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 2

The authors investigated the differences in bacterial community structures from fog water droplet samples collected from Mt. Tai in North Plain of China including those clear and polluted days in July and August of 2014. They performed sequence analysis of the samples, and also investigated the effects of environmental factors on the bacterial community structure. Overall, it is interesting to study the bacteria in the fog water samples, especially in higher altitude from a ground. The information developed is useful to understanding the microbial transport and possible roles in atmospheric pollutant transformation. The authors provided a number of different analyses of their results and derived some valuable information. Nonetheless, this reviewer does observe the following drawbacks that need the authors' attention:

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. From their work, it seems they only had one day with higher $PM_{2.5}$ pollution level, i.e., exceeding 100 ug/m^3, and they had more samples from clear days with much lower $PM_{2.5}$ levels. In their work, they compared them and further derived relevant information. I think the authors have to carefully make their conclusions regarding their limited set of data from a single polluted day. Probably, they can use the 24-hour backward trajectories to discuss more on them.

Response of the authors: According to our field observation data on the summit of Mt.Tai in the year of 2014 and 2015(unpublished), the 24 h average $PM_{2.5}$ mass concentration was basically less than 100 ug/m^3, sometimes with relative lower concentration less than 10 ug/m^3. The possible reason was that the Mt. Tai was the

highest mountain in North China Plain (1534 m a.s.l), which was typically as the background of atmospheric quality. The $PM_{2.5}$ was relatively low than other regions in the North China Plain. Similar results were obtained by other studies and suggest the relation of $PM_{2.5}$ and attitude. Gehrig and Buchmann studied the seasonal variations and spatial distribution of ambient PM_{10} and $PM_{2.5}$ concentrations. In comparison to other study area (different attitude), the lowest $PM_{2.5}$ concentrations were observed at the elevated site Chaumont (1140 m a.s.l.) (Gehrig & Buchmann, 2003). Similarly, Fan et al studied the vertical distribution of $PM_{2.5}$ concentration in fog and haze days in Beijing and suggest that $PM_{2.5}$ concentrations decreased with the increase of altitude (Fan et al., 2009).

The 24 h average $PM_{2.5}$ mass concentration according to our field observation data

2014	PM2.5 ($\mu g/m^3$)	2014	PM2.5 ($\mu g/m^3$)	2015	PM2.5 ($\mu g/m^3$)	2015	PM2.5 ($\mu g/m^3$)
2014/7/23	53.9	2014/8/9	30.1	2015/7/6	62.3	2015/7/23	34.8
2014/7/24	49.9	2014/8/10	67.5	2015/7/7	64.8	2015/7/24	34.2
2014/7/25	12.2	2014/8/11	51.1	2015/7/8	112	2015/7/25	36
2014/7/26	44.5	2014/8/12	45.5	2015/7/9	71.6	2015/7/26	33.7
2014/7/27	66.9	2014/8/13	47.4	2015/7/10	61.2	2015/7/27	65.6
2014/7/28	97.5	2014/8/14	49.3	2015/7/11	80.6	2015/7/28	49
2014/7/29	73.4	2014/8/15	47.6	2015/7/12	31.4	2015/7/29	7.9
2014/7/30	23.4	2014/8/16	66.2	2015/7/13	57	2015/7/30	12.6
2014/7/31	56.2	2014/8/17	69.5	2015/7/14	53.5	2015/7/31	14.9
2014/8/1	17.7	2014/8/18	53.7	2015/7/15	52.1	2015/8/1	17.2
2014/8/2	42.5	2014/8/19	64.9	2015/7/16	54.5	2015/8/2	35
2014/8/3	45.5	2014/8/20	62.1	2015/7/17	62.9	2015/8/3	6.1
2014/8/4	86.5	2014/8/21	71.3	2015/7/18	51.9	2015/8/4	5.9
2014/8/5	54.8	2014/8/22	54.2	2015/7/19	44.4	2015/8/5	9.9
2014/8/6	18.2	2014/8/23	48.8	2015/7/20	21.4	2015/8/6	19.3
2014/8/7	44.1	2014/8/23	42.8	2015/7/21	43.1	2015/8/7	7.8
2014/8/8	40.3			2015/7/22	55.8	2015/8/8	26.2

In addition, the listed $PM_{2.5}$ concentration in Table 1 was the average value during a fog process, not the 24 h average concentration. The 24 h $PM_{2.5}$ concentration in fog days was lower than non-fog days which possible due to the wet deposition. During fog episodes, $PM_{2.5}$ concentration varied with fog process. The mass concentration was high in the initiation of fog episode, with the development and dissipation of fog, the concentration steadily reduced due to the reduced input (nighttime) and wet deposition.

