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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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Abstract. Fungi are ubiquitous throughout the near-surface atmosphere, where they represent an important component of primary biological aerosol particles. This study combined the 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. The fungal abundance was 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 was from Ascomycota and Basidiomycota which are known to discharge actively spores into the atmosphere. The fungal community showed a significant seasonal shift across different size fraction based on the metastats analysis and kruskal-wallis rank sum test. The abundance of *Glomerella* and *Zasmidium* increased and decreased as the particle size got bigger in autumn. Nevertheless, *Penicillum*, *Bullera*, and *Phaeosphaeria* increased in smaller particles in winter. This work may serve as an important reference for the fungal contribution to primary biological aerosol particles.

1 INTRODUCTION

The inhaled particulate matter (PM), categorized as $PM_{2.5}$ and PM_1 (aerodynamic equivalent diameters of ≤2.5 μm and ≤1 μm, respectively) is proved to be associated with the increasing morbidity and mortality from the cardiovascular system and respiratory system diseases (Wang et al., 2014; Brauer et al., 2013). Primary biological aerosol particles (PBAPs, about 10^4-10^8 cells cm^{-2}), constitute an important component of PM. They can actively metabolize with the mass concentration ranged from 5.49 to 102 ng m^{-3} (Zhong et al., 2016) and play an important role in agriculture, 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 PBAP, are broadly distributed in rural and urban environments with approximate 1.5 million unique species (Hawksworth et al., 2001). They eject their spores actively with aqueous jets or droplets into the atmosphere. The global emissions of fungal spores are estimated as the largest sources of organic aerosol (Elbert et al., 2007).

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Pioneering researches reported that the global fungal emission reached to 28 Tg per year and contributed to about 4-13% of $PM_{2.5}$ mass concentration (Heald et al., 2009; Womiloju et al., 2003). In addition, diverse fungi 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). More recently, several fungal species (potential pathogens and allergens for human, animal, and plant) are 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 fungal abundance, diversity, and community structure associated with PM have received limited attentions.

Quantitative assessment of fungal community can be achieved by various techniques such as cultured methods, terminal restriction length polymorphism analysis, nucleic acid sequencing, and single-molecule sequencing technologies (Huang et al., 2002; Bowers et al., 2013; Almaguer et al., 2014). Earlier study on the airborne fungal community, primarily based on the cultured techniques, found the most frequent phyla Ascomycota (AMC) and Basidiomycota (BMC). Some of them are considered as major pathogens and allergens of plant, animal, and human such as *Hemileia vastatrix*, *Aspergillus*, *Cryptococcus*, and *Pneumocystis spp* (Despres et al., 2012; Smets et al., 2016). While the cultivable species (typically <<100) occupies only a tiny minority of the whole species and the vast majority of fungal species arestill unknown. Advances in nucleic acid sequencing allow the accurate determination of uncultured fungal communities. Frohlich-Nowoisky (2009) described the diverse fungal community in coarse (>3 μm) and fine (≤3 μm) particulate matter using internal transcribed spacer region sequencing in Mainz, Germany. Afterward 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) and the fall season, whereas the pathogens were most abundant in the spring season and typically observed in particles with aerodynamic diameters <4.7 μm. Subsequently, DeLeon-Rodriguez (2013) discussed the effect of tropical storm or hurricanes 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 by 18S rRNA gene sequencing. Xu et al. (2017a) investigated the abundance and community of bacteria in submicron particles during severe haze episodes in Jinan, China based on the 16S rDNA sequencing, meanwhile Xu et al. (2017b) discussed the diurnal variation of diverse bacterial community in cloud water. However, the relevant study commonly focused on the microbial communities in total suspended particles (TSP), PM_{10}, and $PM_{2.5}$ and conducted over the Earth's surface. The investigation on the seasonal variation of ambient fungi in PM_1 especially at a high-elevation site remained uncharacterized. Hence, we selected a typical mountain, Mt. Tai, which is the highest in the North China Plain, China, as the sampling site in this area.

