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Interactive comment on "Microbial food web dynamics in response to a Saharan dust event: results from a mesocosm study in the oligotrophic Mediterranean Sea" *by* E. Pulido-Villena et al.

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Review of the manuscript "Microbial food web dynamics in response to a Saharan dust event: results from a mesocosm study in the oligotrophic Mediterranean Sea" by E. Pulido-Villena et al.

Authors' Responses to referees

We thank both referees for their relevant comments which have helped to improve our manuscript.

Anonymous Referee #2 Received and published: 5 March 2014

This is a mesocosm study of the effects of dust addition to the aquatic microbial community with special emphasis on the heterotrophs. The paper is well written and the subject is timely to address the complexity of interactions between aerosols and marine biota. The study shows heterotrophs (heterotrophic bacteria) to be one of the main groups affected by dust addition, at least under the conditions tested in this experiment, as has already been shown in other studies. One of the novelties of this manuscript is to test the effect of a second dust addition, although there is no reasoning decribed in the manuscript for such an experimental design and there are statistical problems to test its effect. It should be clearly decribed why this design was chosen, what is its context in natural conditions, what is the hypothesis behind the design and what were the expected results. In addition, 1 D mesocoms was clearly very different from the other D mesocosms but the data was nevertherless averaged without reason. There are also a number of incoherences and inconsistencies in several methodologies and the references cited. Below follows a list of detailed issues that need to be thoroughly addressed before publication is warranted.

RESPONSE: All these general comments are answered below.

p 340, line 3. An important reference has been omitted throuout the manuscript. Sala et al. 2002. Seasonal and spatial variations in the nutrient limitation of bacterioplankton growth in the northwestern Mediterranean. Aquatic Microbial Ecology. 27: 47-56.

RESPONSE: This reference has now been added.

p 340, lines 19- 21. Sentence sounds strange. What is exactly "poorly documented" related to dust pulses? The fate of bacterial production? the dynamics of the microbial food web? the dynamics of viruses? of heterotrophic nanoflagellates?

RESPONSE: The sentence has been reworded to avoid confusion.

p 341, lines 16-17. Again, Sala et al. 2002 needs to be included.

RESPONSE: Done.

p 341, lines 18-23. What is the reasoning behind the two consecutive dust pulses? This comes out of the blue. Please relate to the natural dynamics of dust events. Put them into the frame of the time of the year the experiment was carried out. Also state your hypothesis in terms of what is to be tested with the second amendment after the first amendment theoretically has alleviated macronutrient limitation of bacteria.

RESPONSE: The reasoning behind the two consecutive dust pulses is described in the Introduction article of this Special Issue by Guieu et al. (2014). The strategy of two successive seedings was decided following DUNE-1 results and comes from the evidence of 'post-depositional' processes influencing biogeochemical conditions and, in turn, the impact of successive pulses. For instance, Wagener et al. (2010) showed that dust addition during DUNE-1-P was followed by a decrease of DFe concentration likely due to DFe scavenging on settling dust particles, giving evidence that large dust deposition events may be a sink for surface ocean dissolved iron. Combining the mesocosm experiment with a batch dissolution experiment, Wagener et al. (2010) then showed that after biological activity was enhanced following dust addition and Fe-binding ligands have been produced, then DFe increased. Although two successive deposition events of this importance have, to our knowledge, not been reported for the Mediterranean region, this strategy was planned in order to explore how dust deposition may impact biogeochemistry under different in situ biogeochemical initial conditions. The goal was, therefore, to get deeper knowledge on such biogeochemical process rather than to reproduce natural conditions. In the revised MS., we have added a short justification and a reference to the Guieu et al. (2014) introductory paper.

p 342, line 11. 10 g m- 2 is a large addition of dust. 20 g m-2 (two additions) is a very large flux that occurs in the area tested only through wet deposition and on rare occasions with this magnitude. Please put in context.

