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Biogeosciences Discussions

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

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We have completed a thorough review of all of the referee comments and we would like to thank both referees for an obviously detailed review of our manuscript. Having considered the referee comments we would like to offer the following response to address all of the comments made both referees.

Referee 1 (C. Morris) had some general concerns about comparing the 16S rRNA Phylochip results and morphological and biochemical characterization. To our knowledge, there have been no previous attempts to make such a comparison. In principal, the thesis of the work was examining what relationships could be made between bacteria that were cultivable from an air sample and what molecular markers of bacteria could found using 16S rRNA microarrays. Many studies have used one or the other (as was

pointed out in the text), but no study has attempted to compare the two. There are several factors that prevent a more rigorous comparison of the 16S rRNA results with the results of cultivation. First, there may be multiple copies of 16S rRNA genes in a single organism that may not be homogeneous. This is the primary reason that no attempt was made identify organisms below the order level. Order is conservative in this case. Case et al., 2007 found that there could be significant confusion in establishing phylogeny at the species level and below. However, in preliminary studies for this work, we noted that freshly cultivated Yersinia rodeii colonies were identified in more than one OTU category that diverged at the order level (this data can be included as an appendix, if desired, in the final revision). Further, another concern that we have had with 16S rRNA analysis is that molecular signatures of this kind are not necessarily associated with a living organism. This is highlighted in our problems with background RNA in the refill water for the OMNI 3000. The water was sold as sterile by the manufacturer; however, numerous RNA signatures for bacteria existed in the water although no bacteria could be cultivated. Also, the quantity of nucleic acid recovered from the air samples was too small to be analyzed directly by the Phylochips, and was therefore amplified by PCR, as described in the text. This amplification, although necessary, introduces a potential bias into the samples if not every piece of nucleic acid is amplified equally. Finally, the survivability of bacteria in the aerosol collection equipment is difficult to determine. In preliminary studies, we found that the OMNI 3000 might kill a significant fraction of the fragile bacteria, but it killed less than other methods available to us at the time (such as dry filtration and impaction). This also introduces an unknown bias into the samples. For all these reasons, a broad characterization metric (gram stain) was chosen to attempt to compare these methods. There are significant limitations to this choice, but it has merit. Gram reaction is a phenotypic marker that is conserved fairly uniformly in bacterial phyla, and so makes a reasonable first comparison point. We would also like to point out an apparent misconception about our analysis. The referee indicates disappointment that we did not randomly sample colonies from the plates, but chose which colonies to sample for further analysis based

on morphological characteristics. This is, in fact, incorrect. Every colony that grew on every initial media plate was sampled for further analysis (with the exception of those colonies that could not be removed from the plate that are noted in the tables). In most cases, there was only one colony of each morphology type represented on each plate, but in the cases where multiple colonies displaying the same morphology were observed all were sampled and replated on all media types. Therefore, the comparison between the two techniques is derived from a complete sampling of all the molecular and viable colonies available in each sample (assuming that they were distributed uniformly in the collected liquid sample), rather than any subset of either. Back-trajectory analysis was used to attempt to explain the breath of the diversity that was found in the molecular data, which we could not straightforwardly attribute to local sources. Without including some discussion of the potential sources of the observed molecular bacterial signatures there would be no context for why those signatures should exist.

We would also like to address the first referee's specific comments.

LINE 135-136: There may be a problem with the PDF reader. We have checked the discussion paper, and it seems to be clear. For reference, the equation is MT = 13 N2.

LINE 162-165: We did measure the DNA content of the samples using a NanoDrop 2000c spectrophotometer (Thermo Fisher Scientific, Inc.) and found that the samples had the following DNA concentrations prior to amplification: Collection 1 - 4.7 ng/ul, Collection 2 - 2.8 ng/ul, Collection 3 - 4.6 ng/ul, Collection 4 - 3.0 ng/ul. Collection 2 and Collection 4 had the lowest DNA concentrations. Collection 4, however, had the broadest diversity. The lack of correlation between indicated diversity and measured DNA concentration supports our finding that diversity increased in Collection 4. I am happy to look into reformatting the figure to improve clarity. Since submitting this publication we have found other formats that may be helpful in combination with these suggestions.

LINE 181-231: Unfortunately, at the time this work was being performed we did not

have the funds for sequencing of these samples. In the time since then, the cost of sequencing has dropped and availability of facilities has increased, but we have not been able to take advantage of them with these isolates. We retain the isolates for characterization in the future, as a part of a library of airborne isolates that we are collecting, but we have not yet identified funds for sequencing. We have addressed the plate sampling issue above. To address the concerns of the knowledge-base of the typical readership we will follow our discussion above to develop a more detailed discussion regarding the use of gram stain as a comparison point in the final manuscript (if invited).

