Biogeosciences Discussions

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Comment on bg-2021-190

Anonymous Referee #1

Referee comment on "N_2 fixation in the Mediterranean Sea related to the composition of the diazotrophic community and impact of dust under present and future environmental conditions" by Céline Ridame et al., Biogeosciences Discuss., https://doi.org/10.5194/bg-2021-190-RC1, 2021

GENERAL COMMENTS:

Ridame et al. present an interesting set of N_2 fixation rates measurements, and the associated diazotrophic community composition, mostly assessed in short term dust addition experiments, in order to determine to what extent the newly fixed nitrogen can support primary and bacterial productions, and evaluate how the composition of the diazotrophic community and environmental factors both in present and future climate conditions can influence the spatial distribution of N_2 fixation rates in the western and central Mediterranean Sea.

The manuscript is well written and constitutes a relevant contribution to the current knowledge. The experiments are well designed, and the interpretations are in general well supported by the data.

However, there are some concerns that the authors should take into consideration:

- It is now generally accepted that minimum detectable uptake rates (N_2 and CO_2) should be determined for every individual incubation experiment, so that rates under their specific detection limit can be reported as such (<DL). Because every sampling site and sampling depth (and sampling time) have their own original substrates concentrations and associated isotope compositions (PN, POC, dissolved N_2 and dissolved inorganic carbon), it makes it important to compute incubation-specific minimum detectable uptake rate, based on the minimum increase in isotope composition detectable by the isotope ratio mass spectrometer. The authors should confirm that all reported N_2 fixation rates are indeed truthful (particularly at depths ≥ 200 m).

- The authors should be more skeptical and critical when comparing high-throughput sequencing of *nifH* gene from different sampling depth, sites and time points

- Primary production rates measurements (based on the ^{13}C incubation method), although mentioned all along the manuscript (with relation to corresponding N_2 fixation rates) are not described or discussed. The authors invite the reader to report to the manuscript by Maranon et al. (2020), who used a different methodology (^{14}C incubation technique). The authors should inform the reader (e.g., in the supporting information) about how the results from the two methods compare? Whether they show similar trends across the sampling sites and dust seeding experiments, despite the contrast in gross versus net rate assessments? This would support the authors' decision not to further discuss primary production in their manuscript and invite the reader to report to Maranon et al. (2020) for more detailed insights.

SPECIFIC COMMENTS:

Materials and Methods:

Lines 139-140: The authors should indicate in the supplementary information how consistent the results from the two methods are (^{13}C-PP and ^{14}C-PP). As of now, no other

manuscript in the Special Issue describes or discusses the ^{13}C-PP rate measurements. Unless a manuscript comparing the data from the two methods is envisioned, having a brief comparison in the Supplementary Material would support the authors' choice not to discuss ^{13}C-PP further in this manuscript and focus on N_2 fixation and diazotrophic community compositions.

Lines 139-140 and 152-153: Please clarify for the reader that the contribution of N_2 fixation to primary production and to bacterial production where estimated using C:N Redfield ratio (6.6) and ratio from Nagata (1986), respectively.

Line 149: The authors chose to use of the $^{15}N_2$ bubble addition method for their incubation experiments, which has been shown to underestimate in situ N_2 fixation activity due to incomplete tracer dissolution. The authors clearly stated that. However, to alleviate some of this uncertainty, the authors could consider in the future, sampling the incubation bottles at the end of the experiment (before filtration) to determine the final ^{15}N%-N_2 enrichment, which can then be used to compute N_2 fixation rates. Although these rates would likely still underestimate the true activity (due to dissolution kinetics taking place during the 24-hour incubation), they would however reduce the uncertainty and inform on the gap between N_2 fixation rates based on measured versus theoretically estimated ^{15} N-N_2 enrichments.

Lines 149-150: The authors should confirm that all reported N_2 fixation rates are indeed truthful (particularly at depths ≥ 200 m), by computing incubation-specific minimum detectable uptake rates, based on the minimum increase in isotope composition (relative to natural abundance), detectable by the isotope ratio mass spectrometer (Fonseca-Batista et al., 2017; White et al., 2020).

Line 152: for the sake of clarity, please inform the reader that BP measurements, which methodology has at this stage not yet been described, are complementary data presented in companion manuscripts (Gazeau et al., 2021b; Van Wambeke et al., 2021)

Lines 152-153: have the authors considered citing Fukuda et al. (1998) (manuscript with Nagata Toshi himself as co-author), to support their choice of C:N conversion factor. In fact, the cell collection in Fukuda et al., seems more appropriate for bacteria than the GF/F filtration used in Nagata (1986), thereby leading to a more reliable estimate of the bacterial C:N ratio in oceanic settings of 6.8 ± 1.2.

Line 176: please explain what influenced the decision to truncate the reads at 350 bp? (no need to report in the manuscript)

Results

Line 273: "CV%" not previously defined

Line 281: please clarify, "low overall **relative** abundance".

