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Interactive comment on "Microbial functional signature in the atmospheric boundary layer" *by* Romie Tignat-Perrier et al.

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We thank the Referee 1 for reviewing our paper and for the constructive comments that, we think, contributed to make the paper much more comprehensible especially the methodology.

-Comment 1: "The authors give a method description for qPCR (starting L133) on air filter samples but the results for the air samples appear to be already published somewhere else as stated in L242/243 and caption of Table S4. Table S4 gives also some qPCR results for soil samples. Where are these data from? Can the authors provide details in the method section about collecting/extracting/qPCR for the soil samples or a reference? To which air samples/location do these soil samples belong? Were they

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taken at the same time/location as air sampling was done? It is also not clear to which unit (L, m3, gram?) the gene copy numbers given in Table S4 refer and how the authors calculated the cell concentrations as stated in L133, L146, L243/244 and where these values can be found. The authors refer in L243 to Table S4 for cell concentration ratios, but this table only includes gene copy numbers and their ratios. Overall, the motivation for the qPCR seems not clear in terms of the purpose of the study. The qPCR is not mentioned in the abstract or introduction/motivation and the discussion is confusing as the term "gene copy numbers" seems to be used also as "cell concentrations". Given the multicopy nature of ribosomal genes and different copy numbers in different organisms this should be corrected in the text."

Answer: Some qPCR results are actually already presented in our previous paper (Tignat-Perrier et al., 2019). Instead of giving all the qPCR methodology again, we modified the text and referenced the previous paper for method details (line 138). We made it clear in the Material and Methods section that the gene copy numbers were used as an approximation of the cell concentrations and added a reference, Louca et al., 2018, that explains why attempts to correct for metagenomic datasets are unproductive (while we agree of the multiple nature of ribosomal genes and different numbers in different organisms) (line 141). We agree that we forgot to give information on the soil samples and we gave details in the Material and Methods section and result section accordingly (line 129, line 138, line 225). The qPCR results on the soil samples, even partial, were used in comparison to the results obtained from the metagenomic data to see if they show similar results and/or the same trend. The soil is the Côte Saint André agricultural soil (France) that we also used for the metagenomic analyses. We agree that it would have been better to have samples from the surrounding landscape at each of our air sites, and we think that a study would be interesting to do to confirm our results and support the new conceptual model we proposed (Fig 6). We modified the text (line 129, line 138, line 225, line 453).

-Comment 2: "The bioinformatic analysis appears to be focused only on fungi and

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bacteria, but there are also other microbial organisms such as e.g., Protozoa, Archaea, Algae in the atmosphere. Can the authors add information about the numbers of non-fungal and non-bacterial reads and explain how and why they were excluded although they are/many of them are microorganisms (see title!). Overall, it might be straighter to separate fungi and bacteria in all figures as they belong to different domains of life. For example, Fig 4a and Fig 5a seem not to provide any additional value to panles b and c, if panels a include only fungi plus bacteria but no other mircoorganisms. Âż

Answer: We agree that non-fungal eukaryotic organisms are certainly present in air and we think that looking at them would be very interesting. Still, our sampling method is not suitable to collect these bigger microorganisms. We used an impaction technique that mostly collected particulate matter whose diameter is inferior to 10 μm. We checked our data for non-fungal eukaryotic reads and they actually seem really low. As an example, a typical puy de Dôme sample (site relatively vegetated) showed 11900 over 12400 annotated sequences belonging to the Fungi reign. Non-fungal eukaryotic sequences and archaeal sequences were very low. We think that the panel showing bacterial and fungal sequences altogether ((a) panel) is still informative. The ratio between fungal and bacterial sequences is specifically high in air and this ratio drives the stress functions based results as shown when comparing the (a) panel including all sequences and the (b) and (c) panels including the fungal and bacterial sequences separately. Considering together bacterial and fungal reads is common place in metagenomic studies while it might be important to separate them, as evidenced here.

-Comment 3: “How can the data be normalized to 10000 sequences (L225) when the filtering cut-off was 6000 sequences (L173) before?”

Answer: We agree that it might have been not clear and we added information in the Material and Methods section in this regard (line 201). It was a deliberate choice to give the relative ratio of a specific function per 10000 sequences. It could have been per 100 sequences and we would have said that for example “0.2 % or 0.2 sequence

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over 100 given sequences is related to this specific function". Still 0.2 sequence means less than 1 sequence and thus sounds odd. Thus we chose per 10000 sequences to have sequence numbers above 1 (0.2 sequences per 100 sequences would be 200 sequences per 10000 sequences).

