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## ***Interactive comment on “Planktonic dinitrogen fixation in the Mediterranean Sea: a major biogeochemical process during the stratified period?” by S. Bonnet et al.***

**S. Bonnet et al.**

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Dear Editor, dear Reviewer,

We are pleased to provide a revised version of our manuscript bg-2011-37. We made our best to take into consideration the different comments pointed out, which have been very constructive and hopefully improved the quality of the manuscript.

You will find below a point by point response to the comments.

Reviewer #2

General comments

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In the introduction, the paragraph mentioning the two hypotheses which explain the anomalous high N:P ratio has been shortened to make them clearer.

Since the submission of this manuscript, other manuscripts cited from the same issue have been submitted and are available on line on the BG website (Ridame et al.). The draft Moutin et al., which is the introduction paper of the special issue is already online on our internal BOUM website. Because it is the introduction paper, it should include a synthesis all other papers and will be the last published of the BOUM special issue. The paper by Cuypers et al. will be published in BGD in the following weeks.

Discussion. The section 4.1 relative to the diazotrophic communities has been shortened and reference is made to the manuscript Le Moal et al (same issue) for more details.

Sections 4.2 and 4.3 have been merged and this section has been shortened as well. More attention is paid to the western Med basin where N<sub>2</sub> fixation rates are the highest.

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.

The title has been modified as: 'Planktonic Dinitrogen Fixation along a longitudinal gradient across the Mediterranean Sea during the stratified period'

Abstract

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

This sentence 'representing a variety of trophic conditions' has been removed in the first sentence and added below combined to the range of rates measured in each basin, to help the reader. 'This study provides extensive data on planktonic N<sub>2</sub> fixation rates across the whole Mediterranean Sea. They show that N<sub>2</sub> fixation occurs

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in Mediterranean waters during the stratification period, with a clear decreasing trend from the oligotrophic western basin ( $10 - 76 \mu\text{mol.m}^{-2}.\text{d}^{-1}$ ) to the hyper oligotrophic eastern basin ( $0 - 0.4 \mu\text{mol.m}^{-2}.\text{d}^{-1}$ ).’ Moreover, new temperature and chlorophyll data have been added in the manuscript and nutrient conditions are shortly described to help the reader to catch the variability of biogeochemical conditions from west to east.

I 14-16: the need to assess N<sub>2</sub> fixation is not only given at other seasons, but more in general at a higher spatial and temporal resolution, including less oligotrophic areas

The last sentence of the abstract has been replaced by ‘These results finally point out the need to assess N<sub>2</sub> fixation at a higher temporal resolution in order to better understand the diazotrophs’ dynamic under contrasted biogeochemical conditions’.

Introduction

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

Has been done

P 1200 I 2-4: there is some confusion in this sentence to which  $\delta^{15}\text{N}$  (in which compound) the authors refer to

There was a mistake in the sentence, which has been repaired as followed: ‘Pantoja et al., (2002) revealed an eastward decrease in surface  $\delta^{15}\text{N}$  (‰ of suspended particulate organic nitrogen (PON) ( $2.7 \pm 1.2\%$  to  $-0.2 \pm 0.7\%$ , chlorophyll a ( $2.6 \pm 2.3\%$  to  $-7.1 \pm 1.3\%$  and deep-water nitrate ( $3.4 \pm 0.5\%$  to  $2.5 \pm 0.1\%$ ’

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

We have removed ‘according to the hypothesis considered’, which refers to the article giving this range (Bethoux and Copin-Montegut, 1986). If the reader wants more information about those calculations, he can refer to this reference.

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P 1200 I 18: it should be mentioned that *Richelia intracellularis* is a symbiont It has been done

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

‘strong trophic gradients’ has been replaced by ‘exhibiting nutrient and chlorophyll gradients’.

P 1201 I 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

We have added the following sentence to explain briefly the seasonal variability of biogeochemical conditions encountered in the Med Sea. Moreover, new hydrographic and nutrient data from the cruise have been added, as explained above: ‘The Mediterranean Sea has long been recognized as a low nutrient concentration basin (Mc Gill, 1965; Krom et al., 1991), exhibiting increasing oligotrophy from west to east. It is characterized by seasonal variability in hydrological structure and trophic regimes, ranging from a strong thermal stratification with a sharp thermocline (10–20 m deep) during the summer and fall, associated with an efficient pycnocline acting as a physical barrier. During the mixing period in winter, nutrients are brought to the surface layer and can allow phytoplankton to bloom in the early spring.’

P 1201 I 202: section 2.1: were there any replicates done? Specify whether incubation bottles were shaken/agitated and how much/long

At each LD station, the vertical profile (9 depths) has been done twice (at day 1 and day 3), and all measurement and incubation have been done with one replicate. Bottles have been shaken 10 times after gas injection. The following sentence has been added ‘Each bottle was then shaken 10 times’.

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

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There were one DCM and the sampling has been performed in the upper part of this DCM. It has been specified in the text ‘corresponding to the subsurface and the upper part of the deep chlorophyll maximum (DCM+)’

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

It is actually a 10% dilution of 32% HCL, so 3% final. It has been corrected in the text ‘10 % dilution of 32% HCl’.

