

General comments:

In this manuscript, Sophie Bonnet and colleagues describe N_2 fixation rate measurements on a transect through the Mediterranean Sea. This dataset represents nice and valuable information as N_2 fixation has so far not been extensively studied in the Mediterranean. Further, the presented data is somewhat contrasting to previous studies which were mainly based on N isotopic signatures which generally suggest higher N_2 fixation rates in the Eastern basin of the Mediterranean Sea.

The introduction gives a comprehensive overview of the relationship between N:P ratios, however, the two hypotheses which explain the anomalous high N:P ratios could more structured, for example, by a short, one or two-sentence summary followed by the detailed descriptions.

The 'Material and Methods' as well as the 'Results' sections are concisely written and are well separated from the discussion.

The citations or presentations of data from other manuscripts which have been/will be submitted to the same issue should be carefully assessed depending on the availability of these data.

The discussion is in general a bit too long, specifically the sections 4.1 and 4.2. In section 4.1, there is a lot of discussion on data which is not presented here but rather in manuscripts submitted to the same issue. This part could be shortened and the diazotroph community could be described more concisely. In section 4.2, the N_2 fixation rates measured in the Eastern Basin are here demonstrated to be "negligible" in terms of contribution to new N but the focus of the manuscript is on the importance of N_2 fixation as a major biogeochemical process in the Mediterranean during the stratified period. Thus the focus of the discussion should not necessarily be on the area where rates are low. Section 4.3 may precede 4.2 or be combined with 4.2 as the 'longitudinal variability' is the basis for the distinction between Western and Eastern Basin. Further, longitudinal variability is not discussed to a larger extent in section 4.3.

In general, this manuscript presents novel data and I suggest publication in Biogeosciences considering the above and below comments.

Specific comments:

Title: Right now, the title is a question but the manuscript actually gives the answer. The title would have a more powerful statement without the question mark.

Abstract

l 3: specify variety; is that nutrients, chlorophyll, hydrography?

l 14-16: the need to assess N_2 fixation is not only given at other seasons, but more in general at a higher spatial and temporal resolution, including less oligotrophic areas

Introduction:

P 1198 l 20-21: write out nitrate and phosphate upon first use

P 1200 l2-4: there is some confusion in this sentence to which $\delta^{15}\text{N}$ (in which compound) the authors refer to

P 1200 l 8: I would take out “(7-40% according to the hypothesis considered)” or explain, it is not clear to which hypothesis this refers

P 1200 l 18: it should be mentioned that *Richelia intracellularis* is a symbiont

P 1200 l 29: strong trophic gradients should be specified, see above

P 1201 l 4: “stratification period” should be specified at least once, this would be a good place; e.g. how long is the stratification period and how is it characterized specifically for the Mediterranean

P 1201-1202: section 2.1: were there any replicates done? Specify whether incubation bottles were shaken/agitated and how much/long

P 1201 l 23: is that the upper part of the deep chlorophyll maximum or were there two deep chlorophyll maxima? Please specify

P 1202 l 2: verify if truly 10% HCl or if it was a 10% dilution of a 32% HCl which would be a 3.2% HCl

P 1202 l 4-5: Sentence a bit confusing, maybe “Incubations were always started before dawn by tracer additions and lasted 24 hours.”

P 1202 l 5-8: rephrase to make the sentence clearer

P 1203 l 7: “background $\delta^{15}\text{N}$ ” do you mean natural abundance of ^{15}N ?

P 1203 l 10: “eight time zero samples” are those for each station or are the eight samples from the entire cruise?

P 1203 l16: substitute “global” by overall or in general throughout the manuscript as the rates here are not referred to “global rates”, i.e. around the world

P 1203 l 17: use “meridional” or “longitudinal” throughout the manuscript

P 1203 l 17: specify whether the rates refer to $\text{nmol “N” L}^{-1} \text{d}^{-1}$ or to $\text{nmol “N}_2\text{” L}^{-1} \text{d}^{-1}$; this is very important throughout the manuscript

P 1203 l 19: what do you mean by fluxes? Rates would probably be better to use

P 1204 l 4: replace “over the vertical” by “throughout the water column”

P 1204 l 11: “over the euphotic zone” does this mean “within the euphotic zone”? specify

P 1204 l 19: “over the vertical” do you mean “throughout the water column”?

P 1204 l 23: “diazotrophic communities” here refers to data which is not presented in this paper, I would leave this out here and give a brief community composition in this part of the discussion

P 1205 l 1: “Data” means “The presented data”?

P 1205 I 6: “U-CYN₂-Fix” change to “unicellular diazotrophic cyanobacteria” or refer specifically to U-CYN A, B or C throughout the manuscript

P 1205 I 12-19: there seems to be some disagreement between the results mentioned here and the ones presented in the cited study; check the results and make sure they are correctly cited

P 1205 I 18: change “must be responsible” to “are likely responsible” or similar as this could only be verified by single-cell analysis like nanoSIMS which cells actually have been responsible for the measured N₂ fixation

P 1206 I 5: “and could also be located above” it is unclear what this means

P 1206 I 9: a citation of a personal communication implies that it comes from a person, so the citation should only be a single person rather than a group of people; personal communications do not go into the reference list, so no one knows who “et al.” is; a personal communication is not cited with a year

P 1206 I 9 and 11: are those cell numbers referring to Richelia or to Hemiaulus?

