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## ***Interactive comment on “Strong stimulation of N<sub>2</sub> fixation in oligotrophic Mediterranean Sea: results from dust addition in large in situ mesocosms” by C. Ridame et al.***

### **Anonymous Referee #1**

Received and published: 5 August 2013

GENERAL COMMENTS Ridame et al’s paper analyzes the effects of simulated atmospheric dust wet and dry deposition events on N<sub>2</sub> fixation, primary production and new production on the oligotrophic Mediterranean Sea waters through the use of sophisticated large mesocosm devices. The effects of atmospheric dust deposition on the activity and diversity of marine microbes is a current topic in marine N<sub>2</sub> fixation research which is not completely resolved and has important implications for biogeochemical cycling of the main elements (C, N, P). Therefore, this paper is timely and of interest for the scientific community. However, there is a need for clarification and improvement of several parts of the manuscript before it is suitable for publication. The methods section is incomplete, more details are needed on the characteristics of the

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mesocosms (volume, height, width, depth of anchoring point, materials, etc) and the protocols followed to measure primary production and new production, nutrient concentrations and chlorophyll concentrations. This reviewer is aware that this paper is part of a special issue in Biogeosciences and therefore many of these details are explained in accompanying papers or alike. However, I think that a brief description of the mesocosms devices and techniques applied is needed. The discussion section could be better organized and merging sections is advised, since some of the discussion in different sections could be linked allowing a smoother reading of the paper. The conclusions stated are solid, but a reinforcement of the poor contribution of N<sub>2</sub> fixation to new production is needed. Other issue I find in this paper is the lack of citation to some key articles in this field. E.g. Marañón et al. (2010; L&O) discussed the responses of marine microbes to Saharan dust deposition by measuring the abundance, biomass, community structure, and metabolic activity. Other recent papers about the contribution of N<sub>2</sub> fixation to primary production and heterotrophic diazotroph abundance in the Mediterranean Sea are not cited. Furthermore, the potential underestimation of N<sub>2</sub> fixation rates due to the use of <sup>15</sup>N<sub>2</sub> bubbles instead of <sup>15</sup>N<sub>2</sub> previously dissolved in seawater is not sufficiently discussed. The authors could argue how much could the N<sub>2</sub> fixation rates measured increase according to the range of differences between the bubble and the dissolved method reported in the literature, and how it would change the % contribution of N<sub>2</sub> fixation to primary and new production estimated. The composition of the diazotrophic community could be considered for this discussion (as pointed out in Großkopf et al., 2012; Nature), given that the authors have access to that data (Biegala et al's work on DUNE project, in prep.). Finally, a thorough revision of the English language by a native speaker or professional translation service is strongly recommended (see Technical corrections).

## SPECIFIC COMMENTS

### Abstract

Page 10583, line 13: please add the concentration of NO<sub>3</sub><sup>-</sup> added as a consequence

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of simulated wet deposition in parenthesis.

Page 10583, line 16: please indicate what the authors define here as “new production” (the sum of what rates? Or is it obtained by considering that NP is 15% of PP, as stated on Table 3?).

Page 10583, line 24: how much higher? Add a number.

## Introduction

Page 10584, line 5: change for “reservoir”, which is more appropriate if you are describing the importance of N<sub>2</sub> fixation over geological time scales.

Page 10584, line 8: Eugster and Gruber’s paper (2012) deals with probabilities rather than real numbers (experimentally derived rates). I recommend citing compilations of real data instead, like the Supplementary Material of Großkopf et al. (2012; Nature).

Page 10584, line 9: reference needed at the end of the sentence.

Page 10584, line 14-17: consider mentioning that P and Fe are amongst the limiting factors, temperature, turbulence, etc may be equally important, depending on the type of diazotroph. Maybe just say “limiting elements” or “nutrients”.

Page 10585, line 5: although the strict definition of Surface Mixed Layer (SML) and Mixed Layer Depth (MLD) may be slightly different, for the purpose of this paper, please consider using the more commonly used MLD term.

