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

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 writen 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.

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.

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?

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

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.

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.

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

p 343, 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.

p 344, 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.

p 344, 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?

p 344, lines 19-20. Was temperature held constant? Tap water can be cold in summer depending on the reservoir it is coming from.

p 346, 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.

p 348. 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.

p 351, l 7. Why were data from D3 not eliminated after the second seeding?

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.

p 351, l 16 "D-mesocosms"

p 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.

p 352, line 16. *Posidonia **o**ceanica*.

p 352, 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.

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

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).

p 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?

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

p 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.