21,~~After day 14, *Rhodobacteraceae* other than *Thalassobium* became ~~the dominant~~more numerous again ~~heterotrophs~~ both in M1 and the lagoon (Fig. 3c, 4c).

Within alphaproteobacteria, SAR11 abundance dynamics were completely uncorrelated between M1 and the lagoon, whereas other groups were slightly positively correlated (~~Pearson correlation ~ 0.5,~~ Suppl. Fig. S2c). Within gammaproteobacteria, *Alteromonadaceae* and SAR86 together constituted between 75 % and almost 90 % (Fig. 3d) and whereas the former showed very similar dynamics between M1 and the lagoon waters, SAR86 did not (Suppl. Fig. S2d). A second abundance peak of SAR86 occurred only in M1 on days 16 and 18 and coincided with similar peaks of ~~the~~ less abundant heterotrophic classes~~ *Deferribacteres* (SAR406), *Betaproteobacteria*, and *Cytophagia*~~ (Fig. 3b, 3d). While in M1, SAR86 never decreased below ~~7.5 %~~30000 cells mL^{-1} with peaks of 80000 and 110000 cells mL^{-1}~~in M1~~, these bacteria ~~constituted below~~ only 2.5 - 6 % of the 16S community were mostly around or below 30000 cells mL^{-1} in the lagoon after day 4~~throughout P2 and dropped to 2.5 % on day 21~~.

Within *Flavobacteriia*, the dominant families exhibited highly correlated temporal dynamics in M1 and the lagoon and also between the different families (Suppl. Fig. S2e). The *Flavobacteriaceae* family dominated, increasing ~~more~~ from 4000 to 32500 cells mL^{-1} ~~than three-fold~~ between day 10 and 1~~8~~2 in M1 (Fig. 3e), in the period of DIP consumption~~, and~~ coinciding with higher Chl concentrations from day 12 onward (Fig. 1b, 1~~2~~c). ~~The~~ A maximum ~~relative~~ abundance of ~~almost 15 %~~38000 cells mL^{-1} was reached in the lagoon also on day 18 (Fig. 4e). ~~In M1, *Flavobacteriaceae* constituted at most 9 %. Two other families, the NS7 and~~The NS9 marine group, second most abundant flavobacterial groups~~,~~ had very low abundances until day 1~~6~~8, ~~then~~ and increased ~~afterwards~~towards the end of the experiment in both locations.

Generally, a steep increase (>2-fold) of total non-photosynthetic cells (abundant classes except cyanobacteria and chloroplasts) from day 12 to day 16 was seen in M1 (Fig. 3a, 3b), which was not seen in flow cytometry counts of total heterotrophic bacteria (Fig. 2b) but was accompanied by a moderate increase (50 %) in BP (Fig. 1e), supporting the former. Spearman rank correlation between BP and inferred cell counts for non-photosynthetic bacteria was 0.56, p=0.09 (0.72, p=0.03 when omitting day 21 with its prominent BP peak). N_2-fixation rates were also substantially higher throughout this period (days 12 to 16) than before and stayed high until the end of the experiment on day 23 (Fig. 1f). Preceding and overlapping this reaction of heterotrophs was a constant increase in *Synechococcus* from its minimum of ~ 20000 cells mL^{-1} on day 9 to maximally 104000 cells mL^{-1} on day 19-20 (Fig. 2a).

~~3.2.3 Cyanobacteria~~

~~*Synechococcus* sp. (OTU-9) was the dominant OTU throughout the experiment in M1 and the lagoon, being almost an order of magnitude more abundant than the second ranked OTU at some time points. The steep drop of *Synechococcus* relative abundance on day 4 in M1, two days after the mesocosm closure, was equally seen in absolute counts by flow cytometry (from ~ 78000 to ~ 44000 cells mL^{-1}, Fig. 2a, 3f). At the same time in M1, *Prochlorococcus* gained in relative and absolute abundance reaching a maximum of 8 % of the 16S community on day 6, corresponding to ~ 16000 and ~ 18000 cells mL^{-1} on day 6 and 8, respectively (Fig. 2a and 3f). Variations in *Synechococcus* abundance correlated with changes in Chl concentrations, except for days 2-5 with high *Synechococcus* cell counts but low Chl concentrations (Fig. 1b, 2a). N_2-fixing cyanobacteria were generally one to two orders of magnitude less abundant than *Prochlorococcus* and *Synechococcus* and were in M1 most prevalent on days 2 and 4, owing mainly to *Trichodesmium* with a maximum of 1.5 % of the 16S community~ 5000 cells mL^{-1} on day 2 (Fig. 3f). After the DIP fertilization, *Trichodesmium* 16S tags dropped quickly by a factor of ten, leaving *Calothrix* as the most abundant detected diazotroph until day 12. Important to mention, the SILVA database used for 16S classification lists all *Richelia intracellularis* 16S sequences as descendants of the *Calothrix* node. This is discussed in section 4. Candidatus *Atelocyanobacterium thalassa* (UCYN-A) constituted the third most abundant diazotroph until day 14. UCYN-A may be seen as a heterotrophic diazotroph, as it misses photosystem II, cannot fix CO_2 into biomass (Tripp et al., 2010), and lives in symbiosis with unicellular algae (Thompson et al., 2012). Between day 14 and 16, there was a steep increase of *Cyanothece* (UCYN-C) 16S tags increased by an order of magnitude, which had been increasing steadily after day 10, but at generally low levels~~exhibiting significantly higher levels in M1 during P2

than during P0/P1 and in the lagoon (ANOVA followed by Tukey's Honestly Significant Difference test, all p-values <0.0001). Nevertheless, UCYN-C was the most abundant diazotroph on day 16 in M1 with ~400 cells mL^{-1} (Fig. 3f). Heterotrophic diazotrophs like *Bradyrhizobium* and *Mesorhizobium* were, with a maximum of 0.00013 % of the community for the former, not present in numbers anywhere close to those for N_2-fixing cyanobacteria (Suppl. Dataset). In the lagoon samples, the three two dominant diazotrophs (*Trichodesmium* and *Calothrix*/*Richelia*)groups were the same, but with different dynamics (Fig. 4f). Notably, *Cyanothece* OTUs did not increase in abundance in the lagoon.

After day 4, the temporal evolution of relative (16S community) and absolute (flow cytometry) *Synechococcus* abundances in M1 diverged substantially (compare Fig. 3f and 2a). As *Synechococcus* and *Prochlorococcus* together constituted over 90 % of the cyanobacterial 16S community and thus up to 30 % of all 16S reads, changes in absolute cell counts for these groups can highly influence the temporal evolution of relative 16S data. To elude possible ambiguous interpretation of these data, we used the combined *Synechococcus* and *Prochlorococcus* cytometric counts to calculate a %16S/cell ratio for each sample (see section 2.2). This ratio was then used to calculate absolute cell numbers from relative 16S abundance in M1 for all other groups investigated here (Suppl. Figs. 3, 4). We assumed equal 16S gene copy numbers.Different 16S copy numbers per genome for different OTUs/phylogenetic groups were taken into account (Suppl. Dataset).

Interestingly, this approach revealed a steep increase (> 2-fold) of total non-photosynthetic cells (sum of all abundant classes except cyanobacteria and chloroplasts per sample) from day 12 to day 16 in M1 (Suppl. Fig. 3)that was not seen in flow cytometry counts of total heterotrophic bacteria (Suppl. Fig. 3) but was accompanied by a moderate increase (50 %) in BP (Fig. 1ed), supporting the former. Spearman rank correlation between BP and 16S-derived numbersinferred cell counts for non-photosynthetic bacteria was 0.5661, p=0.09 (0.72, p=0.033 when omitting day 21 with its prominent BP peak). N_2-fixation rates were also substantially higher throughout this period (days 12 to 16) than before and stayed high until the end of the experiment on day 23 (Fig. 1fd). Preceding and overlapping this reaction of heterotrophs was a constant increase in *Synechococcus* from its minimum of ~ 20000 cells mL^{-1} on day 9 to maximally 103000-104000 cells mL^{-1} on day 19-20.