Page 10585, lines 18-19: “. . . low availability of DIP in the Mediterranean Sea”. Please add a reference to support this statement

## Materials and methods

Page 10586, lines 25 and next: please add a more precise (though brief) description of the mesocosms (height, depth of place of anchoring, etc).

Page 10587, line 11: include the absence of lateral advection inside the mesocosms

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in a more detailed description of the mesocosms earlier in this section.

Page 10588, line 20: indicate how many replicates were used for the measurement of N<sub>2</sub> fixation rates.

Page 10588, line 26: so the water was collected in the morning during routine sampling of the mesocosms, and the 15N<sub>2</sub>-amended bottles incubated for 24h until the next morning? The bottles were replaced at the relevant depths inside/outside the mesocosm during the incubation period using arrays? This is not clear.

Page 10588, line 28: the filters were dried at 40°C for how long?

Page 10589, line 8: I would rather cite the original paper by Mohr et al. here (2010; PLoS ONE).

## Results

Page 10589, line 20: there are no details in the materials and methods section on how chlorophyll a was measured. Please add.

Page 10589, line 20: Exactly the same with primary production, there is no explanation in the materials and methods section.

Page 10589, line 21: Chlorophyll a concentration units  $\mu\text{L m}^{-1}$ , is this meant to be  $\mu\text{g L}^{-1}$ ?

Page 10589, line 22: No details on DIP measurements either. At least state in the materials and methods section that DIP concentrations were measured as described in Pulido-Villena et al., 2010, etc (the same with primary production rates, chlorophyll concentrations, etc).

Page 10590, line 5: there are no details on DFe measurements either.

## Discussion

Page 10592, section 4.1.: why is the stimulation of N<sub>2</sub> fixation rates similar between

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wet and dry dust seedings? Is it caused by the similarity in the P and Fe composition between both types of dust? (Table 1). The amount of N between wet and dry dust was different. Did N inputs not affect N<sub>2</sub> fixation rates in this case? (as stated in the Abstract). Is this related to the composition of the diazotrophic community in these waters? Please add discussion on the possible reasons to explain the lack of differences found.

Page 10593: cite Biegala et al. (2013) as “in prep.”, here and elsewhere throughout the manuscript.

Page 10593, section 4.1.1.: the lack of correlation between N<sub>2</sub> fixation rates and UCYN abundance after dust seedings needs to be discussed in more detail. Please cite Zehr et al. (2007; L&O) where some of these issues are discussed. Please indicate which technique is used in Biegala et al. (in prep.) to determine the abundance of UCYN (TSA-FISH? qPCR? RT-PCR?), discuss the possibility that diazotrophs were present, but not necessarily active. Regarding the presence of diazotrophs other than UCYN, please discuss differential responses to atmospheric dust inputs by different types of diazotrophs (Benavides et al., 2013; L&O). Please also discuss the importance of heterotrophic diazotrophs in the Mediterranean Sea in the light of recent studies (e.g. Rahav et al., 2013a; JGR: Biogeosciences; Rahav et al., 2013b; Ocean Sci Discuss), and the potential underrepresentation of the diazotrophic community if only UCYN are taken into account.

Page 10593, line 23: The discussion of differences between dust-mesos due to variability in the spatial distribution of diazotrophs presented here could be combined with differences in responses to N inputs between wet and dry deposition experiments and potential different responses by different diazotrophic groups, as indicated above.

Page 10594, section 4.1.2.: consider merging this section with section 4.1.1. in order to merge the discussion on N inputs, diazotrophic community composition and variability of N<sub>2</sub> fixation rates between mesocosms as indicated above.

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Pages 10594 and 10595, section 4.1.3.: the temperature range observed is quite broad. Why wouldn't this change in temperature affect N<sub>2</sub> fixation rates at all? Please provide an explanation or speculation.

Page 10595, lines 4-7: these lines belong in the materials and methods section, where an explanation of the estimation of new production rates is also required.

Page 10595, line 5: Ridame et al.'s paper should be also noted here as "in prep."

Page 10595, line 11: consider citing recent results by Rahav et al. (2013; Ocean Sci Discuss).

