Biogeosciences Discuss., 10, C5357–C5380, 2013
www.biogeosciences-discuss.net/10/C5357/2013/

Biogeosciences
Discussions
Open Access

Interactive comment on "Strong stimulation of N_2 fixation in oligotrophic Mediterranean Sea: results from dust addition in large in situ mesocosms" *by* C. Ridame et al.

C. Ridame et al.

celine.ridame@upmc.fr

Received and published: 26 September 2013

We would like first to thank the reviewers for their relevant comments and suggestions which helped us to improve our manuscript.

Specific reply to referee 1

GENERAL COMMENTS

Ridame et al's paper analyzes the effects of simulated atmospheric dust wet and dry deposition events on N2 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 N2 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 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.

RESPONSE: We have added in section 2.1 some characteristics of the mesocosms ‘Briefly, six mesocoms (height: 12.5 m, diameter: 2.3 m, surface area: 4.15 m2, volume: 52 m3) entirely designed in plastic were deployed. The bags were made of polyethylene mixed with vinyl acetate and the holding structure of PVC and polyethylene. The screw anchors were installed at the sea floor 25–30m deep. Underwater, the mesocosms were closed systems without lateral advection.’ ‘Each day, three different depths (0.1-, 5- and 10m-depth) were sampled in the six mesocosms using a system of permanent PVC tubing placed at the center of the bags and connected to a Teflon pump.’

RESPONSE: Our paper is focused on the response of N2 fixation to simulated dust deposition events. The responses of chlorophyll-a, primary and new production to dust seedings are presented in the papers of Guieu et al. (2013) and Ridame et al (2013). In order to evaluate the contribution of N2 fixation to primary and new production, we used the data from Ridame et al., 2013. Also, in order to explain our results and propose some hypothesis, we used the nutrients concentrations (DIP, NO3-, DFe) measured during DUNE which are presented in Wagener et al., 2010; Pulido-Villena et al., 2010, 2013; Wuttig et al., 2013; Ridame et al., 2013. We have added a new section ‘Complementary parameters from DUNE companion papers‘ in Material and Methods where some details about the measurements of chlorophyll a, nutrients (including dissolved Fe), primary production and estimates of new production are given: ‘In this paper, data of chlorophyll-a, nutrients concentrations (NO3-, DIP, DFe), primary production (PP) and new production (NP) are used to characterize the biogeochemical conditions of the seawater before and after seedings. These data are fully described in DUNE companion papers (Wagener et al., 2010; Pulido-Villena, 2010, 2013; Ridame et al., 2013; Guieu et al., 2013; Wuttig et al., 2013) including the methodology and the analytical procedures. Briefly, the chlorophyll-a concentration was measured after extraction in 90% acetone on a Turner Trilogy laboratory fluorometer (see Guieu et al., 2013; Ridame et al., 2013). Nitrate (NO3-) concentrations were analyzed on 0.2μm-filtered seawater according to classical methods using the automated colorimetric technique; the detection limit was 30 nM (see Ridame et al., 2013). DIP was analyzed on 0.2μm-filtered seawater by spectrophotometry using a long waveguide capillary cell (LWCC); the detection limit was 2 nM (see Pulido-Villena et al., 2010, 2013). The concentration of DFe (< 0.2μm) was measured by flow injection analysis with online preconcentration and chemiluminescence detection (FIA-CL); the detection limit was 10 pM (see Wagener et al., 2010). PP (in Ridame et al., 2013) was determined by the 13C uptake after addition of NaH13CO3. This was done simultaneously to N2 fixation determination using the dual 13C/15N2 isotopic label technique. As atmospheric deposition constitutes a source of external nutrients to the surface layer, it induces by definition NP. Thus, the increase in PP in the Dust-meso 24 hours after seeding observed in DUNE P and R experiments can be associated with NP (NPseeding). We consider that after 24 hours, the increase in PP could be partly supported by regenerated nutrients. NP was estimated before seeding and over the course of the experiments in the Control-meso considering that NP represents 15% of PP during periods of stratification in the western Mediterranean Sea (Marty et al., 2002; Moutin and Raimbaut, 2002; L’Helguen et

Full Screen / Esc

Printer-friendly Version

Interactive Discussion

Discussion Paper

al., 2002). We estimated the NP in the Dust-meso (NPdust) 24 hours after seeding using the following equations: NPdust=NPcontrol+NPseeding and NPseeding=PPdust-PPcontrol. The contribution of N2 fixation to PP was estimated using measurements of PP (that are reported in the companion paper, Ridame et al., 2013) and average molar particulate C/N ratios calculated for each experiment (7.5±0.4, 7.5±0.5 and 7.8±0.6 respectively over P, Q and R experiments, whole data set in Ridame et al., 2013).

