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

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Abstract. Fungi are ubiquitous throughout the near-surface atmosphere, where they represent an important component of primary biological aerosol particles. This study combined internal transcribed spacer region sequencing and quantitative real-time polymerase chain reaction (qPCR) to investigate the ambient fungi in fine ($PM_{2.5}$) and submicron (PM_1) particles at the summit of Mt. Tai located in the North China Plain, China. Fungal abundance values were 9.4×10^4 and 1.3×10^5 copies m^{-3} in $PM_{2.5}$ and PM_1, respectively. Most of the fungal sequences were from Ascomycota and Basidiomycota, which are known to actively discharge spores into the atmosphere. The fungal community showed a significant seasonal shift across different size fractions according to Metastats analysis and the Kruskal-Wallis rank sum test. The abundance of *Glomerella* and *Zasmidium* increased in larger particles in autumn, whereas *Penicillium*, *Bullera*, and *Phaeosphaeria* increased in smaller particles in winter. Three environmental factors, namely, Ca^{2+}, humidity, and temperature, were found to be crucial for the seasonal variation in the fungal community. This study might serve as an important reference for fungal contribution to primary biological aerosol particles.

1. INTRODUCTION

Inhaled particulate matter (PM), categorized as $PM_{2.5}$ and PM_1 (aerodynamic equivalent diameters of ≤2.5 μm and ≤1 μm, respectively), has been proved to be associated with the increasing morbidity and mortality from cardiovascular and respiratory diseases (Brauer et al., 2013; Wang et al., 2014;). Primary biological aerosol particles (PBAPs; about 10^4–10^8 cells cm^{-2}) constitute an important component of PM. They can actively metabolize in the atmosphere with their mass concentrations ranging from 5.49 to 102 ng m^{-3} (Zhong et al., 2016). Furthermore, they play an important role in agriculture, the biosphere, cloud formation, global climate, and atmospheric dynamics (Brodie et al., 2007; Despres et al., 2012; Christner et al., 2008; Zhou et al., 2014; Jaenicke et al., 2005). Fungi, the primary group of PBAPs, include 1.5 million unique species, distributed across rural and urban environments (Hawksworth et al., 2001). They actively eject their spores with aqueous jets or droplets into the atmosphere. The global emissions of fungal spores are estimated as the largest source of bioaerosols (Elbert et al., 2007). Pioneering studies have reported global fungal emissions to reach 28 Tg per year and contribute to about 4–13% of the mass concentration of $PM_{2.5}$ (Heald et al., 2009; Womiloju et al., 2003). More recently, some specific fungal species have been verified to be linked with the occurrence of public health problems (Morris et al., 2002; Yadav et al., 2004; Bowers et al., 2012; Bowers et al., 2013; Cao et al., 2014; Ryan et al., 2009). Despite their importance, the abundance, diversity, and community structure of fungi associated with PM have received limited attention in terms of research.

Earlier studies on airborne fungal communities, primarily based on culturing methods, found the dominant phyla to be Ascomycota (AMC) and Basidiomycota (BMC). Some of the species are considered major pathogens and allergens of plants, animals, and humans, e.g., *Hemileia vastatrix*, *Aspergillus*, *Cryptococcus*, and *Pneumocystis* spp. (Despres et al., 2012; Smets et al., 2016). While most of the fungal species remain unknown because cultivable species (typically less than 100) occupy only a tiny minority of all existing species, advances in nucleic acid sequencing allow the accurate determination of both cultured and uncultured microbial communities in environmental samples. For bacterial community composition, Xu et al. (2017a) investigated the abundance and community of bacteria in submicron particles during severe haze episodes in Jinan, China. Later, they discussed the diurnal variation of diverse bacterial communities in cloud water at Mt. Tai, China (Xu et al., 2017b). For diverse fungi in Mainz, Germany, Frohlich-Nowoisky (2009) described the fungal community in coarse (>3 μm) and fine (≤3 μm) PM using internal transcribed spacer (ITS) region sequencing. Yamamoto (2012) reported the crucial influence of aerodynamic diameter and season on the fungal taxonomic composition in the northeastern United States by 454 pyrosequencing. The fungal allergens clustered in the largest size ranges (>9 μm) in the fall season, whereas the pathogens were most abundant in the spring season and were typically observed in particles with aerodynamic diameters of <4.7 μm. Subsequently, DeLeon-Rodriguez (2013) discussed the effect of tropical storm or hurricane periods on the shift of airborne fungal species over the upper troposphere. Gou et al. (2016) described the fungal abundance and taxonomic composition of fungi in PM_1 and PM_{10} in winter in China by 18S rRNA gene sequencing. However, that study focused on the fungal communities in total suspended

particles (TSP), PM_{10}, and $PM_{2.5}$, and was primarily conducted over the ground's surface; therefore, fungal populations in PM_1 at high-elevation sites were not well accounted for. Diverse microbes at high altitudes (such as in cloud water and precipitation) can act as nucleating agents for cloud and ice condensation, influence precipitation patterns (Xu et al., 2017b; Pratt et al., 2009; Creamean et al., 2013; Bower et al., 2013), and drive the biogeochemical cycling of elements in ecosystem processes. Hence, it is essential to advance the knowledge of microbes in PM, especially across the East Asian region which are frequently ravished by dust, haze or other weather phenomenon. During 2013, 2014, and 2015, serious air pollution events associated with the inadequate use of clean energy in the transport, domestic, and industrial sectors affected Northern China, which includes several areas with severe air pollution, namely, Beijing, Tianjin, Shijiazhuang, Jinan, and Qingdao. Most researchers focus attentions on the case study of bacterial abundance and diversity (Gao et al., 2017; Xu, et al., 2017a; Wei et al., 2017; Cao et al, 2014). Because the various physical, chemical and biological factors caused by the severe haze or dust episodes may shifts on the bacterial community structure. Moreover, the microbial abundance and diversity in bioaerosol is also effected by season and meteorological factors, However the investigations on the seasonal variation of fungal characteristics in aerosol particles have been very limited.

Mt. Tai (36°15′N, 117°06′E, 1534 m above sea level), the highest site in the North China Plain, is a tilted fault block mountain, its height increasing from the north to the south, facing the Japanese Islands, Korean Peninsula, East China Sea, and Yellow Sea. The vegetation cover is 80%, with nearly 1000 kinds of plants growing in this area. The number of tourists, from both China and abroad, visiting this mountain increased from 5.5 million in 2014 to 5.9 million in 2015. Past investigations in this region mainly concentrated on the physicochemical characteristics of aerosol particles and cloud water and their influence on air quality and human health. Thus far, there have been no studies addressing the diverse fungal community in aerosol particles at Mt. Tai, necessitating the development of a reliable knowledge base on the atmospheric aerosols in such scenic destinations.

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

2. MATERIALS AND METHODS

2.1 Sample collection

At Mt Tai, spring occurs from March to May; summer, June to August; fall, September to November; and winter, December to February, according to the environmental temperature. Two middle-volume (100 L min^{-1}) inertial impactors (TH-150A; Wuhan Tianhong Instruments Co. Ltd., Wuhan, China), corresponding to cut-off diameters of 2.5 μm and 1 μm, were employed to collect $PM_{2.5}$ and PM_1 samples, respectively. Sixty quartz membrane filters (88 mm) (PALL, NY, USA) were obtained for 23

h (9:00 am to 8:00 am the next day) over 8–13 days during each season from 2014 to 2015 at the summit of Mt. Tai (Table 1). The blank filters were obtained by placing sterilized quartz microfiber filters inside the sampler without any operation. Before sampling, all the filters were baked in a muffle furnace at 500 ℃ for 5 h, placed into sterilized aluminum foil, and then deposited into a sealed bag. To avoid contamination, the sampling filter holder and materials used for changing filters were treated with 75% ethanol every day. After sampling, the samples were stored at −80 ℃ until the next analysis. $PM_{2.5}$ and PM_1 mass concentrations were monitored by a synchronized hybrid ambient real-time particulate monitor (Model 5030; Thermo Fisher Scientific, Wilmington, DE, USA). Half of the $PM_{2.5}$ and PM_1 filters were used to analyze water-soluble inorganic ions (NO_3^-, SO_4^{2-}, NH_4^+, K^+, Ca^{2+}, Na^+, and Mg^{2+}) by an ambient ion monitor (URG-9000; UGR Corporation, Chapel Hill, NC, USA). The remaining filters were analyzed in the same batch of laboratory experiments, including DNA extraction, PCR amplification, quantitative real-time PCR (qPCR), and Illumina sequencing, except for sample A29 in December 9, 2014 (accidentally omitted in the first batch of Illumina sequencing). Considering that a part of the sequences in the 2 batches of experiments differed, we removed this sample before quality control. Meteorological data, including relative humidity, wind speed, wind direction, and temperature, were obtained from http://www.underground.com at a resolution of 3 h during the sampling period. The visibility was monitored online by a visibility sensor (Model PWD22; Vaisala, Finland) with a maximum limit of 20 km.