Mt. Tai (36°15′N, 117°06′E, 1534 m a.s.l), 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. However, the vegetation is limited to bushes and the ground surfaces of sampling site are mostly covered with rocks. In 2014 and 2015, the number of tourists from both China and abroad has increased from 5.5 to 5.9 million. The existing studies are mainly concentrated on the physicochemical characteristics of particles and their influence on the air quality and human health. To evaluate the primary biological aerosol particles in such scenic outlook, it is essential to build a sophisticated finished knowledge on the atmospheric aerosol. The aim of this study is to demonstrate the framework of fungal community in $PM_{2.5}$ and PM_1 across summer, autumn, and winter. Meanwhile we calclulated the fungal abundance and its

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contribution to the mass concentration of $PM_{2.5}$ and PM_1. Combined the chemical characteristic and meteorological factors, we evaluated four crucial factors associated with the seasonal variation of fungal community in $PM_{2.5}$ and PM_1.

2 MATERIAL AND METHOD

2.1 Sample collection

In this region, spring was from March to May; Summer including June, July, and August; fall includes September, October, and November; and winter was from December to February next year according to the environmental temperature. The $PM_{2.5}$ and PM_1 were collected on the quartz membrane filters (PALL, NY, U.S., 88mm) by two middle volume samplers (TH-100A, Wuhan Tianhong Instruments Co., Ltd., China, flow rate: 100 L min^{-1}) 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. The blank filters were obtained by placing a sterilized quartz microfiber filters inside of the sampler without operation. Before sampling, all the filters were baked at a Muffle furnace at 500 ^oC for 5 h, and then placed into sterilized aluminum foil before deposited into a sealed bag. To avoid the 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 ^oC until the next analysis. The $PM_{2.5}$ and PM_1 mass concentration were monitored by synchronized hybrid ambient real-time particulate monitor (Model 5030, Thermo Fisher Scientific, USA). Half of $PM_{2.5}$ 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 ambient ion monitor (URG-9000, Chapel Hill, NC) and the remaining filter was used for DNA extraction for real-time quantity PCR and sequencing. The 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 the maximum limit of 20 km.

2.2 DNA Extraction and PCR Amplification

The DNA was extracted using the Power Water DNA isolation kit (MoBio, USA) according to the manufacturer's instructions. A parallel extraction procedure was performed with the blank filter to check for sample contamination. The DNA concentrations were quantified by NanoDrop 2000 Spectrophotometer (Thermo Fisher Scientific, Wilmington, DE, USA). 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 internal transcribed spacer (ITS) region of the rRNA gene (Manter et al., 2007). The experiment was conducted using an Applied Biosystems® Gene Amp® PCR System 9700 (Applied Biosystems) in a total volume of 50 μL PCR mix containing PCR buffer (1x), 1.5 μM $MgSO_4$, 0.4 μM each deoxynucleoside triphosphate, 0.3 μM forward primer, 0.3 μM reverse primer, 0.5 U of Ex Taq (TaKaRa, Dalian, China), 100 ng template DNA, and double distilled H_2O. The PCR amplification was performed at 94 ^oC for 1 min, followed by 35 cycles (denaturation at 98 ^oC for 20 s, annealing 68^oC for 30 s, and elongation at 72 ^oC for 45 s), and a final extension at 72 ^oC for 5 min. Three replicates of PCR reactions for each sample were combined together. The final products were separated by 1.5% agarose gel electrophoresis and purified using Qiaquick PCR purification kit

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

2.3 Sequence Analyses

The raw reads were deposited into the NCBI Sequence Read Archive (SRA) database under accession number SRR5146156. After quality control, high quality reads were clustered into Operational taxonomic units (OTUs) at 97% similarity cutoff using USEARCH software (version 7.1, http://drive5.com/uparse/) and the chimeric sequences were removed using UCHIME algorithm (Edge et al., 2011). We used the OTUs as a basis for estimating the alpha diversity and beta diversity. The taxonomy of each ITS1 sequence was analyzed by RDP Classifier against the Unite database (Release 7.0, http://unite.ut.ee/index.php, Koljalg et al., 2013) using confidence threshold of 70%. The RDP Classifier was used to the determined the taxonomic composition at the phylum, class, order, family, genus, and species levels (Koiv et al., 2015; Miettinen et al., 2015). The alpha diversity estimators including Chao1, Good's coverage estimator, Simpson's index, and Shannon's index were calculated by Quantitative Insights Into Microbial Ecology software (version 1.8.0, http://qiime.org/scripts/assign_taxonomy.html, Kuczynski et al., 2011).

