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

Response to Reviewer 1

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. The abundant fungal genus and top five orders in our study were listed in the following table. They were also widely found in the suspending particles including TSP, PM_{10}, $PM_{2.5}$, and PM_1. The results we obtained were reasonable and effective.

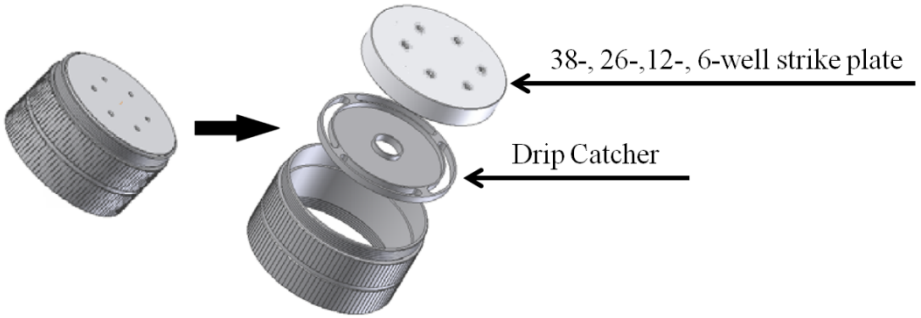
Common Fungi	RAS[a]	RAF[b]	References	Samplers	Sample Type	Concentration or Abundance
Alternaria	11.7	6.2	Hameed et al., 2012	Slit impactor sampler (Model П818N°5587, САЕПАНО, ВССР)	TSP	26.5 CFU/m^3
			Adhikari et al., 2004	Andersen sampler (Thermo Andersen, Smyrna, 300082-5211, USA)	TSP	2.6%
			Oh et al., 2014	High volume air sampler (Model no.5000; E &Instrument, Korea)	TSP	Abundant genera
			Shelton et al., 2002	Andersen N6 samplers (Thermo Andersen, Inc., Atlanta, Ga.)	TSP	
			Hwang et al., 2016	17G9 GilAir Sampler (Gilian Product Sensidyne, Inc., USA)	TSP	
			Dannemiller et al., 2014	High volume PM10 samplers (Ecotech, Knoxfield, VIC, Australia)	PM_{10}	>1%
			Alghamdi et al., 2014	$PM_{2.5}$ samplers (Staplex Air Sampler Division, USA)	$PM_{2.5}$	2.6%
			Gou et al., 2016	Low volume air sampler (BGI, USA)	PM_{10} and PM_1	>1%
Aspergillus	2.3	1.9	Hameed et al., 2012	Slit impactor sampler (Model П818N°5587, САЕПАНО, ВССР)	TSP	103.98 CFU/m^3
			Alghamdi et al., 2014	PM_{10} samplers (Staplex Air Sampler Division, USA)	PM_{10}	13.1 CFU/m^3
			Alghamdi et al., 2014	$PM_{2.5}$ samplers (Staplex Air Sampler Division, USA)	$PM_{2.5}$	7.9 CFU/m^3
			Cao et al., 2014	Air samplers (Thermo Electron Corp., MA, U.S.)	PM_{10} and $PM_{2.5}$	Abundant genera
			Gou et al., 2016	Low volume air sampler (BGI, USA)	PM_{10} and PM_1	Abundant genera
Pleosporales	18.4	45.4	Rittenour et al., 2014	Buck Bioaire Sampler (A.P. Buck, Inc, Orlando, FL, USA)	TSP	46%
			Yan et al., 2016	Air samplers (Air Metrics, USA, 5 L/min)	PM_{10} and $PM_{2.5}$	29.4%
			Gou et al., 2016	Low volume air sampler (BGI,USA)	PM_{10} and PM_1	10-15%