3.2.4 Temporal dynamics of the 16S community

Using all OTUs as a basis, we did unconstrained ordination of all samples on Bray-Curtis dissimilarities calculated from standardized relative abundances. Non-metric multidimensional

scaling (NMDS) showed that inside M1 as well as in lagoon waters, the microbial communities defined three distinct clusters (Fig. 5a). Days 2-8, days 10-16, and days 18-21 (the last day 16S rRNA gene sequencing was done for) were grouped together and the clusters separated along the first MDS axis both in lagoon waters and in M1, whereas the locations *lagoon waters* and *M1* (color coded in blue and orange, respectively) were also clearly separated along the second MDS axis. These clusters-in-time will be called *early*, *mid-time*, and *late* from here on and were time-delayed from the periods P0, P1, and P2 defined by biogeochemical parameters, production rates, and N_2 fixation (see section 2.1). Thus, communities from M1 and the lagoon were clearly different from each other but followed a similar trajectory with time. When we separated the autotrophic community (547 OTUs) from the heterotrophic community (2981 OTUs, all OTUs other than cyanobacteria and chloroplasts) for ordination, it became evident that the heterotrophs showed a pattern very similar to the full community (Fig. 5b). The autotrophic community also clearly separated *early*, *mid-time*, and *late* phases from each other in M1 and the lagoon, but not as far, and separation by location (*M1* vs. *lagoon waters*) only really became evident in the *late* phase (Fig. 5c). Analysis of variance (PERMANOVA) showed that indeed the community structures of the full OTU set, as well as both separate OTU sets, were significantly different between the three phases (predictor *time*) and were also different according to their origin, *M1* or *lagoon waters* (predictor *location*, *p*-values < 0.001, for autotrophs $p = 0.003$). Using the interaction of the predictor *location* with *time* additionally showed that *time* still had a significant effect after taking out the *location* effect but not the other way around. This shows that the effect of *time* was stronger and included the effect of *location*. Depth had no significant effect.

3.2.5 Effect of hydrological and biogeochemical parameters on the community structure of dominant OTUs

The 34 dominant OTUs (in terms of summed relative abundances of all samples) were subjected to constrained ordination within the frame of eight environmental variables (BP, PP, APA, Chl, POC, N_2 fixation, DIP, and temperature (T)), taking all samples from M1 and the lagoon from 1 m and 12 m depth into account (Fig. 6). The sum of all unconstrained eigenvalues was 0.304, the sum of canonical eigenvalues 0.130. Thus, the amount of total variation in the species data that could be explained by the employed environmental variables was 43 % (permutation test, $p < 0.001$ after 999 permutations). The canonical axes F1 and F2 accounted for 50.5 % and 22.3 % of this total explainable variance and 21.6 % and 9.5 % of the total variance, respectively. Both axes were significant and displayed strong species-environment correlations

($p < 0.001$ for both axes). Samples (location and time, Fig. 6b) were mainly separated along F1 with day 6 to 14 towards negative values and day 16 to 21 towards positive values. Interestingly, samples from M1 were separated further apart from each other than those from the lagoon. The main parameters separating samples and OTUs (Fig. 6a) along the F1 axis were DIP, which was clearly higher in M1 samples following the DIP fertilization (days 6-10), and BP, Chl, and PP in opposition to DIP. Highly associated with BP and Chl were the *late* phase samples from M1 and lagoon, together with *Rhodobacteraceae* OTUs 1 and 37 and the flavobacterial NS4 marine group OTU 44, suggesting these directly profited from primary producers at the end of the experiment. Interestingly, another *Rhodobacteraceae* OTU (23) was tightly associated with N_2 fixation and with the OM60(NOR5) OTUs 2 and 18 from the *Halieaceae* family of gammaproteobacteria. Other gammaproteobacterial OTUs, *Alteromonas* OTU 13 and SAR86 OTUs 7 and 26, were positioned oppositely, closer to high DIP values, and together with all three SAR11 OTUs (43, 60, 2922), three of four SAR116 OTUs (16, 20, 34), the *Rickettsiales* group S25(593) OTU 27 and Candidatus Actinomarina OTU 6 (*Actinobacteria*). Most tightly associated with high DIP concentrations was *Defluviicoccus* OTU 40 (*Rhodospirillaceae*). Also in the same quadrant, but forming a separate cluster, were MGII euryarchaea. Two more SAR86 OTUs (98, 1229) were separated from the rest and linked with *Prochlorococcus* and with the very early samples from day 2-4 in M1 and day 2-8 in the lagoon. One MGII and one SAR116 OTU (47 and 19) were also separated from the rest, possibly profiting from higher temperatures. More tightly linked with increasing temperatures and APA was the dominant flavobacterial OTU (*Sufflavibacter* OTU 67) in lagoon samples from days 16 to 21. Noteworthy, there was an opposition between the two dominant autotroph OTUs, *Synechococcus* (9) and *Prochlorococcus* (4), where the former was closely linked to the increase of PP and Chl at the end of the experiment. *Rhodospirillaceae* of the Aegean-169 group were positioned opposite to APA, T, and PP and close to *Prochlorococcus* and two chloroplast OTUs (3 and 1677), suggesting they might profit from these autotrophs specifically.

The quadrants resulting from this CCA nicely correspond to the clusters-in-time found through NMDS (section 3.2.4), with few overlaps between quadrants: the bottom left corresponds to the *early* phase, the top left to the *mid-time* phase, and both right quadrants together to the *late* phase (compare Fig. 6 and Fig. 5).

4 Discussion

4.1 Inference of absolute abundances from relative 16S data

Our results rely on the calculation of absolute cell counts from relative 16S abundance data obtained by MiSeq sequencing. This is possible by supplementing these data with flow cytometry data and 16S copy numbers per genome. Whereas major trends could likewise be observed in the relative data (Suppl. Figs. S4, S5), the inferred absolute data give a much more precise picture of the bacterial community and have the power to reveal absolute abundance changes of specific groups within. However, there are major potential error sources that need to be mentioned. First of all, the curated table of 16S copy numbers per genome is a conscientious estimate and some estimates might well be corrected as more genomes of marine bacteria are being sequenced. Second, flow cytometry might miss some Syn or Pro, if cells are part of aggregates. This would matter if the fraction of Syn or Pro in aggregates changed between samples. To test for biases, we calculated the ratio of Syn to Pro for 16S-copy-number-normalized 16S read counts (16S-norm reads) and for flow cytometry counts. In an error-free scenario, both should give the same ratios. With a systematic error source, e.g. constant percentage of Syn in aggregates, the ratios would differ by a similar value in all samples. However, we observed varying ratio differences (Suppl. Dataset, tab *ratio calculation*). In M1, Pro was either underestimated in flow cytometry or Syn underestimated in the 16S data, leading to higher Syn/Pro ratios in flow cytometry data than in 16S data for most samples. In the lagoon, this was the case until day 16, the samples after that showed the opposite. Apart from differential aggregation between samples, a reason for this might be that the assumed 16S copy number of two for Syn and one for Pro did not reflect the real populations, but that the initial Syn population had only one copy (or the Pro population two copies), leading to Syn underestimation in 16S data. These errors are reduced by using the sum of Syn and Pro for the calculation of the 16S-norm/cells mL^{-1} ratio. However, the reader should be aware that the inferred absolute counts are mere estimates.

4.2 *Synechococcus* as the most numerous primary producer

In the world's oceans, the unicellular cyanobacteria *Prochlorococcus* and *Synechococcus* constitute the most abundant marine picophytoplankton, amounting together up to 10^6 cells mL^{-1}, and ~~are~~ responsible for up to 50 % of the total CO_2 fixation in some regions (Liu et al., 1997; Li, 1994; Veldhuis et al., 1997). Previous studies (Zwirglmaier et al., 2008) showed that *Synechococcus* abundance is low in the oceanic oligotrophic gyres, reaching only 4 x 10^3 cells mL^{-1}, but up to 10^5 cells mL^{-1} in nutrient-rich upwelling regions (see also the data compilation in (Flombaum et al., 2013). Therefore, the cell counts reported here for *Synechococcus* in the

New Caledonian lagoon were with up to 10^5 cells mL^{-1} among the highest reported thus far for marine waters. In contrast, the maximum values for *Prochlorococcus* were relatively low. Several studies indicated that *Prochlorococcus* is adapted to ultra-oligotrophic conditions and is outcompeted by *Synechococcus* in waters of higher nutrient content, such as upwelling and coastal regions (Partensky et al., 1999a, 1999b), which is consistent with the findings in this study (Leblanc et al., 2016)~~(Leblanc et al., 2015)~~. The *Synechococcus* group can be further delineated into several major clades with different ecophysiologies (Scanlan et al., 2009). Due to the insufficient phylogenetic resolution, this was not possible based on 16S rRNA gene sequencing. However, in the accompanying metatranscriptome analysis (Pfreundt et al., 2016)~~(Pfreundt et al., 2015)~~, we found that the *Synechococcus* population was dominated by representatives of clade II and to a lower extent, clade IX, within the picophytoplankton subcluster 5.1A. ~~These may correspond to the *Synechococcus* cells with low and high orange fluorescence, detected during cell sorting by flow cytometry (Van Wambeke et al., 2015), because the phycourobilin:phycoerythrobilin ratio differs between cultured representatives of these clades (Six et al., 2007) and phycourobilin may lead to high orange fluorescence.~~ Recent analyses have suggested that the different frequencies of co-occurring *Synechococcus* ecotypes partition the ocean into four distinct regimes that can be distinguished by temperature, macronutrients and iron availability, suggesting clade II ecotypes are competitive in warmer, oligotrophic conditions (Sohm et al., 2015), which is consistent with the results of our metatranscriptomic analysis (Pfreundt et al., 2016)~~(Pfreundt et al., 2015)~~. Measurements of BP and PP revealed that the organic matter produced by non-diazotrophic phytoplankton was the major carbon source fueling heterotrophic prokaryotes (Van Wambeke et al., 2015). Our community analysis identifie~~d~~s among these primary producers *Synechococcus* as ~~the numerically~~ most ~~abundant~~ numerous contributor.