2.2 DNA extraction and PCR amplification

To optimize the efficiency of DNA extraction, we modified some steps of sample pretreatment and DNA extraction experiments for sufficient DNA yields (Jiang et al., 2015). Half of the filters (about 121.64 cm^2) were cut into small pieces, inserted into 50-mL Falcon tubes filled with sterilized 1 × PBS buffer, and centrifuged at 200 ×*g* for 3 h at 4 ℃. The resuspension was collected into a 0.2-μm Supor 200 PES membrane disc filter. We cut the PES membrane disc filter into small pieces, heated the pieces to 65 ℃ in PowerBead tubes for 15 min, and then vortexed them for 15 min. DNA was extracted according to the standard PowerSoil DNA isolation protocol (Judd et al., 2016) and purified by AMPure XP bead purification. A parallel extraction procedure was performed with the blank filter to check for sample contamination. DNA concentrations were quantified by a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific). The fragments of ITS1 regions were amplified from genomic DNA by PCR using the forward primer ITS1F (5′-CTTGGTCATTTAGAGGAAGTAA-3′) and the reverse primer ITS4 (5′-TCCTCCGCTTATTGATATGC-3′), which target the fungal ITS region of the rRNA gene (Manter et al., 2007). The experiment was conducted using the Gene Amp® PCR System 9700 (Applied Biosystems, CA, USA) in a total volume of 50 μL PCR mix containing PCR buffer (1 ×), 1.5 μM $MgSO_4$, 0.4 μM of each deoxynucleotide triphosphate, 0.3 μM each of the forward and reverse primers, 0.5 U Ex Taq (TaKaRa, Dalian, China), 100 ng template DNA, and double distilled H_2O. The thermal cycling profile was 94 ℃ for 1 min; 35 cycles of denaturation at 98 ℃ for 20 s, annealing 68 ℃ for 30 s, and elongation at 72 ℃ for 45 s; and final extension at 72 ℃ for 5 min. Three replicates of PCR for each sample were combined together. The final products were separated by 1.5% agarose gel electrophoresis and purified using the Qiaquick PCR purification kit

(Qiagen, Valencia, CA, USA). Purified amplicons were quantified by a Qubit 2.0 fluorometer (Thermo Scientific) and pooled with equal molar amounts. Sequencing libraries were generated using the Truseq DNA PCR-Free Sample Prep Kit following manufacturer's instructions. Sequencing was performed on an Illumina MiSeq instrument (Illumina, San Diego, CA, USA) with the MiSeq reagent kit V3 (Illumina) according to the standard protocols.

2.3 Sequence analyses

After high-throughput sequencing, we removed the chimeric and low-quality sequences using the FASTX-ToolKit (http://hannonlab.cshl.edu/fastx_toolkit) and UCHIME algorithm (Edge et al., 2011) before diversity analysis and statistical analysis. The remaining high-quality sequences were normalized to 7973 reads to compare the different samples effectively. They were then clustered into operational taxonomic units (OTUs) at a 97% similarity cutoff using USEARCH software (version 7.1, http://drive5.com/uparse/). We used the OTUs as the basis for estimating the alpha diversity and beta diversity. The taxonomy of ITS sequences was analyzed by RDP Classifier against the Unite database (Release 7.0, http://unite.ut.ee/index.php; Koljalg et al., 2013) using a confidence threshold of 70%. RDP Classifier was used to determine the taxonomic composition at the phylum, class, order, family, genus, and species levels (Koiv et al., 2015; Miettinen et al., 2015). Alpha diversity estimators, including Chao1, Simpson's index, and Shannon's index, were calculated by the Quantitative Insights into Microbial Ecology software (version 1.8.0, http://qiime.org/scripts/assign_taxonomy.html; Kuczynski et al., 2011). The raw reads were deposited into the NCBI Sequence Read Archive database under accession number SRR5146156.

2.4 qPCR for ITS regions

To determine the fungal biomas, we performed qPCR (Gao et al., 2017; Yamaguchi et al., 2016; Lee et al. 2010) using a CFX96 real-time PCR detection system (Bio-Rad, Hercules, CA, USA) in 25-μL reaction mixtures containing 12.5 μL TransStart Green qPCR SuperMix, 1 μL ITS3-KYO2 (5′-GATGAAGAACGYAGYRAA-3′), 1 μL ITS4 (5′-TCCTCCGCTTATTGATATGC-3′), 5 μL sample DNA, and 5.5 μL double-distilled H_2O. The amplification followed a three-step PCR for fungal ITS regions: 40 cycles of denaturation at 95 ℃ for 30 s, primer annealing at 52 ℃ for 30 s, and extension at 72 ℃ for 30 s. A standard curve was created using tenfold dilution series of fungal ITS region plasmids. Assuming that the average fungal genome has about 30–200 rRNA copies, the fungal concentrations were calculated using the methods described by Lee et al. (2010) and van Doorn et al. (2007).

2.5 Fungal contribution to atmospheric organic carbon

The contributions of fungal spores to organic carbon (OC) were calculated using mannitol as a biotracer. We assumed 1.7 pg mannitol and 13 pg OC per spore. To assess the contribution of fungal spores to the OC and to the mass balance of atmospheric aerosol particles quantitatively, we used the weighted-average carbon conversion factor of 13 pg C per spore and of 33 pg fresh weight per spore,

which had been obtained earlier as the average carbon content of spores from airborne fungal species (Bauer et al., 2008; Zhu et al., 2016; Liang et al., 2017).

2.6 Statistical analyses

To determine the differences in the fungal community variations among different size fractions, meta-analyses based on the permutation *t*-test were conducted using Mothur software (version 1.35.1). The program Metastats can produce a tab-delimited table to display the mean relative abundance of the mean, variance, and standard error, together with the *p*-values and *q*-values. Values were considered significant if $p \leq 0.05$ and $q \leq 0.05$. The Kruskal-Wallis rank sum test was used to evaluate the seasonal variation of the microbial community. Boxplots and *q*-values are have been provided for illustration. The relationship between the ambient microbial concentrations and environmental factors, including PM concentrations and chemical compositions, was assessed with nonparametric Spearman's rank correlation coefficients by SPSS 16.0.

3. RESULTS AND DISCUSSION

3.1 Concentration of fungal spores in $PM_{2.5}$ and PM_1

$PM_{2.5}$ and PM_1 samples were collected during summer, autumn, and winter at the summit of Mt. Tai. Temporal variations of the mass concentration and corresponding fungal spore numbers of $PM_{2.5}$ and PM_1 are summarized in Table 1. PM_1 mass concentration was stable over different seasons, while $PM_{2.5}$ demonstrated a high seasonal variation, with much higher concentrations in summer (44.7 μg m^{-3}) than in autumn (37.2 μg m^{-3}) and winter (21.7 μg m^{-3}). The values were much lower than that in the summer of 2006 (123.1 μg m^{-3}; Deng et al., 2011) and comparable with that in the summer of 2007 (59.3 μg m^{-3}; Zhou et al., 2009). The average $PM_1/PM_{2.5}$ ratios were 0.45 in summer, 0.65 in autumn, and 0.84 in winter, implying that fine particles dominated in summer, while submicron particles dominated in autumn and winter.

qPCR revealed an average fungal gene copy number of 9.4×10^4 copies m^{-3} (ranging from 1.0×10^4 to 4.8×10^5 copies m^{-3}) and 1.3×10^5 copies m^{-3} (ranging from 3.7×10^3 to 1.0×10^6 copies m^{-3}) in $PM_{2.5}$ and PM_1, respectively. Assuming an average rRNA gene copy number of 200 per fungal genome (van Doorn et al., 2007; Lee et al., 2010), we obtained an average fungal concentration of 467 spores m^{-3} and 644 spores m^{-3} in $PM_{2.5}$ and PM_1, respectively. The concentrations at Mt. Tai were lower than those at surface ground sites, including those in Korea (ranging from 9.56×10^1 to 4.2×10^4 cells m^{-3}; Lee et al., 2010), Austria (1.8×10^4 cells m^{-3} in urban sites and 2.3×10^4 cells m^{-3} in suburban sites; Bauer et al., 2008), Portugal (ranging from 891 to 964 spores m^{-3}; Oliveira et al., 2009), and the United States (6450 spores m^{-3}; Tsai et al., 2007). Our lower values might be ascribed to an underestimation of the fungal numbers. We used a higher gene copy number of 200 for each microbe studied, whereas DeLeon-Rodriguez et al. (2013) employed a lower number of rRNA copies of fungal genomes (30–100 copies per genome). The discrepancy between our results and those of Lee et al. (2010) might be

because of the differences in sample type, sampling time, and altitude. Lee et al. (2010) focused on the fungal concentration in TSP by a high-volume TSP sampler (0.225 m^3 min^{-1}) 15 m above the ground in autumn and winter, whereas we obtained the $PM_{2.5}$ and PM_1 by middle-volume samplers (0.1 m^3 min^{-1}) 1534 m above the ground in summer, autumn, and winter. It is difficult to explain the disparity between different studies without uniform guidelines for the sampling and quantitative assessment of bioaerosols.

