

Interactive comment on “Planktonic dinitrogen fixation in the Mediterranean Sea: a major biogeochemical process during the stratified period?” by 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 #1

Abstract

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Page 1198, line 2: I would use inputs or rates instead of “fluxes”. line3: “representing a variety of trophic conditions” contains no important information and can be deleted. Instead, this variety of trophic conditions, which is partly mentioned in line 6 (“less oligotrophic”), should explained a bit more in order to enable the reader to better understand in which settings low/high rates were found. It would be also easier for the reader if information on the spatial patterns (east/west) is combined with information on the trophic setting (at present, it’s up to the reader to collect these informations from 2 sentences). Line 6: I’d write “are found within” instead of “are performed within”. Line 7: clarify here whether the estimate of 45-75% of N₂ fixation happening in the <3 size fraction refers to the whole transect or just to the western basin.

The abstract section has been rephrased as followed to take into account the different comments and help the reader to find quickly the information: ‘This study provides extensive data on planktonic N₂ fixation rates across the whole Mediterranean Sea. They show that N₂ fixation occurs 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 $\mu\text{mol.m}^{-2}.\text{d}^{-1}$). Highest rates are measured in the less oligotrophic western basin, between the surface and 75 m-depth, where 45 to 75% of N₂ fixation are found within the picoplanktonic fraction (< 3 μm)’.

Line 9: new production is defined as primary production based on external N-sources. Thus, it is a bit confusing to read that 35% of new production is based on N₂ fixation, while the same process accounts for only 25% of the external N sources. I know that these numbers represent different estimates and that it would be hard to actually reconcile measured rates of primary production and potential primary production based on estimates of different N-inputs, but it’s obviously a bit unlucky to combine these two numbers in one sentence here.

We totally agree with this comment. Those numbers represent indeed different estimates that are explained later in the discussion. We thus only left the following part in

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the abstract: 'N₂ fixation is able to sustain up to 35% of new primary production during the stratified period in the western basin.'

Line 14: the final sentence of the abstract basically says "we need more sampling". This is true, but maybe a bit dry for an abstract. It could be explained that the interplay between diazotrophs and the seasonally changing biogeochemical settings is obviously not fully understood for this region, and that the presented data shed more light on these dynamics.

The final sentence has been modified as followed: 'These results finally point out the need to assess N₂ fixation at a higher temporal resolution in order to better understand the diazotrophs' dynamic under contrasted biogeochemical conditions.'

Introduction

The title of this paper points to the importance of N₂ fixation during the stratification period. Yet the introduction gives only little information on the seasonality of the hydrography in the Mediterranean, and about how the biogeochemistry responds to these changes. This should be shortly explained so that the reader can better grasp how different the conditions really are between seasons, and how the system differs from e.g. the oligotrophic open Atlantic where most studies on diazotrophy have been carried out.

The title has been changed as advised by the second referee as 'Planktonic Dinitrogen Fixation along a longitudinal gradient across the Mediterranean Sea during the stratified period'. Some information regarding the seasonal changes of biogeochemical conditions has been given in the introduction section: '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 summer and fall, associated with an efficient pycnocline acting as a physical barrier. During the mixing

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period in winter, nutrients are brought to the surface layer and can allow phytoplankton to bloom in the early spring'. Moreover, new temperature and chlorophyll data have been added in the manuscript and a detailed description of the nutrient conditions are given as well. Even if some of these data are available in some other papers of the special issue, they will certainly help the reader to understand the biogeochemical context of this N₂ fixation study.

Page 1198, line 23: "some biological processes" is a bit too general; you could shortly explain that non-Redfieldian elevated ratios of NO₃⁻ to PO₄³⁻ produced from regenerated particulate material are indicative of N₂ fixation inputs, since this becomes the focus in the next paragraph.

The sentence has been changed as follows: 'While this canonical value proposed by Redfield is still a reference, several studies conducted in the ocean over the past decades show anomalies in the ratios; for example non-Redfieldian elevated ratios of NO₃⁻ to PO₄³⁻ produced from regenerated particulate material are indicative of N₂ fixation inputs (e.g. Gruber and Sarmiento, 1997).'

Page 1199, line 8: it should read "nutrient budgets for the Mediterranean sea" Lines 10-16: these sentences are a bit complex while giving ambiguous information. After all, the topic is nutrient inputs, so it appears unnecessary to first talk about "Aeolian material deposition" and "anthropogenic aerosols", and then to make the point in line 16 by reporting on N and P inputs. Thus, lines 10-18 could be distilled into 2 concise sentences. I assume that in line 15 it's supposed to read "that represent a background of available N in the Mediterranean"? Definitely check phrasing here.