4.3 Effects of enclosure and DIP fertilization on the microbial community

Apart from investigating the effect of changing diazotroph communities on general community structure, we judged to which extent the enclosure and the DIP fertilization in the deployed large mesocosms changed community composition and development compared to the surrounding lagoon waters. Roughly, the 16S community ~~composition~~ partitioned the M1 samples into three periods, an *early* period of DIP availability (including samples before the spike) until day 8, a *mid-time* period of DIP consumption, and a *late* period of Chl accumulation/P depletion starting with day 18. Remarkably, although we enriched the mesocosms with ~0.8 µM DIP and noticed a significant difference between the communities in

M1 and in lagoon waters (Fig 5a, separation along the second ordination axis, $p < 0.001$), the evolution of ~~the~~ both communities with time was mostly parallel, shown by their parallel separation along the first ordination axis. This indicates an overarching influence of abiotic factors acting on both locations equally, like temperature, which increased during the course of the study. Individual OTUs, like *Sufflavibacter*, were shown to be positively linked with increasing temperatures. These relations may be linked to significantly enhanced chemotactic abilities at higher temperatures, as was recently shown for a coral pathogen (Garren et al., 2015). Despite the parallel evolution of the full community (rare and abundant OTUs had similar weights in NMDS), community composition and dynamics of single abundant groups in M1 were different to those in the lagoon from the beginning. These differences first became evident with the drop in *Synechococcus* ~~cell counts and 16S reads~~ on days 2 to 4 (Fig. 2a, 3a). In this process, cyanophages might have played a role as suggested by the ~~detected~~ high gene expression from *Myoviridae* detected in M1 compared to the lagoon (Pfreundt et al., 2016)~~(Pfreundt et al., 2015)~~. Based on the known host association of these T4-like phages (Frank et al., 2013; Ma et al., 2014; Sabehi et al., 2012), these were likely targeting *Synechococcus*. This event and its putative consequences on the availability of organic material coincided with diatoms sinking out of the water column in the mesocosms, roughly halving in numbers (Leblanc et al., 2016)~~(Leblanc et al., 2015)~~, and overlapped with DIP fertilization on day 4. Thus, it cannot be conclusively determined which had a higher influence on the community differences we observed in the *early* phase of the experiment, decaying phytoplankton or increased DIP concentrations. Van Wambeke et al (Van Wambeke et al., 2015) showed that BP was not P-limited on day 4, suggesting that phytoplankton decay had the greater effect. Indeed, POC concentrations in M1, but not in the lagoon, increased from ~10 µmol L^{-1} on day 2 to 15 µmol L^{-1} on day 4 (Berthelot et al., 2015).,

We correlated the relative abundances in M1 and the Nouméa lagoon for all investigated phylogenetic groups (Suppl. Fig. S2). This approach revealed alpha- and gammaproteobacteria as the least correlated classes between M1 and the lagoon. Investigating this aspect at higher taxonomic resolution showed that SAR11 and SAR86 temporal dynamics differed the most, suggesting that these groups were the most sensitive to changing nutrient sources. SAR11 was present at comparatively low numbers compared to other reports (Morris et al., 2002). We used filters with 0.45 µm pore size, so it cannot be excluded that SAR11 (average size 0.2 µm x 0.4 µm) and the even smaller Candidatus *Actinomarina* were lost at the beginning of filtering and retained when more other cells were already on the filter. Thus, we have possibly missed a substantial fraction of these groups in this study. Nevertheless, we can expect the loss rate to

be very similar ~~in all our~~across all samples, due to the following reasons: 16S and metatranscriptomics analyses were done from DNA and RNA isolated together from the same filter. Transcript~~ome~~ abundances were tightly correlated (Pearson correlation 0.88 in M1, and 0.96 in the lagoon) between SAR86 and SAR11 (Pfreundt et al., 2016)~~(Pfreundt et al., 2015)~~ ~~over~~ across all samples. With different loss rates of SAR11 in different samples this correlation should not be possible.

SAR86, like SAR11, shows metabolic streamlining, but while SAR11 genomes contain putative genes for synthesis of most amino acids (Grote et al., 2012), a substantial fraction of ~~natural~~ SAR86 is probably auxotroph for Met, His and Arg (Dupont et al., 2012). Oppositely, SAR86 genomes encode a disproportionally high number of Ton-B dependent outer membrane receptors (TBDRs) that allow transport of > 600 Da molecules, including carbohydrates and lipids, combined with lipase genes and an expanded sugar utilization metabolism, whereas SAR11 genomes are not known to contain any TBDR genes and have strongly reduced sugar utilization capabilities (Dupont et al., 2012). In our accompanying metatranscriptome study, transcripts for TBDRs and phospholipase (days 14-16) from SAR86 were highly abundant, suggesting membrane lipids were used as an energy source (Pfreundt et al., 2016)~~(Pfreundt et al., 2015)~~. Cultivation experiments of SAR11 representatives have shown their ability to utilize a wide variety of small organic molecules, including peptides, to generate energy (Tripp, 2013). The SAR11 subgroup Ia (surface 1) representative *Pelagibacter ubique* was able to ~~supply~~ substitute one of its ~~obligate required molecules (~~glycine~~)~~ by glycolate (Carini et al., 2013), which is one of the main excretion by-products of phytoplankton and is assumed to be abundant in phytoplankton release. Finally, both SAR11 and SAR86 genomes contain proteorhodopsin genes, potentially providing an additional energy source, but the corresponding transcript levels were much higher in SAR11 than in SAR86 ~~in this study~~during the VAHINE experiment (Pfreundt et al., 2016)~~(Pfreundt et al., 2015)~~.

~~It appears striking that~~Strikingly, for both~~,~~ SAR11 and SAR86, 16S abundance was ~~not well correlated~~ decoupled from ~~with~~ the respective total transcript accumulation presented in the~~a~~ companion paper ~~(Pfreundt et al., 2015)~~, ~~but~~ whereas ~~that the two~~ the transcript abundances of both clades ~~were~~ were highly correlated (Pfreundt et al., 2016)~~(Pfreundt et al., 2015)~~, and specific abundant SAR11 and SAR86 OTUs shared a similar environmental niche (Fig. 6).~~among each other both in transcript abundance and abundance of dominant OTUs.~~ Dupont et al (Dupont et al., 2012) hypothesized that~~,~~ due to their different resource specializations, SAR11 and SAR86 compete only slightly for dissolved organic carbon (DOC), which would allow for their co-existence, albeit not explaining their tight transcriptional correlation.

~~Interestingly, we saw niche partitioning among~~ D~~d~~ominant SAR86 OTUs exhibited niche partitioning in the constrained ordination (Fig. 6). The temporal variation of two OTUs was close to SAR11 and *Alteromonas* and of another two OTUs close to *Prochlorococcus*, suggesting different sources of DOC were utilized by these SAR86 subtypes. On 16S sequence level, pairwise alignments of these four SAR86 OTUs showed sequence identities between 86.3 % (OTU 26 and 1229) and 94.9 % (OTU 7 and 98). Thus, those SAR86 OTUs sharing a niche were not more similar on 16S level than in between niches.

An aspect of special interest was to recognize community changes which could be attributed to the DIP fertilization in M1~~and to possibly explain why~~. CCA suggested that the taxon profiting ~~the~~ most from DIP fertilization was *Defluviicoccus* (*Rhodospirillaceae*). ~~While not showing this apparent reaction to DIP, t~~Apart from the maximum on day 6 in M1, ~~T~~this ~~OTU~~genus had similar abundances also in the Nouméa lagoon (Suppl. Dataset). ~~Interestingly,~~ *Defluviicoccus*-related bacteria were initially described from enhanced biological phosphorus removal (EBPR) systems (Maszenan et al., 2005; McIlroy and Seviour, 2009; Mielczarek et al., 2013). In such systems, used to treat wastewater, *Defluviicoccus* are known as glycogen-accumulating bacteria, competing with polyphosphate accumulating organisms ~~in enhanced biological phosphorus removal from waste water~~ (Burow et al., 2007; McIlroy and Seviour, 2009; Wong et al., 2004; Wong and Liu, 2007). ~~It is therefore interesting that in constrained ordination, the dominant *Defluviicoccus* OTU (40) was associated with DIP for M1 samples on days 6, 8 and 10, immediately following the phosphorus fertilization (Fig. 6). While not showing this apparent reaction to DIP, this OTU had similar abundances also in the Nouméa lagoon.~~ There are only few studies reporting *Defluviicoccus*-related bacteria in marine samples, and if so, these were associated with marine invertebrates (Enomoto et al., 2012; Fan et al., 2013). ~~Based on o~~Our data ~~it is likely that there was~~show a minor population of *Defluviicoccus*-related bacteria in the Nouméa lagoon that could benefit from the addition of fresh phosphorus in M1. Note however, that ~~this~~ the dominant OTU in this genus was only 83 % identical to *Defluviicoccus vanus* 16S, but 99 % identical to environmental sequences that, according to SILVA taxonomy for SSU release 119 and 123, are leaves of the same node as *D. vanus* and were thus classified as *Defluviicoccus* (name of the node). Phylogenetic placement of the here described *Defluviicoccus* OTUs showed their association with marine environmental *Defluviicoccus* 16S (Suppl. Fig. S3~~x~~).~~Therefore, additional work towards the more precise characterization of these *Defluviicoccus* related bacteria is required.~~