The discussion section could be better organized and merging sections is advised, since some of the discussion indifferent sections could be linked allowing a smoother reading of the paper. The conclusions stated are solid, but a reinforcement of the poor contribution of N2 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.

RESPONSE: The discussion section has been reorganized and sections 4.1.1 and 4.1.2 have been merged as advised by the reviewer. The poor contribution of N2 fixation to PP and NP has been added in section 5 ‘Summary and conclusions’. Maranon et al., 2010 was added in discussion section 4.1.

Other recent papers about the contribution of N2 fixation to primary production and heterotrophic diazotroph abundance in the Mediterranean Sea are not cited.

RESPONSE: We have added the following references:

Rahav, E., Herut, B., Stambler, N., Bar-Zeev, E., Mulholland, M.R., and Berman-Frank, I.: Uncoupling between dinitrogen fixation and primary productivity in the eastern Mediterranean Sea, J. Geophys. Res. Biogeosci., 118, doi:10.1002/jgrg.20023, 1-8, 2013a.

Rahav, E., Herut, B., Levi, A., Mulholland, M.R., and Berman-Frank, I.: Springtime contribution of dinitrogen fixation to primary production across the Mediterranean Sea,

Full Screen / Esc
Printer-friendly Version
Interactive Discussion
Discussion Paper

Ocean Sci., 9, doi: 10.5194/os-9-489-2013, 489-498, 2013b.

Furthermore, the potential underestimation of N2 fixation rates due to the use of 15N2 bubbles instead of 15N2 previously dissolved in seawater is not sufficiently discussed. The authors could argue how much could the N2 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 N2 fixation to primary and new production estimated.

RESPONSE: This paragraph has been added in Discussion, section 4.2 'Recently, it has been shown that the gas bubble enrichment method may underestimate N2 fixation rates in surface waters by a factor of 2 to 7 relative to the enriched 15N2 seawater method (Mohr et al., 2010; Großkopf et al., 2012; Wilson et al., 2012). The comparison of both methods in the Mediterranean waters demonstrated a 2–3 fold increase in rates using the enriched seawater method relative to the bubble addition method (Rahav et al., 2013b). Assuming a two-fold underestimation of the N2 fixation rates, the contribution of N2 fixation to PP remained negligible before and after seeding ($< \sim$ 2%) as well as the contribution of N2 fixation to NP ($< \sim$ 6% before and 24 hours after the seedings); the only exception was 24 hours after the Q-seeding where N2 fixation could represent up to about 20% of NP. '

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.).

RESPONSE: We have removed from the manuscript the results on the composition of the diazotrophic community in the DUNE experiments as I. Biegala will probably not publish these data in the SI DUNE and as we do not have access to them. In accordance to I. Biegala, we have chosen to cite briefly the results on the UCYN abundance.

Finally, a thorough revision of the English language by a native speaker or professional translation service is strongly recommended (see Technical corrections).

RESPONSE: A revision of the English language has been performed by a native speaker.

SPECIFIC COMMENTS

Abstract

Page 10583, line 13: please add the concentration of NO3- added as a consequence of simulated wet deposition in parenthesis.

RESPONSE: The nitrate concentration measured in surface waters five hours after the DUNE-R2 seeding has been added in the abstract.

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?).

RESPONSE: We have defined ‘new production’ and the method used for its estimation in Material and methods (see comments above).

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

RESPONSE: The sentence has been completed: ‘The estimated input of new nitrogen (NO3-) from simulated wet deposition was much higher (up to 330 fold) than that associated with N2 fixation.’

Introduction

Page 10584, line 5: change for “reservoir”, which is more appropriate if you are describing the importance of N2 fixation over geological time scales.

