

Response Letter to Referee Comments

„Global analysis of gene expression dynamics within the marine microbial community during the VAHINE mesocosm experiment in the South West Pacific“

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We thank the editor and the referees for their thorough evaluation of our manuscript and valuable comments towards improving it.

Anonymous Referee #1

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This manuscript reports functional metatranscriptome sequences collected every two days from inside and outside a phosphorus-amended mesocosm in New Caledonia during a 3-week period. This work is complementary to a number of other studies undertaken at the same time, which have already been published. Major findings include identifying different community dynamics inside and outside the mesocosm, including a fast decline of *Synechococcus* cyanobacteria inside the mesocosm from the beginning, potentially caused by increased cyanophage lysis.

The authors also used transcript functional information to give hints about the biogeochemical processes occurring inside and outside the mesocosm. In particular, regarding nitrogen requirements, the authors found that certain bacterial species expressed urease genes while others ammonium transporters and glutamine synthetase. Nitrogen fixation was dominated by 5 different cyanobacterial types, again showing temporal dynamics; little heterotrophic N-fixation was found.

Specific comments P 4, L29: sentence missing a word.

Answer: Thank you. The sentence has been corrected to: “*The resulting non-rRNA reads (...) were used as input for de-novo transcript assembly with Trinity (Haas et al., 2013) using digital normalization prior to assembly to even out kmer coverage and reduce the amount of **input data**.*”

Altermonadaceae is misspelled throughout (should be Alteromonadaceae)

Answer: We corrected this throughout.

P9, L29: add “but” after the references

Answer: The “**but**” required in this sentence is in a different place and was likely missed by the referee. However, we added a missing comma before the “but”. “*This group of organotrophs is phylogenetically related to Alteromonadaceae. Gammaproteobacteria such as the Alteromonadaceae occur usually at rather low abundances in oligotrophic systems, but due to their copiotrophic metabolism (Ivars-Martinez et al., 2008; López-Pérez et al., 2012) increase in numbers and activity under eutrophic conditions.*

Anonymous Referee #2

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While a lot of data was obtained in this study, the manuscript would greatly benefit from some revisions. The goal of authors was to link transcription of microbial taxa in the mesocosm to the chemical measurements, diazotrophic abundances and microbial composition shifts obtained by other researchers. Hence, the differential transcription between the treatment and the lagoon community has to be analyzed. While, in the beginning of Results and Discussion section, authors refer to the differentially expressed genes, it is not clear if these genes had differential transcription between the treatment (mesocosm) and the lagoon communities or among different time points within each of the mesocosm and lagoon. The figures show that the second comparison was most likely done.

Answer: Indeed, differential expression analysis was done within M1 and the lagoon, respectively, among the different time points. These results are presented in section 3.1 “Transcripts cluster into distinct groups with similar expression patterns over time in M1 and the lagoon”. The sentence that might have been not clear was probably the following: “*Separate complete linkage clustering of samples based on all differentially expressed transcripts in M1 and the Noumea lagoon, respectively, showed that ...*” This has now been rephrased.

A clear description of the data analysis and a scheme of the analysis flow would help.

Answer: Following this advice, we have added a flow chart (new Fig. 1) to describe the strategy for data analysis more comprehensively.

Overall, the major results and especially their significance are not clear from the analysis, figures and discussion as they are presented now. I suggest breaking down the Results and Discussion into sub-sections following experiment succession: for example, the authors differentiate samples into several clusters, mainly by the time of sampling, with the analysis of what is in these clusters.

Answer: We have changed the flow of Results and Discussion and our new Figure 4 shows the major changes within the three major time clusters (or “phases P0, P1, and P2”) and the major differences between M1 and the lagoon samples within each phase. These phases had been defined previously by other papers within this Special Issue based on biogeochemical measurements and abundances of different diazotrophs.

It would be easier to compare the transcription profiles to other measurements in this way. An alternative separation can be done by metabolism and cellular processes. A comparative analysis between the mesocosm and lagoon communities with a focus on a few findings with major significance to understanding the processes in this experiment would improve this manuscript.

Answer: As suggested we present a comparison of the mesocosm and the lagoon in each of the phases of the experiment (see answer to last question and Figure 4). Additionally, and more specifically, we present an analysis of gene groups attributed to certain metabolisms, like nitrogen and phosphate metabolism, later in the results section.