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Variation of Bacterial and Fungal Community Structures in $PM_{2.5}$ Collected during the 2014 APEC Summit Periods

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ABSTRACT

The biological fraction of $PM_{2.5}$ is considered to be a major cause of various allergies and respiratory diseases. Nonetheless, differences in bacterial and fungal communities in $PM_{2.5}$ under different air quality conditions are not well known. In the present study, we collected $PM_{2.5}$ samples from October 15, 2014 to November 12, 2014 when several successive "Asia-Pacific Economic Cooperation (APEC) blue" days were recorded, following the implementation of strict emission control measures to ensure the APEC summit held during November 5–11, 2014 in Beijing. This study analyzed bacteria and fungi in $PM_{2.5}$ samples through rRNA gene high-throughput sequencing. In total, 690 genera of bacteria and 229 genera of fungi were detected. The variations of species richness and community diversity of bacteria and fungi in $PM_{2.5}$ were not affected significantly by the emission control measures adopted during the summit and different air quality levels. The bacterial and fungal community structures in $PM_{2.5}$ collected during the summit exhibited over 83.7% and 79.6% similarities respectively, with $PM_{2.5}$ collected from air graded as "good" quality (AQI ≤ 100) before the APEC summit. Bacteria and fungi in $PM_{2.5}$ samples collected at AQI levels between 101–200 and 201–300 before the APEC summit had more than 73.4% and 76.3% community structure similarity, respectively, with $PM_{2.5}$ samples collected at AQI ≤ 100. The difference between day and night $PM_{2.5}$ samples was very small for bacterial and fungal community structures. Furthermore, most of the inhalable bacteria and fungi were nonpathogenic and no a clear relationship between air quality levels and pathogens was observed. Our results showed that bacteria and fungi in $PM_{2.5}$ were less affected by emission control measures and different air quality levels. However, due to the limited number of samples, the relationship between air pollution levels and airborne bacteria and fungi still needs further study.

***Keywords*:** APEC; Emission control; Bioaerosols; $PM_{2.5}$; AQI.

INTRODUCTION

With the rapid development of industry and economy in China, exhaust gas and particulate matter (PM) discharged from factories and automobiles are increasing. Beijing, an international megacity, has been suffering from serious $PM_{2.5}$ (aerosol particles with an aerodynamic diameter of ≤ 2.5 μm) pollution (Xu *et al.*, 2005; Zhou *et al.*, 2015; Lang *et al.*, 2017). Although previous studies have shown that the annual average concentration of $PM_{2.5}$ in some cities in China was more than 90 μg m^{-3} (He *et al.*, 2001; Zheng *et al.*, 2005; Meng *et al.*, 2007; Feng *et al.*, 2009), public awareness regarding $PM_{2.5}$ was low until 2011. The relationship between exposure to $PM_{2.5}$ and public health has been well established in a number of studies (Pope III *et al.*, 2002; Boldo *et al.*, 2006; Valavanidis *et al.*, 2008; Wang *et al.*, 2017). Epidemiological data have summarized the correlation between $PM_{2.5}$ exposure and allergy, morbidity, mortality, respiratory, pulmonary, and cardiovascular diseases (Dockery *et al.*, 1993; Pope III *et al.*, 2002; Englert, 2004). Due to their small size, $PM_{2.5}$ can be deposited in the nose, trachea, bronchi, and penetrate the alveoli (Frøsig *et al.*, 2001; Brook *et al.*, 2004). Although $PM_{2.5}$-induced oxidative stress and inflammatory mediator release are considered as important molecular mechanisms by which they influence human health (Becker *et al.*, 2005; Risom *et al.*, 2005; Deng *et al.*, 2013), hitherto, their mechanisms of causation of various diseases have not been well understood.