2.4 Real Time PCR for ITS region

To determine the fungal biomass, we performed the real-time qPCR using a CFX96 real-time PCR detection system (Bio-Rad, Hercules, CA) in 25 μL reaction mixture 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 distillation H_2O. The amplification followed a three step PCR for fungal ITS regions: 40 cycles with denaturation at 95 ℃ for 30s, primer annealing at 52 ℃ for 30s, and extension at 72 ℃ for 30s. A standard curve was created using 10-fold dilution series of fungal ITS region plasmids. Assuming that the average fungal genome has about 30-200 rRNA copies, the fungal concentration were calculated using the method described by van Doorn et al. (2007).

2.5 Fungal contribution to atmospheric OC

Organic carbon (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). The ambient fungi are conventionally considered as a possible OC source in PMs. Previously, Cheng et al. (2009) have estimated that the mean fungal OC concentration were 3.7, 6.0 and 9.7 ng m^{-3}, and they corresponded to 0.1%, 1.2% and 0.2% of the total OC in $PM_{2.5}$, $PM_{2.5-10}$ and PM_{10} in Hong Kong, respectively. The contributions of fungal spores to OC were calculated using tracer-based methods according to the mannitol levels. We assumed that there were 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 use the weighted-average carbon conversion factor of 13 pg C per spore and of 33 pg fresh weight per spore which 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).

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2.6 Statistical Analyses

To describe significant difference of the fungal community variations among different size fractions, the mata-analysis 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 a mean, variance, and standard error together with a p-value and q-value. Generally, we define the significant difference when the p value ≤ 0.05 and q value ≤ 0.05. The Kruskal-Wallis Rank Sum Test was used to evaluate the seasonal variation of the microbial community. Boxplots and p values were given 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. The results were considered to be significant with *P* value lower than 0.05.

3 RESULT AND DISCUSSION

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

Over the sampling period, $PM_{2.5}$ and PM_1 were collected at the summit of Mt. Tai during summer, autumn, and winter. The quantitative PCR of the gene SSU rRNA revealed an average fungal SSUrRNA gene copy number of 9.4×10^4 copies m^{-3} (ranged from 1.0×10^4 to 4.8×10^5 copies m^{-3}) and 1.3×10^5 copies m^{-3} (ranged from 3.7×10^3 to 1.0×10^6 copies m^{-3}) in $PM_{2.5}$ and PM_1, which were much higher that obtained in upper troposphere (6.8×10^2 copies m^{-3}) (DeLeon-Rodriguez et al., 2013). 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. According to the guideline developed by the Chinese Academy of Sciences Ecological Environmental Research Center (800 CFU m^{-3}), the fungal number concentration at Mt. Tai was mainly at a non-polluted level. The concentration was lower than other countries including Korea (ranged from 9.56×10^1 to 4.2×10^4 cells m^{-3}, Lee et al., 2010), UK (ranged from 891 to 964 spores m^{-3}, Oliveira et al., 2009), and US (6450 spore m^{-3}, Tsai et al., 2007). The discrepancy between our results and that of Lee et al. (2010) may be due to the different sample type, sampling time, and altitudes. Lee et al. (2010) focused on the fungal concentration in the total suspended particles (TSP) by high volume TSP sampler (0.225 m^3 min^{-1}) in 15 m above the ground in autumn and winter, while we obtained the $PM_{2.5}$ and PM_1 by middle volume samplers (0.1 m^3 min^{-1}) at 1534 m above the ground in summer, autumn, and winter. Another explanation for that difference was underestimated the fungal number. We used a higher gene copy number of 200 for each microbe we based upon. While DeLeon-Rodriguez et al. (2013) employed a lower number of rRNA copies of fungal genomes (ranging 30-100 copies per genome), thus induced the unified results of fungal number concentration.

