Fungi Diversity in PM_1 and $PM_{2.5}$ at the summit of Mt. Tai: Abundance, Size Distribution, and Seasonal Variation

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Response to Reviewer 2

The authors have studied the fungal diversity in PM_1 and $PM_{2.5}$ collected from Mt. Tai in China using gene sequence method. While some of the results are certainly useful, however the scientific questions they were addressing were not clear, or at least not focused. Its current form more or less looks like a technical report, with most figures developed from commercialized gene sequence method.

Response: We thank the reviewer for the beneficial comments on our manuscript. We have added the description about the scientific questions and redrawn the Figure 2, Figure 3, Figure 4 , and Figure 5 in the revised essay. We respond to the reviewer comments in detail below. The responses to reviewer are in red.

1. In their work, it seems they addressed a variety of issues, e.g., health effects (fungal pathogens), fungal contents in PM_1, seasonal effects, etc., but they did not have a clear scientific question to address.

Response of the authors: We have modified the scientific question in introduction as in Page 3

Line 12-16:

The objectives of the present study were: (i) to fill the knowledge gaps regarding the information on ambient fungi of $PM_{2.5}$ and PM_1 from a high-elevation site over East Asia, (ii) to elucidate the size-resolved differences between the data of ambient fungal concentration, viable fungal community structure in different levels across different seasons, (iii) to estimate whether the environmental factors play a role in the variation of fungal characteristics at Mt. Tai.

2. The reason why they have selected Mt. Tai as a sampling site, but not ground, was not discussed in details. It is hard to use their data to derive its impact on current understanding of the aerobiology, at least not from its current form.

Response of the authors: Thanks for your suggestion. We have added the description about the reason why we selected Mt. Tai as in Page 2 Line 34- Page 3 Line11:

However, the relevant study commonly focused on the fungal communities in total suspended particles (TSP), PM_{10}, and $PM_{2.5}$ and primarily conducted over the ground surface, the attention on the fungal population in PM_1 at a high-elevation site were limited. Specific microbes at high altitudes (such as clouds water and precipitation) can act as nuclei and ice crystals and influence the precipitation patterns (Pratt et al., 2009; Creamean et al., 2013; Bower et al., 2013). Hence, it is essential to advance the knowledge, especially across East Asian region. During 2013, 2014 and 2015, serious air pollution events associated with the inadequate use of energy in the transport, domestic, and industrial sectors attacked the Northern China, which is the seriously air polluted areas including Beijing, Tianjin, Shijiazhuang, Jinan, and Qingdao. Mt. Tai (36°15'N, 117°06'E, 1534 m a.s.l), the highest site in the North China Plain, is a tilted fault block mountain with height increasing from the north to the south, facing to the Japanese Islands, Korean Peninsula, East China Sea, and Yellow Sea. The vegetation coverage reach to 80% and there are nearly 1000 kinds of plants grow in this area. In 2014 and 2015, the number of tourists from both China and abroad has increased from 5.5 to 5.9 million. In this area, the previous investigations mainly concentrated on the physicochemical characteristics of aerosol particles and cloud water and their influence on the air quality and human health. So far there were no researches focused on the diverse fungal community in aerosol particles at Mt. Tai. It is necessary to build a sophisticated finished knowledge on the atmospheric aerosol in such scenic outlook.

3. In addition, they did not do the culturing for their PM samples which is simple. I believe that there will be more fungal spores in $PM_{2.5}$ than PM_1 since fungal species are in general bigger. They only detected sequence copies not the whole fungal spores. It would be much better if they could provide optical images of their detected fungal spores both for PM_1 and $PM_{2.5}$.

Response of the authors: In the present study, samples were collected into 88mm quartz membrane filters. Half of filter was cut for DNA extraction for fungal concentration and community, and the remaining was used for the analysis of water soluble inorganic ions. So It is too pity. We have not enough filters and mature technology for the culturing and optical images. In the future, we will collect more filters for theses two analysis.

4. For their sequence data, it seems they did not perform a robust statistical analysis. Gene sequence results could be very different sometimes if not in the same batch of experiments. How did they address the QC issues in their work?