Physical and chemical properties of $PM_{2.5}$ have been extensively investigated. At present, studies of $PM_{2.5}$ pollution focus on the components, emission sources, and spatiotemporal variation in China. $PM_{2.5}$ components such as organic carbon, elemental carbon, sulfates, nitrates, and geological material have been studied in different cities and

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regions (Xiu *et al.*, 2015; Zhao *et al.*, 2016; Zhan *et al.*, 2016). However, few studies have explored the biological fraction of $PM_{2.5}$ including bacteria, fungi, pollen, viruses, spores, and cell debris (Cao *et al.*, 2014; Li *et al.*, 2015; Yan *et al.*, 2016; Gao *et al.*, 2017). The identification of bacteria and fungi in aerosol particles through culture-based methods and clone library sequencing has been documented (Stoeck *et al.*, 2007; Fahlgren *et al.*, 2010; Haas *et al.*, 2013, 2014; Tarigan *et al.*, 2017), but these results do not realistically reflect the microbial community composition and diversity due to the limitation of cultivatable microorganisms and number of constructed clones in an aerosol sample (Amann *et al.*, 1995; Nocker *et al.*, 2007). Particulate matters of biological origin may contribute 15% to 30% of the total aerosol particles in the atmosphere (Matthias-Maser *et al.*, 1995; Ruprecht, 2005). Fungal spores, pollens, endotoxins, and viruses represent a major part of the biological particles and may elicit diseases such as allergies, asthma, airway obstruction, and inflammatory reaction (Bush and Portnoy, 2001; Degobbi *et al.*, 2011; Arteaga *et al.*, 2015). Due to limited understanding of the variation in composition and dynamics of microorganisms in $PM_{2.5}$ at different air quality levels, a critical knowledge gap on the effect of $PM_{2.5}$ on human health exists.

The Chinese government set the Technical Regulation on Ambient Air Quality Index (AQI) in 2012, which included $PM_{2.5}$ concentration for the first time. Air quality were divided six grades: excellent, good, slight pollution, moderate pollution, heavy pollution, and severe pollution according to AQI $\leq$ 50, 51–100, 101–150, 151–200, 201–300, and > 300, respectively. The corresponding $PM_{2.5}$ concentrations of these grades were $\leq$ 35 μg m^{-3}, 36–75 μg m^{-3}, 76–115 μg m^{-3}, 116–150 μg m^{-3}, 151–250 μg m^{-3}, and > 250 μg m^{-3}, respectively. Cao *et al.* (2014) first investigated different species of the airborne microbes in $PM_{2.5}$ using metagenome analysis during a severe smog event in Beijing city and suggested that majority of them were nonpathogenic to humans. Subsequently, correlation between various factors and cultivable bacteria or fungi during haze and non-haze days in Beijing have been studied (Gao *et al.*, 2015b; Gao *et al.*, 2016) with increasing focus on the species composition and dynamics of bioaerosol particles (Wei *et al.*, 2016). Recently, high-throughput sequencing technology based on the rRNA gene of microorganisms and metagenomes are being used to survey the composition and diversity of microbial communities in atmospheric particles (Nonnenmann *et al.*, 2010; Bowers *et al.*, 2011a; Cao *et al.*, 2014; Gao *et al.*, 2017). However, there is little information regarding the transformation in the biological components of the $PM_{2.5}$ in relation to different pollution levels.

In order to ensure good air quality during the 2014 APEC summit hosted in Beijing during November 5–12, 2014, the government implemented a series of strict measures to control air polluting emissions, such as reducing vehicles on roads by executing odd-and-even license plate traffic bans on alternate days, suspension of activities at construction sites, and shutting down polluting factories. The term "APEC blue" was coined to describe the "good" air quality during the period. This intervention provided an interesting opportunity to research the impact of emission control measures on atmospheric aerosol particles. Latest researches have demonstrated that inorganic and organic aerosols significantly decreased during the APEC summit (Chen *et al.*, 2015; Tang *et al.*, 2015; Sun *et al.*, 2016). Reduction in pollutants during the summit was observed and the relationship between pollutant concentrations and AQI was noted (Tang *et al.*, 2015; Sun *et al.*, 2016). Nevertheless, the variation in the composition patterns of chemical or biological fractions of $PM_{2.5}$ was not explored deeply. To the best of our knowledge, our study firstly demonstrated the impact of emission control on bioaerosols in $PM_{2.5}$ during the APEC summit.