RESPONSE: This has been changed in the text

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).

Full Screen / Esc

Printer-friendly Version

Interactive Discussion

Discussion Paper

RESPONSE: We have changed the reference of Eugster et al., for Codispoti, 2007, Gruber, 2008 and Großkopf et al., 2012

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

RESPONSE: Dore et al., 2002; Karl and Letelier, 2008; Subramaniam et al., 2008 have been added

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".

RESPONSE: We have changed the sentence for: 'In the contemporary ocean, it is assumed that the nutrients limiting N2 fixation are mainly phosphorus (P) (Sanudo-Wihlemy et al., 2001; Sohm et al., 2008; Ridame et al., 2011), iron (Fe) (Berman-Frank et al., 2001; Kustka et al., 2003; Moore et al., 2009; Jacq et al., 2013) or both (Mills et al., 2004). Recently, it has been shown that trace elements other than Fe could also exert a control on N2 fixation (Ridame et al., 2011; Ho, 2013).'

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.

RESPONSE: To our knowledge, SML is the body of water comprised between the sea surface and the MLD, so the definition is indeed different. We believe SML has been correctly used throughout the text and one cannot be replaced by MLD.

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

RESPONSE: Pulido-Villena et al., 2010 has been added

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

RESPONSE: We have added in Material and methods in section 2.1 some characteristics of the mesocosms: 'Briefly, six mesocoms (height: 12.5 m, diameter: 2.3 m, surface area: 4.15 m2, volume: 52 m3) entirely designed in plastic were deployed. The bags were made of polyethylene mixed with vinyl acetate and the holding structure of PVC and polyethylene. The screw anchors were installed at the sea floor 25–30m deep. Underwater, the mesocosms were closed systems without lateral advection.'. . ..'Each day, three different depths (0.1-, 5- and 10m-depth) were sampled in the six mesocosms using a system of permanent PVC tubing placed at the center of the bags and connected to a Teflon pump.'

Page 10588, line 20: indicate how many replicates were used for the measurement of N2 fixation rates. Page 10588, line 26: so the water was collected in the morning during routine sampling of the mesocosms, and the 15N2-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.

RESPONSE: This has been clarified in Material and Methods: '. . .One sample per depth of unfiltered seawater was collected in the morning at two depths (0.1m and 5m-depth) during DUNE-1-P and -Q and at 5m-depth during DUNE-2-R for determination of N2 fixation rates'. . .. 'Immediately after sampling, 15N2 tracer was added to obtain a final enrichment of the N2 pool of about 10 atom% excess and each bottle was well shaken. Then, the 15N2-amended bottles were incubated under in situ conditions on a mooring line, outside the mesocosms for 24 hours at the corresponding sampling depths (0.1m and 5m depth). Incubations were terminated the following morning by filtration onto pre-combusted 25mm GF/F filters, and filters were stored at -20°C.

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

RESPONSE: This has been clarified in Material and Methods: …'Sample filters were dried at 40°C for 48 hours before analysis.'

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

RESPONSE: This has been done

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.

RESPONSE: This has been done, see above response

Page 10589, line 21: Chlorophyll a concentration units μL m-1, is this meant to be μg L-1?

RESPONSE: This has been changed for μg 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.

RESPONSE: This has been done, see above response

Discussion

Page 10592, section 4.1.: why is the stimulation of N2 fixation rates similar between wet and dry dust seedings? Is it caused by the similarity in the P and Fe composition between both types of dust? (Table 1).

RESPONSE: The stimulation of N2 fixation rates was similar after both wet and dry dust seedings. So, it means that both simulated wet and dry dust deposition events brought a sufficient amount of the nutrient(s) which controlled N2 fixation. During the DUNE experiments, we did not determine which nutrient(s) is (are) limiting for N2 fixation. Based on our results and previous studies, we can speculate that it was controlled by DIP or co-limited by DIP and a trace element released by dust (different from Fe) (section 4.3). The similarity in the content of P, Fe and trace metals between EC and non-EC dust (Guieu et al., 2010; Desboeufs et al., 2013) could well explain the similarity in the stimulation of N2 fixation between experiments. The following sentence has been added in section 4.1: ‘As a consequence, the mode of deposition and the type of dust did not influence the magnitude of the stimulation of the N2 fixing activity in the DUNE experiments indicating that both wet and dry dust deposition events brought a sufficient amount of the nutrient(s) that was (were) limiting N2 fixation. The similarity in the contents of P, Fe and trace metals between both types of dust could well explain the similarity in the stimulation of N2 fixation after all the seedings.’

