

## ***Interactive comment on “Assessing the role of dust deposition on phytoplankton ecophysiology and succession in a low-nutrient low-chlorophyll ecosystem: a mesocosm experiment in the Mediterranean Sea” by V. Giovagnetti et al.***

**V. Giovagnetti et al.**

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We are grateful for the referees' comments, which focus on aspects either related to plankton (heterotrophic and autotrophic) succession/competition dynamics, or phytoplankton physiological changes, after atmospheric pulses of new micro- and macronutrients. We agree with all the critics and suggestions made by the first referee, and please find below our responses. Please take notice that line numbers refer to the revised version of the manuscript. We changed the manuscript accordingly and we will submit it in agreement with Biogeosciences journal timing.

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Best regards,

Vasco Giovagnetti and Christophe Brunet, on behalf of all the co-authors.

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General comments The paper deals with the effects of atmospheric dust inputs on the phytoplanktonic community, addressing both changes in ecophysiological traits and the succession of autotrophic organisms after dust pulses. The results are part of a large experimental effort covering numerous aspects of atmospheric dust deposition, and I believe it represents a valuable scientific contribution within the scope of Biogeosciences. Positive aspects of the manuscript are: - The specific focus on surface waters of low-nutrient, low-chlorophyll (LNLC) areas, because surface communities in oligotrophic regions are often overlooked in favour of their counterparts within the deep chlorophyll maximum; - The discussion on the physiological mechanisms underlying the response of phytoplankton to dust pulses; - The accurate methods, with large clean mesocosms and three replicate tanks per condition. Good combination of flow cytometry and HPLC pigment analysis to distinguish different groups within the pico- and nanoplankton size fraction.

We are grateful to the referee for the positive appreciation and consideration of our study. We would like to thank her/him for the critics and comments she/he made, which have been thoroughly considered during the revision of the manuscript. Changes and additions in the text are in blue colour (see below).

However, I feel that the manuscript has some flaws that require additional work: (1) Although the authors refer to a number of companion papers in the materials and methods section, many of them are not yet published and so the reader lacks important information on the experimental design and the physico-chemical background. It is necessary to include at least one table summarizing basic environmental conditions at the beginning of the experiment (temperature, irradiance, mixed layer depth, background nutrient concentrations, mean nutrient inputs coming from the dust additions).

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We agree with the referee and we would like to thank her/him for this suggestion. A table summarizing the initial environmental conditions, as well as the evolution of the main environmental properties of the studied system during the course of the experiment, has been included in the manuscript (Table 1), in order to give proper information on the physico-chemical conditions of the area (Lines 972 and 1037). Few sentences have also been modified and implemented, while referring to Table 1 (e.g. subsection 3.1 and line 292).

Likewise, they provide information on the grams of dust added (line 4, page 19206) but we have no information whatsoever on the magnitude of this addition: does it correspond to a large dust storm? It is on the order of mean dust pulses over the western Mediterranean? How often occur similar pulses in the study area? Some more information on the general context of the study would be desirable.

We would like to thank the referee for this comment. Few sentences have been modified in order to provide more information on the magnitude of the dust additions performed during the experiment, as well as on the area of study (Lines 145 and 157).

(2) The discussion gives an interesting overview on phytoplankton succession after dust pulses, and provides valuable reasoning on nutrient uptake and physiological traits involved in phytoplankton shifts. In my opinion, however, the discussion would gain insights if the authors considered the effects of competing heterotrophic organisms. In oligotrophic regions, bacteria have been shown to quickly respond to dust pulses (Pulido-Villena et al. 2008, Marañón et al. 2010, Romero et al. 2011), depleting available resources and somehow delaying the transfer of nutrients to larger phytoplankton cells (Romero et al. 2011). The potential interaction with bacteria is briefly mentioned with regard to the consumption of phosphate during the second dust pulse, but to what extent that may explain the delay in the response of phytoplankton shall be further discussed.

