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Interactive comment on "Mechanisms of *Trichodesmium* bloom demise within the New Caledonia Lagoon during the VAHINE mesocosm experiment" *by* D. Spungin et al.

Anonymous Referee #3

Received and published: 21 March 2016

This manuscript is interesting but needs revisions. The initial intent of the study was to follow the demise of a Trichodesmium bloom in a mesocosm. While the mesocosms failed, a bloom developed in the wild, so Trichodesmium samples were taken in carboys and bottles. The authors follow the collapse and crash of this Trichodesmus bottle/carboy bloom. The authors measure phenotypic and genetic parameters to describe the bloom. The work is interesting but not always clear to the reader.

Introduction lacks sufficient context to understand the relevance of the VAHINE project which is simply referenced, but not described at all. Although the whole issue is about VAHINE it should have a sentence or so to connect the manuscript to the general experimental framework. The Introduction is weak. It lacks a general rationale for the

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experiment and background about Trichodesmium responses to stress. Furthermore some sentences are difficult to follow and understand, e.g., “While Trichodesmium was initially present and conditions in the mesocosms appeared favorable, no Trichodesmium blooms developed within the mesocosms with other diazotrophs (such as diatom-diazotroph associations, and unicellular types mainly UCYN-C, as well as UCYN-A and UCYN-B) instead developing and dominating at different phases of the experimental period (Turk-Kubo et al., 2015).”

Methods section is well written and appropriate, although it wasn’t very clear for referee N1, who felt more detail was needed, specifically replication of experiments which many times appear in the results. Additionally, further details about the experimental design would improve the manuscript. A figure showing sampling times, type of assessment (chlorophyll, transcript, nutrients, etc.) will improve the readability of the manuscript.

A central assumption from Spungin et al. is that the microcosms "carboys" experiments is representative of the natural environment and therefore the phenomena observed during the experiment, i.e., the collapse and its causes, are equivalent to the natural sea bloom. While this might be true, the reviewer disagrees with this interpretation. Indeed Spungin et al. mention in line L312 that “Based on previous experience (Berman-Frank et al., 2004), resuspension of Trichodesmium cells in the extremely high densities of the surface blooms (Fig. 2a-c) would cause an almost immediate crash of the biomass.” If this is true why would these blooms be equivalent? Furthermore, no data is presented nor mentioned about the timescale, cell physiology and causes of the eventual collapse of the bloom at its natural environment. No control about the behavior of Trichodesmium in the bottle environment is provided (Did the natural bloom also collapse in 22 hours? How? Levels of nutrients?). A deep and thorough discussion about the differences between the microcosms environment with respect to the natural environment is expected, however it was not provided. For example, PCD phenotype cells were quantified in the bottle setup, but not necessarily this mechanism would be

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preset at the natural environment.

Another main weak point of the manuscript is the large amount of the results described, without biological interpretation. Some examples are: L361-L368, interpretation of DIP turnover. L376-402. Gene expression changes for several pathways are described, but no physiological interpretation is provided.

Another result than needs further insight is the low number of reads mapping to Trichodesmium genome for T8 and T22 samples (line L277). While T0 has a percentage of 52% mapped reads (low rate but within reasonable range), only 5% and 3% mapped for T8 and T22 samples. An explanation for this result is expected. If they did not match to Trichodesmium genome, to what other organism do they map (use BLAST)? This result needs to be explained, interpreted and discussed.

The transcript expression figures should not display RPM, but log2 changes from FPKM with respect to initial conditions. RPM is not a good measure to compare different transcripts because different transcripts have different transcript lengths and counts are biased by transcript length. I suggest to use cufflinks package to quantify transcript abundance (FPKM, not RPM) and statistical tests for differential expression. Final figures should present log2 fold changes to illustrate expression dynamics or log10 FPKM if absolute value needs to be illustrated.

The use of important terms like “bloom” or “demise” should be use much more strictly. It is hard to intuitively recognize when the description of the phenomena happens in the lagoon or in the carboys. As an example the sentence “TEP concentration exceeded 700 GX L- 1 on day 23 during the collapse of the bloom (4 h to 20 h after T0) and then declined to 420 GX L- 1 44 h after T0 (Fig. 8b).” may appear to refer to the actual bloom in the lagoon, while it actually may refer to the description of the phenomena in the carboys. It is unclear to the reader. Clear differentiation about the description of the phenomena in the lagoon versus the carboys should be expressed more clearly. Maybe a table would help?

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Discussion: generally the discussion is too long and many irrelevant hypotheses that were not tested are discussed. I recommend to shorten the section and specifically address issues directly related to the work, not tangential hypotheses that were not addressed by the current work, e.g., L576-L596.

Finally, figures resolution is low, and font size of text within figures should be increased. Also acronyms should be fully spelled first time they appear in the main text, e.g, DIP (Dissolved Inorganic Phosphorus) is never defined at the manuscript.

Other suggested changes are:

L369 mentions “The density of Trichodesmium filaments and colonies within the carboys/bottle incubation experiments was maintained lower than the in situ densities found in the intense surface accumulations“, however no actual numbers are shown.

L372 mentions “While we did not directly determine nutrient concentrations within the surface patches, it would be reasonable to assume that nutrient pressure on these dense surface populations (i.e. competition for nutrients and utilization rates) exceeded that in the bottles.”, but no further explanation for this hypothesis is given.

L435-L438, when comparing pre- to bloom values, a coherent measure should be used, e.g., average to average or max to max. Instead several ranges are used and it is not clear to the reader how the fold increase was calculated.

L439, cellular internal components do not “collapse”, they rather “structurally degrade”.

L476. It is unclear to the reader to what experiment the authors refer to when they mention a “for the three weeks of the experiment”. In their work, two experiments were conducted of 22 and 44 hours respectively. Maybe authors refer to the monitoring period of the lagoon?

L493. The use of term “fastidious” is inappropriate.

L506/L516. While L506 states that no specific Trichodesmium phage has been isolated

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or characterized to date, L516 mentions that authors could not identify Trichodesmium-specific phages on their experiments. Probably the the use of term “found” or “characterized” is more appropriate than “identify”.

L514-L516. The arguable hypothesis about “Nonetheless, virus infection itself may be a stimulant for community N2 fixation perhaps by releasing key nutrients (i.e., P or Fe) upon lysis of surrounding microbes” should be further elaborated or at least referenced.

L526-535. Authors conclude that increase of phoA transcripts can be interpreted as an acclimation to low P availability, however, P availability was not measured and other causes are equally plausible as, e.g., general stress, which were not tested.

L618. Capace activity not necessarily means PCD. What is the expression profile of the measured caspases during necrotic cultures? Do those specific caspases display a similar expression pattern during necrosis and PCD? A control is needed where transcripts of necrotic cells are quantified.

L680-L685. Authors mention hypotheses, not conclusions.

L688-L691. Conclusion should be rewriten to be more accurate. What happens in bottles can it be transferred to what happens in situ? Could a shallow lagoon be comparable to the ocean, are these systems comparable?

Other typos include: L94 “bloom to crash” instead of “bloom crash” L151 extra “%” symbol L152 “at” should be removed L170 “then” instead of “than” Figure 4, Y-axis label is wrong. It reads “% of 16S tags”, but scale is from 0-1. L323 “High respective rates” should be changed to “Relatively high rates”. L351 “2 d” should be “2 day” L432 ug is missing between 700 and GX

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