

Biogeosciences Discuss., referee comment RC1  
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## Comment on bg-2022-155

Anonymous Referee #1

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Referee comment on "The response of diazotrophs to nutrient amendment in the South China Sea and western North Pacific" by Zuozhu Wen et al., Biogeosciences Discuss., <https://doi.org/10.5194/bg-2022-155-RC1>, 2022

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### General comments

In their manuscript bg-2022-155, Wen et al. present the results of a series of nutrient additions experiments conducted in the South China Sea and western North Pacific, where in addition to bulk N<sub>2</sub> fixation rates, species composition based on nifH gene abundance was analyzed in response to Fe and P amendments.

Overall, I enjoyed reading the manuscript, and I find the dataset is a useful addition to our understanding of the regulation of N<sub>2</sub> fixation rates and how it is linked to species composition. The manuscript is well written, and the discussion insightful. Yet, there are a few points that I believe should be discussed/improved, specifically I have some concerns/queries with regard to replication and interpretation of qPCR results, as outlined below.

### Specific comments

- Apparently, in several of the experiments there were only 2 replicates – I wonder whether the statistical analysis procedures are valid for two replicates? Could the authors at least indicate in each of the figures which data are averages of 2?
- The authors acknowledge that 15N label% was not measured, which is indeed a shortcoming, but as they state that the experimental procedure and the results were comparable to their previous study I believe it is acceptable. An average and stdev of label% in the previous study are given, but could the authors add any further details to help us understand how reproducible the approach was (number of replicates etc)?
- I wonder how the reports on Trichodesmium polyploidy (ca 100 genome copies per cell in field samples, e.g. Sargent et al. 2016 <https://doi.org/10.1093/femsle/fnw244>) affect the estimates of species composition based on nifH gene copies, as well as the

trends observed in bioassays. Was polyploidy taken into account when the 'dominant species (e.g., l. 351)' were determined? And, taking into account the high level of polyploidy in *Trichodesmium* compared to the other species, couldn't shifts in the species composition explain the mismatch in responses of N<sub>2</sub> fixation vs nifH abundance (e.g., l. 343)?

- Related to this, I would suggest being more cautious about the use of the terms 'abundance' (e.g. abstract l. 26 'abundances of specific diazotrophs', l. 119 'abundances of specific diazotroph phyla') and 'growth' where actually gene copy number was measured. Specifically, in supplementary figure 1, I would suggest replacing 'growth rate' by 'gene abundance', since growth rate might imply that measurements of cell density or C concentration were made.

#### Minor comments

- 34-35 'the largest responses were always dominated by either *Trichodesmium* or UCYN-B': this is not clear (responses in what?) – can it be clarified?
- 110-112 it is not completely clear from this why high spatial resolution is necessary - can this be justified better?
- 164 can more details on the gas-tight plastic bags (supplier) be added?
- 221 why were those Fe and P concentrations chosen – are there any references to add on how these relate to in situ concentrations in this area?
- 223 Can the authors supply some more details on the incubation system? Do I understand correctly that 10L carboys were placed in the on-deck incubator, and there was some kind of water jacket flushed with seawater for temperature control?
- Please supply more detail or a reference on the method for chl measurement
- 441 I believe the biochemical substitution of Fe and P deserves some more explanation (either here or at a later stage) - how could this work, are there specific mechanism/enzymes that can substitute Fe for P?
- 450-451 also the 'serial limitation' of N<sub>2</sub> fixation by another resource deserves a few more words for explanation I believe – it is not clear how this would work
- 476 I think there might be more specific references for the Fe demand of *Trichodesmium* (e.g., Kustka et al., [https://doi.org/10.1016/S0923-2508\(02\)01325-6](https://doi.org/10.1016/S0923-2508(02)01325-6), 10.4319/lo.2003.48.5.1869, <https://doi.org/10.1046/j.1529-8817.2003.01156.x>)
- Could it be specified how the data in Fig. S1 was calculated? Is the growth rate in units d<sup>-1</sup> the total increase in gene abundance over the 3 day bioassay experiment divided by 3?
- In the supplementary table showing parameters involved in nitrogen fixation rate calculations, the units for several of the parameters (e.g., depth, chl a, PON, NFR) are missing in the headers, please add these.

#### Technical corrections

- 62-63 check the order of references: in sequence of publishing year?
- 69 \*did\* not quantitatively match
- 72 \*is\* potentially crucial
- 76 I assume this should mean 'contribute differently to the sinking flux carbon that

small unicellular species (i.e., delete 'that of')?

- 110 remain\*s\*
- 242 metal bound (remove hyphen)
- 266 below
- 313 and others: remove brackets around station names in the text