This study investigates the abundance and similarities of bacteria and fungi in $PM_{2.5}$ using high volume sampling and high-throughput genome sequencing technology. Additionally, the impacts of air pollution emission control and different air quality levels on microorganisms in $PM_{2.5}$ were also analyzed.

METHODS

Sampling Site and $PM_{2.5}$ Collection

$PM_{2.5}$ samples were collected on the roof of a two-story building (~8 m high) between October 15 and November 12, 2014 at the Institute of Atmospheric Physics (IAP), Chinese Academy of Sciences (39°58′28″N, 116°22′16″E). The site is located between the North 3rd and 4th Ring road and air pollution was monitored at this site for several years (Sun *et al.*, 2012). $PM_{2.5}$ was collected using 203 mm × 254 mm quartz microfiber filters (WhatmanTM, GE, USA) with a high volume air particulate matter sampler (TE-6070VFC, Tisch, USA) at a flow rate of 1.13 m^3 min^{-1}. The quartz filters were individually wrapped in aluminum foil and sterilized by calcinating in a muffle furnace at 550°C for 5 h. $PM_{2.5}$ samples were collected both during the daytime (7:00 AM–18:00 PM) and nighttime (18:00 PM–7:00 AM). Each filter capturing $PM_{2.5}$ was immediately wrapped in the original aluminum foil packing after the completion of each sampling and stored in a sealed bag. A blank control sample was set by placing a new sterile quartz filter in the air particulate matter sampler for five minutes under the shutdown mode. All $PM_{2.5}$ samples were stored at –20°C until subsequent analyses were performed.

Sample Groups

A total of 58 $PM_{2.5}$ sample filters were collected during the study period. We excluded two sample filters collected on October 25th as the samples had AQI values above 300 and failed in DNA extraction, presumably due to severe pollution. We divided the 40 sample filters collected before the APEC summit into six sample groups according to the AQI and day or night sample timing. The letters D and N were used to represent the day and night sampling periods, respectively, and letters of G, M, and H represented excellent and good air quality (AQI $\leq$ 100), slight and moderate pollution (100 < AQI $\leq$ 200) and heavy pollution (200 < AQI $\leq$ 300), respectively. Therefore, the six sample groups were GD, GN, MD, MN, HD, and HN. The 16 sample

filters collected during the APEC summit were divided into two sample groups based on the sampling period conducted during the day or night, and were named as AD and AN, respectively. Thus, a total of eight sample groups were established and each group consisted of four or more individual filter membranes (Table 1). The AQI data used in our study were acquired from the Olympic Sports Centre Air Monitoring Station, which was the nearest monitoring station, situated 2 km to the northeast of our sampling site. Fig. 1 presents the AQI values and the dominant pollutants on various days during the sampling period.

DNA Extraction and Sequencing

We merged the filters in the same sample group to extract DNA of the biological components in $PM_{2.5}$. Based on the different number of $PM_{2.5}$ sample filters in each sample group, pieces of different areas were cut from each filter as follows: GD (4 cm^2), GN (4 cm^2), MD (10 cm^2), MN (10 cm^2), HD (6.6 cm^2), HN (6.6 cm^2), AD (5 cm^2), and AN (5 cm^2). The total area of the quartz filters in each sample group was about 50 cm^2. The filter pieces from the same sample group were ground together to powders using an agate mortar, which was sterilized using an autoclave sterilizer. The quartz filter powders were loaded into a bead tube provided with a PowerSoil DNA isolation kit (MoBio Laboratories, Carlsbad, CA, USA). The remaining steps of the DNA extraction were performed according to the manufacturer's instructions except for the vortex adapter oscillation step, which was replaced with FastPrep instrument (MP Biomedicals, Solon, USA) for enhancing mechanical cell breaking to improve DNA yield. We detected the DNA concentration using NanoDrop 2000 (Thermo Fisher Scientific, Wilmington, DE, USA). The range of DNA concentration was 10–80 ng μL^{-1}, and it was generally sufficient to ensure the completion of subsequent experiments.