Xylariales	5.0	14.4	Womack et al., 2015	SKC Biosamplers (BioSampler SKC Inc.)	TSP	Abundant order
			Gou et al., 2016	Low volume air sampler (BGI, USA)	PM_{10} and PM_1	0-5%
Eurotiales	4.8	13.3	Yan et al., 2016	Air samplers (Air Metrics, USA, 5 L/min)	PM_{10} and $PM_{2.5}$	10.6%
			Gou et al., 2016	Low volume air sampler (BGI, USA)	PM_{10} and PM_1	10-15%
Capnodiales	4.4	12.5	Yan et al., 2016	Air samplers (Air Metrics, USA, 5 L/min)	PM_{10} and $PM_{2.5}$	27.96%
			Gou et al., 2016	Low volume air sampler (BGI, USA)	PM_{10} and PM_1	~25%
Polyporales	2.5	6.4	Womack et al., 2015	SKC Biosamplers (BioSampler SKC Inc.)	TSP	Abundant order
			Yan et al., 2016	Air samplers (Air Metrics, USA, 5 L/min)	PM_{10} and $PM_{2.5}$	3.6%
			Yamamoto et al., 2012	Eight-stage Andersen sampler (New Star Environmental, Roswell, GA, USA)	PM with aerodynamic diameter is 2.1-3.3, 3.3-4.7, 4.7-5.8, 5.8-9.0 and >9.0 μm	Abundant order
RAS[a] indicates Relative Abundance in Submicron particles. RAF[b] indicates Relative Abundance in Fine particles.						

1. The authors did not really address my point about particle bounce. The authors insisted no particle bounce, but they did not provide evidences or reasons of why they can insist so. What measures were taken to prevent particle bounce from the fractionating inlets? I assume the fractionating inlets (i.e., impactors) remove all particles larger than 1 or 2.5 μm, and the remaining fractions (i.e., PM_1 and $PM_{2.5}$) were collected on after filters. This point is very important since the study is intended to report fungal communities in PM_1 and $PM_{2.5}$ fractions. It is possible the authors merely measured accumulations of bounced particles from the fractionating inlets that were not really of PM_1 and $PM_{2.5}$ portions.

Response of the authors:

The samplers we used were commercial instruments and the design and quality control were qualified. Actually the particle bounce phenomenon existed in the inertial samplers. To prevent particle bounce from the fractionating inlets, we smear some silicone oil over the inside of drip catcher in every stage (as shown below) before sampling. And the samplers were operated strictly according to the manufactures' direction. We assured that the collected particles were $PM_{2.5}$ and PM_1 rather than the accumulation of bounced particles.



2. The authors seems to misunderstand the definition of sharpness of cutoff diameters by impactors. Cutoff diameter and sharpness of impactors are different. See, for example, Huang, J Air Waste Manag Assoc. 2005;55(12):1858-65 for a definition of sharpness of cutoff diameters.

Response of the authors:
The cutoff aerodynamic diameter(da50) is defined as the aerodynamic diameters of particle when the collection efficiency was 50%.
The sharpness (GSD) were defined as follows: GSD= $\sqrt{\frac{da84\%}{da16\%}}$.
*da84% means the aerodynamic diameters of particle at 84% collection efficiency.
*da16% means the aerodynamic diameters of particle at 16% collection efficiency.

For the $PM_{2.5}$ sampler, the cutoff and sharpness diameter were 2.5μm and 0.80, respectively.
For the PM_1 sampler, the cutoff and sharpness diameter were 1μm and 0.71, respectively.
We have revised as in Page Line:
Two middle volume inertial impactors (TH-150, Wuhan Tianhong Instruments Co., Ltd., China, 100 L min-1), corresponding to the cut-off diameter of 2.5μm and 1μm, were employed to collect $PM_{2.5}$ and PM_1 samples, respectively. We obtained sixty quartz membrane filters (PALL, NY, USA, 88mm) for 23 h (9:00 am to 8:00 am next day) over 8-13 days during each season from 2014 to 2015 at the summit of Mt. Tai as shown in Table 1.