The fungal abundance varied with seasons during the near-surface atmosphere. Saari et al. (2015) reported the increase of coarse fluorescent bioaerosol particles (FBAP, 1.5-5 μm) in summer. While in winter, the fine FBAP primarily existed in smaller particles (0.5-1.5 μm). The snow cover and biological activity in winter resulted in the coarse FBAP's vanishment. In this study, the highest fungal concentration in $PM_{2.5}$ (641 spores m^{-3}) was observed in summer, whereas the highest value in PM_1 (1033 spores m^{-3}) was found in autumn indicated different origins of ambient fungal spores. The fine FBAP was considered to be influenced by long-range transport and anthropogenic sources such as combustion (Huffman et al., 2010). We hypothesized that the straw combustion in north China during

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the harvest season may be the main reason of the fungal enhancement of PM_1. In addition, the phenomenon may be explained by the meteorological diversity (Abdel Hameed et al., 2012). The low wind speed hinders the fungal dispersal due to the accumulation effect. Almaguer et al. (2014) presented the calm winds coming from the southwest direction (rural areas) induced the accumulation of the fungal spore at the Northern coast in the island of Cuba. Lin et al. (2000) observed the wind speed (< 4 m/s) strongly negative correlated with fungal concentration, and the fungal concentration increased as the wind speed higher than 5 m/s. Herein the fungal abundance in PM_1 showed no obvious increase under the breeze condition (wind speed < 2 m/s) mainly come from south direction (Figure 1). When wind speed higher than 2 m/s, the fungal abundance increased under the influence of west wind clearly. With the strength of west wind velocity, the growth velocity of fungal concentration decreased. While in $PM_{2.5}$, the fungal abundance increased under condition that the wind speed higher than 2 m/s mainly from northwest direction from continental areas where various vegetation and plants are grown. The fact that airflow blows from west and northwest direction acted on the fungal abundance implied that the fungal concentration at Mt. Tai highly depend on weather conditions.

3.2 Contribution of spores to OC concentrations and PM mass

The range and average concentrations of fungal contribution to atmospheric OC and mass concentration in $PM_{2.5}$ and PM_1 observed were listed in Table 1. The daily averaged concentrations of fungal OC in $PM_{2.5}$ and PM_1 were 6.1and 8.3 ng C m^{-3}, while the respective contribution to PMs were 0.067% and 0.096%, respectively, suggesting that the fungi are a non-negligible source of carbonaceous aerosol at Mt. Tai. The fungal contribution to OC obtained at Mt. Tai was comparable with another result of 3.7 ng C m^{-3} obtained at an urban site in Hong Kong (Cheng et al., 2009), but lower than that calculated 117.9 ng C m^{-3} at an urban site in Austria (Bauer et al., 2008), a forest site in Japan (Zhu et al., 2016), and 147-923 ng C m^{-3} at a forest site on Hainan Island (Zhang et al., 2015). The discrepancy between the abovementioned studies can be justified by the different particle type (TSP, PM_{10}, $PM_{2.5}$, and PM_1), fungal concentration, spore carbon content value, and assessment method, e.g., sugar alcohol, cultivation, mannitol, and light microscope. Based on the same conversion factor of 13 pg C $spore^{-1}$ by mannitol, the results were much lower that obtained 0.3 ± 0.2 μg C m^{-3} at an urban site in Beijing (Liang et al., 2017) which 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 at urban areas in the North China Plain.

3.3 Ambient fungal diversity and taxonomic composition

In this study, about 509 and 475 OTUs were obtained in $PM_{2.5}$ and PM_1, respectively. It was slightly higher than the previous fungal studies associated with the PMs in Beijing (China, 34-285, Yan et al., 2016) and Rehovot (Israel, 121-178, Dannemiller et al., 2014). Furthermore, the OTUs associated with $PM_{2.5}$ was slightly higher than those associated with PM_1, implying higher richness of fungal spores in larger PMs. Meanwhile, the Shannon and Chao1 index which showed the same trends in $PM_{2.5}$ and PM_1 also indicated a higher diversity in larger PMs (Figure 2). The ambient fungi showed a highest richness and diversity in winter followed by autumn and summer. The low temperature and low biological activity may favor to the growth and development of specific cryophilic fungal spores in winter.