The amount of N between wet and dry dust was different. Did N inputs not affect N2 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.

RESPONSE: Difference in the atmospheric supply of NO3- between wet and dry deposition did not affect the response of N2 fixation as the increase in N2 fixation rates was similar after wet (P, R) and dry (Q) dust seedings suggesting that the diazotrophic community in the DUNE experiments is mainly dominated by unicellular diazotrophs (no access to the data on the composition of the diazotrophic community). We moved the §on the impact on nitrate on the diazotrophic activity (previously in section 4.3 ‘Biogeochemical factors controlling N2 fixation’) into section 4.1: ‘Dissolved N2 constitutes by far the largest pool of nitrogen in the ocean (Gruber, 2008) and it can be used only by diazotrophs for their metabolic processes. Thus, diazotrophs should not be limited by the availability of fixed N in the environment which represents a major ecological advantage relative to non-diazotrophic phytoplankton in N depleted waters. Moreover, as N2 fixation is more energy consuming than NO3- reduction, the input of NO3- could inhibit partially N2 fixation. This hypothesis has been validated on the cultured filamentous diazotroph Trichodesmium which decreased its N2 fixing activity (up to -70%) after 10 μM NO3- addition (Mulholland et al., 2001; Holl and Montoya, 2005). However, the response of UCYN could be different as addition of 10 μM of NO3- did not change the N2 fixation activity of the UCYN Crocosphaera (Dekaezemaker and Bonnet, 2011). An increase of about 10 μM in NO3- concentration (equivalent to that used in the culture experiments) was observed in surface waters (0.1 m-depth) five hours after wet deposition in DUNE-2-R (Ridame et al., 2013). Despite this strong increase, N2 fixation rates in the Dust-meso increased after the wet deposition events (P, R) and the intensity of the increase was similar to that recorded after a dry deposition (Q). This indicates that N2 fixation activity was not inhibited by the atmospheric input of new nitrogen (NO3-) suggesting that the diazotrophic community was mainly dominated by unicellular diazotrophs'

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

RESPONSE: This paper is not cited in the revised version.

Page 10593, section 4.1.1.: the lack of correlation between N2 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.

RESPONSE: The TSA-FISH technique was used by Biegala and collaborators for the determination of the UCYN abundance. This has been added in section 4.1: 'The low values of N2 fixation rates in the Control-meso are in agreement with the low abundances of picoplanktonic (0.2-3μm) unicellular diazotrophic cyanobacteria (UCYN) (TSA-FISH, details in Biegala and Raimbault, 2008) measured before the DUNE seedings (I. Biegala, personal communication 2013)'

RESPONSE: The paragraph on the lack of correlation between N2 fixation and the UCYN abundance has been rewritten (section 4.1) 'In the Mediterranean Sea, the community of the diazotrophic cyanobacteria is mainly represented by UCYN as low concentrations of filamentous diazotrophic cyanobacteria (Trichodesmium and the symbiotic Richelia) have been found (Bar-Zeev et al., 2008; Le Moal et al., 2011; Yogev et al., 2011). Recently, the presence of non-cyanobacterial unicellular diazotrophs such as α- and γ-proteobacteria has been reported in the Mediterranean Sea (Man-Aharonovich et al., 2007; Bar-Zeev et al., 2008; Yogev et al., 2011; Le Moal et al., 2011) suggesting that N2 fixation supported by non-cyanobacterial diazotrophs could be significant in the surface waters (Rahav et al., 2013a). It is therefore probable that during the DUNE experiments, non-cyanobacterial unicellular diazotrophs and to a lesser extent filamentous diazotrophic cyanobacteria have in addition to UCYN contributed to N2 fixation. Others aspects such as the regulation of the diazotrophic biomass development by protist grazers, the viral attack and/or single-cell variability in N2 fixing activity may also explain the larger changes in metabolic rates of N2 fixation to dust addition relative to those of UCYN cellular abundance. This latter hypothesis is probable as a lack of correlation between nifH copy and transcript numbers has been shown previously within the cyanobacterial diazotrophic community (Zehr et al. 2007).

