Temporal-spatial variations of fungal composition in PM$_{2.5}$ and source tracking of airborne fungi in mountainous and urban regions

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Abstract

Fungi are ubiquitous in air and their composition is potentially important for human health. Exposure to fungal allergens has been considered as a significant risk factor due to the prevalence and severity of asthma in humans. However, temporal-spatial variations and potential sources of airborne fungal aerosol have been poorly understood. In this study, 48 PM$_{2.5}$ samples were collected at two sampling sites in Xi'an from April 2018 to January 2019. High-throughput sequencing technology was used to determine the diversity and abundance of fungal composition in all samples. Microbial samples were also collected from leaf-surface and soil to identify the potential sources of fungal aerosols. Results showed that the species richness of fungi in summer and autumn inclined to be higher than that in spring and winter in mountainous and urban regions. Airborne fungal species richness and diversity at Mt. Qinling sampling site were significantly higher compared to Yanta urban sampling site, except in winter. These variations in fungal composition were significantly related to season and location. The influence of atmospheric pollutants (PM$_{2.5}$, ozone, sulfur dioxide and carbon monoxide) on the richness and diversity of airborne fungal composition was higher than meteorological factors (temperature, relative humidity and wind speed). Moreover, it was observed that the leaf-surface was the primary local source of airborne fungi during all seasons at both sampling sites. Back trajectories arriving at both sampling sites showed that a considerable part of airborne fungi might have come from other regions by medium or long-range airflow. This...
1. Introduction

Particulate matter (PM) with different aerodynamic diameters can cause adverse effects on human health, including serious illness or even death. For instance, PM$_{10}$ (<10 µm) can be mainly deposited in primary bronchi (Ren et al., 2016) and PM$_{2.5}$ (<2.5 µm) can penetrate into lungs and embedded in the alveoli, before reaching blood stream (Kai et al., 2013). Long-term exposure to PM$_{2.5}$ might increase the risks of allergic diseases and asthma in preschool children in China (Chen et al., 2018). In recent years, scientific research and public discussion was focused on fine particles, mainly the composition (Khodeir et al., 2012), physical and chemical properties (Shen et al., 2017), sources (Xu et al., 2018) and temporal-spatial variations (Bell et al., 2007) of PM$_{2.5}$. However, there is scarce of information about the variation in bioactive components, especially for fungi.

Fungi are ubiquitous in air and its spores are considered as one of the most common type of airborne biological aerosol particles in various environments, with about 1–1.5 million living fungal species on Earth (Elbert et al., 2007), which include yeasts and molds that can be the sources of both infection and allergic reaction (Green et al., 2003). They actively release spores into the atmosphere through water jets or droplets. It was estimated that the global emission rate of fungal spores was approximately 28–50 Tg·a$^{-1}$ and emission fluxes of average mass were about 6–23 ng·m$^{-2}$·s$^{-1}$ (Elbert et al., 2007; Heald and Spracklen, 2009). Fungi played a key role in the Earth’s system through their interactions with atmosphere, affecting climate and public health. Fungi can act as nuclei and ice crystals and influence precipitation patterns (Pratt et al., 2009). Exposure to fungal allergens has been considered as a strong risk factor for the prevalence and severity of asthma symptoms, and there was a strong evidence supporting the association between increased fungal spores in the environment and asthma attacks (Denning, 2006).

Quantitative evaluation of fungal composition can be achieved through various technologies, such as culture methods (Li et al., 2015), microarray (Brodie et al., 2007), q-PCR and DGGE (Li et al., 2010). However, these methods are not sufficient for the characterization of atmospheric microorganisms. The culture method cannot reflect the actual diversity of airborne fungi because only a few atmospheric microorganisms (<1%) could be investigated on nutrient media (Tham and Zurairi, 2010). The drawback of conventional DNA-based molecular methods is the inadequate classification of organisms. Nonetheless, in recent years, with the advancement of gene sequencing technology, high-throughput sequencing has been developed as an effective method to detect airborne fungal composition and diversity (Bowers et al., 2013; Kumari et al., 2016; Woo et al., 2013; Xu et al., 2017; Yan et al., 2016). Thus, the characterization of fungal diversity by high-throughput sequencing can be significantly improved compared to previous methods.