Fungal abundance varied seasonally with different size particles in the near-surface atmosphere. Saari et al. (2015) found that coarse fluorescent bioaerosol particles (1.5–5 μm) increased in summer, whereas in winter, these particles primarily existed in smaller particles (0.5–1.5 μm). The snow cover and decreased biological activity in winter resulted in the disappearance of microbes from the coarse fluorescent bioaerosol particles. In this study, the highest fungal concentration in $PM_{2.5}$ was observed in summer (641 spores m^{-3}), whereas the highest value in PM_1 was found in autumn (1033 spores m^{-3}), indicating different origins of fungal spores. Huffman et al. (2010) found that long-range transport of aerosols and anthropogenic sources such as combustion influence the fluorescent biological aerosol particles having diameters less than 1 μm. During the autumn sampling, no obvious straw combustion phenomena occurred, and we detected some long-range transportation events in November 2014. Long-range transported airborne PM were mainly derived from the outer Mongolia regions, well-known to be one of the dustiest places in East Asia (November 6), Siberia (November 3 and November 12), and Taklimakan and Gobi desert regions (November 5). Influenced by the air movements from the desert region, the corresponding fungal abundance increased from 6.18×10^4 to 103×10^4 copies m^{-3} (about 16.7-folds). Similarly, the corresponding fungal abundance influenced by air parcels from Siberian regions increased to 22.2×10^4 and 18.3×10^4 copies m^{-3}, respectively. Hence, we hypothesized that the long-range transport of air parcels from north China might have contributed to the fungal enrichment of PM_1. In addition, the increased fungal abundance might be explained by meteorological diversity (Abdel Hameed et al., 2012). Low wind speed hinders fungal dispersal owing to the accumulation effect. According to Almaguer et al. (2014), in Cuba, the calm winds coming from the southwest direction induce the accumulation of fungal spores over the northern coast of the island. Lin et al. (2000) observed a strongly negative correlation between wind speeds of <4 m s^{-1} and fungal concentration; the fungal concentration increased as the wind speed became higher than 5 m s^{-1} in the Taipei area. In our present study, the fungal abundance in PM_1 showed no obvious increase under breezy conditions (wind speed < 2 m s^{-1}) mainly from the southern direction (Figure 1). When the wind speed was higher than 2 m s^{-1}, the fungal abundance increased markedly under the influence of westerly winds. As the westerly wind velocity increased, the fungal concentration increase slowly. Meanwhile, in $PM_{2.5}$, the fungal abundance increased with wind velocities higher than 2 m s^{-1}, mainly from the northwest direction of the continental areas, where diverse vegetation grows. The phenomenon implies that westerly and northwesterly winds might highly induce fungal growth and abundance in PM at Mt. Tai.

3.2 Contribution of spores to OC concentrations and PM mass

OC, accounting for 7–80% of PM mass, constitutes a significant fraction of atmospheric aerosols (Yu et al., 2004; Ram et al., 2012; Ho et al., 2012). Ambient fungi are considered a possible source of OC in PMs. Cheng et al. (2009) estimated the mean fungal OC concentrations in Hong Kong to be 3.7, 6.0, and 9.7 ng m^{-3}, corresponding to 0.1%, 1.2%, and 0.2% of the total OC in $PM_{2.5}$, $PM_{2.5–10}$, and PM_{10}, respectively. In the present study, the range and average concentrations of fungal contribution to atmospheric OC and mass concentration $PM_{2.5}$ and PM_1 are listed in Table 1. The daily averaged concentrations of fungal OC in $PM_{2.5}$ and PM_1 were 6.1 and 8.3 ng C m^{-3}, respectively, with the respective contributions to PM being 0.067% and 0.096%, indicating that airborne fungal spores as a minor source of carbonaceous aerosols cannot be ignored at Mt. Tai. The fungal contribution to OC obtained at Mt. Tai was comparable with that observed at an urban site in Hong Kong (3.7 ng C m^{-3}; Cheng et al., 2009) but lower than that obtained at an urban site in Austria (117.9 ng C m^{-3}; Bauer et al., 2008) and a forest site on Hainan Island (147–923 ng C m^{-3}; Zhang et al., 2015). The discrepancy between the abovementioned studies can be justified by the difference in particle type studied (TSP, PM_{10}, $PM_{2.5}$, and PM_1), fungal concentration, spore carbon content, and assessment method (e.g., sugar alcohol, cultivation, mannitol, and light microscopy). On the basis of the same conversion factor of 13 pg C $spore^{-1}$ by mannitol, the results were much lower than that obtained at an urban site in Beijing (0.3 ± 0.2 μg C m^{-3}; Liang et al., 2017), implying a lower fungal concentration at Mt. Tai than that in Beijing. More studies are needed to better understand the spatial, temporal, and size distributions of fungal OC contributions to atmospheric particles in urban areas in the North China Plain.

3.3 Taxonomic diversity and composition of ambient fungi

On average, 509 and 475 OTUs were obtained in $PM_{2.5}$ and PM_1, respectively, which were higher than those obtained in earlier airborne fungal studies at the ground level in Beijing, China (34-285; Yan et al., 2016) and Rehovot, Israel (121-178; Dannemiller et al., 2014). The OTUs associated with $PM_{2.5}$ in summer, autumn, and winter were higher than those associated with PM_1, implying more diverse fungal spores in $PM_{2.5}$. However, the Shannon and Chao1 indices showed different trends in $PM_{2.5}$ and PM_1 (Figure 2). The ambient fungi showed the highest richness and diversity in winter, followed by autumn and summer. Although PM_1 mass concentration dominated in autumn and winter, the corresponding fungal diversity was lower than that in $PM_{2.5}$. Similarly, the dominant $PM_{2.5}$ mass concentration in summer presented lower diversity than that in PM_1.

In the fungal community, AMC (89.7%) and BMC (7.0%) were the predominant phyla, and they are known to actively discharge spores into the atmosphere (Figure 3A). The remaining phyla were Zygomycota (ZMC) and Glomeromycota. AMC and BMC present a global pattern across continental (Austria, Arizona, Brazil, and Germany), coastal (Taiwan, Puerto Rico, and UK), and marine sites (Pacific, Indian, Atlantic, and Southern Ocean) (Frohlich-Nowoisky et al., 2012). In continental samples, BMC (64%) seems to be more abundant than AMC (34%), whereas in marine sites, AMC (72%) is about 2.6 times more abundant than BMC. Herein, the abundance of AMC was approximately 12.8 times higher than that of BMC. Members of AMC have single-celled or filamentous vegetative

growth forms that are easily aerosolized, unlike BMC (Womack et al., 2015). Furthermore, 10 classes belonging to AMC, 10 to BMC, and 1 to ZMC were observed (Figure 3B). The preponderant classes belonging to AMC were Dothideomycetes (37.3%), Sordariomycetes (15.0%), and Eurotiomycetes (6.1%). The dominant orders in Dothideomycetes included Pleosporales (14.9%), Capnodiales (5.3%), and Botryosphaeriales (1.6%) (Figure 3C). Pleosporales has been reported to include fungi allergenic to local residents (Rittenour et al., 2014). The values were lower than those reported in Beijing's PM (Pleosporales: 29.39% and Capnodiales: 27.96%) (Yan et al., 2016). Likewise, the dominant classes in BMC were Agaricomycetes (4.4%) and Tremellomycetes (1.5%), including the orders Polyporales (2.5%), Agaricales (1.6%), and Tremellales (1.2%). About 291 taxa from the genus level were determined, including *Alternaria*, *Glomerella*, *Zasmidium*, *Pestalotiopsis*, *Aspergillus*, and *Phyllosticta*. The distribution was discrepant with that at the ground level, wherein *Cladosporium* occupied more than 50% of total fungi, followed by *Alternaria*, *Didymella*, and *Khuskia* (Oh et al., 2014). The top 5 orders (Pleosporales, Xylariales, Eurotiales, Capnodiales, Polyporales) and genera (*Alternaria* and *Aspergillus*) were commonly observed in suspended aerosol particles (including TSP, PM_{10}, $PM_{2.5}$, and PM_1) but showed variable relative abundances, as shown in Table 2. We attribute this disparity to the different sampling approaches, instruments, and analysis methods. This aspect needs to be probed and studied in depth in the future.

To date, about 123 fungal genera (mainly belonging to the phylum AMC) have been identified to be human allergens (Simon-Nobbe et al., 2008). Of the 11 potentially allergy-inducing AMC species and 1 potentially allergy-inducing BMC species found at Mt. Tai, the 3 most common species were *Aspergillus flavus*, *Blumeria graminis*, and *Saccharomyces cerevisiae*. *Aspergillus flavus* is a common human pathogen found in air, and it is also a human allergen and mycotoxin producer (Adhikari et al., 2004). It is associated with invasive aspergillosis and superficial infections (Hedayati et al., 2007). *Blumeria graminis*, found on the surface of plant leaves, causes powdery mildew on cereal plants (Belanger et al., 2003). Such pathogens and allergens are expected to be widely spread around the atmospheric environment in temperate and tropical zones (Vermani et al., 2010). Our results also revealed that the abundance of potential allergenic and pathogenic fungal spores in summer were the highest compared to those in autumn and winter. Clinicians should consider the fungal spores described herein as a possible cause of human and plant disease attacks under long exposure to airborne particles throughout the year, especially in the summer season. Furthermore, the abundance of the abovementioned allergenic and pathogenic fungal spores in PM_1 was about 3.8 times higher than that in $PM_{2.5}$ in summer, implying relatively higher health risks of smaller particles. Residents and even visitors at Mt. Tai should be warned about this phenomenon.