The authors should be more skeptical when comparing relative abundance data from different sampling depth, sites and time points (Gloor et al., 2017): **Lines 290-292, 447-450 and 462-463.**

Line 286: Specify from which condition(s) (Control, Dust and Greenhouse) the average contributions of UCYN-A1 and A3 to the total diazotrophic community were determined from at T0.

Discussion:

Lines 320-321, 361-362, 378-379, 401-402, 404, 409 and 455: data not shown, that could be added to the Supplementary Material, with relation to:

1) correlation between N_2 fixation rates and diazotrophic community composition (for instance, surface N_2 fixation versus UCYN-A and NCD)

2) contribution of N_2 fixation to PP and BP

3) evolution of nutrient concentration in the dust seeding experiments: DIP concentration in Control and Dust experiments at station TYR; requiring citation of the corresponding companion paper (line 404).

Lines 331-332: Sentence not clear, please rephrase.

Line 340: Please explain further why the DFe minimum could not only be the result of uptake by diazotrophs

Line 445: "a decrease in the top-down control on the bacterioplankton which is strongly suspected to increase under future climate conditions" Please explain further why

Conclusion:

Lines 462-463: Because cell specific N_2 fixation rates were not determined, this statement should be less affirmative.

Tables and Figures:

Table 1:

- Why were some average and standard deviation values not included in the two bottom rows?

Table 2:

- Please specify what size fraction (or incubation experiment) is used to compute the C:N (mol/mol) ratio?

Figure 1:

- Station "TYR" labelled as "TYRR"

Figure 2:

- Are data points missing at 1000 m for ST6, ST8 and ST10?
- Authors should consider breaking the scale of the x-axis (N_2 fixation, nmol N L^{-1} d^{-1}) for station 10. This would improve the readability of the graph, and highlight significant N_2 fixation rates, not only at 61 m.

Figure 6

- Please adjust the y-axis to a unique range for all 3 graphs and arrange the graphs side by side.

Figure S5:

- Please consider dissociating the stations either into separate plots, or even just separated series on the same plot.

TECHNICAL CORRECTIONS:

Lines 24-25: "strong longitudinal gradient increasing eastward"

Line 72: "enhance" instead of "enhanced"

Line146: space missing between "and" and "^{13}C"

Line 153: Adapt reference “Nagata, 1986” instead of “Nagata et al., 1986”

Line 158: replace “following” by “as follows”

Line 159: For the sake of clarity, the variable Tx could be removed from the formula, since the term cancels itself being in both the numerator and denominator. On the other hand, “$N_2FIXATION_T$” could be replaced by “$N_2FIXATION_{Tx}$”

Line 244: replace “as” by “or”

Line 318: Add in the parentheses “(**in** this and previously published studies)”.

Line 337:

- delete “and”, to read “… take place, combined with…”
- replace “high stocks” by “higher stocks”

Line 349:

- “whole diazotrophic community in the euphotic zone” instead of “the whole diazotrophs”
- Reference Table S1 at the end of the sentence

Line 384: Data reported here do not support an increase of diazotrophs abundances, so consider replacing “obviously” by “likely”.

Line 398: replace “**to** dust seeding” by “**by** dust seeding

Line 406: please clarify, “heterotrophic prokaryotes, NCD, and photoautotrophs” had to compete for dust-derived DIP

Line 407: “the lower stimulation” instead of “the lowest”

Line 477: “UCYN-A remain” instead of “remained”

Line 480: “are expected” instead of “would expect”

References:

Fonseca-Batista, D., Dehairs, F., Riou, V., Fripiat, F., Elskens, M., Deman, F., … Auel, H. (2017). Nitrogen fixation in the eastern Atlantic reaches similar levels in the Southern and Northern Hemisphere. Journal of Geophysical Research: Oceans, 122(1), 587–601. https://doi.org/10.1002/2016JC012335

Fukuda, R., Ogawa, H., Nagata, T., & Koike, I. (1998). Direct determination of carbon and nitrogen contents of natural bacterial assemblages in marine environments. Applied and Environmental Microbiology, 64(9), 3352–3358. https://doi.org/10.1128/aem.64.9.3352-3358.1998

Gloor, G. B., Macklaim, J. M., Pawlowsky-Glahn, V., & Egozcue, J. J. (2017). Microbiome datasets are compositional: And this is not optional. Frontiers in Microbiology, 8(NOV), 1–6. https://doi.org/10.3389/fmicb.2017.02224

White, A. E., Granger, J., Selden, C., Gradoville, M. R., Potts, L., Bourbonnais, A., … Chang, B. X. (2020). A critical review of the 15N2 tracer method to measure diazotrophic production in pelagic ecosystems. Limnology and Oceanography: Methods, Vol. 18, pp. 129–147. https://doi.org/10.1002/lom3.10353