RESPONSE: Please, see comment above.

p 343, lines 11- 12. There is no indication in Lebaron et al. (2001) of using formalde-

hyde as fixative. To my knowledge the standard fixative is a mixture of paraformaldehyde and glutaraldehyde (P+G). Please give correct reference for your methods. Also, samples need to be quick frozen in liquid N (-186 °C), before long term storage at -80. Please explain the use of these alternative methodologies and the goodness of the fixation and storage method.

RESPONSE: The information on the fixative is provided in Lebaron et al (2001), on p. 1776, in the Material & Methods Section Âń Leucine incorporation followed by cell sorting by flow cytometryÂż: "... Incubation was stopped by adding formaldehyde (final concentration, 2%) ...". We cited this publication as our reference, because the protocol we applied in the present study was developed in the same lab, and used in several published studies. The reviewer points out an important step in the fixation of the samples that is the quick freezing in liquid nitrogen. In the present study, all samples for flow cytometric analyses were indeed quick-frozen. We have added this information where it was missing.

p343, lines 26-27. No mention of quick-freezing in liquid-N is made. Christaki et al (2011), explicitely recommends NOT freezing directly at -80. So again the reference is wrong.

RESPONSE: As stated above, according to the protocol described in Christaki et al (2011), the samples for HNF abundance were flash-frozen in liquid nitrogen. The text has been changed accordingly.

p344, lines 4-5. The volume of water and the flow speed is important, but this says nothing about the number of cells of interest that were counted. HNFA were at the most 1000 ml-1. Since 1 ml was analyzed, and the vast majority of particles in 1 ml are NOT HNFA, I would like to know how many hits did actually correspond to HNFA and not to other particles. In epifluorescence microcopy, 200-300 HNFA have to be counted per sample.

RESPONSE: 200-1000 cells/ml in situ The HNFA abundances are based on flow cy-

tometric events ranging between ≈ 600 to 1000. If 10 min of analyses (or 1 ml) were not sufficient to obtain this number of events, the time of analysis (and volume) was extended to up to 30min (or 3ml). This is now clarified in the text.

p344, lines 6-8. Here, for viruses the authors did deep-freeze in liquid nitrogen. Why did they not do it for the other FC samples when it was prescribed in the methods they mention?

RESPONSE: As described above, all samples for flow cytometric analyses were quick-frozen in liquid nitrogen.

p344, lines 19-20. Was temperature held constant? Tap water can be cold in summer depending on the reservoir it is coming from.

RESPONSE: Unfortunately, we had no way to keep the temperature strictly constant. However, since BR measurements were compared between treatments (control vs. dust) the important point here is that samples from both treatments were incubated at identical temperature conditions.

p346, section 2.6 statistical analyses. The second seeding ("D2") can not be compared to C, since it is not its control. It could be compared to the first seeding ("D1") in terms of "before" and "after" the second seedding. The ideal would have been to have 2 C, 2 D1, 2 (D1 + D2). That is, 4 mesocosms D where a first addition was given, and then use two of them for the second addition so that the D1+D2 would have a control (D1) to be compared with. Right now we can not know how much of the effect of D2 is owing to a preconditioning from D1 and how much to a preconditioning of the enclosure effect. The authors assume that C at time 7 days is identical to C at time 0 and this is clearly not true since there was a dynamical response in the C containers from time 0 on.

RESPONSE: We agree with the first part of this comment. The C mesocoms can not strictly be considered as a control for the D2 experiment (second addition of dust) and mesocosms with one addition of dust (No addition after the D1 experiment) should have

been used ideally to have a Âń control Âż for the D2 experiment. The reviewer propose an experimental design which would have been 2 C, 2 D1, 2 (D1 + D2). However, we know from the experience DUNE-1 and DUNE-2 that triplicates are really necessary to make a robust interpretation of the results. The ideal experimental design would thus have been 9 mesocoms deployed (3 C, 3 D1, 3 (D1 + D2)). For logistical and financial reasons, this was not possible and we have therefore opted for the present experimental design.