For the fungal community, AMC (89.7%) and BMC (7.0%) were the predominant phyla, the most of which were known to discharge spores into atmosphere actively. The remaining phyla were Zygomycota (ZMC) and Glomeromycota. The abundance of AMC and BMC herein were similar with previous fungal researches observed at

high altitudes at North American (3200 m m a.s.l, Bowers et al., 2009) and South Germany (990 m a.s.l, Despres et al., 2007). Moreover, AMC and BMC showed 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, the BMC (64%) seems to be more abundant than AMC (34%). The abundance of AMC sampled in marine sites (72%) was about 2.6 times higher than BMC. While at Mt. Tai, the abundance of AMC was approximately 12.8 times higher than that of BMC. It may be affected by the Yellow Sea and Bohai Sea. Moreover, the AMC have single-celled or filamentous vegetative growth forms that are help to be aerosolized compared to BMC (Womack et al., 2015). Further a total of 10 classes belonging to AMC, 10 classes belonging to BMC, and 1 class belonging to ZMC were observed. The preponderant classes belonging to AMC were Dothideomycetes (37.3%), Sordariomycetes (15.0%), and Eurotiomycetes (6.1%) (Figure 3). The dominate orders affiliating to Dothideomycetes class include the Pleosporales (14.9%), Capnodiales (5.3%), and Botryosphaeriales (1.6%). These three orders were often detected in atmosphere as described by Oh et al. (2014). Pleosporales was reported as important allergenic fungi to local residents (Rittenour et al., 2014). Likewise, the dominate class in BMC were Agaricomycetes (4.4%) and Tremellomycetes (1.5%) including orders Polyporales (2.5%), Agaricales (1.6%), and Tremellales (1.2%). Nearly all the Agaricomycetes were terrestrial and widely found in atmospheric environment.

About 291 taxa from the genera level were determined including *Alternaria*, *Glomerella*, *Zasmidium*, *Pestalotiopsis*, *Aspergillus*, and *Phyllosticta*. To date, about 123 fungi genera (mainly affiliating to Ascomycota phylum) were considered as members of humans allergies (Simon-Nobbe et al., 2008). Of the 11 potentially allergy-inducing AMC species and 1 potentially allergy-inducing BMC species found at Mt. Tai, three most common species were *Aspergillus flavus*, *Blumeria graminis*, and *Saccharomyces cerevisiae*. *Aspergillus flavus* is a common human pathogen found in air as well as human allergen and mycotoxin producer (Adhikari et al., 2004). It is associated with invasive aspergillosis and superficial infection (Hedayati et al., 2007). *Blumeria graminis* which found in the surface of plant leaves is considered as the cause agent of powdery mildew on cereal plants (Belanger et al., 2003). Such pathogen and allergens are expected to be widely dispread around atmospheric environment in temperate and tropical zones (Vermani et al., 2010; Yamamoto et al., 2012). Our results also revealed that the abundance of potential allergenic and pathogenic fungal spores in summer were highest compared to autumn and winter. The clinicians should consider the fungal spores described herein as a possible cause of human and plant disease attacks under long time exposure to airborne particles throughout the whole year, especially from summer season. What's more, the abundance of 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 the smaller particles. The phenomenon should be drawn a high attention from all residents even the visitors at Mt. Tai.

3.4 Size distribution and seasonal variation of fungal communities

Not just the fungal abundance, but the fungal community showed seasonal mode across different size fractions (Awad et al., 2013). Yamamoto et al. (2012) presented that the pathogenic fungi 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. Previous researchers identified many environmental factors which have significant influence on the fungal release and growth (Almaguer et al., 2014) such as temperature, NO_2, PM_{10}, SO_2, CO, relative humidity (Yan et al., 2016), and accidental events, e.g., dust (Prospero et al., 2005), rainfall (Zhang et al., 2015), hurricane (DeLeon-