We would like to thank the referee for the positive comment, and for the suggestion

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concerning the effects of competing heterotrophic organisms. Several sentences have been added to the text, giving few insights on the competition between the autotrophic and heterotrophic components of the community (lines 492). The response of heterotrophic prokaryotes (hereafter 'bacteria', including both bacteria and archaea) to dust addition is reported in Pulido-Villena et al. this issue. Basically, dust addition enhanced bacterial respiration quick after the first seeding and then it remained constant and higher than in C-mesocosms until the end of the experiment. However, no additional increase was observed after the second dust seeding. Bacterial abundance was top-down controlled by viruses and, to a lesser extent, by heterotrophic nanoflagellates. This top-down control was enhanced after the second dust addition resulting in lower bacterial abundance in D-mesocosms than in C-mesocosms. The high cell-specific bacterial respiration rates and viral lysis observed in the D-mesocosms, particularly during the second part of the experiment, might have caused an increased remineralisation of nutrients, further available for the phytoplankton community.

A few lines on how the effects observed in this experiment might change under some other conditions (nutrient-replete waters in winter, for instance) would also be interesting.

The comment of the referee is pertinent and interesting, but we did not find any precise report concerning the Mediterranean Sea addressing such conditions. In order to prevent any speculative discussion on this aspect and to maintain the focus of our paper on summer oligotrophic ecosystem responses to dust additions, we prefer not to modify the text.

(3) The positive effect of atmospheric inputs on phytoplankton in the Mediterranean region has been recently challenged (Jordi et al. 2012). Accumulation of elements such as copper can progressively inhibit algal photosynthesis by altering electron transport and by inactivating a fraction of the PSII reaction centers. The authors provide data on dissolved Fe (Table 1), are there Cu measurements available? Could toxic elements such as Cu relate to the observed results of some photosynthetic parameters?

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We would like to thank the referee for this comment, and few sentences touching these recent findings (Paytan et al., 2009; Jordi et al., 2012) have been added within the manuscript introduction (line 85). Copper (Cu) data are provided by Wuttig et al., 2012. Cu was measured (ETAAS) at three time points (Table 1 and 2; Wuttig et al., 2012). Even if we don't know Cu concentration soon after the dust addition (but only at t0-1, given the fact that no measurement was performed in the first 24-48h post-seeding, Table 1; Wuttig et al., 2012), we might assume no Cu toxic effects on phytoplankton physiological state, in relation to stable and similar, and much lower, Cu concentration, relative to Jordi et al. data (2012). Indeed Cu concentrations ranged between 1.24 and 2.16 nmol L<sup>-1</sup> (i.e.  $\leq 0.14$  ng m<sup>-3</sup>), either in dust-amended or control mesocosms (Wuttig et al., 2012).

(4) Statistical information is scarce; the authors should include a table providing, for each parameter measured, the statistical significance of the difference between dust-enriched (DM) and control (CM) mesocosms. It would be a helpful summary of the results.

We agree with the comment of the referee, and we would like to thank her/him for this suggestion. Further statistical analysis have been performed, and related information have been added within the text (subsection 2.5, and mainly section 3), while preferring to avoid the addition of a third table.

(5) The text is a little long, in particular the results section, and some paragraphs in the discussion could be merged to shorten the ms (e.g., lines 10-25 page 19218).

We thank the referee for this comment, and we agree with her/him. Text has been changed and few paragraphs of the Introduction, Results and Discussion sections have been deleted or merged to shorten the manuscript.

The authors should also pay attention to the language, because some sentences are hard to understand. The term 'since', for instance, is sometimes misused (see technical comments below), they should check that throughout the manuscript.

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We agree with the referee. We are sorry for the language faults/problems, and related lack of clearness in some parts of the manuscript. In the revised version of the manuscript, language has been polished (changes are in blue colour along the text). We take this chance to thank the referee for all the valuable suggestions given in the subsection "Specific comments and technical corrections". Referee's critics and comments have been of great help to improve the quality and clarity of the manuscript.

Specific comments and technical corrections

Some terms and sentences are not enough specific and/or clear: Page 19202, line 8: "high light pressure": meaning high light conditions/exposure? We thank the referee for this comment. The sentence has been removed in order to convey a clearer message and shorten the introductory section.

Page 19202, line 15: "have often paid limited attention": do you mean that the topic has received relatively less attention? Please rewrite this sentence. We agree with the referee, thus this part of the introduction has been modified (from Line 58 onward).