Page 10595, lines 12-15: This statement is risky. The C:N<sub>2</sub> fixation ratio in diazotrophs varies widely (see section 3 on Mulholland et al., 2007; Biogeosciences). Take advantage of recent publications where C and N<sub>2</sub> fixation were measured simultaneously in the frame of CO<sub>2</sub> enrichment experiments (using the control data). The contribution of N<sub>2</sub> fixation to NP is straightforward and quantifiable (as for example in Raimbault and Garcia, 2008; Biogeosciences), but the contribution of N<sub>2</sub> fixation to PP relying on C:N ratios of in situ POM is questionable, given that diazotrophs often "overfix" C for a number of reasons (see discussion in Mulholland et al., 2007).

Page 10595, lines 19-21: This references have already been mentioned at the beginning of this section, there's no need to repeat them here.

Page 10595, lines 25-26: indicate mesocosm depth and duration of the experiments in parenthesis.

Page 10596, lines 8-9: add some more notions about why the availability of fixed N is not limiting for diazotrophs, for non-expert readers.

Page 10596, line 10: a reference is needed at the end of the Fe-limitation statement.

Page 10597, lines 1-2: consider citing here (and discussing) Luo et al. (2013; Biogeosciences Discuss) where the non-limiting effect of Fe availability on N<sub>2</sub> fixation rates

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in the ocean is discussed. Also discuss the possibility of the lack of relationship between DFe concentrations and N<sub>2</sub> fixation rates by changes in DFe bioavailability, lack of ligands (organic matter), etc.

Page 10597, line 22: given the results presented here, I wouldn't say that the input of NO<sub>3</sub>- is "strongly suspected" to inhibit N<sub>2</sub> fixation. Consider changing for "could".

#### TECHNICAL CORRECTIONS

Page 10583, line 10: please define "N<sub>2</sub>" on first use.

Page 10583, line 16: please define 'N' as 'nitrogen' on first use, as later done with "NP".

Page 10583, line 17: "as a source. . . as shown". Delete one of the two 'as', maybe by putting 'as a source of N' in parenthesis.

Page 10583, line 20: change "as a maximum" for "since only a maximum".

Page 10583, line 22: please change 'those' for 'these'.

Page 10584, lines 1: change "all" for "other".

Page 10584, line 4: place "(N<sub>2</sub>)" after "dinitrogen".

Page 10584, line 4: change "for regulating" by "for the regulation of".

Page 10584, line 5: change "sustaining" for "sustains".

Page 10584, line 8: could change "a part" for "an important part" or "important fraction".

Page 10584, line 10: should define "N" on first use (line 5).

Page 10584, line 14: ". . .their impacts NEED to be achieved".

Page 10584, line 18: change "subjected" for "subject".

Page 10584, line 19: change "since" for "in" or "during".

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Page 10584, line 24: “. . .good candidate as A controlling factor. . .”

Page 10585, line 5: change “phytoplanktonic” for “phytoplankton”.

Page 10585, line 7: consider changing “production” for “productivity”.

Page 10585, line 10: “to THE oligotrophic. . .”

Page 10585, line 13: change “showed” for “have shown”.

Page 10585, line 19: define BOUM.

Page 10585, line 19: “. . .in THE summer OF 2008. . .”.

Page 10585, line 20: change “have shown” for “showed”.

Page 10585, line 26: “. . . in THE tropical Atlantic. . .”.

Page 10585, line 27: change “proved” for “have proven”.

Page 10585, line 27: “N2 fixation rateS”.

Page 10586, line 3: change “limits” for “limitations”.

Page 10586, lines 3-6: sentence is too long, consider rewriting.

Page 10586, lines 4-5: “. . .on A longer time scale” or “on longer time scales”.

Page 10586, line 5: change “and as” for “given”.

Page 10586, line 12: change “quantity” for “quantify”.

Page 10586, line 15: change “nitrogen” for “N”, as previously used in the text.

Page 10586, line 17: consider changing “experiment” for “experimental”.

Page 10586, line 22: change “was shown to be” for “is”.

Page 10587, line 19: change “realized” for “done”, “conducted” or “performed”.

Page 10587, line 26: “. . .a dry deposition EVENT”.