Previously, most of the studies focused on the characteristics of airborne fungi from urban environments, such as Brazil (Bezerra et al., 2014), Beijing (Du et al., 2018) and Urumqi (Gou et al., 2016). However, few studies have been conducted in non-urban environments, such as mountainous region, which is a significant part of the airborne microbial ecosystem. It was demonstrated that bioaerosols could originate from water bodies, soil, plants, wastes, feces, animals and human activity (Gilbert and Duchaine, 2009). However, less information is available about their contribution to airborne fungi and the relationship between fungal aerosols in mountainous and urban regions.

The aim of this study was to investigate the variations of fungal composition in PM$_{2.5}$ of mountainous and urban regions during one year period. Multivariate regression trees were performed to determine the relative influence of environmental factors on the species richness and diversity of airborne fungi. In addition, leaf-surface and soil samples around the sampling sites were collected to study about the source of airborne fungi. Results of this study contribute to a greater understanding about the temporal-spatial variations of airborne fungal composition, and their potential sources.

2. Materials and methods

2.1. Sampling sites and samples collection

Xi’an is a semi-arid inland city, located in the center of Guanzhong Plain and surrounded by Loess Plateau and Qinling Mountain. The city has pronounced differences in seasons, with short spring and autumn and long summer and winter. In this study, in order to analyze the generality and difference of airborne fungal distribution in mountainous and urban regions, aerosol monitoring was performed at two locations in Xi’an (Fig. S1). The distance between the two sampling sites is about 35 km. One location was on the roof of a building of School of Environment Science and Engineering of Chang’an University (34.23’N, 108.96’E, and 424 m above sea level), situated between 2nd and 3rd ring roads in Xi’an City and have a large urban area population. This sampling site was surrounded by urban roads, greenbelts, trees, school buildings, residential areas and business districts. Another sampling site was located at half way up the northern face of Mt. Qinling (34.02’N, 109.01’E, and 969 m above sea level) belonging to temperate zone. This site was surrounded by dense vegetation in suburban mountain area. There were no identified potential industrial pollution sources around both sampling sites.

Sampling was performed from April 2018 to January 2019 using an air particulate matter sampler (ZR-3930, Qingdao, China) at a flow rate of 16.7 L/min to collect PM$_{2.5}$ onto 47 mm (diameter) sterilized polycarbonate membranes (Whatman, UK) for 24 h per day at two monitoring sites with continuous sampling for 6 days in each season. For quality controls, all polycarbonate membranes and experimental vessels were subjected to high temperature sterilization at 121 °C in a pressure steam sterilizer (LDZX-40BL, Shanghai, China) for 20 min. All experimental materials were UV-sterilized and rinsed with 70% ethanol before use. Blank membranes without the air sampling were used as negative controls and were taken to the sampling sites. From each site, samples of two days adjacent to each other were divided into one group for analysis. A total of 48 air samples were obtained and divided into 24 groups for further analysis. In addition, leaf-surface and soil samples were collected near sampling sites to identify the potential sources of airborne fungal aerosols. 24 leaf and 24 soil samples each at two sampling sites were divided into 16 groups according to seasons and locations (Table S1).

2.2. DNA extraction, PCR amplification, and Illumina sequencing

Half of the filter paper samples were excised into small pieces using a scissor to achieve high total DNA content. Genomic DNA
was extracted using PowerSoil DNA isolation kit (MoBio Laboratories, Carlsbad, CA, USA) following manufacturer’s instructions. The internal transcribed spacer 1 (ITS1) region of fungal rRNA gene was amplified using primers 1737F (5′-GGAAGTAAAAGTCGTAACAAGG-3′) and 2043R (GCTGCGGTTCTGGAGGTCGAGC) through polymerase chain reactions (PCRs). PCRs was performed in a 25 μL volume containing 1 × PCR buffer, 0.2 μM primer, 0.2 mM dNTPs, 0.6 Units Taq DNA polymerase, and ~ 10 ng template DNA under following cycling conditions: pre-denaturation at 94 °C for 5 min; followed by 30 cycles of denaturation at 94 °C for 30 s, annealing at 56 °C for 30 s and extension at 72 °C for 45 s, with a final extension at 72 °C for 5 min. Samples were purified using an AxyPrep DNA Gel Extraction Kit (Axygen, USA) following manufacturer’s instructions and quantified using QuantifiFluor™-ST (Promega, USA). Then, purified amplicons were pooled in equimolar and paired-end sequenced (2 × 250 bp) on an Illumina MiSeq platform following standard protocols. Raw sequencing data were deposited into NCBI Sequence Read Archive (SRA) with accession number SRR4963381.