It is true that talking about Aeolian inputs is confusing in this context. This section has been shortened and re written as follows: 'While several 'conflicting' hypotheses have been proposed over the last twenty years to explain this anomaly, it still represents an open issue for the oceanographic community. In the first hypothesis, based on nutrient budgets for the Mediterranean Sea, the anomalous N:P ratio is explained by the excess

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of nitrogen relative to phosphate in all nutrient sources arriving to the basin, associated to low denitrification rates (Krom et al., 2004). In particular, the Mediterranean Sea receives among the highest rates of aeolian material deposition in the global ocean (Guerzoni et al., 1999), which provide dissolved nitrogen and phosphorus in a molar ratio increasing from 60:1 in the western basin to 105:1 in the eastern basin (Markaki et al., 2010).'

Line 22: how about "found" instead of "generally preferred"? Or, "an alternative explanation for elevated N:P ratios"? The sentence has been rephrased as follows: 'An alternative explanation proposes that elevated N:P ratios are due to intense dinitrogen (N₂) fixation'

Page 1200, Line 8: this is confusing: "(7-40% according to the hypothesis considered)". Which hypotheses do you mean? Does this refer to the reference that follows at the end of the sentence?

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.

Lines 15-19: how about "related to" instead of affiliated?

It has been modified as 'related'.

Reading about Richelia "in low abundances" seems to contrast what is cited from Crombet et al. 2010 later in the manuscript.

It is true. We removed 'in low abundance'

Lines 19-25: this sentence is too long and the phrasing is a bit confusing; what is meant with "high and controversial", is that supposed to mean "high but variable"?

The high N₂ fixation rates reported by Rees et al., (2006, GRL) are extremely high, and have been criticized, in particular by Krom et al., (2010). The sentence has been

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rephrased as follows 'recent studies have indeed reported during the summer stratification period either high N₂ fixation rates at one isolated station in the Levantine basin (Rees et al., 2006), or extremely low rates at six stations distributed across the basin (Ibello et al., 2010). This leads to a difficulty in concluding about the biogeochemical importance of diazotrophy in this environment.'

Methods Page 1201, line 17: Here and in a few other cases: Do you mean "these latter stations"? (instead of "these latest").

Yes, we mean these latter. We made the modifications throughout the manuscript.

Page 1202, lines 5-8: this is interesting- were the two methods, i.e. in situ- and on-deck incubations, ever done simultaneously and the results compared?

Incubations have been made in situ on a drifting mooring line at long duration stations. At short duration stations (as we were in transit), incubations were performed in on-deck incubators. The sentence in the Methods section has been modified to explain clearly this: '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'. It would be valuable to perform such a comparison study using triplicates in each condition. Unfortunately, we could not perform such a study onboard due to the scarcity of seawater available for N₂ fixation measurements (4.5 liters for one measurement).

Line 26: constant response to what?

We changed the word 'constant' by 'unchanging', meaning that 15N atom% did not change whatever the particulate nitrogen mass in the samples. The range of particulate nitrogen measured in our samples was 0.45 to 3.65 μ moles; we measured that within this range, 15N atom% was linear (Fisher test, $p < 0.01$). The sentence is now 'we verified the linearity of 15N atom% as a function of increasing particulate nitrogen

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mass on both natural and ^{15}N enriched material, and the unchanging response of ^{15}N atom% within the range of particulate nitrogen in our samples'.

Results

Results Page 1203, line16: Here, in line 25, and on page 1204 line 7, the authors use "global" or "globally" when talking about the overall range of rates measured within the Mediterranean, which is the wrong choice in parts and can also be a bit misleading since the paper also includes comparisons to rates measured in other basins around the "globe" (Table 2). Please use "overall" instead.

Globally has been replaced by 'overall' throughout the manuscript.

Were there any measurable differences in salinity, temperature, nutrients between stations within and outside of these gyres? Shortly commenting on these differences would provide a nice context to the rate measurements. At present the paper completely lacks information on hydrography, I assume since these are supposed to be presented in other papers within the special issue? Still, it would be nice to have a basic set of such data in the paper, e.g. marking mixed layer depths in plots that show vertical distributions.

Temperature and chlorophyll data along the transect have been added to give a first context to this N_2 fixation study. Moreover, nutrient features across the Med (range of nitrate and phosphate concentrations, depth of the nutriclines) are detailed in the text and reference is made to the article already published in the same issue (Pujo-Pay et al., 2011) and the introduction paper by Moutin et al. Finally, the mixed layer depth at the 3 long duration stations A, B, C have been added on the vertical profiles (Fig.3). Those results are now shortly presented in the Results section and used in the discussion to help interpretation on the longitudinal variability on N_2 fixation across the Med Sea.