And a paragraph on the differential responses to dust input by different diazotrophs has been also added (section 4.1): ' In the tropical Atlantic, Maranon et al., (2010) have shown that although bulk abundance tended to remain unchanged, different groups of phytoplankton and bacterioplankton responded differently to Saharan dust additions thereby inducing changes in the structure of the phytoplanktonic and heterotrophic community. This pattern was also observed for the diazotrophic community after dust events (Langlois et al., 2012; Benavides et al., 2013). After a Saharan dust deposition event in the Canary Islands, the abundance of unicellular diazotrophs increased while that of the filamentous Trichodesmium as well as the N2 fixation rate in the $> 50 \mu$m size fraction were not affected (Benavides et al., 2013). Interestingly, the response within the unicellular diazotrophic community was contrasted: the abundance of the small (<1 μm) unicellular diazotrophs (probably represented by UCYN from group A) strongly increased whereas that of medium-sized (1-3μm) cells decreased leading to a shift in the structure of the unicellular diazotrophic community. Langlois et al. (2012) found that although abundances of all diazotrophic cyanobacterial and γ-proteobacteria phylotypes increased after Saharan dust additions in the tropical North Atlantic Ocean, the intensity of these increases varied with the largest increases observed for UCYN from group A. This variability could be explained by variability in the nutrient requirements and mode of acquisition within the diazotrophic cyanobacterial community as demonstrated for Fe and P in cultured unicellular and filamentous cyanobacteria (Tuit et al., 2004; Dyhrman et al., 2006; Berman-Franck et al., 2007; Jacq et al., 2013). Thus, it is likely that in the DUNE experiments, diverse diazotrophs responded differently to the dust additions and that the simulated dust event changed the structure of the diazotrophic community.'

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 N2 fixation rates between mesocosms as indicated above.

RESPONSE: Sections 4.1.1 and 4.1.2 have been merged. The intensity of the increase in N2 fixation to dust seedings was similar after the DUNE-P, -Q, and R seedings ($p>0.05$). So, there is no variability in the response of N2 fixation between all the experiments. Nevertheless, for the P experiment or Q experiment, we observed at some sampling times variability in the magnitude of the increase in N2 fixation between the triplicate dust mesocosms D1, D2 and D3. One hypothesis could be that spatial variability in the abundance of diazotrophs and/or spatial variability in the composition of the diazotrophic community explain the variability in N2 fixation rates between the triplicate dust mesocosms after seeding. The following changes have been made:

‘In the P as in the Q experiment, the relative changes in N2 fixation rates varied between the triplicate dust mesocosms (D1, D2, D3) at some sampling times indicating heterogeneity in the magnitude of the response of the diazotrophic activity to the dust input. For example, the relative changes in DUNE-1-P were 4.4, 10.1 and 1.4 respectively in D1, D2 and D3, 2 days after the seeding (5m depth, Fig. 1). Such a discrepancy could be attributed to a spatial heterogeneity in the composition of the diazotrophic community. The presence of Trichodesmium sp. in D3 relative to D1 and D2 could explain the small increase in N2 fixation after the wet deposition as the input of NO3- would have inhibited the N2 fixing activity of Trichodesmium. Unfortunately, the phylogenetic characterizations of diazotrophs in the tested seawater are currently unavailable. The variability in the relative changes in N2 fixation rates between the three dust mesocosms could also be due to spatial heterogeneity in the abundance of diazotrophs. Indeed, high variability in the abundance of UCYN (5m depth) between the three dust mesocosms in DUNE-P or –Q was observed (I. Biegala, personal communication 2013). During the R experiment, there was less variability in both N2 fixation rates and UCYN abundances (I. Biegala, personal communication 2013) within the Dust-meso after seedings.

Pages 10594 and 10595, section 4.1.3.: the temperature range observed is quite broad. Why wouldn’t this change in temperature affect N2 fixation rates at all? Please provide an explanation or speculation.