4.4 Effects of DIP depletion at the end of the experiment

Some aspects of the taxonomic succession after day 13, when DIP was mainly depleted, can be related to the strategies used for adaptation to P limitation. Among autotrophs, the main trend observed in M1 and the lagoon waters was the opposition of *Prochlorococcus* and *Synechococcus* with *Synechococcus* reaching higher abundances in M1 at the end of the experiment compared to the beginning, when DIP was comparatively low. Both expressed alkaline phosphatase (AP) mainly two days after their respective 16S abundance peak (Pfreundt et al., 2016)~~(Pfreundt et al., 2015)~~, suggesting that the increase in cell numbers led to a higher phosphorous demand that was subsequently adapted to. Incorporation of sulfolipids instead of phospholipids into membrane lipids is an ecological strategy developed by cyanobacteria for an advantage in P deficient environments (Van Mooy et al., 2006, 2009). The ability to synthetize sulphoquinovosyldiacylgycerol in place of phosphatidylglycerol in P-limited cultures can save 5 to 43 % of total cellular phosphorus demand and is highly variable according to the strain tested (Van Mooy et al., 2009). *SqdB*, a gene encoding a sulfolipid synthesis protein, was mainly transcribed by *Synechococcus* and less by *Prochlorococcus* (Pfreundt et al., 2016)~~(Pfreundt et al., 2015)~~ and transcript abundance changes corresponded to ~~their~~ changes in 16S abundance. In the North Atlantic Ocean, *Synechococcus* are also good competitors with *Prochlorococcus* for DIP and ATP uptake (Michelou et al., 2011). A variety of heterotrophs expressed AP mainly after day 10, possibly to the benefit of other populations. Indeed, metagenomic analyses showed that 59 % of marine bacterial APs are extracellular, periplasmic or located at the outer membrane (Luo et al., 2009). Possibly, DIP release through extracellular APA may have provided P to neighboring cells that cannot synthesize this enzyme, and such effects could possibly be emphasized in macro-aggregate gels (TEP) seen during P2 in the VAHINE experiment (Berman-Frank et al., 2016)~~(Berman-Frank et al., n.d.)~~.

4.5 Possible functional roles of bacterial subcommunities in the degradation of organic matter

The concerted increase of *Cytophagia* and *Flavobacteriia* towards the end of the experiment is a recurrent feature seen in older studies based on plateable strains (Fukami et al., 1981b; Painting et al., 1989), and in molecular studies allowing access to the uncultivable community (Teeling et al., 2012). Both *Cytophagia* and *Flavobacteriia* profit from phytoplankton blooms and have often been found in marine snow (DeLong et al., 1993; Fontanez et al., 2015; Teeling et al., 2012). Members of the *Flavobacteriia* are known to degrade organic matter, some even specializing on algal-derived organic matter (Bauer et al., 2006; Bowman, 2006). In the lagoon,

members of the *Flavobacteriaceae* reached even higher ~~relative~~ abundances than in M1, arguing against M1-specific effects being the reason for their increase. The occurrence of different *Flavobacteriaceae* phylotypes seems to depend on the nature of the dominant phytoplankton species (Pinhassi et al., 2004). Information retrieved from analysis of cultured representatives showed~~s~~ a high capacity to degrade high molecular weight molecules, which is in line with the high ectoenzymatic activities generally associated ~~at~~ with the end of blooms (Chróst, 1992; Riemann et al., 2000). The quality of organic matter issued from specific phytoplankton detritus influences bacterial community succession, and while the potential for polymer degradation appears to be widespread among different phyla it is restricted to some ~~very~~ specialized species (Landa et al., 2014; Murray et al., 2007). Our data indicate that both in M1 and the lagoon, ~~there were~~ available organic components ~~available favoring~~ favored Flavobacteria ~~their~~ growth, probably enhanced by increasing temperatures ~~most pronounced~~ during P2.

Our data further indicate that *Rhodobacteraceae*, when abundant, assimilate much more organic matter than other heterotrophs seen in this study, as dynamics in BP were closely linked to this group. This was not only shown by CCA (Fig. 6~~5~~) but is also evident from directly comparing BP ~~(Van Wambeke et al., 2015)~~(Fig. 1e) to *Rhodobacteraceae* ~~16S reads~~abundance. Both striking BP peaks on day 4 and day 21 in M1 correspond to concomitant *Rhodobacteraceae* abundance peaks ~~in *Rhodobacteraceae* 16S~~, while no other heterotrophic groups showed these peaks. *Rhodobacteraceae* are considered ecological generalists, with large gene inventories, but they cannot be easily condensed in an ecologically differentiated cluster (Newton et al., 2010). *Roseobacter*, a common marine member of the *Rhodobacteraceae*, have a variety of metabolic pathways that ~~facilitate interaction and attachment with phytoplankton detritus or give them~~confer advantages among other bacteria and facilitate interaction with, and attachment to phytoplankton detritus. They can produce quorum sensing molecules, vitamins and antimicrobial compounds (Buchan et al., 2014). CCA showed two dominating *Rhodobacteriaceae* OTUs linked with Chl concentrations (Fig. 5), which argues for their ability to access phytoplankton detritus, while one OTU was closely linked with N_2 fixation. Notably, none of these three OTUs represented the *Thalassobius* sp. OTU that was responsible for the *Rhodobacteraceae* peak on day 4.

4.6 The diazotroph community and its impact on the heterotroph community structure

Diazotroph community structure (Fig 3f, 4f) probably changed in M1 due to DIP fertilization and reduced turbulence, with *Cyanothece*-like cells profiting and forming large aggregates during P2 (Bonnet et al., 2015b)~~(Bonnet et al., 2015a)~~. Our 16S based diazotroph abundances compare reasonably well with those found by quantitative PCR of the *nifH* gene (Turk-Kubo et al., 2015), if we assume that those 16S reads classified as *Calothrix* are indeed mostly *Richelia* (Het-1). The SILVA database positions *Richelia* below the *Calothrix* node, and the metatranscriptome data published in this issue show a substantial *Richelia* read fraction and only little *Calothrix* (Pfreundt et al., 2016)~~(Pfreundt et al., 2015)~~, supporting this assumption. Further, UCYN-C *nifH* gene abundance was at maximum 100 copies mL^{-1} in M1 (Turk-Kubo et al., 2015), which matches well the maximal 4~~5~~00 cells mL^{-1} that we ~~calculated~~ inferred from relative 16S values for *Cyanothece* ~~using flow cytometry counts of *Synechococcus* and *Prochlorococcus*~~ (~~Suppl.~~ Fig. 3f). Note that there are also non-diazotrophic *Cyanothece* representatives, thus the second number can be expected to be larger. This is remarkable given the different methods used to produce these numbers.

The higher *Trichodesmium* abundance inside M1 after its deployment might be attributed to entrapment of filaments that were not present in the lagoon anymore when the sampling started, likely due to high tide currents. The reduction in the number of 16S tags belonging to *Trichodesmium* following the DIP fertilization on the evening of day 4 was unexpected. To the contrary, the combined effects of reduced turbulence and P limitation was thought to trigger a *Trichodesmium* bloom. However, a *Trichodesmium* bloom was observed at the end of the VAHINE experiment in the lagoon (Spungin et al., 2016)~~(Spungin et al., 2015)~~, demonstrating that either the small numbers of existing filaments were competent to build up a bloom~~s~~ rapidly or that most *Trichodesmium* resided in deeper waters before the bloom. While we did see cyanophage gene expression in M1 in the accompanying metatranscriptome study (Pfreundt et al., 2016)~~(Pfreundt et al., 2015)~~, the known host ranges of those phages suggest that they rather would act on *Synechococcus*, excluding viral lysis as the reason for the *Trichodesmium* decline. Thus, there were likely other factors involved that deserve further investigation. *Trichodesmium* has comparatively low growth rates (Turk-Kubo et al., 2015), so enhanced competition after DIP fertilization might be such a factor.