Response of the authors: Thanks for your suggestion. Though the sampling experiment lasted almost two years (May 2014-Aug. 2015), all samples were stored at -80^{o}C till the DNA extraction. We selected sixty representative samples (A1-A30, B1-B30) when the field measurements finished. I am assured that the laboratory experiments of $PM_{2.5}$ and PM_{1} were conducted in a same batch of experiments including DNA extraction, PCR amplication, real-time qPCR, and Illumina Sequencing except A29 (accidentally omitted in the first batch of Illumina Sequencing). Considering the fact that sequence varied different in different batches of experiments, we remove the A29 before quality control. A robust statistical analysis of raw sequences were preformed before diversity and taxonomic analysis. After Miseq sequencing, the raw sequences were saved by Fastq files. The Q value (Phred quality score) were calculated by the following equation:

$$Qphred=-10\log_{10}(p)$$

*p indicates the base read error rate

The paired reads were jointed together into sequences by soft FLAST. The quality control were conducted includes: a) removing the primers and barcodes; b) removing the low-quality sequences (length < 250 bp and Q value < 20); c) removing the chimeric sequences. The valid sequences were shown as below.

Table 1 Raw sequences and valid sequences number of samples.

No.	RS	VS	No.	RS	VS	No.	RS	VS
A1	16770	14551	A21	58617	51755	B12	43968	39322
A2	38089	32550	A22	45199	40554	B13	34925	31512
A3	100967	79898	A23	57862	46376	B14	50917	44886
A4	12236	9109	A24	63683	50015	B15	72251	63627
A5	35098	20950	A25	16412	13938	B16	15817	13991
A6	99119	51335	A26	43746	38228	B17	65677	57616
A7	82450	66653	A27	43877	38571	B18	57527	52063
A8	27325	24609	A28	45251	38686	B19	77755	70479
A9	100807	47939	A30	180380	164935	B20	56931	48796
A10	48298	44184	B1	27627	24078	B21	45951	38094

A11	144435	137037	B2	42178	38007	B22	60784	50330
A12	73806	65545	B3	76494	53373	B23	10202	8717
A13	123617	111296	B4	15338	13969	B24	152770	127661
A14	47854	38137	B5	56068	50431	B25	48400	43593
A15	38086	32625	B6	13823	9797	B26	47504	41459
A16	100545	83655	B7	70444	61531	B27	63400	56821
A17	25850	9008	B8	58302	51779	B28	50117	43514
A18	35313	31841	B9	17488	12427	B29	81316	73897
A19	61030	55692	B10	41966	37598	B30	34285	29926
A20	21763	18760	B11	29285	26147			

* RS indicates Raw Sequences number

* VS indicates Valid Sequences number

We have revised as in Page 3 in Line 34-38 and Page 4 Line 32-37:

Page 3 in Line 34-38:

The remaining filters were analyzed in a same batch of lab experiments including DNA extraction, PCR amplication, real-time qPCR, and Illumina Sequencing except the sample A29 in Dec. 9, 2014 (accidentally omitted in the first batch of Illumina Sequencing). Considering the fact that a percentage of sequences in two batches of experiments were different, we removed this sample before quality control.

Page 4 Line 32-37:

After high-through sequencing, We removed the chimeric and low-quality sequences by FASTX-ToolKit (http://hannonlab.cshl.edu/fastx_toolkit) and UCHIME algorithm (Edge et al., 2011) before diversity analysis and statistic analysis. The remaining high quality sequences were normalized to 7973 reads in order to compare the different samples effectively and then clustered into Operational taxonomic units (OTUs) at 97% similarity cutoff using USEARCH software (version 7.1, http://drive5.com/uparse/).

5. For the guideline values (800 CFU/m3), usually they refer to culturable bacterial CFU, while in their report they detected sequences. For fungal concentration levels, 800 CFU/m3 is a lot higher for most places.

Response of the authors: Thanks for your suggestion. The guideline (800 CFU m^{-3}) was developed for the culturable fungal CFU by Chinese Academy of Sciences Ecological Environmental Research Center. To date, there is no uniform guidelines for the fungal concentration based on the qPCR. We have deleted the unreasonable comparison with this guideline value (800 CFU m^{-3}).

6. Last, some sentences were too verbal, e.g., "got" bigger. What does <typically «100) mean? Also it is "cultuirng" not cultured" method.

Response of the authors: Thanks for your suggestion, the expression “typically << 100” means “typically less than 100” and “ got bigger” means “the particle size increased”. I have revised as in Page 2 in Line 19 and Page 1 in Line 27. The remaining verbal sentences were modified by a native English speaker and highlighted in the revised manuscripts.

7. One suggestion to improve their paper is to try differentiate Mt. Tai from ground as a less human impact location (although there are also a lot of visitors). In this way, they might argue that what is fungal level and composition in less polluted higher atmosphere, and further derive potential conclusion about their presence and impact on climate or other things.

Response of the authors: Thanks for your suggestion, we have revised largely the introduction and discussion sections in the revised manuscript.