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

The sentence has been replaced by ‘Incubations were always started before dawn and lasted 24h’.

P 1202 | 5: rephrase to make the sentence clearer

The sentence has been rephrased as followed: ‘At LD stations, incubations were performed in situ on a drifting mooring line situated at the same depth from which the samples were collected. At SD stations, incubations were performed in on-deck incubators equipped with circulating seawater at the specified irradiances using blue screening’

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

Yes, it has been indicated in the text

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

One Time zero has been used for each station (17 time zero in total). But at one station, we measured the variability on eight time zero samples. We then considered the  $^{15}\text{N}$  excess enrichments to be significant were greater than three times the standard deviation obtained on those eight time zero samples.

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P 1203 I16: 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

It has been done.

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

It has been done, we have used ‘longitudinal’

P 1203 I 17: specify whether the rates refer to nmol “N” LâĖĖ1 dâĖĖ1 or to nmol “N2” LâĖĖ1 dâĖĖ1; this is very important throughout the manuscript

Rates as expressed throughout the manuscript as nmol N.l-1.d-1. It is now specified on the graphs and legends.

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

We replaced ‘fluxes’ by ‘rates’ throughout the manuscript

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

It has been done throughout the manuscript.

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

Yes, it means ‘within the euphotic zone’. It has been modified in the text

P 1204 I 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

This part of the discussion has been shortened and reference is made to articles of the same issue for more information (Le Moal et al. and Crombet et al.)

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

Yes, it means ‘the presented data’, and it has been modified in the text.

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P 1205 | 6: “UâĀĀCYN2âĀĀFix” change to “unicellular diazotrophic cyanobacteria” or refer specifically to UâĀĀCYN A, B or C throughout the manuscript

We only refer to Group A once. So UCYN2-Fix has been replaced throughout the manuscript by ‘unicellular diazotrophic cyanobacteria’

P 1205 | 12âĀĀ19: 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

The whole paragraph has been modified and shortened. We actually needed to specify that it was the community of unicellular diazotrophs that was dominated by picoplanktonic cyanobacteria. Knowing that, it is theoretically possible that 45 to 75% of N2 fixation rates are recovered in the picoplanktonic size fraction and the community of unicellular diazotrophs is dominated by picoplanktonic diazotrophic cyanobacteria at 99%, as picodiazotrophs can also be found in higher size fractions (attached to biological or inert particles). The paragraph is now: ‘Our data report that 45 to 75% of N2 fixation rates were recovered in the picoplanktonic size fraction ( $< 3\mu\text{m}$ ), which is in accordance with TSA-FISH data from Le Moal et al. (2010), showing that the community of unicellular diazotrophs was dominated by picoplanktonic diazotrophic cyanobacteria. Further 16S and nifH phylogenies revealed their affiliation to Group A, Bradyrhizobium and ĀĀ proteobacteria’.

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

We totally agree. This sentence is no longer in the paragraph but of course it is more accurate to write ‘are likely’ as we did not do any rate measurement of single-cell.

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

This has been removed as it does not give any interesting information.

P 1206 I9: 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

We agree with this comment. We have removed ‘et al.’. Concerning the date, the BG production office asked us to put a date after citing a pers. Comm.

P 1206 I 9 and 11: are those cell numbers referring to Richelia or to Hemiaulus? They refer to Hemiaulus. It has been specified in the text.

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

The sentence has been changed as follows ‘This study indicates that N2 fixation rates decreased when going eastward, indicating possible different biogeochemical/nutrient forcing on diazotrophs within the different basins’.

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

We totally agree that isotopic signatures cover longer time scale compared to snapshot rates measured using the 15N2 method. We rephrased to say that this is the decreasing overall trend from the west to the east (and not the low rates measured in the east) that is not in accordance with isotopic data which indicate the opposite trend. ‘This decreasing trend from west to east is not in accordance with previous isotopic data (Pantoja et al., 2002) reporting an eastward increase in the contribution of N2 fixation to the water column N budget’.

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

We decided not to perform any dual labelling (using both 15N2 and H13CO3-) because

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H<sub>13</sub>CO<sub>3</sub><sup>-</sup> is usually contaminated by Fe. It would be possible to chelex the <sup>13</sup>C solution but the chelex adds a possible NH<sub>4</sub><sup>+</sup> contamination. So, as <sup>14</sup>C production profiles were done during the cruise, we performed only <sup>15</sup>N<sub>2</sub> labelling, and thus we performed 'N only' analysis on the mass spectrometer (and thus do not have the exact C:N ratios), that is why we used this approximation.

P 1208 I 23: please specify what justifies the 6 months N<sub>2</sub> fixation period; is that the length of the stratification period?

This is approximately the length of the highly stratified period, that is why it has been used in the calculations. It has been specified in the text. The rest of the year, the water column is less stratified and the deep mixing event occurs at the beginning of year.

