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Comment on bg-2021-190

Anonymous Referee #2

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-RC2, 2021

General comments

This manuscript by Ridame and co-authors addresses spatial variability in the activity and distribution of diazotrophs and potential environmental controls, and future potential impacts of ocean acidification and warming across the Mediterranean Sea. The sampling strategy was well planned to address this relevant question: Discrete sampling of the water column down to 1000m to measure rates and determine the diazotroph community composition against ambient nutrient concentrations was supported by incubation studies with dust and increased temperature/CO_2 concentrations to simulate projected future ocean conditions. The manuscript is well structured and enjoyable to read, the methods and results are clearly described and presented in a generally balanced manner and are well supported by a body of relevant literature. The section on complementary data from other PEACETIME papers is a great idea and much appreciated. This made it very easy to connect to other relevant papers.

Hence, overall the quality of the manuscript is high and it makes an insightful contribution to our understanding of diazotrophs and their activity in the Mediterranean Sea. In saying that, I do still have some questions about the data interpretation and in particular, the reliance of one key outcome of the paper on a high N_2 fixation rate measured at 1 station and at 1 depth which is ~x100 higher than any other measured (volumetric) N_2 fixation rate. The dedicated section 4.3 to "Intriguing station 10" aims to explain this observation citing studies with similar magnitude rates and suggesting that this is due to the patchiness often observed with UCYN-A abundances, the dominant diazotroph detected. The authors argue that this is likely due to nutrient inputs from Atlantic water intrusion into the surface and a different diazotrophic community present. Were replicate incubations made for each sampled depth to indicate if this is a reproducible result? If yes, it would be helpful to report the standard deviation of the rates to indicate variability in the measurements. If not, then I would question how robust this finding is. One other limitation of the presented data set that is also acknowledged by the authors, is that no quantitative nitrogenase gene analysis was carried out and all conclusions are based on

qualitative data on community composition.

I would also encourage the authors to make the data openly available, latest at publication, rather than keeping it embargoed until 2023.

Specific comments

Line 31: The Mediterranean Sea is generally considered a desert because of very low surface nutrient concentrations so it is puzzling to see "nutrient rich" here used to describe some stations, as the measured surface concentrations were in the nanomolar range.

Line 37: It isn't clear how N_2 fixation could be "exacerbated". Consider rephrasing to "increased" or similar.

Lines 75-77: The statement that atmospheric inputs would be particularly important for diazotrophic organisms under increased stratification due to ocean warming is not well explained. Why would diazotrophic organisms in particular be affected?

Line 101: Was the metabolic activity of diazotrophs present measured at this station or was this rather referring to anticipated differences in metabolic activity due to differences in oligotrophic conditions?

Line 104: Unfiltered seawater was used for the incubations. Does this mean that larger grazers could have been present and influenced the biomass development or nutrient regeneration inside the incubations?

Lines 116 – 122: A figure or table as an overview of all key steps in setting up the dust incubation experiments from dust preparation, to CO_2/temperature manipulation and final sampling would be helpful.

Line 125: Concentration of HCl used for acid washing is missing.

Section 2.3: Were blank incubations (i.e. without isotope addition) carried out to correct for any incubation effects?

Line 137: The incubation irradiances are reported as "percentages of attenuation", however it seems this might be more accurately reported as "transmittance"? The order of the values from highest to lowest would indicate the lowest irradiance first (70% attenuation). Is this correct? What type of blue filter was used? Also, to what depths do these attenuations correspond to?

Line 150: Here it states the "molar C:N ratio in the particulate matter was calculated and used to estimate the contribution of N_2 fixation to primary production". How was this exactly done? What impact might detritus have on this calculation? How does this compare to the N demand as calculated from the measured PP rates rather than POC concentrations?

Line 233: Here the surface is specifically mentioned as 5m deep, but this distinction isn't clear in other instances e.g. Fig. 3 and the surface mixed layer is also used to define the surface layer for reporting integrated rates and stocks. This is a little confusing and the changing definition isn't justified in the text for each variable, which makes it difficult as a reviewer to accurately scrutinise and assess the results. If different definitions are necessary to highlight key relationships between variables in different water column section, this should be stated and justified more clearly. If atmospheric deposition is a key nutrient input that drives observed variability in diazotroph activity and community composition, I would imagine the surface mixed layer would be the best definition to be used, rather than just 5m. Table S1 suggests the SML was indeed deeper than 5m and up to 21 meters deep. Following from this, I was a little confused by the observation that surface N_2 fixation rates (5m) and euphotic zone but NOT the surface mixed layer nor aphotic N_2 fixation rates correlated with longitude. As far as I could tell, this wasn't picked up in the discussion at all but would be interested to understand this result better. What could be possible explanations for this observation?

Section 2.6: It isn't clear how the missing nanomolar nutrient concentrations at Station 1-4 were taken into account in the statistical analyses and if this may have an influence on the correlation analysis output. Table S1 does indicate that a maximum concentration of 0.05 µmol L^{-1} was used when calculating the NO_3^- stocks. Was the same approach used for the Pearson correlation? If yes, how may this have affected any potential correlations for the surface mixed layer or where depths <50m were included in the calculation?

Line 253: The exclusion of the N_2 fixation rates from Station 10 can be appreciated due to the one depth that has remarkably high rates but this does lack clear justification in the manuscript. Please also see further comments on this one station below.