Average N_2 fixation rates of 27.3 ± 1.0 nmol N L^{-1} d^{-1} were measured inside the mesocosms during P2 (days 15-23), and with 69.7 nmol N L^{-1} d^{-1} very high maximum rates were reached in M1 between days 18 and 21. A short-term experiment performed by Bonnet et

al. (Bonnet et al., 2015b)~~(Bonnet et al., 2015a)~~ indicated that DDN was efficiently transferred to non-diazotrophic microbial communities during P2: after 24 h, 16 ± 6 % of the fixed N2 was released in the dissolved nitrogen pool and 18 ± 4 % was transferred to non-diazotrophic picoplankton. We show that ~~in terms of relative abundance, SAR86, *Deferribacteres*, *Deltaproteobacteria*, *Cytophagia*, and *Flavobacteriaceae* increased during this period (Fig. 3). In terms of pseudo-absolute abundances, calculated from 16S abundances employing the cytometry cell counts of *Synechococcus* and *Prochlorococcus*, it became clear that indeed~~ almost all heterotrophic ~~groups~~ classes analyzed here (> 0.5 % relative abundance) increased during this period (~~Suppl.~~ Fig. 3). Another publication in this issue showed that BP was ~~always~~ stimulated after short-term ammonium plus nitrate enrichments on day 4 and 20 (Van Wambeke et al., 2015). This ~~finding~~ speaks for a positive effect of released DDN on heterotrophic growth during P2, but effects through phytoplankton growth and decay probably also played a role. Indeed, nitrogen ~~coming~~ inputs from N_2 fixation alone ~~was~~ were not sufficient to sustain BP (Van Wambeke et al., 2015). Constrained ordination showed that generally, temporal variation of dominant *Rhodobacteraceae*, *Halieaceae* (OM60(NOR5), in SILVA release 119 within the *Alteromonadaceae*), and *Flavobacteriia* could be partially be explained with changes in N_2 fixation, whereas variation in other alphaproteobacteria (mainly SAR11, SAR116, and *Rhodospirillaceae*), SAR86, euryarchaea, and *Alteromonas* abundances were not explainable by N_2 fixation (Fig. 6), indicating the involvement of additional factors. However, it appears very clear that the shift within the diazotroph community from the dominant diazotrophs *Richelia* and *Trichodesmium* to UCYN-C after day 14, together with very high N_2 fixation rates (Bonnet et al., 2015b)~~(Bonnet et al., 2015a)~~, led to an increase in abundance of almost all heterotrophic groups investigated here, consistent with N limitation of BP (Van Wambeke et al., 2015).

5 Conclusions

We show that despite their large size (>50 m^3), the VAHINE mesocosms led to significant changes in the microbial community structure, probably initiated by decay of *Synechococcus* and diatoms (Leblanc et al., 2016)~~(Leblanc et al., 2015)~~, albeit not exhibiting a typical bottle effect with a copiotroph-dominated community. ~~Interestingly, t~~The accompanying metatranscriptome study for M1 showed gene expression from cyanophages, which might have contributed to *Synechococcus* decay. Fertilization with 0.8 µM DIP on day 4 did not have directly observable effects on the overall community, as samples from before and four days after fertilization clustered together, but likely influenced development of individual

populations, like *Defluviicoccus*-related bacteria or UCYN-C (*Cyanothece*) diazotrophs. The latter increased after day 10 only in the mesocosms, and were probably limited by temperature before that (Turk-Kubo et al., 2015). The shift of the dominant diazotroph from *Richelia* and *Trichodesmium* to UCYN-C after day 14 (Turk-Kubo et al., 2015), together with extremely high N_2 fixation rates (Bonnet et al., 2015b)~~(Bonnet et al., 2015a)~~, led to an increase in abundance of almost all heterotrophic groups investigated here, consistent with N-limited BP (Van Wambeke et al., 2015).

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)~~(Pfreundt et al., 2015)~~, 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. With the results of this work we present for the first time in this ecosystem an in-depth analysis of the prokaryotic diversity and community structure and of their changes over time with a focus on diazotrophic organisms. We correlate the prokaryotic community structure with effects of DIP fertilization, of enclosure versus open lagoon waters ~~in the lagoon~~, and with the fate of DDN and DOC. The determined microbial diversity was very high, and we provide evidence for previously unknown niche partitioning, e.g., among dominant SAR86 OTUs. These results suggest a plethora of still unknown metabolic and regulatory interactions to occur within these marine microbial communities that are worth to be explored further.

The supplement related to this article is available online at the journal's website at http://www.biogeosciences.net.

Data availability

All raw sequencing data can be downloaded from NCBI's BioProject database under the accession number BioProject PRJNA304389.

Author contributions. S. Bonnet is the chief scientist responsible ~~of~~ for the VAHINE program, she designed and executed the ~~experiment in~~ mesocosm~~s~~ experiment and measured N_2 fixation. F. Van Wambeke sampled for and prepared Figure 1 and 6, M. Caffin performed flow cytometry, W.R. Hess and U. Pfreundt took samples for DNA extraction, U. Pfreundt prepared and analyzed 16S rRNA gene amplicons and prepared all other figures, U. Pfreundt, F. Van Wambeke and W.R. Hess drafted the manuscript, all authors reviewed the manuscript.

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Figures

Figure 1. Evolution of selected parameters in mesocosm M1 and the ~~Noumea~~Nouméa lagoon at depths of 1 m and 12 m. **a)** primary production (PP), **b)** chlorophyll *a* (Chl *a*) concentration, **c)** alkaline phosphatase activity (APA) ~~and~~, **d)** heterotrophic bacterial production (BP, measured by ^{3}H-leucine assimilation), **e)** dissolved inorganic phosphorous (DIP) and **f)** N_2 fixation rates as measured by ^{15}N incorporation in M1 and the Nouméa lagoon.

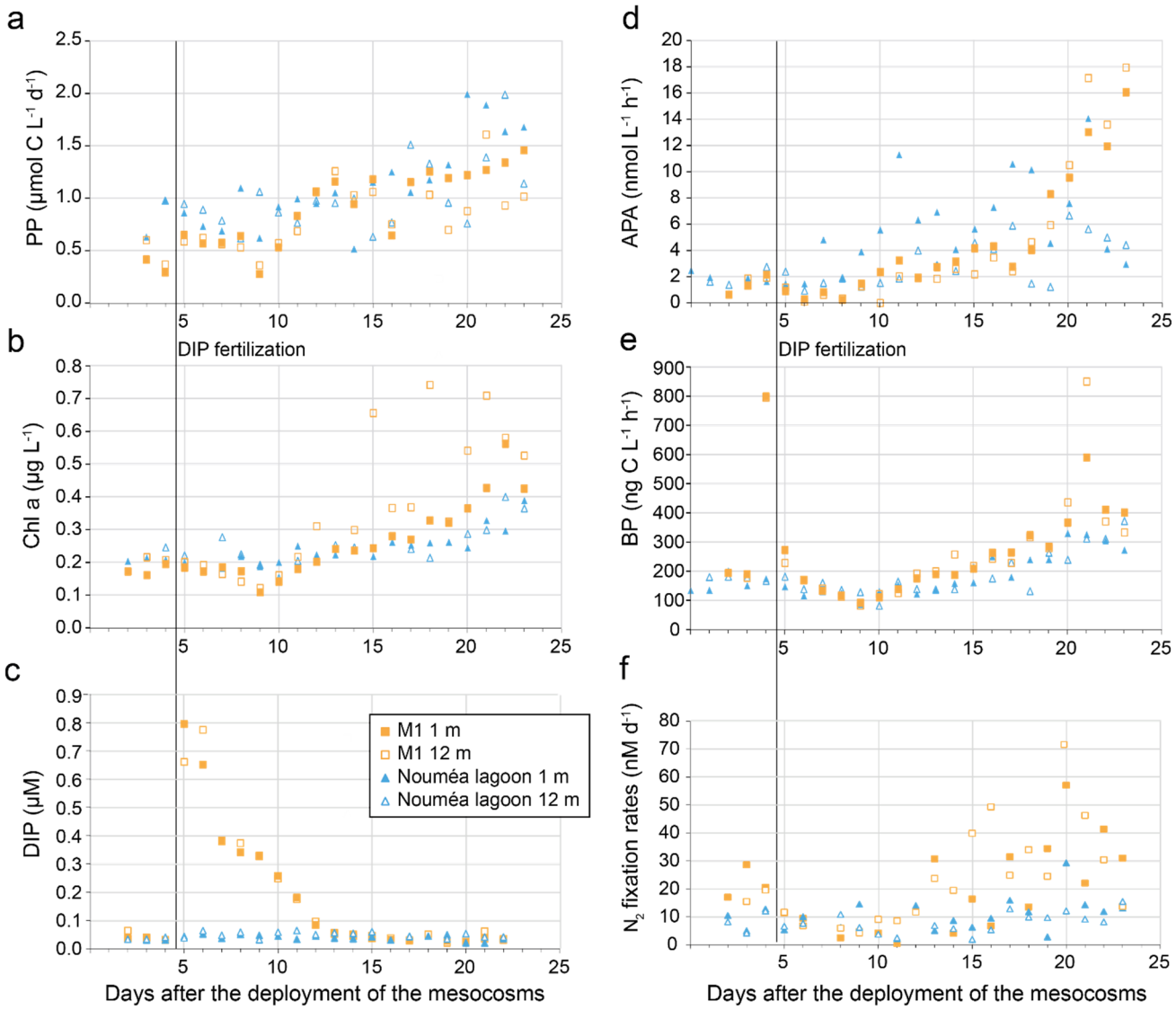


Figure 2. Evolution of absolute abundances as counted by flow cytometry ~~in M1~~ at depths of 1 m and 12 m of ~~a)~~ *Synechococcus* and *Prochlorococcus* in M1 **(a)** and the lagoon **(c)**, and of~~and **b)**~~ non-photosynthetic bacteria (total heterotrophs) ~~as measured by flow cytometry~~in M1 **(b)** and the lagoon **(d)**~~.~~.. ~~Evolution of **c)** dissolved inorganic phosphorous (DIP) and **d)** N_2 fixation rates as measured by ^{15}N incorporation in M1 and the Noumea~~Nouméa ~~lagoon at depths of 1 m and 12 m.~~

