

**Review of paper by Dinasquet et al. entitled “Impact of dust addition on the microbial food web under present and future conditions of pH and temperature” (MS No.: bg-2021-143).**

Dinasquet et al. studied if and how microbial populations may be affected by dust deposition under present and future (warming and acidification) environmental conditions in 3 basins at the Mediterranean Sea during early summer. This work is part of a much bigger project (PEACETIME).

Results suggest that dust amendments changed the microbial ecosystem from being bottom-up limited to a top-down controlled. These changes are likely attributed to induced viral lysogeny rather than grazing. The authors also suggest that the degree of response of the microbial populations will depend on the initial biogeochemical conditions of the receiving environment. Please see below my comments and suggestions:

1. The focus of the manuscript should go a deep revision to highlight its novelty (that is, viral production and lifestyle aspects following dust amendments) rather than basically repeat the Gazeau et al., work and add on that some other measurements. Currently, this paper is hard to follow without looking at the data provided in Gazeau et al. While I did not read the Gazeau et al., paper (as it is currently under review as I understand it...), from reading its title and the rationale provided in lines 311-316 that explains the differences between the studies, I do not really understand the added value of this paper. Thus, the authors should focus more on the viral production and lifestyle aspects which are the most novel, and ignore the rest altogether (which is presented elsewhere). If so, the whole manuscript should be revised accordingly.
2. Seems that much of the results needed to understand what's going on following dust/temperature/pH alterations are, in fact, presented elsewhere (i.e., Gazeau et al.). For example, the temporal dynamics found in microbial variables in the different treatments are not presented at all, but only the change from the control (as  $\Delta$ ) in t24 h in Fig.1 (although measurements at t1, t6, t12, t48, t72h were made). And yet, the authors also discuss other time-points, without showing the data at all (many places throughout). This makes it very difficult to assess what

happened in the different minocosms. Contrary, the relative abundance of viral populations is presented and discussed based on the initial vs. t12 h... Please be consistent and present the whole dataset. The way it is presented now is very misleading. Moreover, from reading the discussion I understand that the dust-borne nutrients were measured (possibly also trace-metals, e.g., lines 307-308), however this data is not presented and thus it is hard to see if the changes were triggered by the added 'goods' or by the temperature+pH alterations in treatment G.

Thus, the authors need to show, even in the supporting information, the temporal changes in *Synechococcus*, heterotrophic bacteria, VLP, HNF, BP, nutrients... all the collected data in all time-points. This could be either added as an excel file or as graphs. Otherwise, it is very difficult to comprehend what happened following dust and/or temperature+pH manipulations.

3. The abstract should be revised to better explain what was done, and what were the main results/outcomes. Currently it is very ambiguous and the 'take home message' is unclear. For example, it is unclear which additions (dust or soils? trace-metals/nutrients as imply in Line 35...? etc.) and manipulations (by how much temperature increased? ditto pH) were made. Moreover, the results are vaguely presented (e.g., "mixotrophs were altered", "...Different responses to dust were observed rapidly after addition..." – it's basically says nothing without putting some numbers or more 'direct' explanations... the results suggest that the responses depend on the initial microbial assemblage and metabolic state of the tested water" – how? etc. Were there any differences in responses between basins? All of this should go into a revised abstract.
4. During atmosphere transport the dust particles are typically acidified, which increases micronutrients availability upon deposition in seawater (e.g., Krom et al., 2016). Contrary, in this study, a 'dust analog' was used for the additions rather than dust that passed these atmospheric processes (as in previous studies, e.g., DUNE, Guieu et al., 2010). Therefore, comparing treatment G with treatment D may not be straightforward. Were the same micro- and macronutrients levels were found

between D and G (as the same amount of dust was added in both)? The authors need to discuss this and show the data.

5. In reality, the changes in temperature and pH are gradual and slow (decades), namely they do not occur at once as tested here (minutes to a few hours). Thus, the experimental setup used do not 'allow' microbes to acclimate to these changes, contrary to the 'real world'. I'm wondering how much the results represent the future oceans and the Med Sea. Please discuss this caveat in climate-change studies – this is especially important for bacteria which have faster growth rates than large animals etc.
6. Growth rates and mortality – the authors used an approach with many caveats, uncertainties and uses many assumptions. For example, why do you assume the cells were in exponential phase of growth (Line 115)? Given that marine microbes grow relatively slowly (see review by Kirchman 2016), how can you assume the cells were in exponential growth after only a few hours/day? Moreover, BP is only part of the cellular carbon needs/demand for heterotrophic bacterial cell. I suggest you calculate the bacterial carbon demand, BCD (BP+BP) assuming respiration is ~10% of the total carbon requirements (or alternatively of someone measured respiration that would be ideal), and thus the bacterial growth efficiency (BP/BCD). This may give you a more accurate estimate for heterotrophic growth than just relying on BP.
7. Moreover, the net growth rates were calculated based on three successive sampling points (lines 114-116), but the sampling times were not linear meaning that some points were close to one another (0-1h, 1-6h...) whereas some are daily (24-48 h, 48-72 h...). Were the same time-points used in all treatments for the growth rates and mortality calculations? Are these rates comparable to other reports from LNLC regions?