LINE 241-242: It is true that several members of Proteobacteria are aerobes; however, as a phylum, it is generally accepted that the majority of its members are either facultative or obligatory anaerobes. The intended focal point of the section in question was only to draw attention to the fact that, if the results associated with this phylum were removed from consideration, the similarity between the two data sets becomes much greater. In addition, this point is made more interesting when considering the primarily anaerobic population of this phylum and the fact that this study did not use anaerobic culture techniques. Rewording this section of the manuscript may better articulate this point in the final revision.

LINES 251-255: We have to concede this point in some respects. We have relied on typical categorizations of bacteria to support the possible origins of the 16S rRNA based on HYSPLIT modeling. We must admit that this type of result is not conclusive of the origins of these RNA signatures, but it is helpful to consider the possible locations from which bacteria (or their ribosomal RNA) might have originated based on back-trajectory modeling. The referee also makes a valid point regarding the species of these bacteria that can exist in other locations. The ubiquity of Cyanobacteria in particular, can not be ignored. In the case of bacterial ecology, there are often more exceptions than rules. We will consider these caveats in future revisions.

LINE 259: The word alarming should probably be removed from future revisions. What

was meant is that the diversity did not appear to be supported by local sources of bacteria.

LINE 262-264: No, it is not surprising that air masses sampled in Maryland might come from the ocean, but it was surprising to us that we could observe (apparently) oceanic bacterial signatures that may have originated from the distance indicated by the HYSPLIT modeling. It is interesting to us that the bacterial structure of an air mass may also indicate the history of that air mass, as well as the chemical composition. We hypothesize that there may be potential uses for this information (if much more work is done) in situations where the source of a particular pollutant is unknown but is carried with bacterial indicators that have specific origins. This work does not provide enough evidence to fully support such a claim, but we hope that it provides motivation for future study.

LINES 266-267: The statement was not intended to be definitive in the way interpreted by the referee. We agree that a much more rigorous set of experiments would be necessary to prove the point definitively. We also agree the 16S rRNA is not specific enough to be used for tracing. Since completing this work we have since begun to investigate the use of shotgun sequencing for this type of environmental metagenomic investigation.

LINES 268-270: Urban environments harbor numerous stress factors and locations of extreme conditions (air pollution, industrial chimneys, etc.) that may cultivate bacteria with very specific mutations. A precedent might be found in the bacteria that have adapted to digest polychlorinated biphenyls, a very problematic anthropogenic pollutant of soils. Furukawa et al., 2004 reviewed the evolution of this degradation that is likely similar to the type of adaptation that could occur in bacteria near other pollution sources.

Upon consideration of referee two's comments, we find that we are in agreement with his suggestion to modify the title and abstract. We did not pursue the characterization

of the general biological aerosol in any depth, we only seriously investigated bacterial aerosol. We also agree that the role of transport is perhaps overstated in the title. Primarily, we used the back-trajectory analysis as a means to aid in the interpretation of the 16S rRNA data. As the referee points out, it is not definitive for sourcing the bacterial aerosols that were identified.

We would now like to address the specific comments of Referee 2.

1. The review of the literature presented here was not meant to be completely comprehensive, but it has missed a few articles of relevance that were published recently and not picked up during the original writing. We will work to incorporate those into a final version.

2. We felt that including detailed methods in this paper was redundant to the referenced papers and would make the manuscript unnecessarily long. We would like to defer this decision to the editor. The inability of this technique to identify unknown organisms is critical and has been important to another study that we are currently pursuing. We will reinforce this point in the text upon revision.

3. Correct, we did not collect enough samples to map out short term variation. The intention was to see if enough nucleic acid could be collected in 4 hours to allow characterization of short-term variation in later studies. Past studies using the Phylochip microarray on air samples relied on pooling of multiple 24-hour samples (e.g. Brodie et al., 2007) to collect enough nucleic acid for analysis. We will reword this to avoid confusion.

4. Thank you for this comment. We will develop a more detailed description of the media and its uses for inclusion in later revision.

5. Agreed. We will remove the emphasis on fungi.

6. This comment has been partially addressed by our response to referee one. As noted in those comments, our measurements of the DNA concentration in each sam-

ple support our findings and will be addressed in more depth in the revision of the manuscript. There was no variation observed in the sampling that would lead us to believe that any one sample differed significantly from any other. The methods for each sample were identical.

7. As discussed above, our use of the word “alarming” needs to be explained. This should have been worded more specifically as: The broad diversity of the organisms found in the aerosol samples was not easily explained by potential local sources of bacterial aerosol.

In general, after reviewing the comments of the referees we would like the opportunity to add strength to the results and methods section using our existing data and the suggestions of the referees. Further, we feel that the referee’s comments on the discussion section of this manuscript need to be addressed in later revisions to provide clarification. We would also be happy to provide both the microscopy and the Phylochip data as online supplements if that would benefit the readers.

References

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