2.3. Measuring environmental parameters

The meteorological parameters of sampling sites included temperature (TEM), relative humidity (RH) and wind speed (WS) that were monitored by portable automatic meteorological station (PH-1, Wuhan, China), which was installed near sampling site. Simultaneously, the concentration of gaseous pollutants (SO2, NO2, O3, CO, PM2.5, and PM10) at Mt. Qinling and Yanta urban sampling site were obtained from Shaanxi environmental monitoring stations (http://www.tianghoubao.com/aqi/xian.html), and time resolution was 1 h. The environmental indices during the sampling period were shown in Table S2.

2.4. Statistical analysis

Least-Significant Difference (LSD) one-way analysis of variation (ANOVA) was used to examine whether the differences of species richness and diversity of fungi in different seasons’ air samples were significant. Spearman’s rank correlation analysis was performed to examine the correlations between environmental factors and diversity of airborne fungal composition. The analyses were performed using SPSS Statistics 20 and p value less than 0.05 was considered statistically significant.

The R (x64 3.4.1) statistical computing software was used to determine all community statistics and generate visualizations. The mvpart package in R was employed to develop multivariate regression trees (MRT) to assess the effects of atmospheric factors on fungal composition richness and diversity. The interpretation quantity of each partition node was obtained in the process of R language drawing. Source tracker was used to explore the species contribution of local sources (leaf-surface and soil) to airborne fungal compositions (Knights et al., 2011).

Backward trajectory analysis was performed to detect the source of air mass by using Hybrid Single-Particle Lagrangian Integrated Trajectory model (HYSPLIT_4) (Ashrafi et al., 2014).

3. Results and discussion

3.1. Airborne fungal richness and diversity in mountainous and urban regions

In total, approximately 745,662 high-quality sequences of fungal ITS gene were obtained after removing unqualified gene sequences. Table 1 shows the number of alpha diversity and OTUs (mean ± standard deviation) of fungal composition in PM2.5 at two sampling sites. At Mt. Qinling sampling site, richness indices (Chao1 and ACE) in summer samples (314.01 and 314.90) were higher than those in autumn (270.61 and 272.06), spring (256.52 and 250.01) and winter (205.61 and 205.33), respectively. The results of statistical analysis showed that there were significant seasonal differences in fungal species richness (ANOVA P = 0.049, F = 3.087). In addition, the order of average Shannon index in four season samples were autumn (6.51), spring (6.36), summer (6.17) and winter (5.21). This indicated that fungal composition diversity was significantly lower in winter compared to other seasons (P < 0.05). Similar result was observed at Yanta sampling site (urban region), with high airborne fungi species richness in summer and autumn and low in spring and winter. Moreover, sequence clustering demonstrated that except for winter samples, the number of OTUs was significantly higher at Mt. Qinling than that of Yanta sampling site (P < 0.05). This finding suggested that airborne fungal richness and diversity were higher at a mountainous site than an urban site.

Fungal spores were mainly affected by plant growth and weather conditions (Fang et al., 2008). It has been reported that plants are an important source of airborne aerosols (Lighthart, 1997). Therefore, diverse plant ecosystem of Qinling Mountains was highly likely to generate different types of fungal communities. In addition, the microbial composition showed uniform distribution pattern and high diversity in areas with high organic carbon content in soil (Zhou et al., 2002). Thus, the large amount of decaying organic matter in the soil of Mt. Qinling was another important reason for the higher abundance and diversity of fungi. (Tang (2009) reported that the survival of microorganisms was prolonged at low temperature; however, the propagation and growth were inhibited. At Mt. Qinling sampling site, soil was covered with snow for a long period in winter and the temperature was low (Table S2), which can reduce the activity of microorganisms in soil and lead to the reduction in the richness of airborne fungi.