3. The details of DNA extraction protocol were provided, but information about extraction efficiency was not provided. If DNA extraction efficiency is

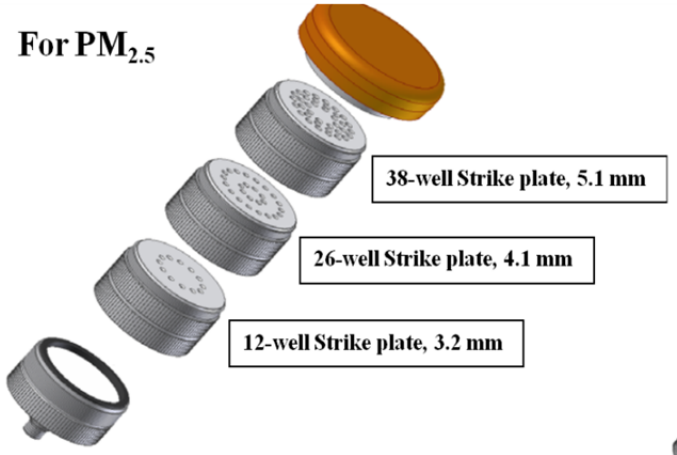
unknown or un-assumed, DNA concentrations in air cannot be back-calculated.

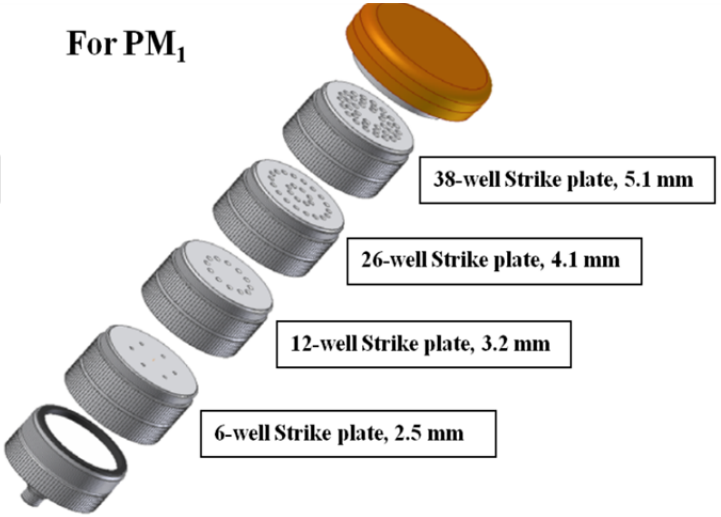
Response of the authors:
Generally, the recover efficiency should be 60%-80%. The value depends on the type of sample. Some turbid samples might come lower efficiency as it is a bit hard to lyse, but for water it will be higher. In the lab experiment, I have added some external control in exact copy number to the sample firstly, and check with the copy number after extraction. The efficiency was 71.29%. Below is the data for the expected yield and specification of the kit.

Sample	Relative Quantity	Relative Quantity SD	Corrected Relative Quantity SD	Relative Quantity SEM	Corrected Relative Quantity SEM	Mean Cq	Cq SD	Cq SEM	Ln (Copy Number)	Copy Number	Copies per µl
Pre OP	0.4191	0.0730	0.0739	0.0516	0.0522	17.4340	0.2462	0.1741	13.3790	646304.6385	64630463.8462
Post OP	0.5862	0.0062	0.0115	0.0044	0.0081	16.9597	0.0149	0.0105	13.7174	906567.4378	90656743.7794
										Efficiency	71.29%

4. The authors explained two possible reasons of why Alternaria can be found in PM_1 and $PM_{2.5}$ fractions. The second explanation of fragmentation by the sampler's inlets is problematic. If it is so, the sizes of Alternaria reported in this study were not really representative of their sizes in air. I assume the purpose of this study was to report their sizes in air, not the sizes of spores fragmented by the sampler's inlets.

Response of the authors:
Based on the guideline developed by the China's Ministry of Environmental Protection (HJ93-2013), the design of samplers were eligible and effective. The PM_1 sampler was composed of the $PM_{2.5}$ sampler and one more stage (6-well 2.5mm strike plate and drip catcher) before the filter. For PM_1 sampler, the da16% is 1.18 µm and da84% is 0.83 µm, respectively. The geometric standard deviation of sampling efficiency (σg) is 1.2±0.1. The fragment of fungal spores caused by the sample's inlets were existed indeed. But this phenomenon was tiny and within a reasonable range. We operated the samplers strictly according to the manufactures' directions and have applied this instruments to the researches on the size distribution of aerosol particles (Zhang et al., 2016; Zhao et al., 2017).