Table 1. Sampling information for the eight sample groups.

Sample group	Sampling duration	AQI range	Pollution Level	Sampling Date
GD	7:00–18:00	0–100	excellent and good	Oct 15, 16, 21, 22, 26, 27, 28, and Nov 1,2, 3
GN	18:00–7:00 (next day)	0–100	excellent and good	Oct 15, 16, 21, 22, 26, 27, 28, and Nov 1,2, 3
MD	7:00–18:00	101–200	slight and moderate pollution	Oct 17, 23, 29, and Nov 4
MN	18:00–7:01 (next day)	101–200	slight and moderate pollution	Oct 17, 23, 29, and Nov 4
HD	7:00–18:00	201–300	heavy pollution	Oct 18, 19, 20, 24, 30, 31
HN	18:00–7:00 (next day)	201–300	heavy pollution	Oct 18, 19, 20, 24, 30, 31
AD	7:00–18:03	0–100	excellent and good	Nov 5, 6, 7, 8, 9, 10, 11, 12
AN	18:00–7:03 (next day)	0–100	excellent and good	Nov 5, 6, 7, 8, 9, 10, 11, 12

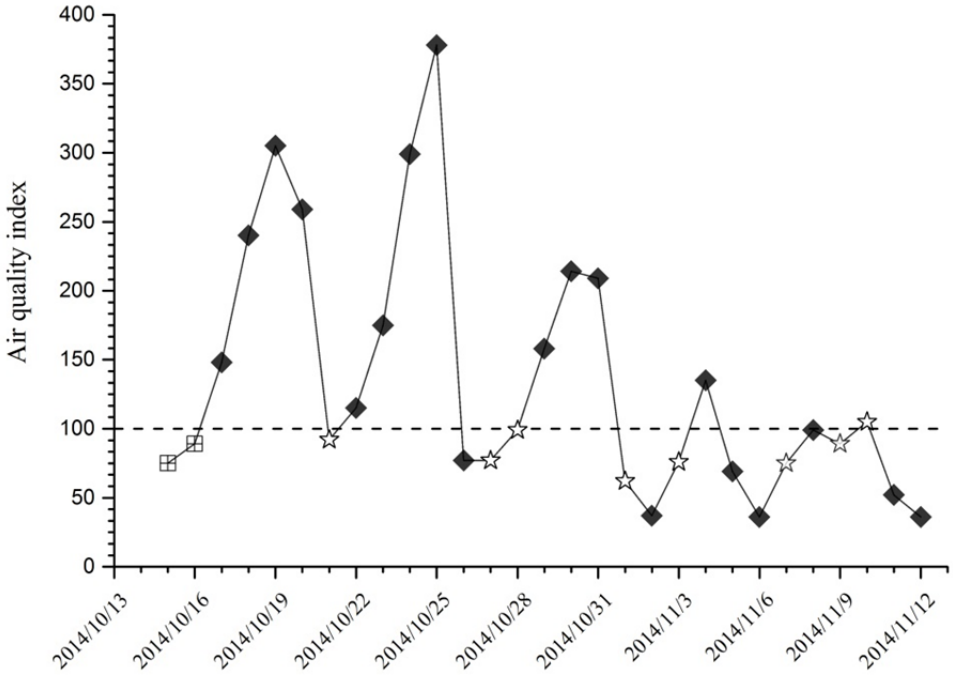

Fig. 1. Air quality indexes and dominant pollutants during sampling period. Labels of dominant pollutants: Square corresponds to PM_{10}; diamond corresponds to $PM_{2.5}$; star corresponds to NO_2. Dash line corresponds to second grade air quality (AQI = 100).