-Comment 4: "Fig2: The numbers in the figure are hard to read. The grey for the air samples appears not to be in the figure. The order seems to be by % of fungal and bacterial sequences. To me it seems more useful to display the different sample groups (air, soil, water,..) so that one might be able to see trends. Does this figure display all sequences for each of the samples/sample sets i.e., different total numbers of reads/sequences per sample/sample/set or is this figure based on rarefied sequences (6000, 2000)?"

Answer: We increased the height of the numbers so that they would be more readable. We do not think that displaying only the average percentage of bacterial and fungal reads per ecosystem is helpful as the difference between sites (especially within soil and air sites) could be very large (due to the fact that the sites are more or less vegetated, affecting the percentage of fungal reads). We think that displaying the average combined with the very large standard deviation would not have been meaningful. The percentages were calculated using all sequences and not rarefied sequences. We added information in order to made it clear in the Material and Methods section (line 201).

-Comment 5: "The authors selected specific stress-related functions with the purpose to identify a specific atmospheric functional potential signature. Stresses like e.g., UV, desiccation, however, are not limited to the atmosphere. Also soil bacteria and microorganisms living on e.g., plant or building surfaces are exposed to these stresses. This might help to explain that the authors did not find a specific signature with their selected genes in the airborne fraction."

Answer: We agree that some stress-related functions we chose like UV and desiccation

could also be stresses experienced by surface microorganisms (i.e. microorganisms found on plant leaves, on soil surface, on sea surface etc.) and this is what we tried to explain in the discussion section line 494 and line 526.

-Comment 6: “The authors state that the methane mono-oxygenase-related functional proteins per 10000 sequences were only detectable when considering all sequences. As all sequences are the sum of fungal and bacterial sequences I wonder why they can only detect it when they sum up the sequences. If they have sequences in the sum, they must have had them for fungi and/or bacteria before. Can the authors clarify? Âż

Answer: Firstly, we used all reads and functionally annotated them (as explained line 172 in the Material and Methods section). In this case, the majority of the reads was related to bacteria and fungi (because of our sampling method), although some reads might belong to other reigns (for example Archaea, Protista. . .) as you have highlighted it in a previous comment. Secondly, we tried to separate bacterial reads from fungal reads (using Kraken, FindFungi and specific complete genome-based databases – line 183), then functionally annotated them separately. Thus, when taking in consideration all sequences, it is not exactly the sum of the bacterial and fungal sequences. We hope that the text in the Material and Methods section is clear enough.

-Comment 7: “Please correct the statement that concentration of fungal spores and fungal hyphae fragments in air are unknown. For example, numbers of spore and hyphae concentration can be found in Després et al., 2012 and references therein. Després, V.R., Huffman, J.A., Burrows, S.M., Hoose, C., Safatov, A.S., Buryak, G., Fröhlich- Nowoisky, J., Elbert, W., Andreae, M.O., Pöschl, U., Jaenicke, R., 2012. Primary biological aerosol particles in the atmosphere: a review. Tellus B 64. http://dx.doi.org/10. 3402/tellusb.v64i0.15598 Âż

Answer: We corrected the statement and made it clear that some numbers of spores and hyphae were measured and could be found in Després et al., 2012. We specified that the number of hyphae and spores, and thus the ratio between the two, has never

been measured at the same site (line 457).

-Comment 8: “Table S1: site should be capitalized, abbreviations in first column should be explained; what is meant with “same hour”? There is no time information in this table.”

Answer: We changed the text accordingly. By “same hour”, we meant that the collection was stopped exactly the same hour as it started (i.e. the sampling lasted exactly 7 days – even if the hour was not given because we thought it was not useful), but we removed this as it was not clear.

-Comment 9: “Figure S1: This is a nice figure, but is only mentioned once and it seems not be used for discussion in the text. I suggest to consider this figure when discussing Fig.3, as it supports the results shown in Fig 3.”

Answer: We added a reference to Fig S1 when discussing Fig 3 (line 417).

-Comment 10: “Figure S2: This figure is very hard to read as a lot of text overlaps”

Answer: We agree that text overlaps and that not all the text is readable. We added this Fig S2 in SI so that readers can have indications on where the sites are situated on the multivariate analysis (as the main Fig 3 shows only the colors and not the text), even if we know that all the text could not be readable. We added colors (based on the “ecosystem”) to make the reading easier in the case that the site names (i.e. the text) is not what interests the reader.

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