3.4 Size distribution and seasonal variation of fungal communities

Both fungal abundance and fungal community show a seasonal trend across different size fractions (Awad et al., 2013). Yamamoto et al. (2012) observed that the pathogenic fungi were mainly detected at $PM_{4.7}$ (PM with aerodynamic diameter < 4.7 μm), while the allergenic fungi existed primarily at PM with aerodynamic diameter > 9 μm. In the present study, a discrepant size distribution of the fungal

community was observed according to the Metastat analysis by permutation *t*-tests (Table 3). *Glomerella*, *Zasmidium*, and *Phyllosticta* were abundantly enriched in $PM_{2.5}$, while the abundance of *Preussia*, *Truncatella*, *Umbelopsis*, *Sebacina*, and *Cordyceps* increased in PM_1. The Kruskal-Wallis rank sum test (Figure 4) showed that 6 fungal genera had apparent seasonal variation. *Glomerella* and *Zasmidium* increased in autumn and decreased as the particle size increased. *Glomerella* was widely found on the surface of leaves, suggesting that leaf senescence is an important source of fungi in $PM_{2.5}$ in autumn (Wang et al., 2015). Some crucial environmental factors having a potential influence on fungal release and growth, such as temperature; NO_2; PM_{10}; SO_2; CO; relative humidity (Yan et al., 2016); radiation, vegetation, and urbanization, and accidental events, e.g., dust storms (Prospero et al., 2005), rainfall (Zhang et al., 2015), hurricanes (DeLeon-Rodriguez et al., 2013), and haze (Yan et al., 2016), (Moreau et al., 2016), have been identified. In the current study, Spearman's rank coefficient analysis indicated that Ca^{2+}, a typical water-soluble inorganic ion from dust, was negatively related to the prevalence of *Glomerella* and *Zasmidium* in autumn (Figure 5). In winter, the abundance of *Penicillium*, *Bullera*, and *Geosmithia* increased owing to their sensitivity to low temperature (Sousa et al., 2008; Abdel Hameed et al., 2012). The results based on Spearman's rank correlation test analysis support this notion (Figure 5, $p < 0.01$). Humidity, another important factor for fungal release into the atmosphere either by active or passive modes, is a crucial factor for the variation in fungal spores such as *Lophium* ($p < 0.01$), *Cenococcum* ($p < 0.05$), *Tricholoma* ($p < 0.05$), and *Candida* ($p < 0.05$). In summer, no distinct difference was observed based on the top 40 fungal genera (Figure 4). However, some trace fungal genera presented inverse correlation with temperature (*Coccomyces*, $p < 0.01$; and *Dictyosporium*, $p < 0.01$) and humidity (*Botryosphaeria*, $p < 0.001$; *Coccomyces*, $p < 0.01$; and *Dictyosporium*, $p < 0.01$). We determined that 4 crucial environmental factors contributed to the variation in the fungal community. As culture studies on the detailed mechanism of the effects of environmental factors on specific fungal spores have been limited, this problem still needs to be surveyed over a longer duration. Moreover, the detailed relationship of bioaerosols with environmental factors require further study.

4. CONCLUSIONS

Information about diverse airborne fungal spores is relevant for studies on the atmosphere, biogeoscience, climate and ecology, environmental hygiene, agriculture, and bioengineering. As the details of fungal spores present at high-elevation sites remain unknown, the detection and characterization of ambient fungi can help elucidate the regional and global distribution of diverse fungi. Herein, we provide a comprehensive framework of the fungal abundance and communities associated with different seasons across $PM_{2.5}$ and PM_1 at Mt. Tai. The results revealed that the concentration and fungal community structure at Mt. Tai differ considerably from those reported for surface ground sites. Over the sampling period, average fungal concentrations of 467 spores m^{-3} and 644 spores m^{-3} in $PM_{2.5}$ and PM_1 were calculated. In addition to long-distance air mass movement events, westerly and northwesterly winds also favor the increase in fungal abundance. Diverse fungal

communities presented significant seasonal variation across different size particles. The prevalence of *Glomerella* and *Zasmidium* increased in autumn and decreased as the particle size increased. In winter, the prevalence of *Penicillium*, *Bullera*, and *Geosmithia* increased with the decrease in particle size. No distinct disparity was observed in summer. The variation in fungal profile can be influenced by environmental factors, including humidity, temperature, wind speed, $PM_{2.5}$, and some chemical components in PMs (including Ca^{2+}). Nevertheless, the detailed specific effects of environmental factors on the ambient fungal community remain poorly explained. In further studies, a combination of traditional culture-based methods and metagenomics may help answer the various unresolved questions.

ACKNOWLEDGEMENTS

This work was supported by the National Natural Science Foundation of China (No. 41375126, 21527814), Taishan Scholar Grant (grant number ts20120552), Cyrus Tang Foundation (No. CTF-FD2014001), the Ministry of Science and Technology of of China (No. 2016YFC0202701, 2014BAC22B01) and the European Union's Horizon 2020 research and innovation programme under grant agreement No. 690958 (MARSU-Project).

REFERENCES

Abdel Hameed, A.A., Khoder, M.I., Ibrahim, Y.H., Saeed, Y., Osman, M.E., Ghanem, S. (2012) Study on some factors affecting survivability of airborne fungi. Sci. Total Environ. 414, 696-700.

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

Almaguer, M., Aira, M.J., Rodriguez-Rajo, F.J., Rojas, T.I. (2014) Temporal dynamics of airborne fungi in Havana (Cuba) during dry and rainy seasons: influence of meteorological parameters. Int. J. Biometeorol. 58(7), 1459-1470.

Awad, A.H., Gibbs, S.G., Tarwater, P.M., Green, C.F. (2013) Seasonal evaluation of fine and coarse culturable bacterial aerosols from residences within a rural and an urban city in Egypt. Int. J. Environ. Heal. R. 23(4), 269-280.

Bauer, H., Schueller, E., Weinke, G., Berger, A., Hitzenberger, R., Marr, I.L., Puxbaum, H. (2008) Significant contributions of fungal spores to the organic carbon and to the aerosol mass balance of the urban atmospheric aerosol. Atmos. Environ. 42(22), 5542-5549.

Belanger, R.R., Benhamou, N., Menzies, J.G. (2003) Cytological evidence of an active role of silicon in wheat resistance to powdery mildew (*Blumeria graminis f. sp. tritici*). Phytopathology 93(4), 402-412.

Bowers, R.M., McCubbin, I.B.; Hallar, A.G.; Fierer, N. (2012) Seasonal variability in airborne bacterial communities at a high-elevation site. Atmos. Environ. 50, 41-49.

Bowers, R.M., Clements, N., Emerson, J.B., Wiedinmyer, C., Hannigan, M.P., Fierer, N. (2013) Seasonal variability in bacterial and fungal diversity of the near-surface atmosphere. Environ. Sci. Technol. 47(21), 12097-12106.

Bowers, R.M., Lauber, C.L., Wiedinmyer, C., Hamady, M., Hallar, A.G., Fall, R., Knight, R., Fierer, N. (2009) Characterization of airborne microbial communities at a high-elevation site and their potential to act as atmospheric ice nuclei. Appl. Environ. Microbiol. 75(15), 5121-5130.

Brauer, M.; Freedman, G., Frostad, J., van Donkelaar, A., Martin, R. V., Dentener, F., van Dingenen, R., Estep, K., Amini, H., Apte, J. S., Balakrishnan, K., Barregard, L., Broday, D., Feigin, V., Ghosh, S., Hopke, P.K., Knibbs, L.D., Kokubo, Y., Liu, Y., Ma, S., Morawska, L., Sangrador, J.L., Shaddick, G., Anderson, H.R., Vos, T., Forouzanfar, M.H., Burnett, R.T., Cohen, A. (2013) Ambient air pollution exposure estimation for the global burden of disease 2013. Environ. Sci. Technol. 50(1), 79-88.

Brodie, E.L., DeSantis, T.Z., Parker, J.P., Zubietta, I.X., Piceno, Y.M., Andersen, G.L. (2007) Urban aerosols harbor diverse and dynamic bacterial populations. P. Natl. Acad. Sci. USA. 104(1), 299-304.

Cao, C., Jiang, W., Wang, B., Fang, J., Lang, J., Tian, G., Jiang, J., Zhu, T.F. (2014) Inhalable microorganisms in Beijing's $PM_{2.5}$ and PM_{10} pollutants during a severe smog event. Environ. Sci. Technol. 48(3), 1499-1507.

Cheng, J.Y.W., Chan, C.K., Lee, C.T.; Lau, A.P.S. (2009) Carbon content of common airborne fungal species and fungal contribution to aerosol organic carbon in a subtropical city. Atmos. Environ. 43, 2781-2787.

Christner, B.C.; Morris, C.E., Foreman, C.M., Cai, R., Sands, D.C. (2008) Ubiquity of biological ice nucleators in snowfall. Science 319(5867), 1214.

Creamean, J.M., Suski, K.J., Rosenfeld, D., Cazorla, A., DeMott, P.J., Sullivan, R.C., White, A.B., Ralph, F.M., Minnis, P., Comstock, J.M., Tomlinson, J.M., Prather, K.A. (2013) Dust and biological aerosols from the Sahara and Asia influence precipitation in the western U.S. Science 339(6127), 1572-1578.