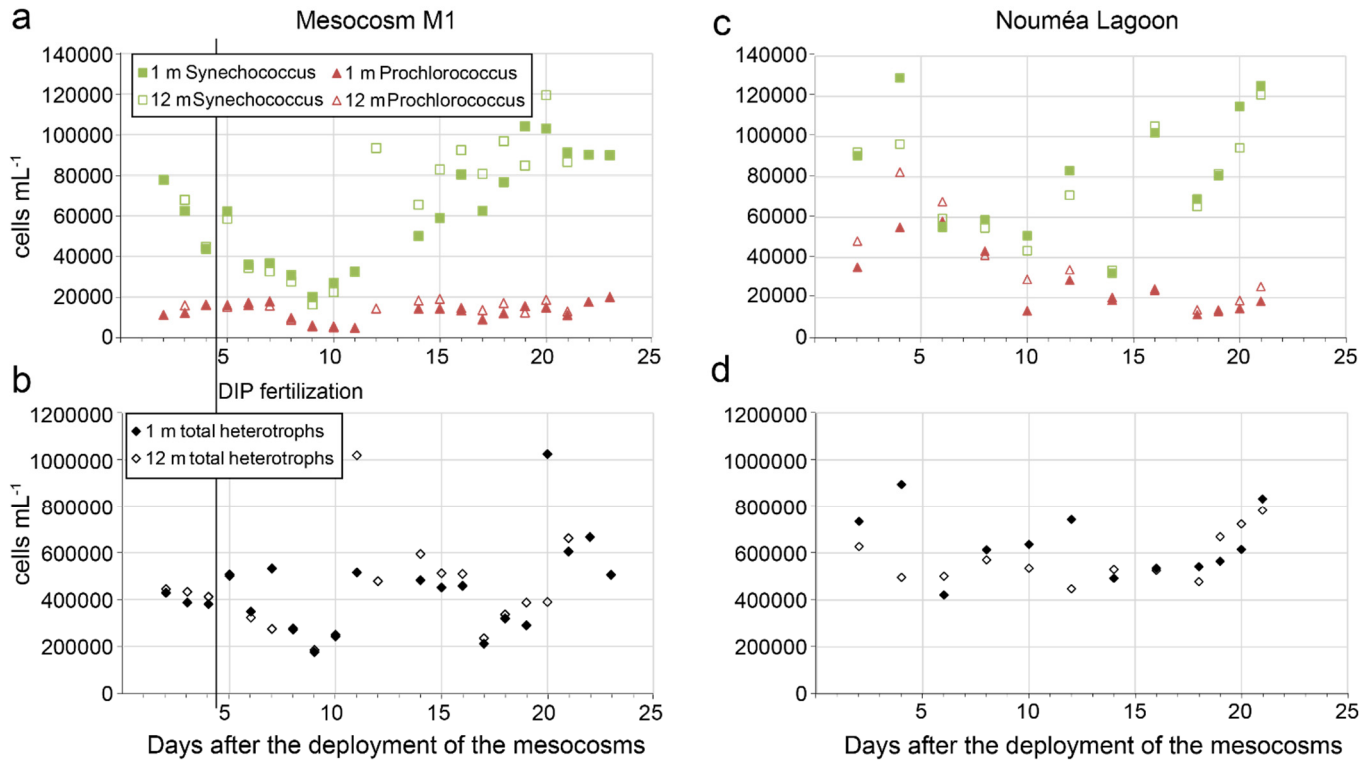


Figure 3. Evolution of the 16S community with time for surface samples (1 m depth) inside mesocosm M1. ~~Pseudo-a~~Absolute abundances in cells mL^{-1} were inferred in three calculation steps. First, raw read counts per OTU were normalized to equal 16S copy numbers ("~~equal~~ 16S ~~raw~~ norm ~~counts~~reads"). These ~~counts~~reads were summed for all *Prochlorococcus* (Pro) and *Synechococcus* (Syn) OTUs in each sample and divided by the sum of flow cytometry cell counts for Syn and Pro in that sample, creating a ratio. For each OTU, the 16S-norm read count ~~equal 16S raw count was~~was then divided by this ratio to produce the ~~pseudo-~~inferred absolute abundance of that OTU. This procedure accounts for library size in each sample. ~~All abundances are given as a percentage of total 16S reads.~~ For better visualization, the classes with **(a)** a maximum abundance above 5 % and **(b)** a maximum abundance between 0.5-5 % were plotted separately. **(c-e)** Families with >~~1%~~5000 ~~of total 16S reads~~cells mL^{-1} (at their respective maximum) amongst the dominant non-photosynthetic classes *Alphaproteobacteria*, *Gammaproteobacteria*, and *Flavobacteriia*. **(f)** All genera with >250 cells mL^{-1} ~~0.05% of total 16S reads~~ (at their respective maximum) within photosynthetic *Cyanobacteria*. Dashed lines were used to connect the data points for ~~better~~ visualization purposes only. The DIP fertilization event in the evening of day 4 is indicated by a black vertical line.