The 16S rRNA gene of bacteria and internal transcribed spacer 1 (ITS1) rRNA gene of fungi were sequenced through high-throughput sequencing to determine the bacterial and fungal community composition in $PM_{2.5}$. The V3-V4 hyper-variable region of the bacterial 16S rRNA gene was amplified using the primers 338F (5'-ACTCCTACGGGAGGCAGCA-3') and 806R (5'-GGACTACHVGGGTWTCTA AT-3'). The ITS1 region of the fungal rRNA gene was amplified using the primers 1737F (5'-GGAAGTAAAAGTCGTAACAAGG-3') and 2043R (GCTGCGTTCTG CATCGATGC). Each primer included a unique barcode sequence used for distinguishing different samples in the subsequent analyses. Polymerase chain reactions (PCRs) were performed in a 25 μL reaction mixture containing 1 × PCR buffer, 0.2 mM dNTPs, 0.2 μM primer, 0.6 Units taq DNA polymerase, and nearly 10ng template DNA under the following cycling conditions: pre-denaturation at 94°C for 5 min; 30 cycles of denaturation at 94°C for 30 s, annealing at 50°C for 30 s (for bacteria) or 56°C for 30s (for fungi), extension at 72°C for 45 s, with a final extension at 72°C for 5 min. Each $PM_{2.5}$ sample group had three repeated PCRs and the products were pooled together on the completion of the PCRs. Blank control samples were treated as above for DNA extraction and PCR amplification and the results showed that nothing was produced after the PCR processing. Subsequent purification and sequencing of the PCR products were performed using the Ilumina Miseq PE300 platform (Illumina, San Diego, CA, USA) by the Majorbio company (Shanghai, China). Sequences obtained from the Miseq sequencing platform were deposited in the NCBI Sequence Read Archive under accession number SRR3822027.

Sequence Analysis

Sequences were processed and analyzed using the open-source software package Mothur v.1.34.4 (http://www.mothur.org/wiki). Sequences were removed if they were < 400 bp or > 470 bp (for 16s rRNA gene), < 200 bp or > 300 bp (for ITS rRNA gene), contained ambiguous bases, or had homopolymer stretches of more than 8 bp. PCR chimeras were filtered out using the Chimer Uchime command in the Mothur (http://www.mothur.org/wiki/Chimera.uchime). The subsequent processing and analysis steps of the 16s rRNA gene were performed as described by Kozich *et al.* (2013). Sequences of the ITS rRNA gene were aligned using MAFFT version 7 (http://mafft.cbrc.jp/alignment/server), which was a target-independent alignment tool as alignment database was unavailable. Sequences were assigned into operational taxonomic units (OTUs) at 97% similarity. In order to compare the alpha and beta diversities in different $PM_{2.5}$ sample groups fairly, the minimum number of sequences in the eight sample groups was selected as the standard of normalization. Abundance based coverage estimator (ACE), Chao1 species richness estimator, Shannon diversity index, and Good's coverage were calculated based on the abundance of each OTU using Mothur. The bacterial sequences were assigned to phylotypes using a Bayesian approach against the Ribosomal Database Project (RDP) 16s rRNA gene training database with a confidence threshold of 70%. The method and database employed in the taxonomic classification process for fungal sequences were the K-Nearest Neighbor algorithm and UNITE ITS database, respectively (Wang *et al.*, 2007; Abarenkov *et al.*, 2010). The alpha and beta diversities of the $PM_{2.5}$ samples were calculated using the operating procedure described at http://www.mothur.org/wiki/MiSeq_SOP (Kozich *et al.*, 2013). Beta-diversity indexes (Bray-Curtis similarity index and Yue & Clayton similarity index) were determined to highlight the difference in each pair of $PM_{2.5}$ sample groups and were calculated using the formulas:

