Characteristics of bacterial community in fog water at Mt. Tai: similarity and disparity under polluted and non-polluted fog episodes

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Response to reviewer 1

The authors have performed taxonomic analysis of the metagenomes from polluted and non-polluted episodes of fog events at Mount Tai. The study has described the key differences in the bacterial composition and associated it with the environmental factors. The results are interesting, however, several key points need to be addresses:

We thank the reviewer for the beneficial comments on our manuscript. We respond to the reviewer comments in detail below. The responses to reviewer are in red.

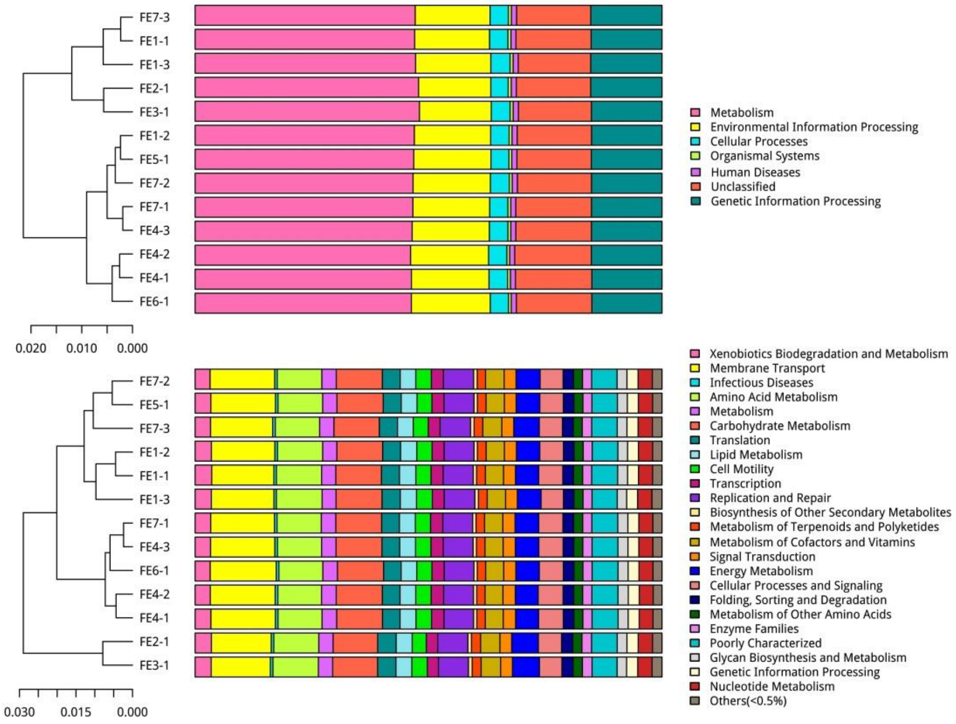
1. The authors performed a 16S analysis of the microbial community which is an excellent and reliable approach to classify the composition. However, the functionality of microbes cannot be assumed on this base. It would be other approaches (e.g. metagenome-assembly based analysis). Therefore, the authors should modify the text accordingly i.e. by not assuming the functional diversity of fog microbes. Please refer to Jiang et. al, Nat Protoc, 2015 for general methodology.

Response of the authors: thank you for your comments.
First, yes, the Miseq 16S rRNA gene sequencing was a powerful tool in microbial diversity investigation, which provides a comprehensive understanding of community composition. The metagenome-assembly based analysis have been widely used in microbial community functional analysis, e.g., Cao et al. (2014) described the microbial communities in $PM_{2.5}$ and PM_{10} using metagenomics during a serious smog event (Cao et al., 2014), Be et al examined aerosolized microorganisms in urban airborne microbes and revealed the metagenomic complexity of urban aerosols and the potential of genomic analytical techniques for biosurveillance and monitoring of threats to public health (Be et al., 2015). We also studied the suggested reference

Jiang et. al for general methodology (Jiang et al., 2015). However, due to the complexity of fog water collection, the amount for each fog episode ranged from 40 to 200 mL based on the duration, mist or dense fog. The sampled volume was inadequate for metagenomic analysis.

Second, community functions are based on community composition, bacterial taxa. For specific functional bacteria, e.g. Rhizobia (*Phyllobacterium myrsinacearum*) are involved in Biological nitrogen fixation, and favorable for plant growth, Methanotrophic Bacteria (*Methylobacterium aquaticum*, *Methylobacterium adhaesivum*) are related to methane oxidation and carbon recycling. We have added the references attached to the bacterial species in Table 3.

Third, to acquire more accurate community function, we performed PICRUST function prediction in the revised manuscript. Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUST) can be used to predict the metabolic function spectrum of corresponding bacteria and archaea based on the 16S rRNA gene sequence (Langille et al., 2013). PICRUST has been used in bacterial diversity and function analysis (Corrigan et al., 2015; Wu et al., 2016) and it was applied in community function prediction in the present study. The predicted function including metabolism and human disease are essential part of atmospheric microbial community function, which are likely attributed to the bacterial gene content from the identified species in Table 3.



Predictive functional profiling of microbial communities, using 16s rRNA gene sequences

2. Quality of English needs to be revised. Grammatical mistakes and use of wrong terminologies is seen throughout the manuscript. For instance, grammatical mistake in introduction (Line 5), Page 9 line 33 (“: : : server infections”) and Page 10 line 5 (“Previous studies has shown : : :”). The author names also have discrepancy in main text and supplement.