3.2. Airborne fungal composition variation with seasons and locations

Fig. 1 shows the airborne fungal composition in PM2.5 at phylum level in different seasons in mountainous and urban sampling sites. In total, 14 fungal phyla were identified in 48 PM2.5 samples during four seasons. The dominant fungal compositions across all samples were Ascomycota and Basidiomycota in both sampling sites. The relative abundance of Ascomycota and Basidiomycota was similar with previous studies (Bowers et al., 2013; Du et al., 2018; Gou et al., 2016; Woo et al., 2013; Xu et al., 2017; Yan et al., 2016). The remaining phyla were mainly Zygomycota, Rozellomycota, GS19 and Glomeromycota. Interestingly, Rozellomycota and GS19 were only detected in winter, which can be attributed to the suitability of cold environments for these two fungi.

At the genus level, nearly 45 different genera (relative abundance > 0.1%) were identified in PM2.5 samples. Fig. 2 shows that airborne fungal composition at genus level was significantly different in different seasons in both mountainous and urban sampling sites (P < 0.05). Fungal species collected in mountainous region (Fig. 2A) in spring were represented by Penicillium (7.69%), Malassezia (6.28%) and Auricularia (4.12%). The primary genera of fungi in summer were Protodontia (7.90%), Trametes (6.01%) and Amanita (4.93%). Similarly, in autumn, the top three genera were Talaromyces (3.66%), Penicillium (2.88%) and Cordyceps (2.62%). Further, the predominant genera in winter were Talaromyces (9.07%), Cutaneotrichosporon (7.20%) and Microsporum (5.14%). Similarly, Fig. 2B shows that there were significant seasonal differences in fungal genus level in urban region (P < 0.05). In spring, the primary genera of fungi were Phoma (5.08%), Mortierella (2.83%) and Bullera (2.35%). Similarly, in summer, the predominant genera were Cryptococcus (11.14%), Trichosporon (8.98%) and Aspergillus (6.73%). The represented genera in autumn were Trechispora (5.01%), Trametes...
local sources (vegetation and others). For instance, differences in fungal composition might be related to the different differences can be attributed to geographical variations. Significant

can show a significant negative correlation with temperature. Penicillium showed a significant negative correlation with temperature (Xu et al., 2017). Therefore, it was assumed that the significant seasonal differences in fungal composition might be related to the differences in environmental factors (TEM, RH and WS) in different seasons.

Fig. 3 shows the endemic fungi and their average relative abundance at Mt. Qining (A) and Yanta urban sampling site (B). At genus level, there were 28 fungi genera with significant differences in both sampling sites. Among them, there were 13 and 15 fungi endemic to mountainous and urban regions, with annual average relative abundance as 11.64% and 10.44%, respectively. These differences can be attributed to geographical variations. Significant differences in fungal composition might be related to the different local sources (vegetation and others). For instance, Ceriporia, Amanita, Cordyceps and Auricularia were observed at mountainous region. Among them, Ceriporia and Amanita can exhibit serious negative effects on plants and human health, respectively. Ceriporia is a genus of wood rotting fungi that can cause white rot (Jang et al., 2012). Amanita muscaria is a highly toxic fungus that can affect nerves in humans. However, Cordyceps and Auricularia were beneficial fungi. Cordyceps sinensis is a parasitic complex composed of fungi and caterpillars, which has been commonly used to tonify kidneys, soothe lungs and cure fatigue, and widely used in China, Japan and other countries (Zhou et al., 2009). Auricularia is found in the dead or decaying wood of broad-leaves trees and it contains polysaccharides, such as d-glucosinol, which has anticoagulant effect in plasma. It has been widely used as medical and/or food supplement in Korea and China (Yoon et al., 2003). In addition, other unique fungal genera observed in urban region included Acremonium and Bipolaris. Acremonium is the main representative fungus of wall mold, which often appears on wet building materials. Bipolaris belonged to common pathogenic fungi of crops, such as maize and can cause maize disease (Guo et al., 2016). In this study, Bipolaris was only detected in summer and autumn, when corn was planted and harvested.