$$S_{bray\text{-}curtis} = 2\frac{\sum_{i=1}^{n}\min\left(S_{A,i}, S_{B,i}\right)}{\sum_{i=1}^{n}S_{A,i} + \sum_{i=1}^{n}S_{B,i}} \quad (1)$$

$$S_{yue\&clayton} = \frac{\sum_{i=1}^{n}a_i b_i}{\sum_{i=1}^{n}\left(a_i - b_i\right)^2 + \sum_{i=1}^{n}a_i b_i} \quad (2)$$

where,

S_{Ai} is the number of sequences of the OTU *i* in community A;

S_{Bi} is the number of sequences of the OTU *i* in community B;

"n" is the total number of OTUs in communities A and B;

a_i is the relative abundance of OTU *i* in community A;

b_i is the relative abundance of OTU *i* in community B.

In order to explore the potential effect of bioaerosol in $PM_{2.5}$ on human health, pathogenic bacteria and fungi were identified at the genus level according to the directory of pathogenic microorganisms infecting humans promulgated by the Ministry of Health of the People's Republic of China. Meanwhile, metabolic function pathway of bacterial communities, involving the human diseases caused by bacteria, was predicted through the PICRUSt (Phylogenetic Investigation of Communities by Reconstruction of Unobserved States). Community metabolic function was annotated against with the Kyoto Encyclopedia of Gene and Genomes (KEGG) database. The variations in species richness and community diversity in different experimental treatments were assessed using analysis of variance (ANOVA) test conducted using SPSS 19.0 software.

RESULTS AND DISCUSSION

Impact of Emission Control and Air Quality Levels on Species Richness and Community Diversity

Nearly 110331 high-quality sequences of bacteria and 103810 of fungi were gathered and the optimized sequences were assigned to different OTUs at 97% similarity. Following segmentation of OTUs, each $PM_{2.5}$ sample was normalized by singling out 11245 and 9181 sequences, which were the least sequence numbers for bacteria and fungi among the eight sample groups, respectively, to ensure fairness in

downstream analyses. A total of 7043 OTUs for bacterial sequences and 1008 OTUs for fungal sequences were obtained and used to calculate the alpha diversity indexes and the coverage index reflecting the sequencing depth. The Illumina Miseq sequencing results for bacteria shown in Table 2 indicate that the depth of sequencing for bacteria was more than 90.3% in most samples, except for AN, which was 88.5%. Table 3 lists the Illumina Miseq sequencing results for fungi and shows that all sequencing depths were greater than 98.0%.

Emission control implemented during the APEC summit had effectively reduced all forms of pollutants and rendered the air to a "good" quality (Chen *et al.*, 2015; Sun *et al.*, 2016). Bacterial and fungal communities in the $PM_{2.5}$ samples collected during that period were compared with the $PM_{2.5}$ samples collected prior to the summit. The results for bacterial communities indicated that community species richness indexes ACE and Chao1 for AN were 8145 and 4915, respectively, and greater than GN (5905 for ACE, 3768 for Chao1). Additionally, the Shannon index for AN was 5.878 and greater than the GN sample (5.786). However, AD collected in daytime during the APEC summit showed lower ACE and Chao1 indexes compared with GD. The result of statistical analysis showed no significant difference between samples collected during APEC summit and samples collected before the summit (ANOVA, P = 0.43, F = 0.95 for ACE; P = 0.39, F = 1.22 for Chao1; P = 0.69, F = 0.22 for Shannon). Similarly, the fungal communities harbored in $PM_{2.5}$ samples collected during APEC summit and before the summit showed no significantly different for indexes ACE (ANOVA, P = 0.78, F = 0.10), Chao1 (ANOVA, P = 0.80, F = 0.08), and Shannon (ANOVA, P = 0.79, F = 0.09). Therefore, emission control measures probably did not affect the species richness and community diversity of microbial community harbored in $PM_{2.5}$.