RESPONSE: The paragraph on the potential impact of temperature changes on N2 fixation has been moved in section 4.3 'factors controlling N2 fixation'. The temperature in the mesocosms ranged between 19 and 27°C. As N2 fixation rates in the control-meso were similar between P, Q, R1 and R2, over the course of the experiments ($p>0.05$), the change in temperature did not affect the N2 fixation activity of the diazotrophic community. This has been added in the revised version: 'As shown by the small R2 between temperature and N2 fixation rates in the DUNE experiments (R2=0.001), temperature was probably not a limiting factor of diazotrophic activity in summer in the northwestern Mediterranean Sea as also mentioned by Yogev et al. (2011) for the eastern basin.'

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.

RESPONSE: These lines have been moved in Material and methods

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

RESPONSE: As recommended by Biogeosciences: the reference of a papier 'in prep' has to be cited in the text as author et al. (YEAR) and added to the reference list as AUTHORS: TITLE, STATUS (in preparation, in review, accepted...), YEAR.

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

RESPONSE: As our experiments were performed during the summer season, we have compared our results on the contribution of N2 fixation to PP to previous results obtained during the same period. As the data of Rahav et al, 2013 (Ocean Science) correspond to the springtime (4-28 April), we have chosen to cite Rahav et al., 2013 JGR.

Page 10595, lines 12-15: This statement is risky. The C:N2 fixation ratio in diazotrophs varies widely (see section 3 on Mulholland et al., 2007; Biogeosciences). Take advan-

Full Screen / Esc

Printer-friendly Version

Interactive Discussion

Discussion Paper

tage of recent publications where C and N2 fixation were measured simultaneously in the frame of CO2 enrichment experiments (using the control data). The contribution of N2 fixation to NP is straightforward and quantifiable (as for example in Raimbault and Garcia, 2008; Biogeosciences), but the contribution of N2 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).

RESPONSE: We have removed the following sentence: ‘Therefore, assuming that diazotrophs used only dissolved N2 as a source of nitrogen, the CO2 fixation from diazotrophs before, as well as after seedings, was negligible as compared to the CO2 fixation from the whole photosynthetic community’ as a significant part of N2 fixation could be attributed to heterotrophic diazotrophs.

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.

RESPONSE: These references are mentioned at the beginning of the section for the contribution of N2 fixation to PP. Here, they are cited for the low contribution of N2 fixation to NP.

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

RESPONSE: This has been provided in the revised text and table 4.

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.

RESPONSE: We have added in the revised version, section 4.1: ‘Dissolved N2 constitutes by far the largest pool of nitrogen in the ocean (Gruber, 2008) and it can be used only by diazotrophs for their metabolic processes. Thus, diazotrophs should not be limited by the availability of fixed N in the environment which represents a major ecological advantage relative to non-diazotrophic phytoplankton in fixed N depleted waters.’

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

RESPONSE: Raven, 1988 has been added in the text as well as ‘Furthermore, the high energetic cost of N2 fixation imposes an additional Fe requirement for increased photosynthetic capacity (Kustka et al., 2003).’

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 N2 fixation rates in the ocean is discussed.

RESPONSE: Luo et al., 2013 (BGD) has been added in Discussion: ‘Luo et al., (2013) concluded from a data-based study that on global scale dust deposition used as a proxy for iron supply, does not influence the spatial patterns of N2 fixation, but that at regional scale like in the North Atlantic, N2 fixation is related to dust input. In the northeast Atlantic, correlations between DFe concentrations, Trichodesmium abundance and N2 fixation have been previously reported (Moore et al., 2009). Culture-based studies have shown that the N2 fixing activity of Trichodesmium was more impacted by Fe limitation than that of UCYN indicating much lower Fe requirement for UCYN (Berman-Frank et al., 2007; Jacq et el., 2013). These findings are in good agreement with our results which highlight the non-limiting role of Fe for N2 fixation in a region strongly impacted by Saharan dust deposition where the diazotrophic community is mostly represented by unicellular diazotrophs.’