3.3. Potential fungal pathogens in mountainous and urban regions

Due to the increase of immunodeficiency in human population and incidence of therapeutic infection, the threat of fungal pathogens to human health has been increased (Andrianopoulos, 2002). In this study, 10 opportunistic pathogenic fungi were detected from PM$_{2.5}$ samples at both sampling sites (Fig. 4). In mountainous region, the top three opportunistic pathogenic fungi were Malassezia (2.70%), Aspergillus (3.02%) and Penicillium (2.78%). However, in urban region, higher relative abundance of Aspergillus (3.86%), Cryptococcus (3.20%) and Trichosporon (1.81%) were observed. These results suggested that predominant pathogenic fungi were different in different regions. This was consistent with previous studies, showing that dominant opportunistic pathogenic fungi significantly varied in different regions and climates, with Cladosporium, Aspergillus and Penicillium as the most widely distributed genera (Table S4).

Cladosporium spp. are common pathogenic fungi in indoor and outdoor environments (Shelton et al., 2002). Aspergillus genera are distributed as dominant fungi in many cities' environments

### Table 1
Estimated number of observed diversity and OTUs (mean ± standard deviation) of fungal compositions in PM$_{2.5}$ at Mt. Qining (QL) and Yanta urban (YT) sampling site.

<table>
<thead>
<tr>
<th>Sites</th>
<th>Treatment</th>
<th>n</th>
<th>Richness</th>
<th>Diversity</th>
<th>OTUs</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Chao1</td>
<td>ACE</td>
<td>Simpson</td>
</tr>
<tr>
<td>QL</td>
<td>spring</td>
<td>6</td>
<td>256.52 ± 24.71</td>
<td>250.01 ± 11.22</td>
<td>0.96 ± 0.01</td>
</tr>
<tr>
<td></td>
<td>summer</td>
<td>6</td>
<td>314.01 ± 33.50</td>
<td>314.90 ± 38.74</td>
<td>0.95 ± 0.03</td>
</tr>
<tr>
<td></td>
<td>autumn</td>
<td>6</td>
<td>270.61 ± 37.32</td>
<td>272.06 ± 38.72</td>
<td>0.97 ± 0.01</td>
</tr>
<tr>
<td></td>
<td>winter</td>
<td>6</td>
<td>205.61 ± 79.91</td>
<td>205.33 ± 74.87</td>
<td>0.95 ± 0.01</td>
</tr>
<tr>
<td>YT</td>
<td>spring</td>
<td>6</td>
<td>181.82 ± 24.60</td>
<td>181.77 ± 25.42</td>
<td>0.93 ± 0.01</td>
</tr>
<tr>
<td></td>
<td>summer</td>
<td>6</td>
<td>220.87 ± 134.36</td>
<td>221.10 ± 133.63</td>
<td>0.96 ± 0.02</td>
</tr>
<tr>
<td></td>
<td>autumn</td>
<td>6</td>
<td>268.00 ± 37.72</td>
<td>268.60 ± 37.40</td>
<td>0.97 ± 0.01</td>
</tr>
<tr>
<td></td>
<td>winter</td>
<td>6</td>
<td>209.90 ± 99.90</td>
<td>213.12 ± 98.61</td>
<td>0.96 ± 0.02</td>
</tr>
</tbody>
</table>
in different seasons, which might be because *Aspergillus* genera are saprophytes commonly found in soil, water, wetland and other ecosystems globally (Dagenais and Keller, 2009). It can cause allergies, respiratory infections, asthma and lung disease (chronic pulmonary aspergillosis) (Brown et al., 2012). Besides, it can cause diseases in important cash crops, such as corn and peanuts and produce mycotoxins (Pasqualotto, 2009). Although relative abundances of these pathogenic fungi genera in the samples were not
high, they are known to be harmful and could cause human disease. Malassezia genera are a normal part of human skin flora, but they have been associated with several common diseases, such as seborrheic dermatitis and dandruff, atopic dermatitis and psoriasis (Gupta et al., 2004). Similarly, Penicillium marneffei as pathogenic fungus can cause fatal systemic mycosis in patients (Hu et al., 2013). Further, it has been reported that Cryptococcus neoformans caused pulmonary infection and pneumonia to form cryptococcosis, which is a life-threatening disease that co-occurred with HIV/AIDS (Kronstad et al., 2011).