Dannemiller, K.C., Lang-Yona, N., Yamamoto, N., Rudich, Y., Peccia, J. (2014) Combining real-time PCR and next-generation DNA sequencing to provide quantitative comparisons of fungal aerosol populations. Atmos. Environ. 84, 113-121.

DeLeon-Rodriguez, N., Lathem, T.L., Rodriguez, R.L., Barazesh, J.M., Anderson, B. E., Beyersdorf, A.J., Ziemba, L.D., Bergin, M., Nenes, A., Konstantinidis, K.T. (2013) Microbiome of the upper

troposphere: species composition and prevalence, effects of tropical storms, and atmospheric implications. P. Natl. Acad. Sci. USA. 110(7), 2575-2580.

Despres, V.R., Nowoisky, J.F., Klose, M., Conrad, R., Andreae, M.O., Poschl, U. (2007) Characterization of primary biogenic aerosol particles in urban, rural, and high-alpine air by DNA sequence and restriction fragment analysis of ribosomal RNA genes. Biogeosciences 4, 1127-1141.

Despres, V.R., Huffman, J.A., Burrows, S.M., Hoose, C., Safatov, A.S., Buryak, G., Fröhlich-Nowoisky, J., Elbert, W., Andreae, M.O., Pöschl, U. (2012) Primary biological aerosols in the atmosphere: A review of observations and relevance. Tellus B 64, 1-58.

Edgar, R.C., Haas, B.J., Clemente, J.C, Quince, C., Knight, R. (2011) UCHIME improves sensitivity and speed of chimera detection. Bioinformatics 27(16), 2194-2200.

Elbert, W., Taylor, P.E., Andreae, M.O., Poschl, U. (2007) Contribution of fungi to primary biogenic aerosols in the atmosphere: wet and dry discharged spores, carbohydrates, and inorganic ions. Atmos. Chem. Phys. 7, 4569-4588.

Frohlich-Nowoisky, J., Pickersgill, D.A., Despres, V.R., Poschl, U. (2009) High diversity of fungi in air particulate matter. P. Natl. Acad. Sci. USA. 106(31), 12814-12819.

Frohlich-Nowoisky, J., Burrows, S.M., Xie, Z., Engling, G., Solomon, P.A., Fraser, M.P., Mayol-Bracero, O.L., Artaxo, P., Begerow, D., Conrad, R., Andreae, M.O., Despres, V.R., Poschl, U. (2012) Biogeography in the air: fungal diversity over land and oceans. Biogeosciences. 9 (3), 1125-1136.

Gandolfi, I., Bertolini, V., Bestetti, G., Ambrosini, R., Innocente, E., Rampazzo, G., Papacchini, M., Franzetti, A. (2015) Spatio-temporal variability of airborne bacterial communities and their correlation with particulate matter chemical composition across two urban areas. Appl. Microbiol. Biotechnol. 99(11): 4867-77.

Gao, J.F., Fan, X.Y., Li, H.Y., Pan, K.L. (2017) Airborne bacterial communities of $PM_{2.5}$ in Beijing-Tianjin-Hebei megalopolis, China as revealed by Illumina MiSeq sequencing: a case study. Aerosol Air Qual. Res.17(3)

Gou, H., Lu, J.J., Li, S., Tong, Y.B., Xie, C.B., Zheng, X. (2016) Assessment of microbial communities in PM_1 and PM_{10} of Urumqi during winter. Environ. Pollut. 214, 202-210.

Hawksworth, D.L. (2001) The magnitude of fungal diversity: the 1.5 million species estimate revisited. Mycol. Res. 105(12), 1422-1432.

Heald, C.L., Spracklen, D.V. (2009) Atmospheric budget of primary biological aerosol particles from fungal spores. Geophys. Res. Lett. 36(9), 269-277.

Hedayati, M.T., Pasqualotto, A.C., Warn, P.A., Bowyer, P., Denning, D.W. (2007) *Aspergillus flavus*: human pathogen, allergen and mycotoxin producer. Microbiol. 153 (6), 1677-1692.

Ho, K.F., Lee, S.C., Yu, J.C., Zou, S.C., Fung, K. (2002) Carbonaceous characteristics of atmospheric particulate matter in Hong Kong. Sci. Total Environ. 300, 59–67.

Huffman, J., Treutlein, B., and Poschl, U. (2010) Fluorescent biological aerosol particle concentrations and size distributions measured with an ultraviolet aerodynamic particle sizer (UV-APS) in central Europe. Atmos. Chem. Phys. 10, 3215-3233.

Jaenicke, R. (2005) Abundance of cellular material and proteins in the atmosphere. Science 308 (5718), 73.

Judd, C.R., Koyama, A., Simmons, M,P., Brewer, P.E., von Fischer, J.C. (2016) Co-variation in methanotroph community composition and activity in three temperate grassland soils. Soil Biology and Biochemistry 95, 78-86.

Koljalg, U., Nilsson, R.H., Abarenkov, K. (2013) Towards a unified paradigm for sequence-based identification of fungi. Mol. Ecol. 22(21), 5271.

Koiv, V., Roosaare, M., Vedler, E., Kivistik, P.A., Toppi, K., Schryer, D.W., Remm, M., Tenson, T., Mae, A., (2015) Microbial population dynamics in response to *Pectobacterium atrosepticum* infection in potato tubers. Sci. Rep-UK. 2015, 5, 11606.

Kuczynski, J., Stombaugh, J., Walters, W.A., GonzAlez, A.; Gregory Caporaso, J., Knight R. (2011) Using QIIME to analyze 16S rRNA gene sequences from microbial communities. Curr. Protoc. Bioinformatics. doi:10.1002/0471250953.bi1007s36.

Lee, S.H., Lee, H.J., Kim, S.J., Lee, H.M., Kang, H., Kim, Y.P. (2010) Identification of airborne bacterial and fungal community structures in an urban area by T-RFLP analysis and quantitative real-time PCR. Sci. Total Environ. 408 (6), 1349-1357.

Liang, L., Engling, G., Du, Z.; Duan, F., Cheng, Y., Liu, X., He, K. (2017) Contribution of fungal spores to organic carbon in ambient aerosols in Beijing, China. Atmos. Pollut. Res. 8(2), 351-358.

Lin, W.H., Li, C.S. (2000) Associations of fungal aerosols, air Pollutants, and meteorological factors. Aerosol Sci. Technol. 32, (4), 359-368.

Manter, D.K., Vivanco, J.M. (2007) Use of the ITS primers, ITS1F and ITS4, to characterize fungal abundance and diversity in mixed-template samples by qPCR and length heterogeneity analysis. J. Microbiol. Meth. 71(1), 7-14.

Miettinen, H., Kietavainen, R., Sohlberg, E., Numminen, M., Ahonen, L., Itavaara, M. (2015) Microbiome composition and geochemical characteristics of deep subsurface high-pressure environment, Pyhasalmi mine Finland. Front. Microbiol. 6, 1203.

Moreau, C.S., Weikl, F., Tischer, C., Probst, A.J., Heinrich, J., Markevych, I., Jochner, S., Pritsch, K. (2016) Fungal and bacterial communities in indoor dust follow different environmental determinants. Plos One 11(4), e0154131.

Morris, C.E., Kinkel, L.L., Lindow, S.E., Hechtpoinar, E.I., Elliott, V.J. (2002) Fifty years of phyllosphere microbiology: significant contributions to research in related fields. Phyllosphere Microbiology 365-375.

Oh, S. Y., Fong, J.J., Park, M.S., Chang, L., Lim, Y.W. (2014) Identifying airborne fungi in Seoul, Korea using metagenomics. J. Microbiol. 52 (6), 465-472.

Oliveira, M., Ribeiro, H., Delgado, J.L., Abreu, I. (2009) The effects of meteorological factors on airborne fungal spore concentration in two areas differing in urbanisation level. Int. J. Biometeorol. 53(1), 61-73.

Pratt, K.A., DeMott, P.J., French, J.R., Wang, Z., Westphal, D.L., Heymsfield, A.J., Twohy, C.H., Prenni, A.J., Prather, K.A.(2009) In situ detection of biological particles in cloud ice-crystals. Nat. Geosci. 2(2), 398-401.

Prospero, J.M., Blades, E., Mathison, G., Naidu, R. (2005) Interhemispheric transport of viable fungi and bacteria from Africa to the Caribbean with soil dust. Aerobiologia 21(1), 1-19.

Ram, K., Sarin, M.M., Tripathi, S.N. (2012) Temporal trends in atmospheric $PM_{2.5}$, PM_{10}, elemental carbon, organic carbon, water-soluble organic carbon, and optical properties: impact of biomass burning emissions in the Indo Gangetic Plain. Environ. Sci. Technol. 46, 686-695.

Rittenour, W.R., Ciaccio, C.E., Barnes, C.S., Kashon, M. L.; Lemons, A.R., Beezhold, D. H., Green, B.J. (2014) Internal transcribed spacer rRNA gene sequencing analysis of fungal diversity in Kansas City indoor environments. Environ. Sci. Proc. Impacts. 16 (1), 33-43.