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Rodriguez et al., 2013), and haze (Yan et al., 2016)), radiation, vegetation, and urbanization (Moreau et al., 2016). In this study, discrepant size distribution of fungal community was observed based on the matastat analysis by permutation t-test (Table 2). The abundance of Glomerella, Zasmidium, and Phyllosticta enriched in $PM_{2.5}$, while the abundance of Preussia, Truncatella, Umbelopsis, Sebacina, and Cordyceps increased in PM_1. Combined with the Kruskal-Wallis Rank Sum test (Figure 4), we found seven fungal genus from the top 40 fungal genera showed apparent seasonal variation. *Glomerella* and *Zasmidium* increased in autumn and decreased as the particle got larger. The *Glomerella* widely found at the surface of leaf, suggesting that the leaf senescence is an important source of fungi in $PM_{2.5}$ in autumn (Wang et al., 2015). The spearman's rank coefficient analysis showed Ca^{2+} which is typical water soluble inorganic ions from dust was negative related to *Glomerella* and *Zasmidium* in autumn (Figure 5). In winter, *Penicillum*, *Bullera*, and *Geosmithia* increased due to the sensitive to low temperature (Sousa et al., 2008; Abdel Hameed et al., 2012). The results based on the spearman's rank correlation test analysis to support this notion (Figure 5, $p< 0.01$). Humidity, another important factor for the fungal release into atmosphere either by active or passive modes, was a crucial factor for the variation 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, we observed some trace fungal genera that presented inverse correlation with temperature (*Coccomyces*, $p<0.01$; and *Dictyosporium*, $p<0.01$) and humidity (*Botryosphaerta*, $p<0.001$; *Coccomyces*, $p<0.01$; and *Dictyosporium*, $p< 0.01$). We determined four crucial environmental factors contributed to the variation of fungal community. As the limited cultured study on the detailed mechanism of environmental factors on the specific fungal spores, thus this problem still need to be surveyed for a longer time and their detail relationships with environmental factors require further study.

4 CONCLUSIONS

The information about the diverse airborne fungal spores is relevant for the researches of atmosphere, biogeoscience, climate and ecology, environmental hygiene, agriculture, and bioengineering. As many fungal spores in the biosphere are still unknown, the detection and characterization of ambient fungi can help to elucidate the regional and global distribution of diverse fungi. Herein we provided a comprehensive framework of the airborne fungal concentration and community associated with different season across $PM_{2.5}$ and PM_1 at Mt. Tai by high-throughput sequencing. Over the sampling period, an average fungal concentration of 467 spores m^{-3} and 644 spores m^{-3} in $PM_{2.5}$ and PM_1 were calculated. The west and northwest wind is more favorable to the increase of fungal abundance. We found diverse fungal community including AMC, BMC, ZMC, and Glomeromycota. They presented a significant seasonal variation across different size fraction. *Glomerella* and *Zasmidium* increased in autumn and decreased as the particle got larger. In winter, *Penicillum*, *Bullera*, and *Geosmithia* increased with smaller particles. The variable fungi among different season and aerodynamic diameter may be explained by environmental factors including humidity, temperature, wind speed, $PM_{2.5}$, and some chemical components in PMs (including Ca^{2+}). Nevertheless, the detail specific environmental roles on the ambient fungal community remain poorly explained. In the further study, the combination of traditional culture-based method and metagenomics may help to investigate the pending questions.

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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		°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

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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

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Table 2.

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

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Figure 1.

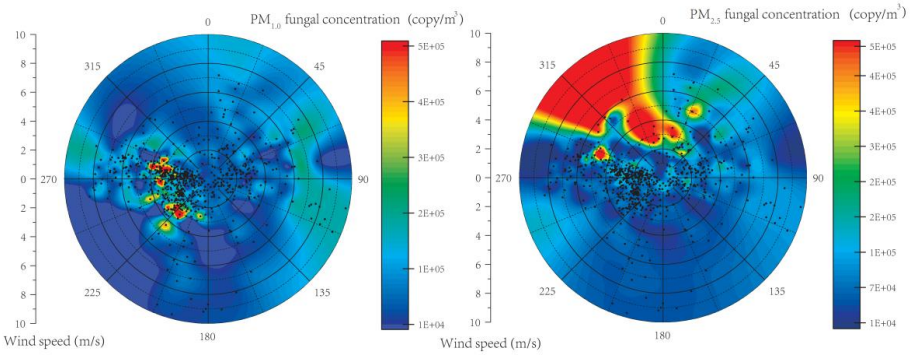

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Figure 2.