In the present study, the polluted fog episodes were defined according to the 24 h concentration of WHO air quality guideline (25 ug/m^3) and the standard was applied

by Australia, New Zealand and European Union.
In the section of 3.4, we have discussed the influence of air mass and meteorological conditions on PM2.5. The Sampling site was 1534 m a.s.l, air pollution was typically effected by air mass over long term transport than local emissions.We use the 24-hour backward trajectories to track the air mass and combined the wind direction and wind speed to deeply discuss the possible driven factors. The main points obtained was that air mass from the contaminated area through long term transport with lower wind speeds, largely reduce the diffusion rate of pollutants and thus lead to the sustained high $PM_{2.5}$ during polluted fog episodes.

2. It seems they did not clearly define what level of PM2.5 for which a day can be classified as a polluted day in their method section. Also they should clearly define what those symbols such as "FE" stand for? although I guess it should be "Fog Episode", but they should appear in all figure captions so that readers can easily understand the figures. They should describe that the characteristics of each Fog Episode are shown in relevant Tables in each Figure.

Response of the authors: thank you to your suggestion, we have clearly define the polluted fog episodes and indicated the abbreviation in the table and figures. The polluted fog episodes were defined according to the 24 h concentration of WHO air quality guideline (25 ug/m^3) and the standard was applied by Australia, New Zealand and European Union. During a fog episode, the average $PM_{2.5}$ concentration higher than 25 ug/m^3 was classified as polluted. WHO proposes $PM_{2.5}$ less than 10 ug/m^3 is safe. Elevated $PM_{2.5}$ concentration will highly increase health risks. The high pollutant and pathogens are detrimental to individuals (Fang et al., 2013). $PM_{2.5}$ concentrations were compared to the 24 h World Health Organization limit of 25 ug/m^3.

3. be aware that they only performed genus level sequence and they cannot derive any particular bacterial species, especially when they discuss about pathogens. For certain genera, not all of their species are pathogens or opportunistic pathogens.

Response of the authors: we have revised the discussion about potential pathogens. Yes, the Miseq sequencing identify bacterial taxa mostly at the genus level. In the present study, the V3-V4 hypervariable region of the bacterial 16S rRNA gene was amplified. Single OTUs were removed and taxonomy was assigned to each OTU using the Ribosomal Database Project (RDP) classifier in QIIME, with a minimum confidence cutoff of 80% against the Silva reference database (silva 119,http://www.arb-silva.de/) to the genus level. Finally, we focused on those

bacterial genera that included species known or suspected to be opportunistic pathogens. To this aim, we performed a systematic literature review to identify potential pathogenic bacteria in water habitats (Bibby et al., 2010; Guo & Zhang, 2012; Luo & Angelidaki, 2014).

Previous studies have discussed the bacterial pathogens based on NGS sequencing (454 pyrosequencing, Miseq, Ion Torrent PGM). Razzauti et al conducted a comparison between transcriptome sequencing and 16S metagenomics for detection of bacterial pathogens in Wildlife (Razzauti et al., 2015) and suggest that 16S approach was able to determine bacterial diversity in each individual. They also indicated that NGS techniques (454-pyrosequencing and MiSeq) are very affordable candidates and could become routine approaches in future large-scale epidemiological studies. Luo and Angelidaki studied the bacterial communities and bacterial pathogens with the high sequencing depth by Ion Torrent PGM (16S rRNA gene sequencing), they suggest the Ion Torrent PGM is also possible to detect the potential bacterial pathogens in biogas reactors. To identify potential pathogens, they use the reference bacterial pathogen database and identified the potential bacterial pathogens at the species level (Luo & Angelidaki, 2014).

4. I did not see any concentration levels for the total bacteria in their fog water droplet samples? Did they perform qPCR for total bacteria for their samples?