This said, we do not agree with the reviewer on the fact that we assume that C at time 7 days is identical to C at time 0, because we always compare between the D and the C mesocoms at the same time. For example at R8 we compare between D and C 143 hours after the first dust addition and for R16 we compare between D and C 311 hours after the first addition. We take into account the evolution in the C mesocosms. We can therefore say that during D1 the comparison between C and D gives information on the effect of the first seeding and during D2 the comparison between C and D, gives information on the effect of the combination of the two seedings. In the revised manuscript, new explanations have been added to better explain the interpretation of the results of the two dust additions.

p348. 3.2 Bacterial abundance and respiration It seems that after the second dust addition one D replicate went balistic and hence the high variability in many of the analyzed parameters. The main reason researchers that use 3 replicates in mesocosm studies give is that if one replicate differs from the other 2, it can be eliminated from the study. I wonder why this is not done here. It may be necessary to see the individual mesocosm data instead of the aggregated data.

RESPONSE: This issue was a topic of discussion within the project team. We finally decided to average the data from all three mesocosms in an attempt to account for inter-mesocosm variability possibly reflecting differences in the enclosed water masses. We still prefer not to eliminate the data from D3 but we could show individual mesocosm data as supplementary information if the referee and the editor consider

it crucial.

p 351, l 7. Why were data from D3 not eliminated after the second seeding? RESPONSE: Please, see comment above.

p 351, line 12 -13. "Assuming a 100% dissolution of nitrogen (which was artificially added as part of the atmospheric transport simulation process)" I do not understand. Was nitrate artificially added? in addition to the N leached from the dust seeding? Please clarify as this is a crucial aspect.

RESPONSE: We apologize for this confusing sentence and we have rephrased it in the revised MS. Briefly, the dust used for the DUNE-R experiment was processed to reproduce chemical aging by cloud water processing. Dust was mixed with acidic model cloud water and evaporated in a clean room (Guieu et al., 2010b). Sulfuric, nitric and oxalic acids were used to simulate air masses under anthropogenic influence that encounter Saharan air masses in frontal situations typical of dust transport over the Mediterranean (Moulin et al., 1998). Dust processing with model cloud water resulted in a significant addition of N compared to initial dust (from âĹij0.1 to âĹij1.4 %) (Guieu et al., 2010b). Dissolution experiments performed under abiotic conditions showed a percentage of nitrate dissolution from dust of 100% (Ridame et al. 2014, this issue).

p351, l 16 "D-mesocosms"

RESPONSE: Corrected

352. 4.2 Pronounced response of bacterial respiration. In this section I am missing important scientific contributions found in the previously mentioned Sala et al. 2002, and also from Lekunberri et al. 2010, Romero et al. 2011. Coastal Mediterranean plankton stimulation dynamics through a dust storm event: An experimental simulation. Estuarine, Coastal and Shelf Science. 93: 27-39.

RESPONSE: Lekunberri et al. 2010 is already cited in this section and at several moments in the manuscript and the study by Sala et al. 2002 has been added to this

section. Romero et al. 2011 is now cited in the introduction but not in this particular section which focuses on the impact of dust on respiration rates.

p352, line 16. Posidonia oceanica.

RESPONSE: Corrected.

p352, line 20-22. The C:N:P ratios for bacteria are questionable. The original papers that are mentioned extensively used enrichment before assessment of C, N, and P content. Most probably this changes elemental stoichiometry. Some analyses were even made in formalin fixed samples!!! In any case, the data in those papers have very large error bars and when marine bacteria are looked at, especially native (even if enriched) they encompass the Redfield ratio, even though it is true that means point towards lower C:N and C:P ratios. But measured C:N for those bacteria in Fagerbakke go as high as 16.7 and for C:P as high as 431. Beware that the data presented in table 4 of Fagerbakke et al. 1996 is in wt:wt not mol:mol. But more importantly, bulk DOC tells us little about the bioavaiabillity of C. Much of the carbon should be little labile or plainly recalcitrant, and as such bacteria may respond to its presence very slowly. Thus, I would certainly not rule out C limitation of bacteria, which you invoke indirectly later on in the section.