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Page 10588, line 1: change “evapocondensed” for “EC” as previously noted in this section.

Page 10588, line 5: change “has not been” for “was not”.

Page 10588, line 12: change “was” for “were”.

Page 10588, line 17: “N2 fixation rateS”.

Page 10588, line 18: “before and after dust addition/seeding”.

Page 10589, lines 1-2: please complete “isotope ratio mass spectrometer (IRMS)”.

Page 10589, line 5: “isotope mass BALANCE EQUATIONS”.

Page 10589, line 11: write “Fisher Least Significant Difference (LSD)” in full.

Page 10589, line 12: “N2 fixation rateS”.

Page 10589, line 18: change “has shown” for “showed”.

Page 10590, line 1: “. . .due to THIS analytical problem. . .”.

Page 10590, line 9: change “was rapidly increasing” by “increased rapidly”.

Page 10590, line 14: “. . .of THE second seeding. . .”.

Page 10590, line 19: “In surface waters” or “At the surface”. Add real depth into parenthesis.

Page 10590, line 19: change “as compared to” by “in comparison with”.

Page 10590, lines 21-22: “N2 fixation rateS WERE. . .”.

Page 10591, line 1: change “In surface” for “At the surface” or “In surface waters”.

Page 10591, line 1: “N2 fixation rateS”.

Page 10591, line 11: “N2 fixation rateS WERE”.

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Page 10591, line 21: “. . .in THE Dust-Meso. . .”

Page 10592, line 1: “THE initial characteristics. . .”.

Page 10592, line 6: change “They” for “These”.

Page 10592, line 7: “during periodS of. . .”.

Page 10592, line 15: There is no need to repeat “from the picoplanktonic size fraction”.

Page 10592, line 15: Finish the sentence after the references in parenthesis. Start next sentence: “Bonnet et al. (2001) reported. . .”.

Page 10592, line 22: “. . .dust addition and LONGER. . .”

Page 10592, line 23: “N2 fixation rateS”.

Page 10592, line 25: “at both THE surface and. . .”.

Page 10593, line 3: “THE dust event. . .”.

Page 10594, line 1: “Nevertheless, THE variability. . .” (again in line 2).

Page 10594, line 21: “(i) THE N2 fixing activity. . .”.

Page 10595, line 1: “unicellular diazotrophs ARE usually found”.

Page 10595, section 4.2.: I believe that PP and NP had been introduced before in the text, please correct (no need to write in full here).

Page 10595, line 19: change “Such low number” for “Such a low contribution”.

Page 10596, line 6: “. . .during stratification PERIODS”.

Page 10596, line 10: delete “process”.

Page 10596, line 14: “DFe concentrationS” (same in line 16-17).

Page 10596, line 17: “. . .concentration in THE. . .”.

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Page 10596, line 23: delete “was”.

Page 10597, line 27: “N2 fixation rateS” or “N2 fixation activity”.

Page 10598, line 1: “N2 fixation rateS”, and “in THE. . .”.

Page 10598, line 2: delete first “was”.

Page 10598, line 9: “..and THE bacterial respiration. . .”.

Page 10598, line 12: “FOR THE summer in THE northwestern Mediterranean. . .”.

Page 10598, line 18: delete “from Sahara”, since “impacted by mineral dust deposition” is indicated at the end of the sentence.

Page 10598, line 18: “N2 fixation rateS”. Again in line 20.

Page 10598, line 19: “dust eventS”.

Page 10598, line 22: “and dust DEPOSITION eventS”.

Page 10598, line 25: “dust eventS”.

Page 10598, line 26: delete “a” and change “is” for “are”.

Page 10598, line 27: “environmentS”. Change dissolved iron for DFe, as previously used in the manuscript. “DFe concentrationS”.

Page 10599, line 1: “ARE likely”.

Page 10599, line 8: Add capital letters (North Atlantic).

Table 2: are the chlorophyll units correct?

Table 4: “integrated over the DEPTH OF THE mesocosm. . .”.

Figure 1: the label of y-axis of the 5m depth graph needs correction of the superscripts. The dots between N. L-1.d-1 could be deleted.

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