Response of the authors: Due to the complexity of fog water collection, the amount for each fog episode ranged from 40 to 200 mL based on the fog duration, mist or dense fog. For the majority fog episodes, e.g. FE1-3, FE2-1, FE4-1, FE4-2, the remained volume was inadequate for other analysis after Miseq sequencing.

The collected fog water samples were processed by genomic DNA extracting, PCR amplification, Miseq sequencing and qPCR. In DNA extraction, some samples DNA cannot be successfully extracted and require repeated extraction, thus consume more sample volume. We have performed qPCR for total bacteria after Miseq. However, after miseq, no remaining sample DNA for the further analysis for certain samples. QPCR was just performed for the samples with sufficient DNA and bacterial concentration are listed in the following table. Therefore, we did not discuss the total bacterial concentration in the manuscript.

Bacterial concentration for different fog episodes

Sample	Collected volume (mL)	Bacterial concentration (cells/mL)
FE1-1	90	8.9×10^{4}
FE1-2	80	1.3×10^{5}

FE1-3	55	Not detected
FE2-1	75	Not detected
FE3-1	100	Not detected
FE4-1	65	Not detected
FE4-2	40	Not detected
FE4-3	40	Not detected
FE5-1	50	Not detected
FE6-1	60	Not detected
FE7-1	210	1.5×10^{5}
FE7-2	200	5.8×10^{4}
FE7-3	120	1.6×10^{5}

5. It would be great if they can provide data for fungal spores. I guess there will be some fungal spores in the fog water droplets.

Response of the authors: We agree that the investigations of fungal diversity in fog water are very important areas for future work. Your suggestions are very helpful for our further study. However, the analysis of fungal spores requires substantial amounts of additional work, including the resequencing and culture-dependent experiments. The remaining parts of the samples are unable to support the above experiments. We therefore decide not to include these in our current manuscript and leave for future work. The next studies on microbial community will consider the fungal diversity in fog water and other aerosol samples.

6. For certain bacteria, when they are stored at 4 degree C, they can still grow. How long did it elapse between the collection and their actual analysis?

Response of the authors: thank you for your comments. We have modified the description and clearly described the storage conditions of the sample for different measurements. Basal analysis of water typically included chemical and biological two parts. For chemical analysis, part of samples were stored in pre-baked glass bottles, immediately preserved with hydrochloric acid (HCl, pH <2.0), stored at 4 ℃ in ice box during transit, and analyzed upon arrival at the Laboratory. Samples for microbial diversity analysis were not preserved with hydrochloric acid and stored with dry ice in transit, and frozen at -80 ℃ until further analysis.

Reference

Bibby, K., Viau, E., Peccia, J.: Bibby K, Viau E, Peccia J.. Pyrosequencing of the 16S rRNA gene

to reveal bacterial pathogen diversity in biosolids. Water Res 44: 4252-4260, Water. Res., 44, 4252-4260, 2010.

Fan, W., Hu, B., Wang, Y., Wang, S., Sun, Y.: Measurements on the Vertical Distribution of $PM_{2.5}$ Concentration in Fog and Haze Days in Beijing City, Climatic & Environmental Research (Chinese) 14, 631-638, 2009.

Fang, W., Yang, Y., Xu, Z.: PM_{10} and $PM_{2.5}$ and health risk assessment for heavy metals in a typical factory for cathode ray tube television recycling, Environ. Sci. Technol., 47, 12469-12476, 2013.

Gehrig, R., Buchmann, B.: Characterising seasonal variations and spatial distribution of ambient PM_{10} and $PM_{2.5}$ concentrations based on long-term Swiss monitoring data, Atmos. Environ., 37, 2571-2580, 2003.

Guo, F., Zhang, T.: Profiling bulking and foaming bacteria in activated sludge by high throughput sequencing, Water. Res., 46, 2772-2782, 2012.

Luo, G., Angelidaki, I.: Analysis of bacterial communities and bacterial pathogens in a biogas plant by the combination of ethidium monoazide, PCR and Ion Torrent sequencing, Water. Res., 60, 156-163, 10.1016/j.watres.2014.04.047, 2014.

Razzauti, M., Galan, M., Bernard, M., Maman, S., Klopp, C., Charbonnel, N., et al.: A Comparison between Transcriptome Sequencing and 16S Metagenomics for Detection of Bacterial Pathogens in Wildlife, Plos. Neglect. Trop. D., 9, 876-887, 2015.