Ryan, P.H., Bernstein, D.I., Lockey, J., Reponen, T., Levin, L., Grinshpun, S., Villareal, M., Hershey, G.K.K., Burkle, J., Lemasters, G. (2009) Exposure to traffic-related particles and endotoxin during infancy is associated with wheezing at age 3 years. Am. J. Resp. Crit. Care 180(11), 1068-1075.

Saari, S. (2015) Seasonal and diurnal variations of fluorescent bioaerosol concentration and size distribution in the urban environment. Aerosol and Air Qual. Res. 15, 572-581.

Simon-Nobbe, B., Denk, U., Poll, V., Rid, R., Breitenbach, M. (2008) The spectrum of fungal allergy. Int. arch. allergy imm. 145 (1), 58-86.

Smets, W., Moretti, S., Denys, S., Lebeer, S. (2016) Airborne bacteria in the atmosphere: presence, purpose, and potential. Atmos. Environ. 139, 214-221.

Sousa, S.I.V., Martins, F.G., Pereira, M.C., Alvim-Ferraz, M.C.M., Ribeiro, H., Oliveira, M., Abreu, I. (2008) Influence of atmospheric ozone, PM_{10} and meteorological factors on the concentration of airborne pollen and fungal spores. Atmos. Environ. 42(32), 7452-7464.

Tsai, F.C., Macherb, J.M., Hung, Y.Y. (2007) Biodiversity and concentrations of airborne fungi in large US office buildings from the BASE study. Atmos. Environ. 41,5181-5191.

van Doorn, R., Szemes, M., Bonants, P., Kowalchuk, G.A., Salles, J.F., Ortenberg, E., Schoen, C.D. (2007) Quantitative multiplex detection of plant pathogens using a novel ligation probe-based system coupled with universal, high-throughput real-time PCR on OpenArrays. BMC genomics 8, 276.

Vermani, M., Vijayan, V.K., Kausar, M.A., Agarwal, M. K. (2010) Quantification of airborne *Aspergillus* allergens: redefining the approach. J. Aasthma. 47(7), 754-761.

Wang, X., Chen, J., Sun, J., Li, W., Yang, L., Wen, L., Wang, W., Wang, X., Collett Jr, J.L., Shi, Y., Zhang, Q., Hu, J., Yao, L., Zhu, Y., Sui, X., Sun, X., Mellouki, A. (2014) Severe haze episodes and seriously polluted fog water in Ji'nan, China. Sci. Total Environ. 493, 133-137.

Wang, L., Song Z.W., Xu, A.L., Wu, D.D., Yan, X. (2015) Phylogenetic diversity of airborne microbes in Qingdao downtown in autumn. J. Appl. Ecol. in Chinese 26, 1121-1129.

Wei, M., Xu, C.H., Chen, J.M., Zhu, C., Li, J.R., Lv, G.L. (2007) Characteristics of bacterial community in cloud water at Mt Tai: Similarity and disparity under polluted and non-polluted cloud episodes. Atmos. Chem. Physic. 17:5253-5270

Womiloju, T.O., Miller, J.D., Mayer, P.M., Brook, J.R. (2003) Methods to determine the biological composition of particulate matter collected from outdoor air. Atmos. Environ. 37(31), 4335-4344.

Womack, A.M., Artaxo, P.E., Ishida, F.Y., Mueller, R.C., Saleska, S.R., Wiedemann, K.T., Bohannan, B.J.M., Green, J.L. (2015) Characterization of active and total fungal communities in the atmosphere over the Amazon rainforest. Biogeosciences 12, 6337-6349.

Xu, C.H., Wei, M., Chen, J.M., Wang, X.F., Sui, X., Zhu, C., Li, J.R., Zheng, L.L., Sui, G.D., Li, W.J., Wang, W.X., Zhang, Q.Z., Mellouki, A. (2017a) Bacterial characterization in ambient submicron particles during severe haze episodes at Ji'nan, China. Sci. Total Environ. 580: 188-196.

Xu, C.H., Wei, M., Chen, J.M., Sui, X., Zhu,C., Li, J.R., Zheng, L.L., Sui, G.D., Li, W.J., Wang, W.X., Zhang, Q.Z., Mellouki, A. (2017b) Investigation of diverse bacteria in cloud water at Mt. Tai, China. Sci. Total Environ. 580: 258-265.

Yadav, R.K.P., Halley, J.M., Karamanoli, K., Constantinidou, H.I., Vokou, D. (2004) Bacterial populations on the leaves of Mediterranean plants: quantitative features and testing of distribution models. Environ. Exp. Bot. 52(1), 63-77.

Yamaguchi, N., Baba, T., Ichijo, T., Himezawa, Y., Enoki, K., Saraya, M., Li, P.F., Nasu, M. (2016) Abundance and community structure of bacteria on Asian dust particles collected in Beijing, China, during the Asian dust Season. Biological and Pharmaceutical Bulletin 39 (1), 68-77.

Yan, D., Zhang, T., Su, J., Zhao, L.L., Wang, H., Fang, X.M., Zhang, Y.Q., Liu, H.Y., Yu, L.Y. (2016) Diversity and composition of airborne fungal community associated with particulate matters in Beijing during haze and non-haze days. Front. Microbiol. 7, 487.

Yamamoto, N., Bibby, K., Qian, J., Hospodsky, D., Rismani-Yazdi, H., Nazaroff, W.W., Peccia, J. (2012) Particle-size distributions and seasonal diversity of allergenic and pathogenic fungi in outdoor air. ISME J. 6(10), 1801-1811.

Yu, J.Z., Tung, J.W.T., Wu, A.W.M., Lau, A.K.H., Louie, P.K.K., Fung, J.C.F. (2004) Abundance and seasonal characteristics of elemental and organic carbon in Hong Kong PM_{10}. Atmos. Environ. 38, 1511-1521.

Zhang, Z., Engling, G., Zhang, L., Kawamura, K., Yang, Y., Tao, J., Zhang, R., Chan, C.Y., Li, Y. (2015) Significant influence of fungi on coarse carbonaceous and potassium aerosols in a tropical rainforest. Environ. Res. Lett. 10(3), 034015.

Zhong, X., Qi, J., Li, H., Dong, L., Gao, D. (2016) Seasonal distribution of microbial activity in bioaerosols in the outdoor environment of the Qingdao coastal region. Atmos. Environ. 140, 506-513.

Zhou, M., Liu, Y., Wang, L., Kuang, X., Xu, X., Kan, H. (2014) Particulate air pollution and mortality in a cohort of Chinese men. Environ. Poll. 186, 1-6.

Zhu, C., Kawamura, K., Fukuda, Y., Mochida, M., Iwamoto, Y. (2016) Fungal spores overwhelm biogenic organic aerosols in a midlatitudinal forest. Atmos. Chem. Phys. 16(11),7497-7506.

Table and Figure Captions

Table 1. Sample descriptions and the associated meteorological characteristics of the atmosphere, including the temperature, relative humidity, visibility, $PM_{2.5}$ mass concentration, PM_1 mass concentration, and fungal cell concentrations on the basis of qPCR analysis of SSU rRNA gene copy numbers in $PM_{2.5}$ and PM_1.

Table 2. The relative abundance of the top 5 orders and 2 genera in TSP, PM_{10}, $PM_{2.5}$, and PM_1 (RAS^a indicates relative abundance in submicron particles, and RAF^b indicates relative abundance in fine particles).

Table 3. Metastats analysis showing the fungal genera that are significantly different among $PM_{2.5}$ and PM_1.

Figure 1. Relationships between fungal number concentrations of $PM_{2.5}$ and PM_1 with wind speed and wind direction.

Figure 2. Statistical comparisons of OTUs, and Chao1 and Shannon indices among summer, autumn, and winter in $PM_{2.5}$ and PM_1.

Figure 3. Relative abundances of fungal communities of $PM_{2.5}$ and PM_1 at phylum (A), class (B), and order level (C).

Figure 4. Variance analysis of fungal genera based on the Kruskal-Wallis rank sum test.

Figure 5. Heatmap analysis of the top 64 fungal genera based on Spearman's rank correlations (***$p < 0.001$; **$p < 0.01$; *$p < 0.05$). Red arrows indicate that the specific fungi varied significantly in different seasons.

Table 1.