Lines 16- 24: Figures 2 and 3 essentially show the same kind of data, i.e. the vertical

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distribution of volumetric N_2 fixation rates. The calculation of contours in Figure 2 is in most cases based on only 2 or 3 measurements within the water column (SD stations), and thus, vertical patterns of N_2 fixation are "invented" for large parts of the transect. Blanking out all areas not supported by data would be necessary here, but would make the plot look very awkward, I assume. Further issues with Figure 2 are that a) the SD stations are not clearly marked on the x-axis, b) rates at station B appear to differ from rates shown in Figure 3 for station B (is this due to averaging?) and c) the caption of Figure 2 includes confusing information (unit is written as $\text{nmol l}^{-1} \text{d}^{-1}$; and what does "distance 11 from station 27" mean?). I therefore think it would be best to delete Figure 1. The spatial patterns would appear much clearer if measurements from all stations were shown as rate profiles (as shown in Fig. 3), with rates from SD stations as single data points and data from the LD stations drawn as profiles, as already done in Figure 3.

As we have added temperature and chlorophyll data as contour plot graphs, we have decided to keep the N_2 fixation as a contour plot but we increased the number of data included. In particular, we included the two replicate profiles performed at each duration stations at day 1 and day 3 instead of the average between both as done in the previous graph. A present, there are 85 data points (clearly indicated on the ODV graph), and we highly reduced the degree of interpolation. At station B, rates presented on the ODV graph are in accordance with those on the vertical profile. We have also indicated the number of each short duration station on the X axis of all ODV graphs. Finally, we have modified the legend accordingly to indicate that the X axis corresponds to the distance (in km) from station 27.

Sentence starting in Line 25: This sentence is very hard to read and understand. The authors should stick to providing one message per sentence- this one has at least 2, it seems.

The sentence has been replaced by 'Data indicate that rates were globally lower at the three LD stations A, B, and C (located in the center of anticyclonic gyres), compared

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to the ones measured at SD stations’.

Page 1204, line 4: “over the vertical” should be replaced by “throughout the water column”.

It has been done.

Lines 6-11: it is difficult to clearly see these vertical differences in N₂ fixation rates at the SD stations, especially since SD stations are not marked in Fig. 2. This is another reason for rather providing the rates as simple 2D graphs instead of a contour plot, as requested above.

Cf above

Line 13: Using “confirmed” here appears inappropriate to me. Of course both the profiles of rate measurements as well as areal rates show the decrease towards the east, since areal rates are calculated by depth-integrating the rate profiles. The areal rates are simply the better choice when comparing diazotrophs inputs between different areas, as done in Table 2, but apart from that, they provide no further information regarding spatial patterns. The authors should delete this sentence; it is enough to discuss areal rates as done on pages 1207 and 1209.

We agree that the term ‘confirmed’ is not appropriated as areal rates are calculated using the rate profiles. So we modified the sentence but propose to keep this useful information in the ‘results’ section as follows: ‘Depth integrated rates were calculated at stations # A, B, C, 15, 19, 24 (Table 1) and show a decrease from the western basin (10.2 ± 2.7 to $76.2 \pm 7.7 \mu\text{mol.m}^{-2}.\text{d}^{-1}$ at station B and 27, respectively, Table 1) to the eastern basin ($0.4 \pm 0.1 \mu\text{mol.m}^{-2}.\text{d}^{-1}$ at station C).’

Line 19: “same trend” refers to what?

The term ‘trend’ refers actually to the ‘vertical’ trend. We have replaced ‘trend’ by ‘vertical pattern’: “N₂ fixation rates in this picoplanktonic fraction followed the same vertical pattern at station B. . .’

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Discussion

In all, based on the presented dataset, the discussion appears a bit overambitious and should be shortened. Points 4.2 and 4.3 could be combined to a discussion about “Biogeochemical significance and potential controlling factors of N₂ fixation in the Mediterranean Sea”

The discussion has been shortened. In particular, lots of details regarding the diazotrophic communities in the section 4.1 ‘N₂ fixation rates, size fractionation and diazotrophic communities’ have been removed and reference is made to the manuscript Le Moal et al (same issue) for more details. Moreover, section 4.2 and 4.3 have been merged and untitled ‘Biogeochemical significance and potential controlling factors of N₂ fixation in the Mediterranean Sea’

Page 1205, line 6: I am unaware of the term “UCYN2-fix”. It is not defined here nor in the LeMoal manuscript- the original papers by Zehr et al. differentiate between the different types (UCYN-A, -B etc), but never use “UCYN2-fix”. Why not use “diazotrophic cyanobacteria” throughout?

UCYN2-fix has been replaced throughout the manuscript by ‘unicellular diazotrophic cyanobacteria’

Line 25-26: the choice of words is suboptimal here; to what do the authors refer when talking about “behaviour”? I assume the sentence is about the different biogeochemical/nutrient forcing on diazotrophs within the different basins.