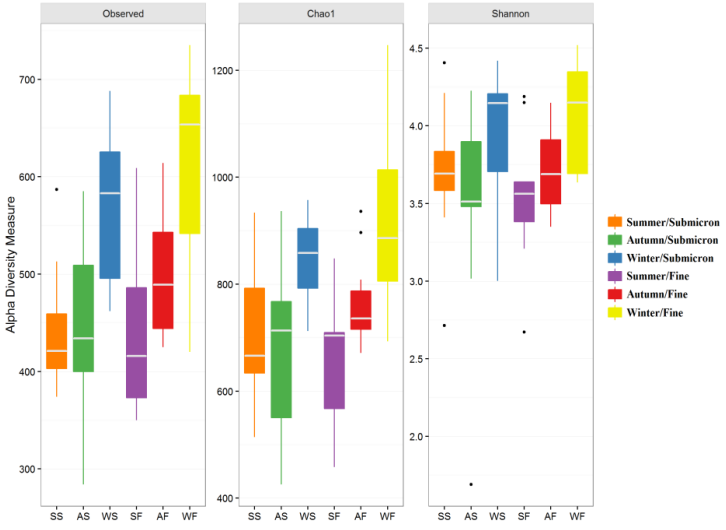

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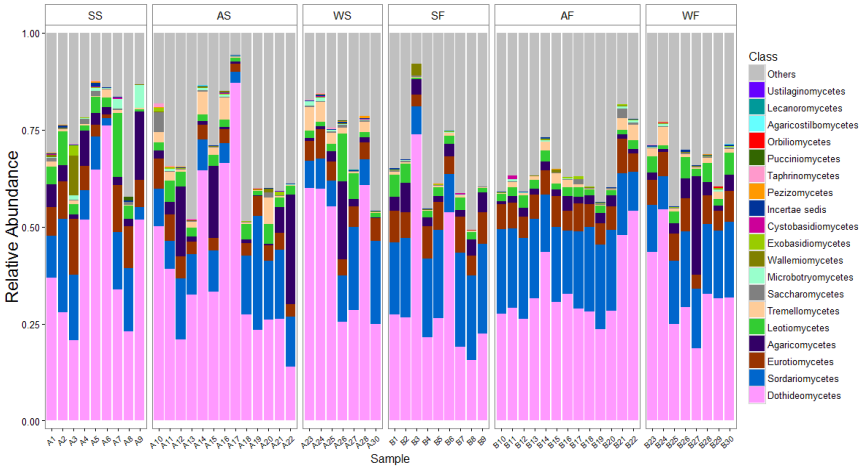
Figure 3.



Atmos. Chem. Phys. Discuss., doi:10.5194/acp-2017-204, 2017
Manuscript under review for journal Atmos. Chem. Phys.
Discussion started: 29 March 2017

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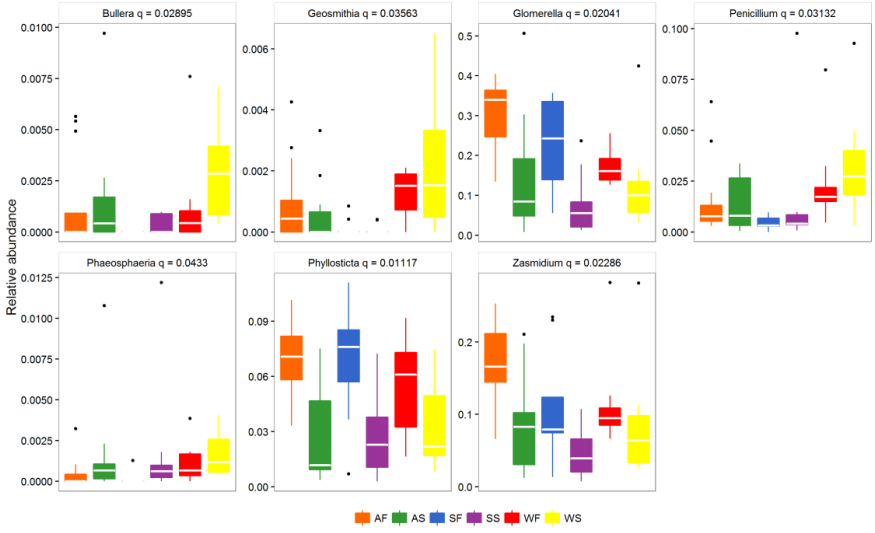
Figure 4.



Atmos. Chem. Phys. Discuss., doi:10.5194/acp-2017-204, 2017
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Figure 5.