3.4. Relationship between species richness and diversity of fungal composition and environmental factors

Fig. 5 shows the effects of environmental factors on structural diversity and richness of fungal compositions during different sampling periods using multivariate regression trees (MRT). Further, Table 2 shows the Spearman’s linear correlations between environmental factors and diversity indices of airborne fungi. Selected
chemical pollutant factors (PM$_{2.5}$, PM$_{10}$, SO$_2$, O$_3$, NO$_2$ and CO) and meteorological factors (TEM, RH, and WS) were evaluated to assess their effects on fungi in PM$_{2.5}$ samples. The richness and diversity indices of different airborne fungal compositions were categorized into different groups according to various environmental factors (TEM, RH, WS, PM$_{2.5}$, SO$_2$, CO, NO$_2$ and O$_3$), with 6 segmentation nodes and 7 branches (Fig. 5). In addition, the hierarchical relationships of tree plots indicated the relative effects of various environmental factors on the diversity and richness of airborne fungal compositions. The tree exhibited 87.75% of variation characteristics of airborne fungal richness and diversity. The error was 0.06 and standard deviation was 0.02, indicating that the multivariate regression trees were accurate and effective in explaining the variation characteristics of fungal richness and diversity. Cross validation error was 0.14, demonstrating that the multivariate regression trees showed a good prediction ability. The order of the degree influence of these factors was as follows: PM$_{2.5}$ (42.80%) > O$_3$ (22.57%) > SO$_2$ (8.78%) > CO (7.77%) > TEM (2.17%). Moreover, the relative effects of humidity and wind speed on the diversity of fungal composition were <1.88%, so, they were not visible in multiple regression trees. These results suggested that atmospheric pollutants (PM$_{2.5}$, O$_3$, SO$_2$ and CO) exhibited higher impact on the richness and diversity of airborne fungal compositions than meteorological factors. Among all factors, the concentration of PM$_{2.5}$ showed most significant effects. There were no consistent correlations observed between PM and fungal diversity from previous studies. High PM and chemical components may inhibit or promote the growth of some microorganisms during haze pollution, and thus lead to the changes in compositions (Gou et al., 2016; Sun et al., 2018). In this study, the species richness of airborne fungi exhibited a negative correlation with PM (Table 2). This was in contrast with previous studies (Dong et al., 2016; Li et al., 2017; Xie et al., 2018). Li et al. (2017) found that the average concentrations of viable fungi on haze days were higher than non-haze days. Similarly, other studies reported a significant increase in total airborne microbial concentration on haze days (Dong et al., 2016; Xie et al., 2018). However, the results of this study were also in agreement with some previous studies. (Gao et al., 2015) reported that there was a negative correlation between bioaerosol concentration levels and PM. In addition, it was found that Sporisorium, Trametes, Cladosporium and Fusarium were highly abundant in non-haze days (Yan et al., 2016). Further, Aspergillus Japonicus, Aureobasidium pullulans and Penicillium Chrysogenum were only detected on non-haze days (Li et al., 2017). It can be ascertained that increase in haze pollution can result into the increase in toxic and harmful chemical substances attached to suspended particles, and their toxic effects. In addition, the excessive growth of different microbial populations on haze days inhibited the growth of other fungal communities. Low inhibition or competition between different kinds of microorganisms on non-haze days allowed more species to coexist in the community. It was further found that the concentration of heavy metals in PM$_{2.5}$ during haze days were approximately 2.5–3 times higher than that on non-haze days in Xi’an (Yan et al., 2015). Heavy metals released from airborne PM with redox potential can catalyze the production of reactive oxygen species (ROS), especially hydroxyl radical (HO$^-$), leading to extensive damage to cellular biological molecules (Estillore et al., 2016). Therefore, the influence of toxic chemical compositions in airborne PM on airborne fungi diversity should be investigated in future studies.

Ozone was another crucial factor influencing the species richness and diversity of airborne fungi in PM$_{2.5}$. There was a negative

**Table 2** Spearman’s correlation between species richness and diversity of fungal composition and environmental factors.

