

Biogeosciences Discuss., referee comment RC2
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Comment on bg-2022-155

Anonymous Referee #2

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-RC2>, 2022

In this paper, Wen et al. reported the patterns and limiting factors of nitrogen fixation activity and diazotroph community in the South China Sea and western North Pacific. Through the iron and phosphate amendment experiments, they delineated the nutrient limitations of nitrogen fixation and diazotrophs for different water bodies in the study region. These findings may facilitate our understanding of the bottom-up controls of the diazotrophs in the study region, while I have some concerns regarding the methods, interpretations, and discussions in the current manuscript.

This manuscript gives me a general impression that the authors kept mentioning another paper of their group (Wen et al., 2022), instead of sticking to the findings of the current study. For example, they discussed a lot about the iron to nitrogen supply ratio, which seems like a highlight in Wen et al., 2022, but this ratio was not analyzed in the current study. Also, the dissolved iron to dissolved inorganic nitrogen ratio was not available in this manuscript. I suggest the authors focus more on the direct and interactive effects of iron and phosphorus availability on the diazotrophs for the discussion.

Given that the iron and phosphorus limitations of diazotrophs are the major focus of this study, the authors should describe the iron and phosphorus availability in the study region based on their real data, like ambient concentrations of iron and phosphate, instead of speculating. Also, the iron and phosphate concentrations in the nutrient amendment experiments should also be reported. This information is particularly important when the authors discuss the reasons and biogeochemical implications for the experimental results.

Besides, the authors should be aware that they only analyzed some commonly observed diazotroph groups (i.e., part of the diazotroph community) with qPCR assays. In other words, some of the unanalyzed diazotrophs might be important N-fixers at some stations. Some unanalyzed diazotrophs might even pop up during the 3-day incubation. So, they need to be cautious when comparing the patterns of nitrogen fixation rates and diazotroph abundances. The authors may also consider conducting nifH amplicon sequencing for

reconstructing the whole diazotroph community in the study region. Also, initial diazotroph abundances of the incubations should be reported as well.

L28-30: It is better to avoid hypothesis/speculation in the abstract. The iron to nitrogen supply ratio was not directly measured in this study as the authors stated in L416.

L34-L35: "the largest" and "always" seem subjective and inaccurate based on Figure 5. Also, there was no significant response at some stations where *Trichodesmium* dominated.

L38-40: Why? I did not see any evidence from this study supporting this speculation directly.

L136-137: This sentence is not informative, as the depths are not labeled in the figure. Nevertheless, the depths of the seafloor are not important here.

L152-156: Were the water samples collected from different depths (2-5m) exposed to different degrees of dissolved iron contamination from the research vessel? Did the authors measure dissolved iron concentration for these water samples?

L160: The results of primary production were not described or discussed. Was primary production also measured in the nutrient amendment experiment? The Chl-a and primary production from the experiments may be helpful when the authors discuss/speculate about the competition between diazotroph and non-diazotrophic phytoplankton (L28; L403).

L226: Did you measure iron concentration in the $^{15}\text{N}_2$ enriched water? The preparation of $^{15}\text{N}_2$ enriched water may introduce iron contaminants (Klawonn et al., 2015).

Klawonn, I., Lavik, G., Böning, P., Marchant, H. K., Dekaezemacker, J., Mohr, W., & Ploug, H. (2015). Simple approach for the preparation of $^{15}\text{N}_2$ -enriched water for nitrogen fixation assessments: evaluation, application and recommendations. *Frontiers in microbiology*, 6, 769.

L307: The description of S3 is confusing. Does "ab" in Figure 4 mean no significant difference with a and b? If that's the case, S3 seems not "independent co-limited". Please clarify.

L317-323: Please clarify the exact number of replicates for each treatment. Also, I doubt the statistical significance based on duplicates (n=2). The limitation of replication should be stated clearly in the manuscript. It is also the same for Figure 5. Were initial nitrogen fixation rates (i.e., the rates of the seawater from the pump) measured?

L350: As you only analyzed part of the diazotroph community, you may consider revising "diazotroph community structure" to "abundances of analyzed diazotrophs".

L367 and Figure 5: How about UCYN-A1? UCYN-A1 was also abundant at K1 and WP based on Figure 3, while they disappeared in the nutrient amendment experiment (Figure 5). Also, the initial diazotroph abundances should also be displayed in Figure 5.

L382: There is no doubt about Kuroshio being a hotspot of nitrogen fixation, while the low rate at K1 is not the "increasing evidence" as stated here.

L385: Abundances of diazotrophs do not necessarily mean their contribution to nitrogen fixation.

L412-431, 453-464, etc.: The contents (mostly iron to nitrogen supply ratio) of Wen et al. 2022 are worth mentioning, but, they should be reduced significantly in the discussion. As said, the iron to nitrogen supply ratio was not analyzed in this study.

L440: I think another reason would be that the analyzed groups did not represent the entire diazotroph community. There could be other diazotroph groups, which were not analyzed in this study, influenced by treatments.

L473-475: However, the nitrogen fixation at S3 and S4 was mostly iron-limited, while the *Trichodesmium* abundances there were not affected by iron addition treatment. All these pieces of finding should be considered when you discuss the relationship between iron and *Trichodesmium* in the NSCS.