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

It has been removed as the sentence is clearer without

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

It has been done

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 turnover times were identical in surface waters in the western and eastern basins. However, there were a deepening of the isoline 10h from 50-60m in the west to 100-120m in the east, indicating a much lower P availability in the eastern basin. The following sentence has been added: 'Uniform phosphate turn over times < 10h were measured in surface waters during the BOUM cruise (Mauriac et al. In prep.), which may prevent Trichodesmium spp. and maybe other N<sub>2</sub>-fixing organisms with high energetic request, to develop extensively. However, these latter authors showed a clear deepening of the phosphate turn over time isoline of 10h from 50-60m-depth in the

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western basin, to 100-120m in the eastern basin, indicating a much lower P availability in the eastern basin’.

P 1211 | 29: “has recently been confirmed” sounds a bit weird as the data is not published, what about “N<sub>2</sub> fixation in *Trichodesmium erythraeum* and *Crocospaera watsonii* cultures appears to be sensitive to N:P ratios (A. Knapp, pers. comm.)”

The sentence has been rephrased as advised

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

‘Recently’ has been removed

P 1212 | 11-16: the authors are here considering reasons for upward estimates of N<sub>2</sub> fixation, they should cite the recently published study on the methodological underestimation of N<sub>2</sub> fixation (Mohr et al. 2010 Plos One) as the direct injection of a 15N<sub>2</sub> gas bubble was used here.

The following sentence has been added ‘Moreover, a recent methodological study indicates that the 15N<sub>2</sub> method used in most of studies dedicated to N<sub>2</sub> fixation do underestimate N<sub>2</sub> fixation (Mohr et al., 2010).’

Tables and figures

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

Primary production are now expressed in  $\text{mmol C m}^{-2} \text{ d}^{-1}$  in table 1. The sentences of the legend have been modified as followed ‘Table 1. Integrated primary production, N<sub>2</sub> fixation, vertical nitrate diffusion (base of the euphotic zone), and atmospheric deposition at stations A, B, C, 15, 19 and 24. Each source of N is expressed in percentage of estimated ‘new’ primary production (New PP). New PP (expressed in  $\mu\text{mol N.m}^{-2}.\text{d}^{-1}$ ) has been considered as 10% of PP (Moutin & Raimbault, 2002) as a maximum value during the stratified period.’

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P 1220 Table 1: in this manuscript, the authors consider the eddies (stations A, B and C) as closed systems to which N<sub>2</sub> 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.

It is true, we added the following sentence in the discussion ‘It has to be noted that at some stations, the sum of all estimated sources of ‘new’ N are not sufficient to sustain 100% of new primary production. This may come from the possible underestimation of N<sub>2</sub> fixation by the 15N<sub>2</sub>-tracer addition technique (Montoya et al., 1996). Mohr et al., (2010) recently demonstrated that the 15N<sub>2</sub> bubble injected in seawater does not attain equilibrium with surrounding water, leading to a 15N<sub>2</sub> concentration lower than assumed in the 15N<sub>2</sub>-fixation calculations. Moreover, estimated new primary production numbers (Table 1) are possibly overestimated; direct measurements are needed in the future to refine those calculations.’

P 1220 Table 2: change “contrasted” to “contrasting”; specify whether the rates are in  $\mu\text{mol N}$  or N<sub>2</sub> m<sup>-2</sup> d<sup>-1</sup>

It has been done. Rates are in  $\mu\text{mol N m}^{-2} \text{ d}^{-1}$

P 1221 Table 3: sentence about K<sub>z</sub> values is twice in the legend; the table headers: “m<sup>-2</sup>” instead of “m<sup>2</sup>” for the N flux min and max

The redundant sentence has been removed in the legend and the units have been corrected in the table.

P 1225 Figure 3: the authors mention in the methods and results the size-fractionated N<sub>2</sub> 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 “N<sub>2</sub>”; I would write “rates” rather than “fluxes”; do

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the red squares belong to the first or third day?

We wrote a mistake in the mat-meth section. Samples have been size fractionated but only the fraction  $<3\mu\text{m}$  has been analysed. Actually, if it is easy to 'wash'  $10\mu\text{m}$  PC filters onto GFF filters, it is much harder on  $3\mu\text{m}$  PC filters because the organic matter was attached to the filter. So analysing the fraction  $>3\mu\text{m}$  would have given underestimated values. We modified the mat-meth section as follows: 'At LD stations at day 3, for each depth, one replicate was filtered following the same procedure and one more replicate per depth was size-fractionated: it was pre-filtered onto  $3\mu\text{m}$  polycarbonate filters for fraction  $>3\mu\text{m}$ , while the filtrate was collected onto a pre-combusted GF/F filter for analysis of the  $<3\mu\text{m}$  fraction ( $0.7\mu\text{m}$  nominal porosity).'

Finally, we performed all technical corrections required.

We would like to sincerely thank you for your advices and constructive comments.

Sincerely,

Sophie Bonnet on behalf on all the authors

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Interactive comment on Biogeosciences Discuss., 8, 1197, 2011.

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