Also discuss the possibility of the lack of relationship between DFe concentrations and N2 fixation rates by changes in DFe bioavailability, lack of ligands (organic matter), etc

RESPONSE: Due to changes in DFe concentration and Fe binding ligand concentrations, it is likely that the concentration of bioavailable Fe has changed after seedings. Nevertheless, in both cases with or without Fe scavenging, N2 fixation was stimulated suggesting that the bioavailable concentration of Fe present after seedings was sufficient to support the increase in N2 fixation. We have modified the §on Fe and N2 fixation in Discussion: ‘After the DUNE-1-P, -Q and DUNE-2-R1 seedings, the DFe

Full Screen / Esc

Printer-friendly Version

Interactive Discussion

Discussion Paper

concentrations dropped to about 1.5-2 nM (decrease of ~1 nM) due to DFe scavenging on settling dust particles and aggregates in the Dust-meso (T. Wagener, personal communication, 2013; Wagener et al., 2010; Wuttig et al., 2013) while after the DUNE-2-R2 seeding, a transient increase in the DFe concentrations in surface waters of about 2 nM was observed (Wuttig et al., 2013). This increase in DFe was correlated with an increase in the Fe binding ligand concentrations produced by phytoplankton and/or heterotrophic bacteria which allowed the maintenance of Fe in solution, thereby increasing probably the concentration of bioavailable Fe (Wagener et al., 2010; Wuttig et al., 2013). Despite these changes in DFe concentration after dust addition and the potential changes associated with the bioavailable Fe concentration, the process of N2 fixation increased after all the dust seedings (P, Q, R1, R2) and the magnitude of this increase was similar in all experiments. This indicates that during the DUNE experiments, the bioavailability of Fe was not a controlling factor of N2 fixation and that DFe concentrations of the order of 1.5 nM and higher did not limit the N2 fixation in the western Mediterranean Sea.'

Page 10597, line 22: given the results presented here, I wouldn't say that the input of NO3- is "strongly suspected" to inhibit N2 fixation. Consider changing for "could".

RESPONSE: This has been done

TECHNICAL CORRECTIONS

RESPONSE: All the corrections listed below have been made in the revised version

Page 10583, line 10: please define "N2" 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".

Full Screen / Esc

Printer-friendly Version

Interactive Discussion

Discussion Paper

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

Page 10584, lines 1: change “all” for “other”.

Page 10584, line 4: place “(N2)” 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”

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”.

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".

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: : :”.

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”.

Full Screen / Esc

Printer-friendly Version

Interactive Discussion

Discussion Paper

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?

RESPONSE: It was a mistake, the good unit is μg L-1

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

RESPONSE: It has been changed

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.

RESPONSE: It has been changed

References

Pulido-Villena, E., Rérolle, V., and Guieu, C.: Transient fertilizing effect of dust in P-deficient LNLC surface ocean. Geophys. Res. Lett., 37, L01603, doi:10.1029/2009GL041415, 2010.

Pulido-Villena, E., Baudoux, A-C., Obernosterer, I., Caparros, J., Catala, P., Georges, C., Harmand, J., Landa, M., and C. Guieu.: Enhanced carbon remineralization by the microbial food web after a dust event in a Low Nutrient-Low Chlorophyll ecosystem: results from a mesocosm experiment in the Mediterranean Sea, in pre., to be submitted to Biogeosciences, 2013.

Wagener, T., Guieu, C., and Leblond, N.: Effects of dust deposition on iron cycle in the surface mediterranean sea: Results from a mesocosm seeding experiment, Biogeosciences, 7, 3769-3781, doi:10.5194/bg-7-3769-2010, 2010.

Wuttig, K., Wagener, T., Bressac, M., Dammshäuser, A., Streu, P., Guieu, C., and Croot, P. L.: Impacts of dust deposition on dissolved trace metal concentrations (Mn, Al and Fe) during a mesocosm experiment, Biogeosciences, 10, 2583–2600, doi:10.5194/bg-10-2583-2013, 2013.

Ridame, C., Dekaezemacker, J., Guieu, C., Bonnet, S., L'Helguen, S.: Phytoplanktonic responses to contrasted Saharan dust events during mesocosm experiments, in prep., to be submitted to Biogeosciences, 2013.

Guieu C., Dulac. F., Ridame C., and Pondaven, P.: Introduction to the project DUNE, a DUst experiment in a low Nutrient, low chlorophyll Ecosystem, Biogeosciences Discuss., 10, 12491-12527, 2013.

Interactive comment on Biogeosciences Discuss., 10, 10581, 2013.