<table>
<thead>
<tr>
<th></th>
<th>TEM</th>
<th>RH</th>
<th>WS</th>
<th>PM$_{2.5}$</th>
<th>PM$_{10}$</th>
<th>O$_3$</th>
<th>NO$_2$</th>
<th>SO$_2$</th>
<th>CO</th>
<th>AQI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chao1</td>
<td>0.37</td>
<td>−0.47*</td>
<td>0.17</td>
<td>−0.37</td>
<td>−0.48*</td>
<td>−0.38</td>
<td>−0.31</td>
<td>0.08</td>
<td>0.01</td>
<td>−0.42</td>
</tr>
<tr>
<td>ACE</td>
<td>0.36</td>
<td>−0.47*</td>
<td>0.19</td>
<td>−0.35</td>
<td>−0.46*</td>
<td>−0.36</td>
<td>−0.29</td>
<td>0.10</td>
<td>−0.01</td>
<td>−0.40</td>
</tr>
<tr>
<td>Shannon</td>
<td>0.11</td>
<td>−0.17</td>
<td>−0.15</td>
<td>−0.26</td>
<td>−0.35</td>
<td>−0.11</td>
<td>−0.36</td>
<td>0.41</td>
<td>−0.31</td>
<td>−0.22</td>
</tr>
<tr>
<td>Simpson</td>
<td>0.17</td>
<td>0.07</td>
<td>0.19</td>
<td>−0.08</td>
<td>−0.17</td>
<td>−0.005</td>
<td>0.04</td>
<td>−0.16</td>
<td>−0.05</td>
<td>−0.14</td>
</tr>
</tbody>
</table>

* $p < 0.01$ (2-tailed).
* * $p < 0.05$ (2-tailed).
correlation between ozone and airborne fungal diversity due to its germicidal properties. High ozone concentrations inhibited fungal damage to plants (Khan and Khan, 1999). Thus, ozone oxidation can effectively inhibit or kill airborne fungi. Although the concentration of ozone was highest in summer, species richness of airborne fungi was found to be relatively high. This can be attributed to the fact that the growth of fungi was affected by PM$_{2.5}$, TEM, O$_3$, and other factors, which weakened the influence of ozone. In this context, it has been reported that SO$_2$, NO$_2$ and CO can provide nutritive raw materials for airborne microorganisms as sulfur, nitrogen and carbon are the basic elements of life, however, they can also produce toxic pollutants like H$_2$SO$_4$ that can inhibit the growth of microorganisms (Fan et al., 2019).

Temperature has been regarded as a key factor that can affect fungal compositions. High temperature can accelerate conductive air movement, promote growth and diffusion of microorganisms into the atmosphere (Lu et al., 2018; Smets et al., 2016). Some studies reported a positive correlation between spore levels and high temperatures, which explained the highest richness of airborne fungi in warmer seasons (Erkara et al., 2008; Hollins et al., 2004; Tang, 2009). RH can also affect the concentration, diversity and community structure of microorganisms in air. In this study, RH showed a negative correlation with the richness of fungi. Although high RH was considered conducive to the release and production of bacteria, it affected the activity of microorganisms (Tang, 2009). Previously, Botryosphaeria, Coccomyces and Diorygosporum exhibited inverse correlation with RH (Xu et al., 2017). When RH was high, pollen and spores were more likely to adhere to the wet surface, thus reducing the release of microorganisms in the source (Knudsen et al., 2017). In addition, under high humidity conditions, biological particles suspended in the atmosphere were more likely to wrap and absorb water vapor, which will increase their weight and size, and thus increasing the chance of deposition to the ground. Overall, the combined effects of meteorological factors and chemical pollutants on the richness and diversity of airborne fungal compositions were significant.

3.5. Potential sources of airborne fungal

Different sources can elucidate the seasonal differences of airborne fungal composition at different sampling sites. In this study, fungal composition of leaf-surface and soil, near the sampling site were analyzed to identify the potential sources of airborne fungi. Fig. 6 shows the local sources of airborne fungi during four seasons at Mt. Qinling and Yanta urban sampling sites. It was observed that in both sampling sites, the main local source of airborne fungi was leaf-surface during all seasons, while soil had less contribution. Highest and lowest contribution of local sources were observed in autumn and winter, respectively. However, the contribution rate of leaf-surface and soil to airborne fungi was higher at Mt. Qinling, compared to Yanta site.