RESPONSE: We agree with this comment and we have removed the corresponding paragraph in the revised manuscript. As correctly pointed out by the reviewer, in the following paragraph we evoke the possibility of a stimulation of bacterial activity by phytoplankton release of labile DOM.

p 354, line 21-23. But why would dust stimulate lysis only after the second seeding?

RESPONSE: As mentioned in the submitted version of the ms (P 355, lines 17 – 18), “the lack of response of the virus community to the first seeding remains puzzling”. We have as yet no explanation for this observation. We did notice a steep decrease in virus abundance in all mesocosms 2 days after the first amendment, which co-occurred with

a transient stratification of the water column. It would be tempting relate these events with the lack of virus production. However such speculative statement would need to be back up with actual rates of virus activities (lysis, lysogeny) or at least previous studies focused on such relationships. We have thus decided to limit the discussion on this topic. Nonetheless, the observed diversity of virus responses to dust amendment points out the need to accurately assess virus activities in future studies.

p 355, lines 11-20. Here the authors seem to be confused about terminology. Lysogeny is when the phages integrate into the DNA of their host and replicate with their host without bursting and producing viral particles (lytic cycle).

RESPONSE: As mentioned in the ms, phages typically replicate through a lytic cycle (they induce host cell lysis upon the release of viral progeny) or a lysogenic cycle (they integrate into their host genome as a provirus or prophage in the case of bacteriophage). The lysogenic bacteria are however "inducible", that is the prophage remains dormant and replicates along with the host until a lytic cycle is activated (or induced) by an "induction" event such as a metabolic change. We have slightly reworded our paragraph to clarify these terminology issues.

362, Table 1. If one considers a C: Chla of 50 (wt:wt). Then you have 0.08 μg chla L-1 d-1. This is a Chla turnover of ca 1 d-1. Isn't that something to think about?

RESPONSE: We agree that a Chla turnover of 1 is a high value for an oligotrophic oceanic system. We suspect the assumed C:Chla ratio of 50 to be responsible of this apparent inconsistency. Indeed, at the beginning of the experiment, particulate organic carbon was measured and the PP:PC ration leads to a Chla turnover ratio of 0.1, a typical value for oligotrophic systems.

363, Fig. 1. D mesocosms after the second addition show a tremedous variability. Could one replicate be eliminated?

RESPONSE: Please, see response to comment to "p348. 3.2 Bacterial abundance

and respiration".

Also the plots for the different depths are really not very informative and could be omitted. I would leave the 5 m data because this is the only depth for some of the parameters. And then perhaps give a vertically intergrated value from the 3 depths (probably in a table).

RESPONSE: The differences between C-meso and D-meso for most studied parameters are not equal for the different depths. For this reason we prefer to keep the plots for the three depths.

369. Fig 7. Ratios of virus to bacteria are much lower OUT than in the mesocosms. On the contrary HNF are higher OUT than in the mesocoms. These seem to be enclosure effects, and I do not recall discussing them. Probably in mesocoms top microbial predators are limited and HNF much more controlled by larger flagellates and ciliates. I would like to see all these issues addressed.

RESPONSE: We partially agree with this comment. It is true that enclosure effects may explain the difference between virus:bacteria ratios and HNF inside and outside the mesocosms, and we have added a sentence on this issue in the revised MS. However, we think that the mesocosm approach constitutes a step forward compared to the classical microcosm incubation techniques and it allow extracting valuable patterns of response to the dust addition.

Interactive comment on Biogeosciences Discuss., 11, 337, 2014.