Season	Date of Collection	T	RH	$PM_{2.5}$				PM_1			
				MC	Fungal SSU rRNA gene copy number	Fungal spore OC MC	Fungal spore MC	MC	Fungal SSU rRNA gene copy number	Fungal spore OC MC	Fungal spore MC
Unit		^{o}C	%	μg/m^3	E+04 copy/m^3	ng C /m^3	μg /m^3	μg/m^3	E+04 copy/m^3	ng C /m^3	μg /m^3
Summer	06/25/15	12.6	98	5.5	11.00	7.15	0.02	BDL	18.00	11.69	0.03
	06/26/15	14	97	2.8	3.58	2.33	0.01	BDL	6.51	4.23	0.01
	06/27/15	15.1	94.6	52.7	1.01	0.66	0.00	18.1	0.40	0.26	0.00
	06/28/15	16.9	84.4	91.1	6.85	4.45	0.01	40.0	3.10	2.02	0.01
	06/29/15	17.3	62.6	16.8	4.71	3.06	0.01	13.3	2.79	1.81	0.00
	07/03/15	17.7	31.0	15.3	12.20	7.92	0.02	12.7	11.40	7.42	0.02
	07/07/15	16.9	84.4	94.0	47.70	31.00	0.08	39.9	4.20	2.73	0.01
	07/08/15	17.3	62.6	110.9	22.60	14.71	0.04	42.0	5.31	3.45	0.01
	08/07/15	17.4	97.6	13.5	5.64	3.67	0.01	11.4	6.94	4.51	0.01
Autumn	10/22/14	6.7	60.7	40.1	9.34	6.07	0.02	28.1	0.37	0.24	0.00
	10/25/14	10.3	80.7	48.1	7.28	4.73	0.01	34.6	45.70	29.73	0.08
	10/26/14	11.4	73.6	50.8	6.78	4.41	0.01	31.9	8.26	5.37	0.01
	11/03/14	0.3	21.6	4.9	1.57	1.02	0.00	BDL	22.20	14.44	0.04

	11/04/14	2.6	33.7	31.6	7.95	5.17	0.01	24.5	6.18	4.01	0.01
	11/05/14	4.1	30.9	33.1	3.70	2.41	0.01	25.6	103.00	66.75	0.17
	11/06/14	5.1	19.3	22.7	12.70	8.24	0.02	18.0	8.86	5.76	0.01
	11/07/14	2.6	34.0	19.8	7.89	5.13	0.01	16.0	6.39	4.15	0.01
	11/08/14	2.4	45.7	22.4	14.70	9.54	0.02	17.8	2.92	1.90	0.00
	11/09/14	1.1	73.1	77.1	5.56	3.61	0.01	33.5	3.61	2.34	0.01
	11/10/14	3.0	49.0	49.2	9.38	6.10	0.02	37.2	16.70	10.87	0.03
	11/11/14	2.7	65.4	32.7	27.50	17.85	0.05	25.3	26.30	17.07	0.04
	11/12/14	1.0	50.1	51.7	7.50	4.87	0.01	25.7	18.30	11.87	0.03
Winter	12/03/14	-8.9	24.4	13.7	5.03	3.27	0.01	9.7	6.84	4.45	0.01
	12/04/14	-11	39.1	35.0	8.68	5.64	0.01	30.6	2.78	1.81	0.00
	12/05/14	-10.6	23.4	14.5	1.09	0.71	0.00	13.3	16.20	10.52	0.03
	12/06/14	-5.7	11.0	9.1	6.32	4.11	0.01	8.3	4.15	2.70	0.01
	12/07/14	-5.4	45.7	38.8	7.90	5.14	0.01	30.9	9.36	6.08	0.02
	12/08/14	-7.9	35.7	36.5	1.33	0.86	0.00	29.0	7.17	4.66	0.01
	12/09/14	-5.3	16.1	16.5	10.10	6.55	0.02	13.5	5.73	3.72	0.01
	12/10/14	-5.6	58.3	9.3	3.24	2.10	0.01	8.1	7.10	4.62	0.01

*MC-Mass Concentration *T-Temperature *RH-Relative Humidity *BDL-Below the detection Line

Table 2.

Taxonomic level	Common Fungi	RAS[a]	RAF[b]	References	Samplers	Sample Type	Concentration or Abundance
Genera	Alternaria	11.7	6.2	Adhikari et al., 2004	Andersen sampler (Thermo Andersen, Smyrna, 300082-5211, USA)	TSP	2.6%
				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_1	>1%
	Aspergillus	2.3	1.9	Cao et al., 2014	Air samplers (Thermo Electron Corp., MA, U.S.)	PM_{10} and $PM_{2.5}$	Abundant
				Gou et al., 2016	Low volume air sampler (BGI, USA)	PM_{10} and PM_1	Abundant
Order	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^{-1})	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
				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^{-1})	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^{-1})	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
				Yan et al., 2016	Air samplers (Air Metrics, USA, 5 L min^{-1})	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

Table 3.

Taxa	PM_1			$PM_{2.5}$			*p* value	q value
	Mean	Std.err	Variance	Mean	Std.err	Variance		
Glomerella	10.51984	0.021813	0.013798	22.49025	0.01807	0.009796	0.000999	0.025543
Zasmidium	6.523201	0.011769	0.004017	12.71881	0.012239	0.004494	0.000999	0.025543
Phyllosticta	2.507228	0.004038	0.000473	5.948659	0.004366	0.000572	0.000999	0.025543
Preussia	0.039161	0.000195	1.10E-06	0.009109	6.81E-05	1.39E-07	0.002322	0.042885
Truncatella	0.030579	0.00024	1.67E-06	0.005152	2.44E-05	1.79E-08	0.002784	0.046274
Umbelopsis	0.027549	0.000252	1.84E-06	0.005369	2.55E-05	1.95E-08	0.001669	0.034675
Sebacina	0.021306	0.000196	1.11E-06	0.001261	1.26E-05	4.77E-09	0.000550	0.022857
Cordyceps	0.020939	0.000137	5.45E-07	0.002518	1.75E-05	9.18E-09	0.001392	0.030848

Figure 1.

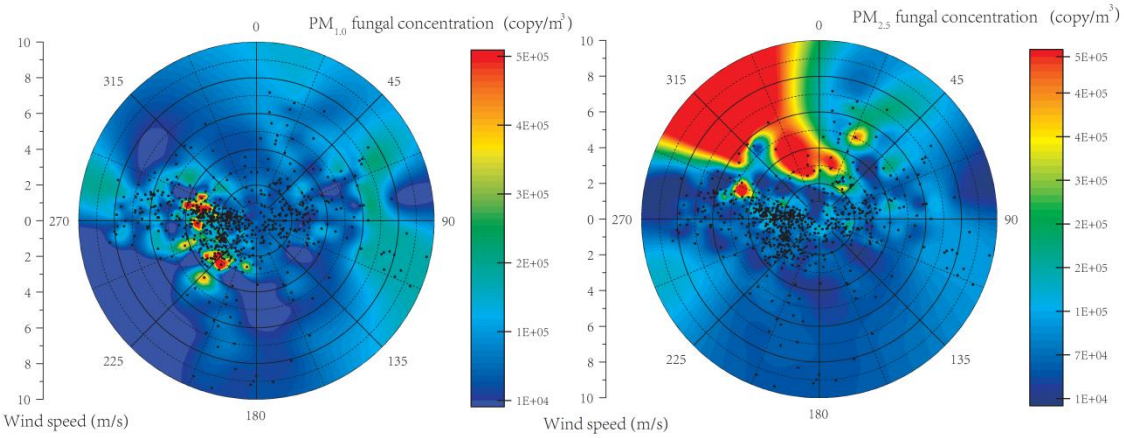

PM1.0 fungal concentration (copy/m3)
PM2.5 fungal concentration (copy/m3)
0
45
90
135
180
225
270
315
Wind speed (m/s)
5E+05
4E+05
3E+05
2E+05
1E+05
1E+04
7E+04

Figure 2.

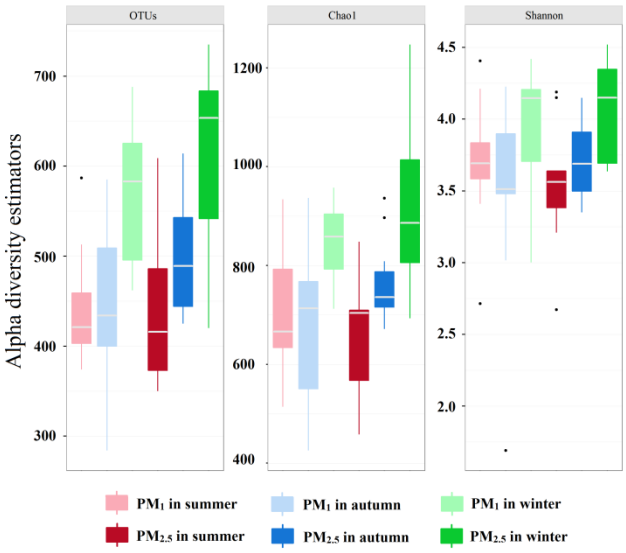

OTUs
Chao1
Shannon
Alpha diversity estimators
700
600
500
400
300
1200
1000
800
600
400
4.5
4.0
3.5
3.0
2.5
2.0
PM1 in summer
PM1 in autumn
PM1 in winter
PM2.5 in summer
PM2.5 in autumn
PM2.5 in winter

Figure 3.