Page 19202, lines 20-23: "causing a paucity of data by which defining. . .": this sentence is confusing, please rephrase it. We thank the referee and agree with her/his comment. The sentence has been therefore changed (Line 66).

Page 19202, line 26: "due to the important amount (. . .)": I am not sure whether this sentence is correct. 'given the important amount'? We thank the referee for this suggestion, and we changed the sentence (Line 72).

Page 19204, line 4: "since their greater capacity (. . .) than bigger ones.": this part of the sentence is hard to understand, please rewrite it. We agree with the referee, and we rephrased the sentence in order to make it clearer (Line 114).

Page 19204, line 14: "since their different types of adaptation (...)": do you mean that *Synechococcus* and *Prochlorococcus* present varying relative proportions as a function of their different adaptation to physico-chemical conditions? Please rewrite

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this sentence. We thank the referee for this comment, and we agree with him. Please refer to the changed sentence (Line 120).

Page 19204, line 24: "to deeply investigate the ecology of picoeukaryotes" sounds a bit extreme to me. Although the combination of pigment analysis and flow cytometry gives useful information on the groups of picophytoplankton present in the water, it is not the ultimate method to assess the ecology of picoeukaryotes. Please clarify this. We agree with the referee, and we changed the sentence accordingly (Line 132).

Page 19204, line 29: "better able": most able? We thank the referee for this comment, and we changed the sentence accordingly (Line 136).

Page 19206, line 24: "two solvent mixtures: methanol, aqueous ammonium acetate (70:30) and methanol". Methanol appears twice, is it a mistake? We thank the referee for this comment. The word "methanol" must appear twice, and we decided to slightly modify the sentence to prevent any misunderstanding (Line 182).

Page 19207, line 11: "since the low phytoplankton": meaning given the low phytoplankton? We thank the referee for this comment, and we changed the sentence accordingly (Line 198).

Page 19207, line 19: "would have induced any stress": could induce any stress? We agree with the referee. The sentence has been modified accordingly (Line 205).

Page 19210, section 3.2. Statistical information (p-values) should be given with regard to the difference between CM and DM mesocosms for the different parameters measured (see previous comment). We agree with this comment of the referee. As mentioned above, statistical information have been added within the text (subsection 2.5, and mainly section 3).

Page 19211, lines 1-3: "It can be highlighted (. . .) after the dust additions (DM)": I do not quite understand this sentence. Please rephrase it. We agree with the referee that such sentence was not clear, and was not adding any further insights to the argument.

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We therefore decided to delete it (Line 292).

Page 19216, lines 15-16: "since the size-fractionation of samples for pigment analysis". This sentence is unclear. We agree with the referee, and modified the sentence (Line 421). Page 19217, line 28: (0.0091  $\mu\text{g Chl L}^{-1} \text{d}^{-1}$ ): Where does this value come from? Isn't it 0.0077 as appears in Table 1? We agree with the referee that the way we presented this value in the text is misleading. The value 0.0091  $\mu\text{g Chl a L}^{-1} \text{d}^{-1}$  was not shown in Table 2, and it corresponds to the daily increase in Chl a concentration at 23h. We therefore modified the sentence in order to be clearer (Line 447).

Page 19219, line 18: "constrains": constraints. Check this word throughout the ms. We thank the referee for this comment, and we corrected the word accordingly, wherever it was used (Line 636).

Page 19220, line 1: "strongly respond": meaning significantly respond? We thank the referee for this comment. The sentence has been modified accordingly (Line 498).

Page 19220, line 22: "prevented us a meticulous": did not allow a meticulous. . .? The whole sentence is confusing. We agree with the referee that this sentence was not clear, thus we rephrased it (Line 528).

Page 19220, line 25: "the increase in greater cell size community biomass": this expression is not clear. Please rephrase this sentence. We thank the referee for the comment, and we rephrased the sentence to gain in clarity (Line 531).

Page 19221, line 9: "nitrogen (NO<sub>3</sub>+NO<sub>2</sub>)/phosphate (PO<sub>4</sub>) ratio": what about NH<sub>4</sub>? NH<sub>4</sub> can be the preferential N source for small cells in oligotrophic waters.

