Biogeosciences Discussions

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# Comment on bg-2021-349

Anonymous Referee #1

Referee comment on "Controlling factors on the global distribution of a representative marine non-cyanobacterial diazotroph phylotype (Gamma A)" by Zhibo Shao and Ya-Wei Luo, Biogeosciences Discuss., https://doi.org/10.5194/bg-2021-349-RC1, 2022

Shao and Luo compile published abundance data of Gamma A (qPCR nifH gene counts), a putatively heterotrophic diazotroph widespread in the oceans. Using ancillary data, atlas, satellite products and models, they perform a thorough statistical analyses to infer relationships between Gamma A and environmental variables. Their results suggest that Gamma A benefits from primary production by-products and is mostly dominant in warm and iron-poor waters of the ocean. The data analyses are extensive and the results worth publishing. However, the authors should improve the comparison between their study and Langlois et al. 2015, who also compiled Gamma A data and performed statistical analyses to define their niche. How does the present study build up on previous ones? I also found several mis-citations, where the wrong citations are given to justify a statement or where the message of a given paper was not well understood. In all, the exercise seems statistically correct but of relatively poor ecological interpretation significance unless several points are improved. Below I provide a list of comments.

ABSTRACT

L7: Delete “the” in “to the global marine”.

L10: What is the carrying capacity? This term is used throughout the manuscript whereas it is never really explained.

L15: “in addition” not “in additional”.

L17: Eddies are not short-term features, they may last several months. Please choose another term.

L18: “organic matter” not “organic matters”

L19: Weird wording, please rephrase.

L20: “sampling” not “samplings”, and delete “better” from the end of the sentence.

INTRODUCTION

L32: “oxygen deficient zones”

L33: “heavy” sounds weird, please rephrase.

L33: I would not say heterotrophic $N_2$ fixation is “not well quantified”, it’s just not quantified at all. There is -currently- no assay able to isolate heterotrophic $N_2$ fixation from autotrophic $N_2$ fixation.

L41: This is not what these papers really say. In Benavides 2018b Gamma A was not detected, so it does not necessarily mean it was not stimulated by DOM, it just was not present in the samples at all. In Benavides 2015 $N_2$ fixation in dark waters was stimulated by amino acids.

L42-43: NCDs are thought to be attached to particles, but they haven't been found to be attached to particles.

L45: Bonnet 2016 find that N released by Trichodesmium is taken up by diatoms. Not N released by NCDs.

L45: "to equip" sounds weird.

L52-53: How did those studies look at DIN inhibition of individual NCDs strains? It seems this is not what these studies really did.

L58: This is quite unfair to say, please cite:

Bombar, Deniz, Ryan W. Paerl, and Lasse Riemann. 2016. "Marine Non-Cyanobacterial Diazotrophs: Moving beyond Molecular Detection." *Trends in Microbiology* 24 (11): 916–27.

Cornejo-Castillo, Francisco M., and Jonathan P. Zehr. 2020. "Intriguing Size Distribution of the Uncultured and Globally Widespread Marine Non-Cyanobacterial Diazotroph Gamma-A." *The ISME Journal*. https://doi.org/10.1038/s41396-020-00765-1.

Langlois, Rebecca, Tobias Großkopf, Matthew Mills, Shigenobu Takeda, and Julie LaRoche. 2015. "Widespread Distribution and Expression of Gamma A (UMB), an Uncultured, Diazotrophic, γ-Proteobacterial NifH Phylotype." *PloS One* 10 (6): 1–17.

Moisander, Pia H., Mar Benavides, Sophie Bonnet, Ilana Berman-Frank, Angelicque E. White, and Lasse Riemann. 2017. "Chasing after Non-Cyanobacterial Nitrogen Fixation in Marine Pelagic Environments." *Frontiers in Microbiology*. https://doi.org/10.3389/fmicb.2017.01736.

Riemann, Lasse, Hanna Farnelid, and Grieg F. Steward. 2010. "Nitrogenase Genes in Non-Cyanobacterial Plankton: Prevalence, Diversity and Regulation in Marine Waters." *Aquatic Microbial Ecology: International Journal* 61 (3): 235–47.

L59: change "can" to "may".

L63: You may cite Benavides, M., and J. Robidart. 2020. "Bridging the Spatiotemporal Gap in Diazotroph Activity and Diversity With High-Resolution Measurements." *Frontiers in Marine Science* 7. https://doi.org/10.3389/fmars.2020.568876.

L69: "suggesting" would be fairer than "revealing" here. Note that nif genes can be used for other purposes.

METHODS

L84: "the upper 100 m of the water column".

L87: there is no common qPCR detection limit, it depends on the essay, the machine, the lab, the volume of water filtered.

Table 1: I am a bit puzzled at 0 m depths, this is unlikely. Please check.

L103-104: I wonder why an artificial neural network was considered for DOC concentrations when there is a now a global database available https://odv.awi.de/data/ocean/dom-compilation-hansell-et-al-2021/ Please reconsider using it instead.

L116: It is unclear how SLA data was used, data was extracted from the same days as Gamma A samples were taken? Please explain.

RESULTS AND DISCUSSION

L149: nifH abundance also decreases with depth in the North Pacific (see work from Church at station ALOHA).

L170: please explain what the carrying capacity is.

L172: Please cite Bombar 2016.

L175: How are biogeographic patterns biased by the sparse and uneven sampling in different ocean regions? Can this be assessed statistically?

L190-191: This negative correlation is because low temperature anticorrelates with NPP, right?

Section 3.4. The first sentence belongs in the methods. Why even show linear regressions at all if the model is deemed better? I would suggest just mentioning the correlations, maybe move them to the supplementary, and dive into the GAM directly in the main text. Why are, in any case, the effects found using GAM so different to the ones obtained with linear correlations? (e.g. L219).

L223: fuel with what?

L224: I suggest replacing Farnelid 2019 for Riemann 2010.

L235: This seems quite a speculative conclusion to make. DOC concentrations alone do not inform about lability, and, to date, we don't know anything about the metabolism of Gamma A or which kind of DOM molecules they may use.

L243-244: note that NCDs also need P.

L301: this seems quite different from observations.

Figure 6: why is the abundance "annual"? it's not a rate.

Section 3.5. the connection or justification of why the effect of SLA is tested here is hard to follow.

L316: eddies are not short term phenomena.

L320: but also the number of data points in the NH is much higher than in the SH, potential bias, how can it be assessed?

SUMMARY AND OUTLOOK

L367: “confirming heterotrophy” seems quite risky. We need genomic data and tracer experiments to confirm that.

L379: Unclear here, nifH primers are universal. There is no primer for cyanobacterial diazotrophs only. These primers target all diazotrophs with Mo nitrogenases.