We agree. The sentence has been replaced by ‘this study indicates that N₂ fixation rates decreased when going eastward, indicating possible different biogeochemical/nutrient forcing on diazotrophs within the different basins.’

Page 1207, line 10: more precise information on the possible temperature effects would be nice here (too low I assume? How do temperatures differ between seasons?)

We modified this sentence by removing the possible effect of temperature in the surface

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waters of the eastern basin (22-27°C) as they are relatively high and probably not limiting for the development of N₂ fixers such as *Trichodesmium* or *Crocospaera*. During the mixing period in winter, surface temperature drop below 15°C, which might prevent those types of diazotrophs to develop extensively at that season. However, we did not add this information in the manuscript as the BOUM cruise took place during the summer season.

Lines 15-20: this sentence is too long, confusing, and the brackets are not closed.

The sentence has been modified as follows 'On the basis of a C:N = 6.6, the contribution of N₂ fixation to the nitrogen demand of 'new' primary production at station C (considered as 10% of primary production presented in Christaki et al., 2010) is negligible (0-0.3%, Table 1), indicating on the opposite a minor contribution of N₂ fixation at the studied period.'

Pages 1207-1208: Comparison of N₂ fixation and diffusive fluxes: Unfortunately, neither Moutin et al. 2010 nor Cuypers et al. 2010, both supposed to be found as open discussion papers in the same special issue, are (yet?) accessible on the Biogeosciences website at the time of this writing. This makes me assume that they have either not been submitted yet, or have been initially rejected. It would be necessary to know about the contents, the status, and the outcome of these manuscripts, otherwise the present paper would reference to a lot of "unpublished data". In table 3, I think the truly interesting data are the Nitrate fluxes; but these are presented in Table 1 already and are just listed here in more detail- if Cuypers et al. is published in the same special issue, presenting the data in 2 separate tables would be unnecessary/excessive here.

We apologize for having referred to 2 manuscripts (Moutin et al. and Cuypers et al.) that were not yet online when this manuscript was submitted. 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

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Cuypers et al. will be published in BGD in the following weeks. We decided to keep the Table 3 as they present, in addition to NO₃ fluxes, the minimum and maximum values of K_z. Very few data are yet available for this area and it could be interesting for readers to be able to refer to these K_z values and compare them to the ones measured (or calculated) in oligotrophic gyres. They will not be presented in such a way in the manuscript of Cuypers et al.

Page 1209, line 12: "confirmed since ever": what does that mean? Line 14: I assume it should read "subject to" instead of "submitted to"?

The sentence has been rephrased as follows 'Recent studies conducted in the tropical North Atlantic confirmed that inputs of low $\delta^{15}\text{N}$ -NO₃- from the atmosphere have to be taken into account when investigating the present-day N-cycle in oceanic environments subjected to high atmospheric inputs (Baker et al., 2007; Knapp et al., 2005).'

Line17: stating that rates of N₂ fixation were higher in the western part has been said many times before; I would suggest starting right with the comparison of rates with results from the other oceanic areas.

We agree that this sentence is written too many times. We modified accordingly: 'N₂ fixation rates in the western basin were 10 to 76 $\mu\text{mol N.m}^{-2}.\text{d}^{-1}$. They are in the same orders of magnitude than those commonly measured in the tropical and subtropical Atlantic and Pacific oceans (Table 2).'

Page 1210, line 21: again the same information at the beginning of a paragraph (N₂ fix higher in the west). I would rather start right away with discussing possible explanations for the spatial patterns (phosphate availability is the topic of this paragraph).

We changed the sentence as: 'The decrease of N₂ fixation from West to East is probably due to decreasing phosphate availability towards the eastern Mediterranean Sea. ...' in order to introduce that we talk about the longitudinal variability of N₂ fixation

Page 1211, line 5-7: it is unclear how "despite stimulation was higher with Saharan

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dust” relates to the first part of the sentence. Please explain.

This has been removed for more clarity as it is not the main purpose of this paragraph and confusing. We consider that if readers want more details on this nutrient fertilisation study, they can refer to Ridame et al., (same issue).

Lines 13-29: this paragraph discusses the interesting finding of elevated N₂ fixation in waters having detectable N concentrations, and suggests that a low N:P ratio, i.e. a surplus of P relative to N, is important in promoting the growth of certain diazotrophs. These findings can be backed up further by some other recent studies which report on the same scenario, i.e. elevated N₂ fixation by planktonic diazotrophs in estuarine/coastal waters having high N concentrations but low N:P ratios (Grosse et al. 2010, L&O, Bombar et al. 2011, MEPS); these papers should be cited here as well, in addition to Short and Zehr 2007.

We apologize. These 2 references have been added in the text and in the reference list.

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

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