Response of the authors: We have polished the manuscript with a professional assistance in writing. The mistakes have been corrected.

3. Few major statements are not supported by references e.g. the authors mentioned that the recruitment of bacteria from various sources to air is harmful to humans upon deposition back to land via fog.

Response of the authors: thank you for your comments.

Numerous studies are focused on the potential pathogens identified in atmospheric particulate matter ($PM_{2.5}$ and PM_{10}) (Cao et al., 2014; Creamean et al., 2013), rain water (Kaushik & Balasubramanian, 2012; Simmons et al., 2001), and indicated that health risk-related bacteria in atmospheric samples should be concerned. For fog water, studies of health risks to individuals are typically focused on the chemical characteristic, e.g. the low pH (acid fog) (Hackney et al., 1989), PAH (Ehrenhauser et al., 2012), etc. Limited literatures discussed the microorganism in fog/cloud water suggested the potential pathogens in fog/cloud water(Vaïtilingom et al., 2012), they find potential plant pathogens such as *Pseudomonas syringae* and *Xanthomonas campestris*. In their study, they suggest that the wet deposition play a major role in the dispersion of microorganisms, which appear as an extension of the phyllosphere and carry living species of plant pathogens that could then infect new hosts through precipitation.

In the present study, potential human pathogens were identified in the fog water samples. However, the detailed health risks should be prudently assessed. Further study depending on the culture-dependent method and biochemical experiments will perform to check the pathogenicity. We have revised the relevant discussion.

4. Figure captions need to a bit more descriptive/proof read.

Response of the authors: More detailed descriptions have been added to the figure captions.

Figure 1 Bacterial community variation for the fog episodes at the phylum (A) and class (B) level. FE refers to the fog episodes. Bar graphs for each sample represent the percentage of taxa assigned to each phylum with 80% bootstrap confidence.

Taxonomic summary of the most abundant taxa (more than 1%) across all fog samples are indicated in the bar graphs.

Figure 2 Bacterial taxa are related to KEGG functional pathways. Bacterial gene functions were predicted from 16S rRNA gene-based microbial compositions using the PICRUSt algorithm to make inferences from KEGG annotated databases. Spearman's correlation coefficients were estimated for each pairwise comparison of genus counts and KEGG pathway counts. Selected KEGG pathways relating to metabolism and disease infection and predominant genera are included in the heatmap. Red color refers to the positive correlation, and green indicates a negative correlation. Correlation is significant at $^{*}P < 0.05$, $^{**}P < 0.01$.

Figure 3 Schematic representation of bioaerosols life cycle and potential influence on atmosphere, ecosystem and human health, modified from Poeschl (Poeschl, 2006). The predominant identified bacterial species with potential ecological functions are indicated in the figure. Bioaerosols emissions and resuspension from various terrestrial environments, e.g., soil, water, plants, animals or human beings, may include pathogenic or functional species. These bacteria can be attached to particles or incorporated into water droplets of clouds/fog. Certain species can serve as biogenic nuclei for Cloud Condensation Nuclei (CCN) and Ice Nuclei (IN), which induce rain formation, precipitation, and wet deposition of gases and particles. For the potential pathogens and functional bacteria, during fog process, they can be deposited back to land via deposition and possibly induce infections to human health and impose effect on the diversity and function of aquatic and terrestrial ecosystems.

Figure 4 Bacterial taxa significantly differentiated between the polluted and non-polluted fog episodes identified by linear discriminant analysis coupled with effect size (LEfSe). The LDA effect sizes (left) were calculated using the default parameters. The taxonomic cladogram (right) with LDA values higher than 3.5 comparing all bacterial taxa and significantly discriminant taxon nodes are colored and branch areas are shaded according to the highest-ranked variety for that taxon. Taxa with significant difference in polluted and non-polluted fog episodes are indicated in red and green circles, respectively. Bacterial taxa with nonsignificant differences are represented as yellow circles and the diameter of the circles are proportional to relative abundance.

Figure 5 Biplot of the environmental variables and genus-level community structure using a redundancy analysis (RDA) model, describing the variation in bacterial community explained by environmental variable. Species data are listed in Table S2. The selected environmental variables are significant ($P < 0.05$) using Monte Carlo permutation testing. Species are labeled with triangle, the closer of which indicates existence in similar environment. Environmental variables are showed by arrows; the relative length is positive correlation with the importance in influencing bacterial community structure. The angle between the arrow and the ordination axis suggest the variable response respect to the RDA gradient. The axes explain 73.3% of the variability in the data for bacteria and $PM_{2.5}$ seems to be the most important environmental variable shaping the bacterial community.

Figure 6 Air mass transport pathways for each fog episodes using the Hybrid Single Particle Lagrangian Integrated Trajectory (HYSPLIT) model. 24-hour backward trajectories were calculated for air parcels arriving at the summit of Mt. Tai ($36^o18'$ N, $117^o13'$ E, and 1534 m a.s.l). The polluted fog episodes are indicated in red lines, and green lines are non-polluted fog episodes. Wind Rose Diagram to quantitative analysis of wind speed and wind direction during sampling time. The frequency of winds is plotted by wind direction, the color bands show wind speed ranges. The direction of the longest spoke shows the wind direction with the greatest frequency.

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