8. Some of the methods used should be better described. For example, no information is given on how pico-phytoplankton and heterotrophic bacteria were fixed, processed, which flow cytometer was used, which stain was used for the prokaryote's enumeration (this in contrast to the viruses...). Similarly, how did you measure mitomycin C concentration?
9. The authors concluded that the initial biogeochemical conditions of the receiving environment (based on oligotrophy? Microbial populations?) are important in understanding the responses of the microbial populations to dust deposition (nowadays and in the future). However, I am not convinced it can be deduced based on only 3 stations (rather than across a nutrient or chlorophyll-a gradient etc.).
10. The conclusion section is a repetition of the discussion and/or refer to other studies from PEACETIME and does not really add much.

Additional comments

Line 24	Dust deposition may also have anthropogenic components ('European dust', e.g., Tsagaraki et al., 2017 FMS).
Lines 24-25	There are numerous studies dealing with the influence of dust deposition on microbial processes and community composition, including from the Mediterranean Sea (many of them by the co-authors).
Lines 27, 491, 501	Wet dust deposition sounds like rain mixed with dust. Is that what the authors meant? What is the difference? Is this a technical issue result from the soil's aging (as in Guieu et al., 2010)? Please explain this in more details in the M&M.
Line 35	It reads like trace metals and nutrients were also manipulated... please revise.
Lines 37-38	How were the mixotrophic community altered? This is a vague description of the results.
Lines 33-35	An ambiguous sentence.
Lines 38-40	"Overall, these results suggest that the effect of dust deposition on the microbial loop is dependent on the initial microbial assemblage and metabolic state of the tested water" – How? Unclear.
Lines 48-51	A very long sentence. Moreover, BP is relatively high (to primary production) in oligotrophic environments such as the Med Sea during summertime. I suggest rephrasing this part.

Line 52	What does it mean "...to this microbial ecosystem..."? Is there other microbial ecosystem in the oceans?
Lines 53-55	The word 'degree' appears twice in the same sentence.
Line 56	If I remember correctly, Ridame's paper showed that dust does not always stimulate N <sub>2</sub> fixation (depending on the basin, incubation time, amount added, etc.).
Lines 66-68	The fact that dust events will become more prominent in the future does not necessarily mean that microbial food web might become more dependent on atmospheric deposition of nutrients. You need to better connect with the previous sentence saying that LNLC regions are expanding... enhanced stratification... Currently it reads weird and the flow is not sound.
Line 77	microbial growth and controls (remove the comma).
Line 85	Remove the question mark after "Pourquoi Pas".
Line 95	How much dust was added eventually (in mg/L)?
Line 111	Define HB.
Lines 114-118	Please back up this approach by citing other studies who used it. To me, this approach has many caveats and uses many assumptions that must be discussed.
Lines 139-140	How were the samples preserved before they were run? Did you have an onboard flow cytometer (and thus preservation may not be required)?
Line 143	How mitomycin C was measured?
Line 150	Please give the BS number you used based on the paper cited.
Line 192	Figure 1 does not show the t <sub>72h</sub> time-point.
Lines 192-193	Were these changes significant?
Lines 195-196	"Heterotrophic bacterial mortality was also higher than in C..." – Which treatment/s? Unclear.
Lines 208-209	You cannot establish a gradient based on 3 points.
Line 217 and/or discussion	Please explain what Girus is, and define its size (how was it done FSC?)
Lines 222-225	This is basically true for all variables tested, not only HB viruses' production and life strategy.
Lines 238-241	Which time-point/s? t <sub>24</sub> ?
Line 293	Please explain how May to June are considered 'late spring' (oceanographically-wise) in the Mediterranean area.
Lines 299-300	Bosc et al (line 299) show satellite data and do not present any nutrients data. Ditto D'Ortenzio – it does not present nutrients data but mainly discuss the thermal stability of the water upper column in the Med Sea. Thus, both citations are inappropriate.
Line 301	Define PP.

Lines 307-308	Please show this data. Also – were dust leaching experiments done? If so, how did it differ relative to the values measured in the minicosms?
Lines 308-310	Following what? D or G amendments? Also, which changes? What are you referring to?
Lines 396-397	How do you know? Did you run HPLC analyses and looked for <i>E. huxleyi</i> pigment markers?
Discussion in subsection 4.2	Suggested paper to consider – Sharoni et al., (2015). Infection of phytoplankton by aerosolized marine viruses. PNAS. doi/10.1073/pnas.1423667112
Line 443	The Rahav et al paper is not about dust-borne metals toxicity (unlike Paytan et al., 2009), but on airborne viruses delivered with dust and affect cyanobacterial populations. In fact, this paper should also be considered in subsection 4.2 and/or in lines 461-463.