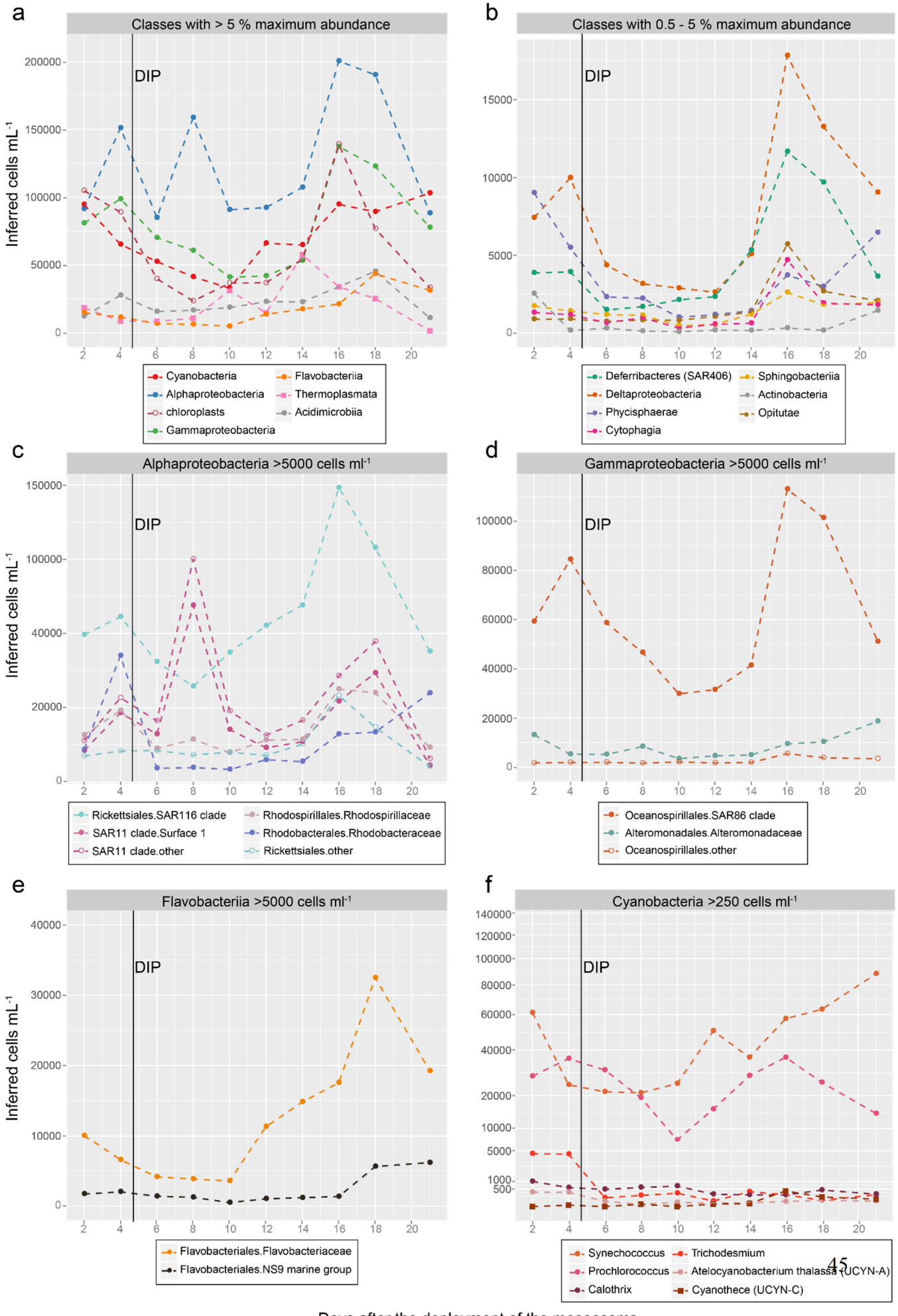

a
Classes with > 5 % maximum abundance
DIP
Inferred cells mL-1
Cyanobacteria
Alphaproteobacteria
chloroplasts
Gammaproteobacteria
Flavobacteriia
Thermoplasmata
Acidimicrobiia
b
Classes with 0.5 - 5 % maximum abundance
DIP
Deferribacteres (SAR406)
Deltaproteobacteria
Phycisphaerae
Cytophagia
Sphingobacteriia
Actinobacteria
Opitutae
c
Alphaproteobacteria >5000 cells ml-1
DIP
Inferred cells mL-1
Rickettsiales.SAR116 clade
SAR11 clade.Surface 1
SAR11 clade.other
Rhodospirillales.Rhodospirillaceae
Rhodobacterales.Rhodobacteraceae
Rickettsiales.other
d
Gammaproteobacteria >5000 cells ml-1
DIP
Oceanospirillales.SAR86 clade
Alteromonadales.Alteromonadaceae
Oceanospirillales.other
e
Flavobacteriia >5000 cells ml-1
DIP
Inferred cells mL-1
Flavobacteriales.Flavobacteriaceae
Flavobacteriales.NS9 marine group
f
Cyanobacteria >250 cells ml-1
DIP
Synechococcus
Prochlorococcus
Calothrix
Trichodesmium
Atelocyanobacterium thalassa (UCYN-A)
Cyanothece (UCYN-C)
Days after the deployment of the mesocosms

Figure 4. Evolution of the 16S community with time for surface samples in the Nouméea lagoon waters. All abundances are given as ~~pseudo-~~inferred absolute abundances in cells mL^{-1} as described for Figure 3.~~[a] percentage of total 16S reads.~~ Data was plotted in the same way as for Figure 3~~2~~.

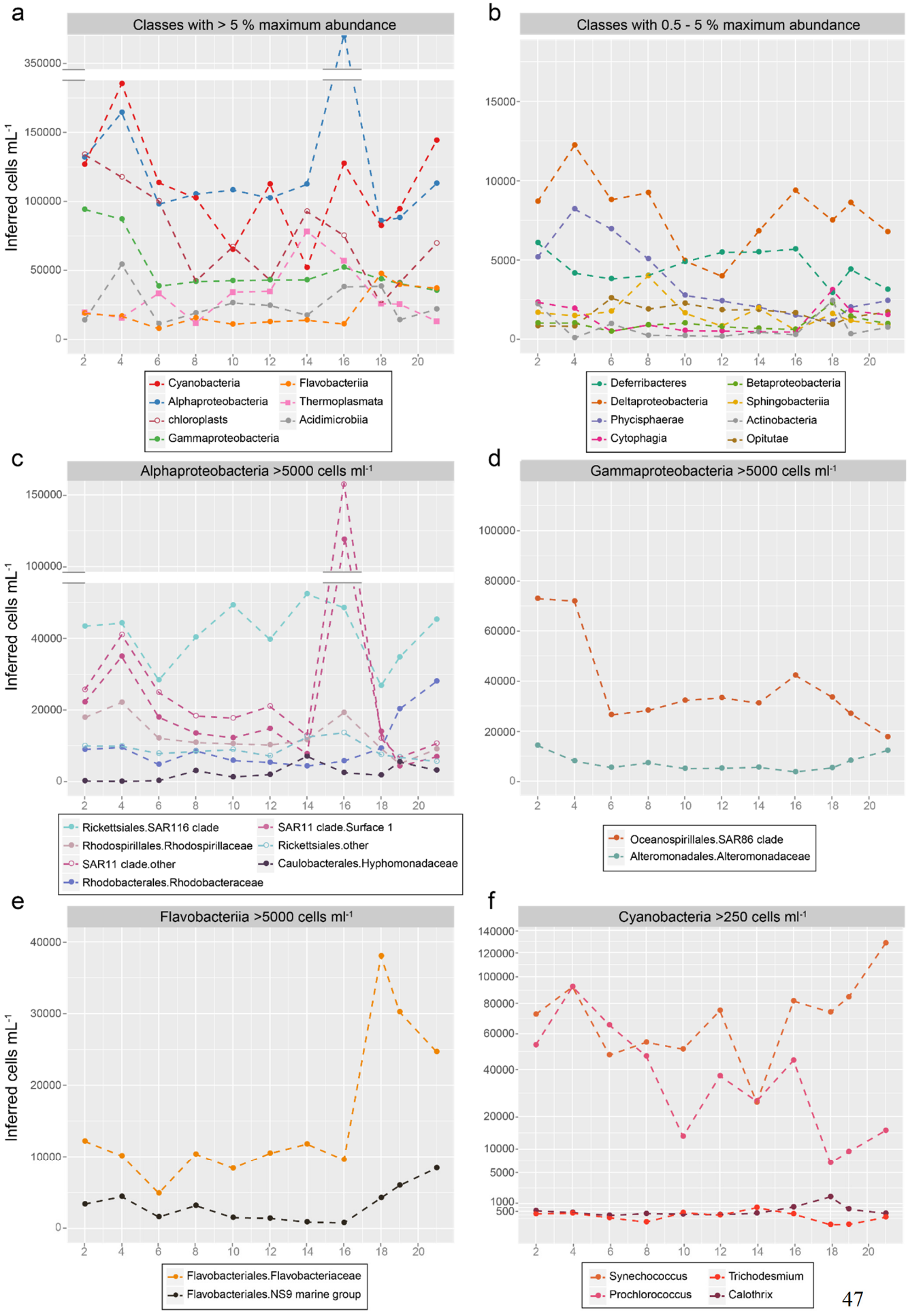

a
Classes with > 5 % maximum abundance
Inferred cells mL-1
Cyanobacteria
Alphaproteobacteria
chloroplasts
Gammaproteobacteria
Flavobacteriia
Thermoplasmata
Acidimicrobiia
b
Classes with 0.5 - 5 % maximum abundance
Deferribacteres
Deltaproteobacteria
Phycisphaerae
Cytophagia
Betaproteobacteria
Sphingobacteriia
Actinobacteria
Opitutae
c
Alphaproteobacteria >5000 cells ml-1
Rickettsiales.SAR116 clade
Rhodospirillales.Rhodospirillaceae
SAR11 clade.other
Rhodobacterales.Rhodobacteraceae
SAR11 clade.Surface 1
Rickettsiales.other
Caulobacterales.Hyphomonadaceae
d
Gammaproteobacteria >5000 cells ml-1
Oceanospirillales.SAR86 clade
Alteromonadales.Alteromonadaceae
e
Flavobacteriia >5000 cells ml-1
Flavobacteriales.Flavobacteriaceae
Flavobacteriales.NS9 marine group
f
Cyanobacteria >250 cells ml-1
Synechococcus
Prochlorococcus
Trichodesmium
Calothrix
Days after the deployment of the mesocosms

Figure 5. NMDS ordination of all samples using Bray-Curtis distances based on the relative abundances of (aA) all OTUs, (bB) only non-cyanobacterial OTUs ("heterotrophic bacteria"), or (cC) only OTUs classified as photosynthetic cyanobacteria. The samples are color-coded as follows: orange - M1 surface, red - M1 12 m, light blue – Nouméea lagoon surface, blue - Nouméea lagoon 12 m. Ellipses are color coded in a similar way to distinguish M1 sample clusters and Nouméea lagoon sample clusters. Ellipses denote a confidence interval of 0.95 around the weighted average of each cluster. Thus, clusters can be considered significantly different with alpha=0.05, if ellipses do not overlap.

