

***Interactive comment on “Determining the hierarchical order by which the variables of sampling season, dust outbreaks occurrence, and sampling location, can shape the airborne bacterial communities in the Mediterranean basin” by Riccardo Rosselli et al.***

**Anonymous Referee #4**

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General Remarks: The authors present the results of an interesting experiment, sampling airborne microbial communities at two locations, Sassari and Cagliari, located at the opposite ends of the Mediterranean island of Sardinia. The first facing Europe in the north, the second facing north Africa, in the south. The study of airborne microbial communities it is of great interested nowadays as the roil of air dispersal in determining the biogeography of micro-organisms is slowly being discovered. The authors collected samples in two different seasons, at the two different locations and considering differ-

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ent dust events/wind direction shifts and compared the results of these variables on the sampled airborne microbial community through the use of amplicon sequencing. The idea of using these two locations is very interesting, since it can clearly capture the airmasses coming from two different continents with less interference. The results show interesting patterns, defining the season as the most important variable in defining alpha and beta diversity differences among the samples. Moreover, the location, although less clearly, seem also to influence combined with the timing of the samples used as control (before and after the dust event). In general, I think the experiment and the results are interesting but I would advise to authors to review certain sections before publication.

Specific comments: 1)The manuscript presents itself with a title that seems way more inclusive, as “Determining the hierarchical order by which the variables of sampling season, dust outbreaks occurrence, and sampling location, can shape the airborne bacterial communities in the Mediterranean basin” points to a much larger study, where the whole Mediterranean basin (and not only the island of Sardinia) is considered, and more observations and samples are available. In this case the experiment is quite small, only having two seasons, and one year time span, with only two locations, and it should be rather presented as a case study, since one cannot draw any certain conclusions from these observations, but only hypotheses. This is not meant in diminishing the value of the study since sampling airborne microbial communities presents technical difficulties that make the design of bigger studies quite challenging. Nevertheless, a better title should be considered.

2) The experimental design is not really clear until the results section. A table or a schematic of the design should be included at the beginning of the material and methods section.

3) The result section seems very messy and hard to read. First the authors introduce the readers to the microbial composition: From line 259 to 309 the authors make a great efforts in describing the different microbial taxa that compose the samples. Nev-

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ertheless, it is hard to find the logic the authors followed to write this section. Such analysis should start from a clear graph showing the different phyla composing the different samples. The graph provided (figure 3) is extremely hard to read. From there you could enter specific phyla that you want to further comment. Then the authors present a method to identify the taxa that form a common core in the samples. The method they use, in my opinion, is not clearly explained: Line 319 to 321: “the criterion was to set a cutoff value with respect to the percent of variation occurring between the first 12 h of the collection time and the second half of it. Only the taxa which displayed a mean variation higher than  $\frac{1}{2}$  of the corresponding standard deviation were considered” So why variation WITHIN the dust event should provide this information? Shouldn't it be better to just analyze the OTUs found in all samples? Not clear why this method was used. Generally, the results section is hard to follow and confusing and needs a deep restructuring. Further comments below in the technical corrections.

4)The discussion part is well organized. It very well explains the hypotheses of our authors to explain the results observed in relationship to the geographical and meteorological conditions. The authors could nevertheless improve the ecological value of the discussion, better linking previous experiments and observations done with microbial dust dispersal. Moreover, the discussion is mainly based on the measurements of alpha and beta diversity but does not include much of taxonomical data. So, the authors fail to discuss the results explained in the results section (259 to 309, tab 4) Only few lines are dedicated to this purpose (554-556).

Technical corrections:

Line 52: Dispersal not Dispersion. Dispersal is the act itself; dispersion is the result of dispersal.

Line 63: Here it seems the topic changes suddenly. Entering deep into the microbiology. I would put the paragraph ending here and not in line 60

Line 182: OTU

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Figure 3: Replace it with a stacked barplot

Line 310 to 314. This might go up in the methods. Once again, a clear section of the methods describing the methodology is needed.

Line 315: OTUs or taxa? Along all the manuscript I had the feeling these words were not used consistently, please check.

Tab1: It is unclear to me what you are reporting here, please explain better.

Tab2: Are you reporting the number of orders? If it is just the count, that is a measure of richness, and not diversity. Also, are these results adding important information compared to the bray Curtis similarity table? It seems quite redundant and not helpful for the discussion, since the number of orders is not really helpful for any results. For quantifying community variations, Bray-Curtis is the right instrument, as used later.

Tab3: Simpson and Shannon are both diversity indexes, and the use of two of them does not add much information to the discussion. Why not using richness instead of Simpson?

Tab4: should be connected to the results at line 259 to 309. But it is not.

Figure 5A, the PCoA does not add any info to the study. Moreover, the dissimilarity matrix used for the PCoA should be reported.

Tab5: This is hard to read. I suggest a heatmap style table.

565: How was it verified? You would need additional controls after and before the dust events.

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