Biogeosciences Discuss.,
https://doi.org/10.5194/bg-2020-236-AC2, 2020

Biogeosciences Discussions

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

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Received and published: 1 October 2020

We thank the Referee 2 for reviewing our paper and the constructive comments.

We agree that it would have been very interesting to have samples from the ecosystems that directly underlie the air sampling. Our study is a preliminary comparative metagenomic study that gave a first insight on the microbial functional genes present in air compared to the ones commonly found in other environments, and we think that the next large-scale metagenomic study should include air samples as well as samples from the direct underlying environments (especially if we consider that short-range transport of microorganisms is more likely than long-range transport in air; Tignat-Perrier et al., 2019). This sampling, that should also include "simple" sites that are

surrounded by one type of environment over a long distance (such as a desert site or polar site), would provide interesting information on the aerosolization process (i.e. microbial populations that would be more likely aerosolized and thus found in air compared to others). Here, the non-air environmental samples were chosen on different public databases based on their ecosystem type and diversity within an ecosystem type (i.e. different forest ecosystems, different seas etc.) as well as based on the sequencing technique used (i.e. we wanted metagenomic datasets made from the Miseq, HiSeq and 454 technology). We downloaded the free-access datasets (database and reference number in the Table S2) and analyzed the fastq files in the same pipeline as our fastq files.

We agree that looking at non-fungal eukaryotic microorganisms in air 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, in France) showed 11900 over 12400 annotated sequences belonging to the Fungi reign. Non-fungal eukaryotic sequences, as well as archaeal sequences were very low. Our study evidenced that fungi could be an important part of airborne microorganisms (quantitatively, compared to bacteria), and that is why our paper mostly focused on fungi.

Contamination in aerobiology is very hard to prevent but still, like the Referee 2 observed, very critical to control. Our sampling strategy, i.e. sampling a very large volume of air per sample using a high volume air sampler, made contamination effect, if existing, much less significant on our samples and allowed us to get a microbial biomass suitable for molecular analyses. The contamination level of our air sampling was accessed in Dommergue et al., 2019. The large air sampling done at different sites around the world allowed us to collect large samples on which we cut sub-samples to do chemical analyses (Dommergue et al., 2019 and Tignat-Perrier et al., 2019 and

2020) and analyses on the DNA such as qPCR and amplicon sequencing (16S rRNA gene and ITS; Tignat-Perrier et al., 2019 and 2020) as well as metagenomic sequencing (this paper). Controls (filters just put in the pump without functioning and transport filters that were transported in an aluminium fold and a plastic bag at the same time as the real samples but never opened) were collected and were processed at the same time as the real samples for the organic carbon concentration, qPCR and amplicon sequencing analyses. In the methodological paper (Dommergue et al., 2019), we gave results on the processing of the controls (organic carbon concentration and 16S rRNA gene qPCR) and identified the potential contamination in our air sampling. Only for the Antarctica site, the real samples showed DNA concentration so low (not detectable) that they could not be differentiated from the controls, and we decided to remove the Antarctica dataset altogether. For all sites, we verified that the DNA concentration on the controls was not detectable, and that the 16S rRNA gene qPCR and amplicon sequencing gave little and low-quality sequences compared to the corresponding samples, respectively. From Dommergue et al., 2019 we can read: "Except for the polar sites and CHC, the concentration of 16S rRNA gene copies in blank samples were < 0.3% that in the corresponding atmospheric samples. The blanks at CHC were up to 7% of the average number of copies in the atmospheric samples, due to the low concentrations of DNA sampled from air at this high altitude site. At both polar sites (DMC and Villum) the 16S rRNA gene concentrations were similar to controls, indicating very low biomass."

qPCR results based on our air samples are actually already presented in our previous paper (Tignat-Perrier et al., 2019) and in this paper we used only some qPCR results given as examples. Instead of giving all the qPCR methodology again, we modified the text and referenced the previous paper for method details (line 138). We agree that we forgot to give information on the soil samples (on which we did also qPCR analysis) and we gave details in the Material and Methods section and result section accordingly (line 129, line 138, line 225). The soil is the Côte Saint André agricultural soil (France) that we also used for the metagenomic analyses. Here, the partial qPCR

results were used in comparison to the results obtained from the metagenomic data to see if they show similar results and/or the same trend (especially regarding the ratio between fungi and bacteria). We agree that it would have been better to have samples (soil, sea samples etc.) from the surrounding landscape at each of our air sites (and do qPCR analysis but also metagenomic sequencing), and we think that a future study would be interesting to do to confirm our results and support the new conceptual model we proposed (Fig 6). We modified the text to give more information on the partial qPCR analysis and its purpose (line 129, line 138, line 225, line 453).

We agree that a few SI figures are difficult to read, especially the Fig S2. In the Fig S2 some text overlaps and 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 type) to make the reading easier in the case that the site names (i.e. the text) is not what interests the reader. For the SI tables, we agree that they are large tables with lot of text, but we tried to do everything to make them readable.

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