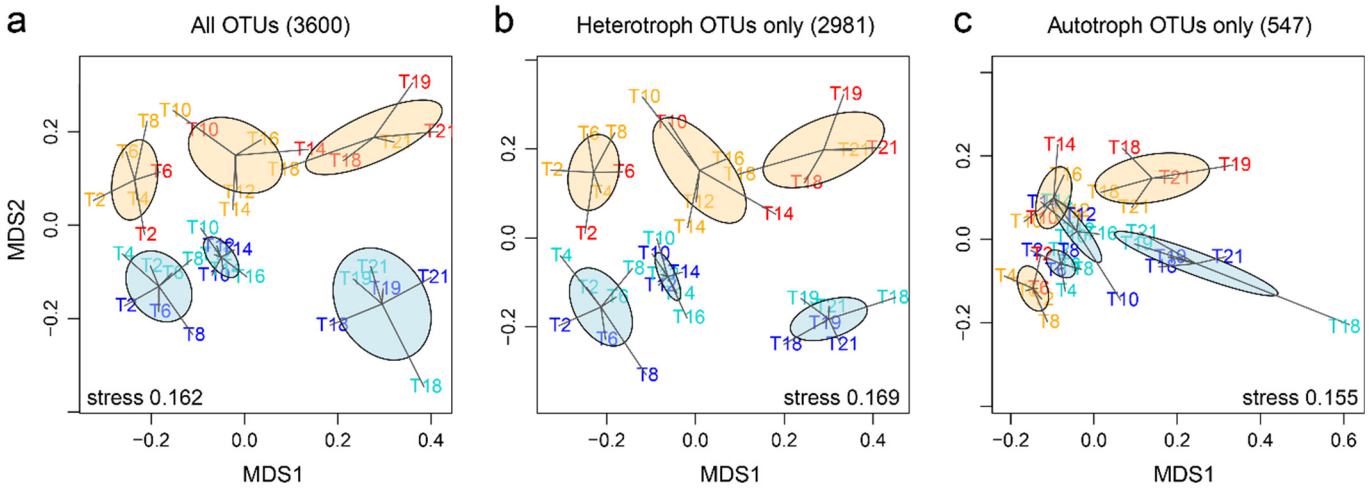
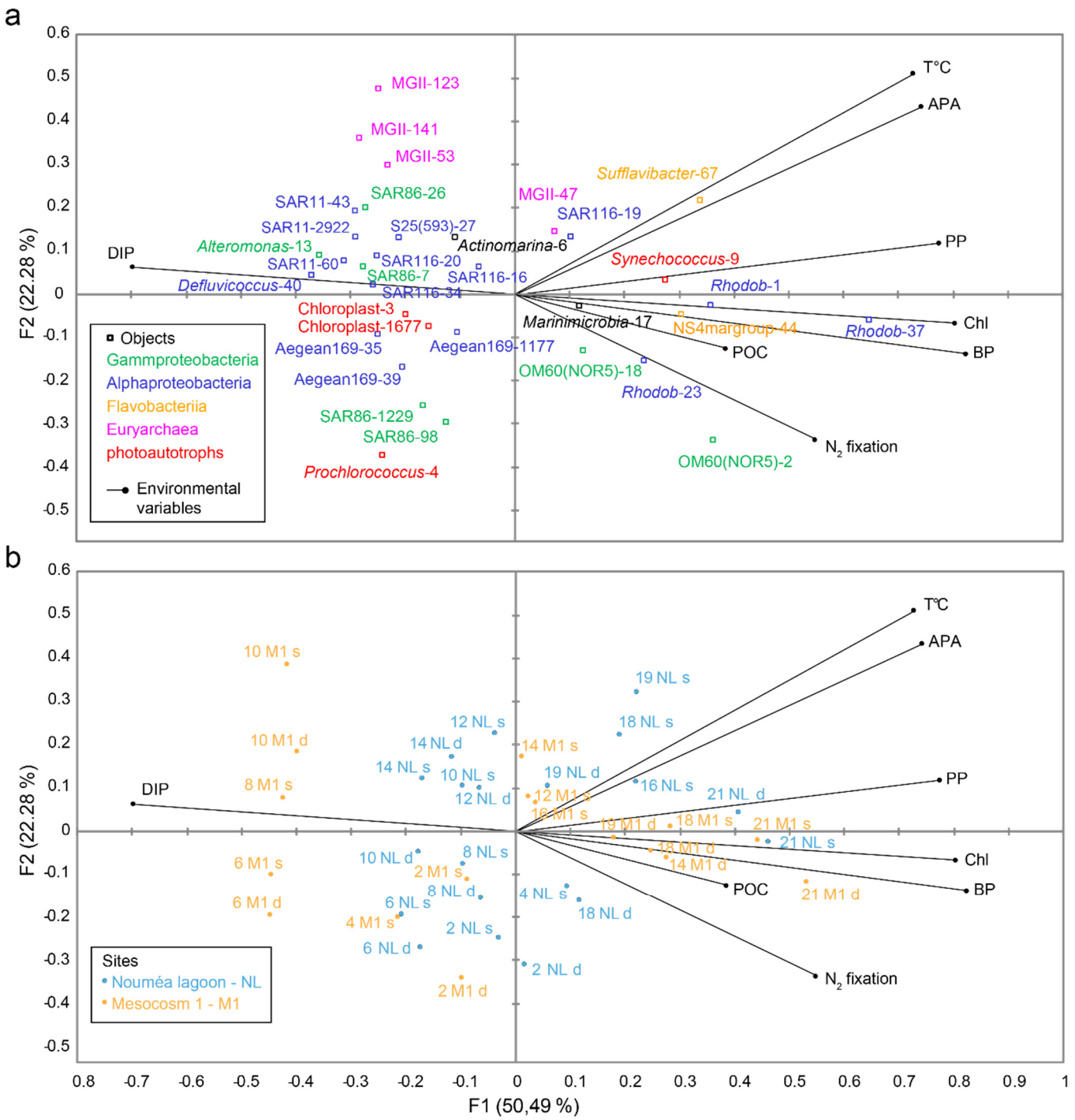


Figure 6. Canonical correspondence analysis (CCA) biplot of bacterial 16S community structure. Correlations between environmental variables and the first two CCA axes are represented by the lengths and angles of the arrows. The position of OTUs or samples relative to arrows indicates the extent to which the distribution of that OTU or the community composition of that sample is influenced by the environmental parameter represented by that arrow. **a)** Ordination of OTUs constrained by the given environmental variables (black lines). ~~Gray full circles are "sites" coded "M1" and "NL" for their origin from mesocosm M1 and the Nouméa lagoon, then "s" or "d", according to the depth sampled (1 m and 12 m, respectively). These codes are prefixed by a number according to the day of sampling (from day 2 to day 21).~~ Color coded pen squares mark the 34 most abundant OTUs in the two-dimensional space of the plot. These were one *Synechococcus*, one *Prochlorococcus*, and two chloroplast OTUs (photoautotrophs, red); three SAR11, four SAR116, three Aegean169 marine group, three not further classified *Rhodobacteraceae* (Rhodob), one *Rickettsiales* S25(593), and one *Rhodospirillaceae* (*Defluviicoccus*) OTU (A~~a~~lphaproteobacteria, blue); four SAR86, two OM60(NOR5) clade (*Alteromonadaceae)*, and one *Alteromonas* OTU (G~~g~~ammaproteobacteria, green); one *Sufflavibacter* and one NS4 marine group OTU (*Flavobacteriia*, orange); four Marine Group II archaea (MGII, E~~e~~uryarchaea, pink); one Candidatus Actinomarina OTU (Acidimicrobiales, black) and one unclassified *Marinimicrobia* OTU (black). ~~Correlations between environmental variables and the first two CCA axes are represented by the lengths and angles of the arrows. The position of OTUs or samples relative to arrows indicates the extent to which the distribution of that OTU or the community composition of that sample is influenced by the environmental parameter represented by that arrow.~~ **b)** Ordination of sampling sites in the same two-dimensional space. Sites are coded "M1" and "NL" for their origin from mesocosm M1 and the Nouméa lagoon, then "s" or "d", according to the depth sampled (surface-1m and deep-12m, respectively). These codes are prefixed by a number giving day of sampling (from day 2 to day 21).


a
b
F2 (22.28 %)
F1 (50,49 %)
DIP
T°C
APA
PP
Chl
BP
POC
N2 fixation
MGII-123
MGII-141
MGII-53
SAR11-43
SAR86-26
SAR11-2922
S25(593)-27
Alteromonas-13
SAR11-60
SAR116-20
SAR86-7
SAR116-16
Defluviicoccus-40
SAR116-34
Chloroplast-3
Chloroplast-1677
Aegean169-35
Aegean169-1177
Aegean169-39
SAR86-1229
SAR86-98
Prochlorococcus-4
Actinomarina-6
MGII-47
SAR116-19
Sufflavibacter-67
Synechococcus-9
Rhodob-1
Marinimicrobia-17
NS4margroup-44
Rhodob-37
OM60(NOR5)-18
Rhodob-23
OM60(NOR5)-2
Objects
Gammproteobacteria
Alphaproteobacteria
Flavobacteriia
Euryarchaea
photoautotrophs
Environmental variables
Sites
Nouméa lagoon - NL
Mesocosm 1 - M1