We agree with the comment of the referee, and we are aware of the relevance of NH<sub>4</sub><sup>+</sup> as N source. NH<sub>4</sub><sup>+</sup> was under detection limit (5 nM) in control mesocosms over the first and second dust additions (Ridame et al., in preparation, this issue). After dust seeding, an increase in NH<sub>4</sub><sup>+</sup> was observed in dust-amended mesocosms (up to 22 ± 6 nM after the first dust addition, Ridame et al., in preparation, this issue),

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rapidly decreasing toward concentrations under detection limit. For this reason, we could not take its effect/role into account, in our attempt of parameterization of the onset of phytoplankton blooms after dust additions.

Page 19224, line 1: “after the second rather than the first dust addition”: remove this part of the sentence. We thank the referee for the suggestion, and we removed this part of the sentence accordingly (Line 611).

Page 19225, line 8: “distinctively”: differently? We thank the referee for this suggestion. The word “distinctively” has been therefore changed into “differently” (Line 651).

Page 19225, line 15: “abiotic and biotic energy”: could you please clarify this? We thank the referee for this comment. The sentence has been changed to be more clear (Line 658).

Page 19225, line 17: “the first group responding”: you should clarify that it is the first group among the autotrophic organisms. We agree with the comment of the referee, thus we modified the sentence accordingly (Line 659).

References: I am not sure whether unpublished papers (manuscripts in preparation) shall be cited as XXX et al. (in prep.) instead of XXX et al. 2012, because the latter is confusing. There are many of them throughout the ms.

We agree with the referee, and we are sorry for these three “confusing citations” that appear within the manuscript, papers belonging also to the Special Issue: “Impact of atmospheric inputs on an oligotrophic ecosystem - the DUNE experiment”. Citations have been corrected by using “XX et al., in preparation” within the text, and related references have been changed accordingly (Lines 814, 911, and 922). In order to be clearer, we also modified the text, accordingly to this comment as well as previous comments (on physico-chemical information and on bacterial activity).

Figures: Fig. 7: I would include the p-value of the correlation in the figure. Although the correlation in Fig. 7b is significant (line 3, page 19216), it looks strongly dependent

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on just one point (aprox.  $X=110$ ,  $Y=13$ ). Please verify that it is a valid measurement.

We would like to thank the referee for this comment. We modified the equation describing the linear correlation between  $^{13}\text{C}$  PP and  $\text{relETR}_{\text{max}}$  (Fig. 8b), by forcing it to pass by the intercept  $(x, y) = (0, 0)$ , and be less biased by this single point. p-values of the two correlation have been also included in the figure (see Fig. 8b and c).

Additional references:

Jordi A, Basterretxea G, Tovar-Sánchez A, Alastuey A, Querol X (2012) Copper aerosols inhibit phytoplankton growth in the Mediterranean Sea. *Proceedings of the National Academy of Sciences*; doi:10.1073/pnas.1207567110. Marañón E, Fernández A, Mouriño-Carballido B, Martínez-García S, Teira E, Cermeño P, Chouciño P, Huete-Ortega M, Fernández E, Calvo-Díaz A, Morán XAG, Bode A, Moreno-Ostos E, Varela MM, Patey MD, Achterberg EP (2010) Degree of oligotrophy controls the response of microbial plankton to Saharan dust. *Limnology and Oceanography* 55(6): 2339-2352. Pulido-Villena E, Wagener T, Guieu C (2008) Bacterial response to dust pulses in the western Mediterranean: Implications for carbon cycling in the oligotrophic ocean. *Global Biogeochemical Cycles* 22, GB1020, doi:10.1029/2007GB003091. Romero E, Peters F, Marrasé C, Guadayol O, Gasol JM, Weinbauer MG (2011) Coastal Mediterranean plankton stimulation dynamics through a dust storm event: An experimental simulation. *Estuarine Coastal and Shelf Science* 93(1): 27-39.

We would like to thank the referee for these selected references of interest. These studies have been cited within the text, and thus the reference list updated accordingly, by also adding other references related to different key aspects the study deals with.

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Interactive comment on *Biogeosciences Discuss.*, 9, 19199, 2012.

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