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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.

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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 2

GENERAL COMMENTS The factors that control the distribution and magnitude of nitrogen fixation (iron supply through dust deposition among them) is a currently discussed issue in oceanography. Ridame et al. shows the effects of simulated wet and dry dust depositions on nitrogen fixation and its relevance in the supply of N for primary production on Mediterranean low nutrient low chlorophyll waters. The experi-

mental approach used large mesocosms to establish the effects through the web chain from bacteria to zooplankton in enclosed and controlled systems, which allowed extending the period of sampling further in time, and also adding more variables, in order to improve the amount of data retrieved, in comparison to microcosms experiments or field surveys. Thus, this manuscript provides interesting information, and represents a substantial contribution to the scientific community, presenting consistent conclusions about the control of nitrogen fixation by dust deposition in this area. The manuscript is generally structured with clarity and the logical flow is correct, but the use of English needs revision, as pointed by Referee #1. Besides some parts are slightly confusing and could be smothered, in particular, points 3.2, 3.3 and 3.4 of the results section. After a few changes, it will be suitable for publication in BG.

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

SPECIFIC COMMENTS p10586 – section 2.1. The reviewer understands that the experimental design is completely described in another paper but more details on the dimensions (width, diameter, depth) and characteristics of the mesocosms are required, also a brief description of the methods of estimation of primary production and new production. Because this manuscript will be read on its own, a brief description of the mesocosms design and the primary production methods will help to fully understand the relations with nitrogen fixation.

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: We have added a new section: 'Complementary parameters from DUNE companion papers' in Material and Methods where some details about the measurements of primary production and estimates of new production are given: 'PP was determined by the 13C uptake after addition of NaH13CO3. This was done simultaneously to N2 fixation determination (Ridame et al., 2013) 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 have considered that after 24 hours, the increase in PP could be partly supported by regenerated nutrients. NP was estimated before seedings 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 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$\pm$0.4, 7.5$\pm$0.5 and 7.8$\pm$0.6 respectively over P, Q and R experiments, whole data set from Ridame et al., 2013). '

p10588 – section 2.2.What was the limit of detection of nitrogen fixation? Is it possible to estimate it according to Montoya et al. (1996).

RESPONSE: The limit of detection of N2 fixation was added in the Material and methods section: 'The 15N uptake rates were considered as significant when 15N excess enrichments of PON were greater than three times the standard deviation obtained on natural samples. According to our experimental conditions and equations from Montoya et al. (1996), the detection limit for N2 fixation, calculated from significant enrichment and lowest particulate nitrogen is estimated to be 0.05 nmol N L$-$1 d$-$1.'

And, if possible, a extended explanation on the methodology will be helpful: number of replicates per depth, type of incubation (refrigerated, simulation of light?)...

RESPONSE: The following paragraph has been added: '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. Samples were collected in the six mesocosms and outside the mesocosms before and after dust seeding'. . ...' 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).'

The underestimation of nitrogen fixation due to the addition of 15N2 as a bubble is a key issue, which needs to be discussed further. It is not extremely important for the comparison of changes between control-meso as dust-meso in nitrogen fixation. As long as the difference of diazotroph species between replicates and treatments is not large, the underestimation should be of the same order and the % of change remains valid. But it may be relevant when estimating the fraction of N supply for primary production using absolute values of nitrogen fixation. Besides, a couple of key papers are missed in the citation: Mohr et al. (2010), PLoS ONE, which could be addressed to apply corrections to the estimated dissolution of the bubble of 15N2 during DUNE experiments; and Grosskopf et al. (2012), Nature, which showed that the underestimation in communities dominated by UCYN in the Atlantic was up to 7-fold in certain regions, suggesting that the % of N to primary production, estimated during DUNE, may be higher that currently estimated. Due to this, p10596 - section 4.2, needs also revision and a slightly cautious interpretation because of possible underestimation.

RESPONSE: We totally agree that this is an important issue. The following sentence has been added in section 2.2 in Material and Methods: 'In spite of this potential underestimation, the relative changes in N2 fixation (N2 fixationDust/N2 fixationcontrol)

after seeding should not be impacted'. And the following paragraph has been added in Discussion in 4.2 section: '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. '

p10597 – line 25. Please also cite Mulholland et al. (2001) J. Phycol.

RESPONSE: This reference has been added in the revised version: '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).'

TECHNICAL COMMENTS p10586 – line 23. It is a mere suggestion, but it is helpful to explain abbreviations again the first time it appears in a new section. It allows readers understand what we are telling them, without going back to look for the meaning of the abbreviation. So, the reviewer would recommend the same for the results and discussion. p 10593 – line 3. Please, explain the meaning of UCYN (unicellular cyanobacteria) before introducing the abbreviation, not specialists could find it confusing. I will not add any other technical comment, as most of the issues I detected are mentioned in the thorough revision of Referee #1.

RESPONSE: This has been changed in the text in section 2.1: 'More precisely, the experiments were realized in the Elbo Bay located in the Natural Preservation Area of Scandola (8.554°E, 42.374°N) which is representative of the low nutrient low chloro-

phyll (LNLC) conditions of the open western Mediterranean Sea (Guieu et al., 2010b).' and in section 5: 'Our results from original mesocosm experiments demonstrate that atmospheric dust deposition may greatly influence N2 fixation rates in Low Nutrient and Low Chlorophyll environments impacted by mineral dust deposition.'

RESPONSE: We have defined UCYN as unicellular diazotrophic cyanobacteria at the beginning of the Discussion: 'The low values of N2 fixation rates in the Control-meso are in agreement with the low abundance of picoplanktonic (0.2-3μm) unicellular diazotrophic cyanobacteria (UCYN) measured (TSA-FISH, details in Biegala and Raimbault, 2008) before the DUNE seedings (I. Biegala, personal communication 2013)'.

References

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