$PM_{2.5}$ samples collected before the summit, in the absence of emission control measures, were compared based on different pollution levels and sampling times. The results of bacterial communities showed that no significant variation occurred between sample groups collected under different pollution for indexes ACE (ANOVA, P = 0.29, F = 1.89), Chao1 (ANOVA, P = 0.26, F = 2.16), and Shannon (ANOVA, P = 0.29, F = 1.96). Likewise, the fungal communities showed no significant difference between them for fungal species richness and community diversity indexes (ANOVA, P > 0.05 for every index). Furthermore, the results of ANOVA analysis showed that bacterial and fungal communities had no significant difference between daytime and nighttime (ANOVA, P > 0.05 for every index). On the whole, at a short period of time, the species richness and community diversity of microbial communities in $PM_{2.5}$ would not vary significantly because of the impacts of emission control measures, air quality levels, and sampling time.

Previous studies have briefly discussed the relationship between bioaerosol and air pollution. Gao *et al.* (2015a, b) showed that the concentration of viable bioaerosols decreased with increasing haze severity in Beijing city. However, Huang *et al.* (2017) showed that airborne bacterial and fungal concentrations were positive correlated with particle mass and number concentrations. Li *et al.* (2015) suggested that bioaerosols exhibited higher concentration on hazy days than on non-hazy days in Xi'an city. Thus, previous

Table 2. Richness and diversity indices of bacteria in the eight sample groups.

Sample group	Number of sequences [a]	OTUs [b]	ACE	Chao 1	Shannon	Coverage
GD	11245	1762	5934	3939	5.506	0.907
GN	13521	2034	5905	3768	5.786	0.906
MD	15300	2028	6498	3868	5.435	0.909
MN	15823	1894	3967	2863	5.525	0.926
HD	11790	945	1148	1178	5.227	0.976
HN	12620	1705	4429	3038	5.460	0.922
AD	15268	2212	5892	3913	5.599	0.903
AN	14764	2457	8145	4915	5.878	0.885

[a] sequences that passed quality controls.
[b] The operational taxonomic units (OTU) were determined at 97% similarity.

Table 3. Richness and diversity indices of fungi in the eight sample groups.

Sample group	Number of sequences [a]	OTUs [b]	ACE	Chao 1	Shannon	Coverage
GD	9181	421	868	672	3.147	0.980
GN	13704	277	714	461	1.854	0.986
MD	13876	252	552	399	1.883	0.988
MN	10269	252	571	396	2.012	0.987
HD	12777	233	319	298	1.999	0.991
HN	17783	186	551	282	1.525	0.992
AD	13444	419	878	634	2.273	0.980
AN	12776	378	765	564	2.331	0.983

[a] sequences that passed quality controls.
[b] The operational taxonomic units (OTU) were determined at 97% similarity.

studies have presented inconsistent results on the relationship between the concentration of airborne bioaerosols and pollution levels (Gao *et al.*, 2015a, b; Li *et al.*, 2015). Our results revealed that the community richness and diversity were not affected by the air quality levels and emission control measures. This might be because community richness was independent of the concentration of airborne bioaerosols. Several factors may have influenced the bioaerosol concentration. For example, chemical pollutants, meteorological conditions, and biological sources affected the characteristics of airborne microbes (Mouli *et al.*, 2005). Some air pollutants might have potential to impair some sensitive bioaerosols (Gingell *et al.*, 1976; Adhikari *et al.*, 2006). Additionally, physiological function of plants, which were major sources of bioaerosols (Bertolini *et al.*, 2013; Bowers *et al.*, 2013), might be influenced by atmospheric particle pollution, and this might cause change of biological activity bacteria and fungi inhabiting the surface of the plant. These impacts would result in changes on the concentration of culturable microorganisms and their biological activity. However, community richness calculated through gene sequencing technology was independent of concentration and activity of microorganisms, and it estimated that how many different kinds of microorganisms in the community. Therefore, even though the concentration of micororganisms in $PM_{2.5}$ varied according with the air pollution level, the community richness would still remain stable in a short period of time.

