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## ***Interactive comment on* “Transport and characterization of ambient biological aerosol near Laurel, MD” by J. L. Santarpia et al.**

### **Anonymous Referee #2**

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#### General remarks

In the presented paper “Transport and characterization of ambient biological aerosol near Laurel, MD”, Santarpia and colleagues studied bacteria and fungi in different aerosol samples. Next to the analysis of 16S rRNA genes with Affymetrix PhyloChips, they did biochemical tests with culturable bacteria and included back-trajectory calculations in their interpretation.

Overall this study is placed in a scientific field, which has increasing research activity and still many open questions. The attempt to study especially bacterial bioaerosols has been made several times but mainly based on microscopic analysis, cultivation, and sequence analysis of 16S rRNA genes. In addition to this technology this study includes several biochemical tests which might enable a better characterization of the

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ambient bacteria. The study also attempts to include back-trajectory calculations in the analysis of their results, which is important and still not consequently done in other airborne particle research. However, although the aims of the study are well thought through, the results very interesting and the methods used promising, I see some general problems which should be addressed.

First, the title should be changed. In the title the authors promise the characterization and transport information of the “ambient biological aerosol”. In fact, the authors characterize culturable bacteria very well and culturable fungi very little. Unculturable bacteria are not “characterized” but “identified” through the 16S analysis but all other bioaerosols like unculturable fungi, plant and animal tissue fragments, archaea, protists, and viruses are not characterized or even discussed at all. Thus, the title is misleading. I also would not include the fungal analysis in the title, as the analysis of the fungi is very poor but restrict the title to the analysis of bacteria in air. The second major issue is the proclamation of the paper to interpret back-trajectories and thus get insights of the transport and source of biological particles. The authors also claim in their abstract that the analysis of their data with back-trajectories is one of their main results. However, I find that the analysis of the trajectories is done, but not very intensively. Information of the back-trajectory analysis is not given in the introduction and the results, but only in methods and in the summary and conclusion. The authors discuss possible sources of the detected bacteria from open water as they found Planctomycetes and Cyanobacteria. However, their analysis of back-trajectories only implies that in general the sampled air parcels traveled across water areas and were close to the ground. This information is in my opinion not informative enough to draw conclusions about the sources of bioaerosols or even give the promise to study the transport as done in the title of the study. To uptake bioaerosols into an air parcels, the air parcel must not only travel over a specific area and have the correct height, but there are also other parameters which are important and have not been discussed at all, such as the speed with which the air parcel traveled, the information if there was precipitation between the water location and the sampling site etc. Cyanobacteria and

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Planctomycetes also need not necessarily to originate from the ocean as interpreted but might also originate from other open water sources. If the authors have good reasons to believe that they are from an oceanic origin they should state that more clearly. Thus, I believe the authors should stick more to the actual results they have, which are very interesting and not promise highlights their data cannot hold.

Finally, one positive issue in this study is the intensive study of culturable bacteria with biochemical tests. The authors give information of their results in the results section. However, they miss to interpret these results in-depths and to combine the results from their phylogenetic analysis with the results from their biochemical analysis. I would encourage the authors to strengthen more the discussion on this topic.

#### Specific remarks

1. Although actual references are used, the authors should screen the literature again, because several publications from the recent biological aerosol analysis are missing.
2. The authors use the Affymetrix PhyloChip technology for the analysis of bacteria based on 16S rRNA genes. In the method section they do not give any method details but only refer to the publications from which they adapted their method. Although this is formally correct it would read much better, if some information like for the PCR would be given directly in the text, so that possible readers do not need to read DeSantis et al., 2003 and Brodie et al., 2007 parallel to the manuscript. Concerning the results of the PhyloChip the authors should make clear that in this attempt one only can find the organisms provided on the chip, other organisms cannot be identified, even if they occur in high numbers.
3. In the method section the authors explain their sampling strategy and their aim to examine short-term variation. As the authors only took 4 samples on 2 different days I believe from a statistical point of view there is no chance to draw any conclusion on short-term variation.

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4. The authors describe very detailed which media were used and how the analysis based on bacteria culture techniques was pursued. In a journal like Biogeosciences which addresses a broad readership not every reader is familiar with culture techniques, it would be very helpful if the authors explained in one sentence which Agar is used to identify which kind of organisms especially discriminating bacteria and fungi.

5. As mentioned before, the analysis of fungi and bacteria is promised in the abstract of the paper: “we show comparisons between the diversity of culturable bacteria and fungi”. In fact the authors do not mention the analysis of fungal cultures in the method section at all. In the results section they give on p 6732 in l 20ff the information “. . .growth on SDA, along with macroscopic and microscopic morphology consistent with fungal isolates, and were therefore classified as fungal and not further characterized”. Thus, the “diversity” of fungi was not studied at all or not described in the manuscript. The authors should either increase their work on fungi or promise less about the fungal analysis in the abstract.

6. In the results sections the authors claim to see an increase in biological diversity in the course of the 4 collection periods. Besides the fact that the authors can only interpret a possible diversity of “bacteria” and not general “biological particles”, this result cannot easily be followed, even with the given table. It would be easier to read if the authors could give some numbers, e.g. that in the 4th sample xy numbers of phyla were detected, while in the 3rd only xy were present. Or they could give this information in percentages, so that the reader can see if the “increase” in diversity is high (e.g. doubling) or only very little (a few percent) and thus not informative based on the number of samples analyzed. The authors discuss in-depth the possible impact of the weather in their results, which can be discussed obviously. Still, they should also discuss the possibility of just sampling variation, thus the variation in diversity observed might also just have a method reason.

7. On p 6735, l 17-18, the authors write that the observed bacterial diversity was “alarming”, is there any reason why this observation should be “alarming”? If yes, the

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authors should explain this, if not the authors should change to a less “drastically” word.

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