P 1206 I 26: ““potential contrasted behaviours” change to e.g. “contrasting environments” or “contrasting conditions”

P 1207 I 12: low rates and isotopic data do not necessarily need to disagree as 24-h incubations during a rather short period (few weeks) may not represent the same data as isotopic signatures which usually cover a much longer time scale; maybe this sentence needs to be rephrased to make the intended statement clear

P 1207 I 16: the authors could use the C:N ratios measured in this study

p 1208 I 23: please specify what justifies the 6 months N₂ fixation period; is that the length of the stratification period?

P 1209 I 12: “since ever” what does that mean? Please clarify

P 1209 I 14: change “submitted” to, for example, “subjected”

P 1211 I 2: were the turnover times for phosphate different between the Western and the Eastern basin? This section is about the longitudinal variability and if there were substantial differences in the phosphate turnover times they should be presented here

P 1211 I 29: “has recently been confirmed” sounds a bit weird as the data is not published, what about “N₂ fixation in *Trichodesmium erythraeum* and *Crocospaera watsonii* cultures appears to be sensitive to N:P ratios (A. Knapp, pers. comm.).”

P 1212 I 1-2: citation of personal communication, please see comment above

P 1212 I 2: there is “recent” or “recently” twice in that sentence

P 1212 I 9: citation of personal communication, please see comment above

P 1212 | 11-16: the authors are here considering reasons for upward estimates of N₂ fixation, they should cite the recently published study on the methodological underestimation of N₂ fixation (Mohr *et al.* 2010 Plos One) as the direct injection of a ¹⁵N₂ gas bubble was used here

Tables and figures:

P 1220 Table 1: here primary production should also be presented in μmol or $\text{mmol C m}^{-2} \text{ d}^{-1}$, the first sentence is very long, could be divided into two shorter sentences

P 1220 Table 1: in this manuscript, the authors consider the eddies (stations A, B and C) as closed systems to which N₂ fixation, atmospheric deposition and vertical nitrate flux are considered the only 'new' sources of nitrogen; in some cases (e.g. stn A), all three sources only add up to 34% of 'new' primary production; are there explanations for other sources of 'new' N into these systems; should be included into the discussion

P 1220 Table 2: change "contrasted" to "contrasting"; specify whether the rates are in $\mu\text{mol N}$ or $\text{N}_2 \text{ m}^{-2} \text{ d}^{-1}$

P 1221 Table 3: sentence about K_z values is twice in the legend; the table headers: " m^{-2} " instead of " m^2 " for the N flux min and max

P 1225 Figure 3: the authors mention in the methods and results the size-fractionated N₂ fixation measurements; did the two fractions add up to the same rate as the bulk measurements? This has not been reported in the manuscript so far but would be valuable information as the authors here used another approach for the size-fractionated rates than most other studies; "N" or "N₂"; I would write "rates" rather than "fluxes"; do the red squares belong to the first or third day?

Technical corrections:

In general: references are now mixed in capitalized and not capitalized, either one

P 1199 | 23: "mediated" instead of "meditated"

P1199 | 25: "not associated" instead of "non associated"

P 1200 | 28: "fixation" instead of "uptake"

P 1200 | 14: "reaches" instead of "reach"

P 1200 | 17: "Archaea" instead of "Archeae"

P 1201 | 9: "20 July 2008" instead of "20 July"

P 1201 | 13: "CTD" instead of "CTDO"

P 1201 | 17: "LD stations" instead of "These latest LD stations"

P 1203 | 24: "...Mediterranean Sea, respectively"

P 1204 l 12: parentheses are missing around “Table 1”; “...(Table 1) confirming that” instead of “...(Table 1) data confirmed that”

P 1204 l 24: “datasets” instead of “dataset”

P 1205 l 16: “clone libraries” instead of “clones libraries”

P 1207 l 21: “system” instead of “systems”

P 1209 l 13: “atmosphere” instead of “atmosphé”

P 1213 l 25: the “2” in “N₂” should be subscript

P 1215 l 31: “*nifH* phylotypes” instead of “nifH 4 phylotypes”; gene names should be italicized throughout the manuscript

P 1218 l 20: *Hemiaulus* should be italicized

P 1221 Table 3: “m⁻²” instead of “m²” for the N flux min and max in the table

P 1222 Table 4: the “9” in 10⁹ should be superscript

P 1223 Figure 1: the letters (A, B and C) are hard to read

P 1224 Figure 2: “distance in km from station 27” (?) instead of “distance 11 from the station 27”

P 1225 Figure 3: “μg l⁻¹” instead of “μg l⁻¹” (i.e. only the -1 should be superscript); no dots between nmol and l and d; the dark and light blue are not distinguishable