For $PM_{2.5}$
38-well Strike plate, 5.1 mm
26-well Strike plate, 4.1 mm
12-well Strike plate, 3.2 mm


For PM_1
38-well Strike plate, 5.1 mm
26-well Strike plate, 4.1 mm
12-well Strike plate, 3.2 mm
6-well Strike plate, 2.5 mm

References

Hameed A A A, Khoder M I, Ibrahim Y H, et al. Study on some factors affecting survivability of airborne fungi. Science of the Total Environment, 2012, 414(1): 696-700.

Adhikari A, Sen M M, Gupta-Bhattacharya S, et al. Airborne viable, non-viable, and allergenic fungi in a rural agricultural area of India: a 2-year study at five outdoor sampling stations. Science of the Total Environment, 2004, 326(1–3):123-141.

Shelton B G, Kirkland K H, Flanders W D, et al. Profiles of Airborne Fungi in Buildings and Outdoor Environments in the United States[J]. Applied & Environmental Microbiology, 2002, 68(4):1743.

Hwang S H, Cho J H. Evaluation of airborne fungi and the effects of a platform screen door and station depth in 25 underground subway stations in Seoul, South Korea. Air Quality, Atmosphere & Health, 2016, 9(5):561-568.

Dannemiller KC., Lang-Yon N, Yamamoto N, Rudich Y, Peccia J. Combining real-time PCR and next-generation DNA sequencing to provide quantitative comparisons of fungal aerosol populations. Atmospheric Environment, 2014, 84(84):113–121.

Almaguer M, Aira M J, Rodríguez-Rajo F J, et al. Temporal dynamics of airborne fungi in Havana (Cuba) during dry and rainy seasons: influence of meteorological parameters. International Journal of Biometeorology, 2014, 58(7):1459-1470.

Gou H, Lu J, Li S, et al. Assessment of microbial communities in PM1, and PM10, of Urumqi during winter . Environmental Pollution, 2016, 214:202.

Cao C, Jiang W, Wang B, et al. Inhalable Microorganisms in Beijing's PM2.5 and PM10 Pollutants during a Severe Smog Event. Environmental Science & Technology, 2014, 48(3):1499.

Rittenour W R, Ciaccio C E, Barnes C S, et al. Internal transcribed spacer rRNA gene sequencing analysis of fungal diversity in Kansas City indoor environments. Environmental Science Processes & Impacts, 2013, 16(1): 33-43.

Yan D, Zhang T, Su J, et al. Diversity and Composition of Airborne Fungal Community Associated with Particulate Matters in Beijing during Haze and Non-haze Days. Frontiers in Microbiology, 2016, 7:487.

Yamamoto N, Bibby K, Qian J, et al. Particle-size distributions and seasonal diversity of allergenic and pathogenic fungi in outdoor air. ISME Journal, 2012, 6(10):1801.

Womack A M, Artaxo P E, Ishida F Y, et al. Characterization of active and total fungal communities in the atmosphere over the Amazon rainforest. Biogeosciences Discussions, 2015, 12(10):1176-1176.

Huang C H. Predicting cutoff aerodynamic diameter and sharpness of single round-nozzle impactors with a finite impaction plate diameter. Journal of the Air & Waste Management Association, 2005, 55(12):1858-65.

Zhang J, Yang L, Chen J, et al. Influence of fireworks displays on the chemical characteristics of PM 2.5, in rural and suburban areas in Central and East China. Science of the Total Environment, 2016.

Zhao T, Yang L, Yan W, et al. Chemical characteristics of PM1/PM2.5 and influence on visual range at the summit of Mount Tai, North China. Science of the Total Environment, 2017, 575:458-466.