Line 307-309: The detection of UCYN-A3 and -A4 sublineages is an exciting new discovery for the region. Is there a particular reason why these groups were now detected? Is this due to methodological developments or rather due to the oceanographic conditions present?

Line 340: Why is this low DFe not explained solely by diazotroph uptake? As no quantitative data is reported on abundances, this is difficult to assess.

Line 398: What is considered the limiting factor for N_2 fixation at TYR and ION that was not considered limiting at FAST? Final rates in the dust incubations were actually quite similar between the three stations but the difference in trends in % difference in rates appears to be driven by the different baseline at the different stations e.g. the baseline at FAST is higher (~0.5 vs ~0.2 nM N L^{-1} d^{-1}). Could this mean the diazotrophs at all stations have the same potential to fix N but are just limited under ambient conditions (without dust/nutrient inputs). It seems like the ION community are only nutrient limited, yet in TYR and FAST are below their thermal optimum conditions. The idea of temperature optima is brought up in regards shifts in the diazotrophic community within a station (lines 450-454) but could this also be important between the three studied regions of the Mediterranean Sea?

Lines 406-408: The final sentence in this paragraph was confusing to me, in particular how the three group (heterotrophic prokaryotes, NCD, photoautotrophs) would outcompete and thereby reduce the DIP taken up by each cell. I'm not sure "outcompete" is the right word here and would recommend rephrasing this sentence in a more simple manner.

Lines 409-412: Could the symbiosis be stimulated the other way around i.e. dust enhances N_2 fixation in UCYN-A which then relieves N-limitation in the photoautotrophic host? This would fit better with the "potential N limitation" and nutrient ratios reported e.g. lines 264-267.

Lines 419-423: It is true that changes in CO_2 concentrations would not directly affect diazotrophs that do not fix CO_2 however there are associated changes in seawater pH under ocean acidification (OA) that may affect cellular metabolism on short time scales in both autotrophic and non-autotrophic organisms. This, probably more relevant, change in seawater chemistry for non-autotrophs is not acknowledged here in this introductory paragraph although mentioned later in lines 441-443. I would suggest pH should be more clearly stated as the key factor rather than CO_2, even if observations indicate OA does not seem to affect communities dominated by UCYN-A.

Line 453-458: The shift to larger diazotrophs with a higher cell-specific N_2 fixation rate under ocean warming could explain the stimulation in N_2 fixation rates but is difficult to determine from relative nifH-based community composition. I feel the that here, care needs to be taken to not to confuse increase in absolute abundance (as observed in the cited study by Henke et al. 2018 for UCYN-A2) with an increase in the relative abundance which was what was measured in this study. Any increase in N_2 fixation would still need sufficient resources (e.g. nutrients) to fuel this unless there was an underlying change in organism metabolism with temperature/pH. Differences in community composition may drive changes in observed activity but differences in abundances would arguably have a larger impact on measured rates. Furthermore, could the increase in N provided by stimulated N-fixation by NCDs not account for the increase in PP also observed for the

FAST station? As NCDs were the dominant diazotroph detected, this, to me at least, would make more sense.

Line 469: The phrase "triggered N_2 fixation" implies that there was no N_2 fixation happening before. Instead, this is probably referring to the observed increase in N_2 fixation in the dust/warming/acidification incubations. Please consider rephrasing.

Figure 2: It is intriguing that the N_2 fixation rates were highest below the surface rather than in the top 5 m considering the importance of atmospheric deposition. Is this related to other processes such as diffusive nutrient supply? It might be helpful to indicate the nutrient depth profiles, perhaps in the supplementary material, to explain this.

Figure 6: It would be helpful if the x-axis had the same scale for each site i.e. proportionally longer for FAST as the incubation lasted 4 days instead of 3. Also, why were some days excluded from the analyses? These seem to be robust rate estimates. Was only the nutrient replete period of interest here? It would be useful to know why a linear relationship was expected by the authors that lead them to a linear regression analysis rather than an approach such as mixed models.

Figure S5: The use of a line plot connecting the Shannon H index is a little misleading as it suggests all data are connected along the x-axis and I would recommend revising this figure. As there are multiple stations included in this figure, I would recommend breaking the line between stations to highlight the change in diversity over time which is, what I understand, the most important aspect here but is not clearly portrayed in the current figure. Different symbols for the different stations would also be helpful to demonstrate the increase in diversity for FAST vs. decrease in diversity over time for TYR and ION. An x-axis label would also be helpful.

Table 1: Consider reporting the standard deviation for the three distinct areas to further highlight

In general, addition of important information to panels such as the station (e.g. Fig. 8) would also make it easier for the reader to grasp the figure, rather than keeping it in the figure captions. A table with an overview of the Pearson correlation coefficients would be a useful addition.

Technical corrections

Overall these suggested corrections are minor and do not impede reading or understanding but I would recommend a careful check of plurals throughout the text.

Some of these are detailed here:

Line 79: “nutrients repleted” should be “nutrient repleted”.

Line 84: “nutrients” should be “nutrient” and “diazotrophic communities” should be “diazotrophic community”.

Line 168: Individual stations should have a capital S i.e. “Station 10” or “Stations 1 and 10” on Line 226, whereas “stations”, when referring in general (e.g. Line 255) do not need capitalisation.

Line 217: Please consider adding citations to specific R packages used within the software to acknowledge the package authors.

Line 336: “exchanges” should be “exchange”.

Line 340: “diazotrophs uptake” should be “uptake by diazotrophs” or “diazotroph uptake”.