It was demonstrated that local sources, such as leaf-surface and soil could explain some of the seasonal variations in airborne opportunistic pathogenic bacteria composition between autumn and winter (Fan et al., 2019). In addition, it was reported that leaf surfaces were usually colonized by many microorganisms (Mercier and Lindow, 2000). Therefore, high contribution of the leaf-surface to airborne fungi can be attributed to the high leaf coverage at both sampling sites. The leaf area and soil coverage levels were considerably higher at Mt. Qinling, compared to Yanta site. This explained the relatively higher contribution of leaf-surface and soil to airborne fungi in mountainous region. Previous study showed that the separation density of fungi in leaf-surface increased with leaf age (Bao et al., 1992). Meanwhile, it was reported that the abundance and density of fungal epiphytes were positively correlated with soluble sugar content in leaf-surface (Zhang et al., 1994). Moreover, high soluble sugar content was observed in October (Wang et al., 2014). Thus, the richness and diversity of fungi in leaf-surface were highest in autumn (Table S3). However, filamentous fungi were found to be temporarily present in the leaf environment as spores (Andrews and Harris, 2000). Hence, higher wind speeds could easily release more fungal epiphytes into the air in autumn (Table S2). This confirmed that leaf-surface had the highest contribution to airborne fungi in autumn. In addition, Microsporum as a dermatogenic fungus, mainly isolated from human and animal hair, can cause psoriasis and other diseases in human and animals (Han et al., 2018). This suggested that human and animal could also contribute to the spread of ambient fungi.

Moreover, a long-distance transport of dust in the atmosphere is an effective process of spreading viable and non-viable microbes at local, regional and global scales (Hara and Zhang, 2012). Fig. S2 shows clustering back trajectories of the air masses arriving at Mt. Qinling and Yanta urban sampling sites. In each season, 72 trajectories arriving at same sampling site were clustered together and then divided into 3 clusters according to the similar direction of airflow. The percentage on each line represents the trajectories number arriving at each sampling point. Red lines represent the direction of dominant clusters in spring and winter, and blue lines represent the direction of dominant clusters in summer and autumn. It was observed that the source direction of long, medium and short-distance transport air mass at Mt. Qinling was similar to that of Yanta urban sampling site. Airflow transmission paths were similar in spring, autumn and winter, which originated from deserts in northwest. Air currents from dust areas typically contain more UV-resistant and psychrophilic microbes, such as Mortierella, which showed significantly higher average relative abundance in spring, autumn and winter than in summer. Long-distance transmission originates from the East China Sea in summer. The average atmospheric residence time of biological aerosols released by marine sources is shorter than that of land surface, which are rapidly removed by precipitation formed over the ocean, thus, making the marine air cleaner, with low microbial diversity index (Mayol et al., 2014). Thus, the low diversity index of airborne fungi in summer might be due to the influence of long-distance airflow from the East China Sea. In addition, the cyclonic circulation was observed at both sampling sites in autumn, which is not conducive to the spread of airborne fungi, leading to uniform distribution of air particles and fungi. As a result, fungi can be enriched in air, increasing the airborne fungal richness and diversity. These might be the important reasons for high diversity and richness index of fungi in this season. Thus, it was ascertained that long-range air-
flow affected airborne fungi during the sampling period. The airborne fungi may have originated from the environments surrounding the sampling sites and transported over long-distance.

4. Conclusions

The present study showed that species richness of airborne fungi was high in summer and autumn, particularly in mountainous regions. There were significant seasonal and location differences in airborne fungal composition, with different dominant genera observed in each season. In addition, predominant opportunistic pathogenic fungi were different in mountainous and urban regions, and their long-term exposure might create various health risks to local people. It was further observed that atmospheric pollutants (PM$_{2.5}$, O$_3$, SO$_2$ and CO) showed more variations in fungal richness and diversity than meteorological factors (TEM, RH and WS). Moreover, the main local source of airborne fungi was leaf-surface during the four seasons at both sampling sites, while soil showed little impact. Back trajectories arriving at both sampling sites showed that airborne fungi might have originated from other regions through medium or long-range airflow.

This study only focused on the contribution of soil and leaf-surface to the airborne fungal community. However, fungi aerosol could originate from any environmental reservoir of microorganisms, such as soil, leaf-surface, water, feces, animals and human activities. Hence, future research should focus on collecting more source samples to extensively explore the potential sources of bioaerosols. Further, this study investigated the characteristics of pathogenic fungi at genus level. Thus, in order to accurately explore the health hazards of airborne pathogenic fungi, species level characterization will be required.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2019.135027.

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