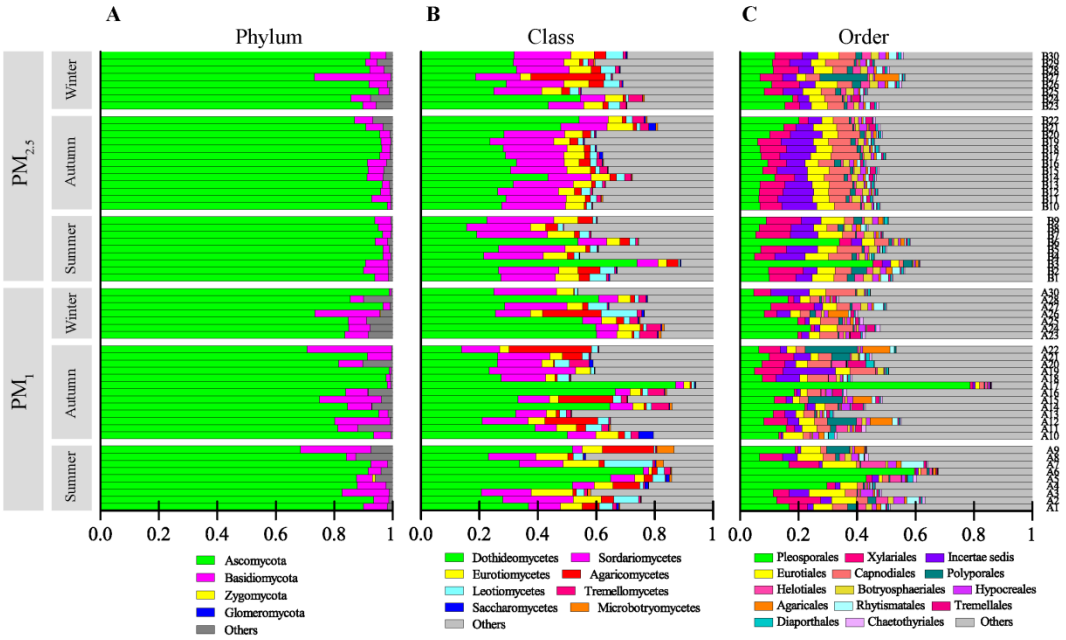

A
Phylum
B
Class
C
Order
PM2.5
PM1
Winter
Autumn
Summer
Winter
Autumn
Summer
0.0
0.2
0.4
0.6
0.8
1
Ascomycota
Basidiomycota
Zygomycota
Glomeromycota
Others
Dothideomycetes
Sordariomycetes
Eurotiomycetes
Agaricomycetes
Leotiomycetes
Tremellomycetes
Saccharomycetes
Microbotryomycetes
Others
Pleosporales
Xylariales
Incertae sedis
Eurotiales
Capnodiales
Polyporales
Helotiales
Botryosphaeriales
Hypocreales
Agaricales
Rhytismatales
Tremellales
Diaporthales
Chaetothyriales
Others

Figure 4.

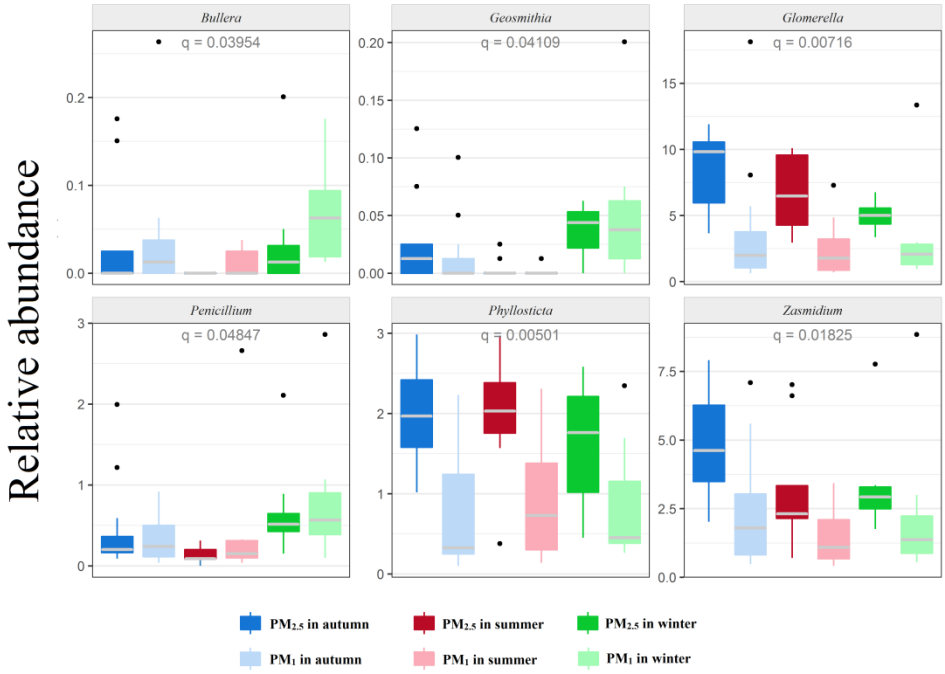
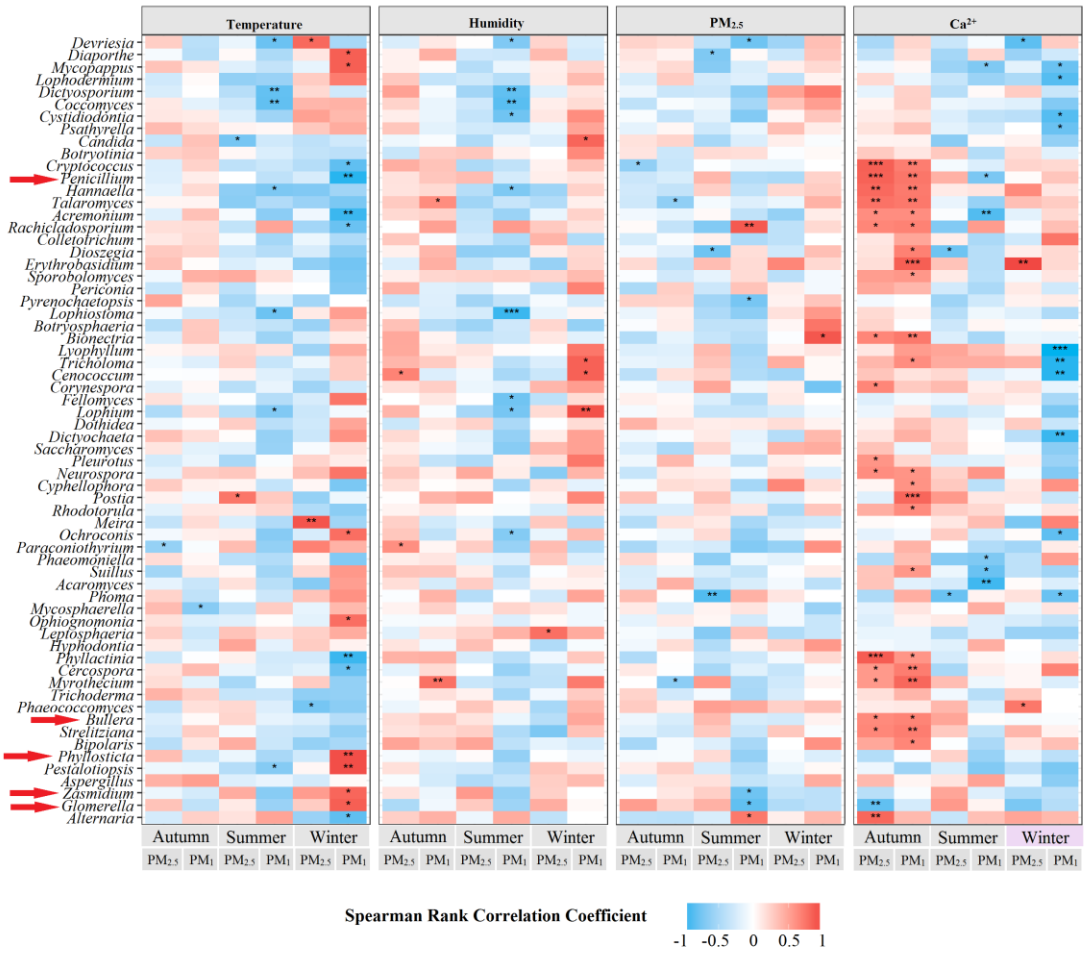

Relative abundance
Bullera
q = 0.03954
Geosmithia
q = 0.04109
Glomerella
q = 0.00716
Penicillium
q = 0.04847
Phyllosticta
q = 0.00501
Zasmidium
q = 0.01825
PM2.5 in autumn
PM2.5 in summer
PM2.5 in winter
PM1 in autumn
PM1 in summer
PM1 in winter

Figure 5.


Temperature
Humidity
PM2.5
Ca2+
Devriesia
Diaporthe
Mycopappus
Lophodermium
Dictyosporium
Coccomyces
Cystidiodontia
Psathyrella
Candida
Botryotinia
Cryptococcus
Penicillium
Hannaella
Talaromyces
Acremonium
Rachicladosporium
Colletotrichum
Dioszegia
Erythrobasidium
Sporobolomyces
Periconia
Pyrenochaetopsis
Lophiostoma
Botryosphaeria
Bionectria
Lyophyllum
Tricholoma
Cenococcum
Corynespora
Fellomyces
Lophium
Dothidea
Dictyochaeta
Saccharomyces
Pleurotus
Neurospora
Cyphellophora
Postia
Rhodotorula
Meira
Ochroconis
Paraconiothyrium
Phaeomoniella
Suillus
Acaromyces
Phoma
Mycosphaerella
Ophiognomonia
Leptosphaeria
Hyphodontia
Phyllactinia
Cercospora
Myrothecium
Trichoderma
Phaeococcomyces
Bullera
Strelitziana
Bipolaris
Phyllosticta
Pestalotiopsis
Aspergillus
Zasmidium
Glomerella
Alternaria
Autumn
Summer
Winter
PM2.5
PM1
Spearman Rank Correlation Coefficient
-1
-0.5
0
0.5
1