Impact of Emission Controls and Air Quality Levels on the Bacterial Community Structures

The Bray-Curtis similarity index and Yue&Clayton similarity index were calculated based on the OTUs to quantify the impact of emission control and air quality on the bacterial community structure in the $PM_{2.5}$ samples. There were 7 dominant OTUs (abundance > 1% in all $PM_{2.5}$ sample groups), 13 major OTUs (abundance > 0.5% in all $PM_{2.5}$ sample groups), and 60 common OTUs (abundance > 0.1% in all $PM_{2.5}$ sample groups) in the bacterial communities of the $PM_{2.5}$ samples (SI Table S1). Based on the dominant OTUs, the Bray-Curtis similarity and Yue & Clayton similarity between AD and GD was 91.5% and 95.6%, respectively, and the Bray-Curtis similarity and Yue & Clayton similarity between AN and GN was 94.8% and 98.3%, respectively (Table 4). Additionally, based on the major or common OTUs, the similarity of AD and GD was more than 86.8%, and the similarity of AN and GN was more than 83.7%. The high similarity between AD-GD and AN-GN demonstrated that the emission control measures implemented during the summit had little effect on the bacterial community structure. Chen *et al.* (2015) suggested the aerosol chemical composition pattern was relatively similar before and during APEC summit although various aerosol pollutants decreased significantly by 40%–80% following the emission control measures. Furthermore, the similarities of GD-MD, GD-HD, and MD-HD were more than 84.2%, 82.3%, and 78.9%, respectively. At the same time, the similarities of GN-MN, GN-HN, and MN-HN were more than 83.1%, 73.4 %, and 65.2%, respectively. Thus, high similarity between bacterial community structures at different pollution levels was demonstrated. Hence, the impact of air pollution levels on bacterial structures was very little. Coincidentally, Wei *et al.* (2016) indicated that no significant difference was discovered for the dominant bacterial genera between haze and sunny days and this might be due to transformation of bacteria depending on their source (Bertolini *et al.*, 2013). Additionally, bacterial communities in $PM_{2.5}$ collected during day and night times were compared for four pairs of sample groups (AD-AN, GD-GN, MD-MN, HD-HN), and the average value of similarities calculated based on three levels of OTUs using two methods was 86.9%. These results demonstrated that the bacterial community structures in $PM_{2.5}$ altered slightly between day and night times. The concentration of bioaerosol is likely to vary (Dong *et al.*, 2015; Gao *et al.*, 2015a; Li *et al.*, 2015) along with the haze pollution levels, but the main biological composition pattern in $PM_{2.5}$ was probably relatively stable within different pollution levels. This

Table 4. Similarities of bacterial communities in $PM_{2.5}$ collected under different air pollution levels.

Pair-wise comparison	Bray-Curtis[a]	Yue & Clayton[a]	Bray-Curtis[b]	Yue & Clayton[b]	Bray-Curtis[c]	Yue & Clayton[c]
AD-GD	0.914912	0.955613	0.916032	0.957033	0.868348	0.936608
AN-GN	0.947849	0.982889	0.919668	0.963863	0.837341	0.910845
GD-MD	0.908037	0.961903	0.850908	0.89565	0.841817	0.886187
GD-HD	0.932844	0.968033	0.920558	0.955089	0.822717	0.884695
MD-HD	0.853416	0.91819	0.838573	0.90311	0.789131	0.85567
GN-MN	0.90302	0.961346	0.851547	0.872949	0.83129	0.857736
GN-HN	0.759697	0.784182	0.753448	0.749472	0.74311	0.73434
MN-HN	0.697861	0.651964	0.717455	0.65651	0.74909	0.675205
AD-AN	0.891519	0.922154	0.861354	0.902938	0.834444	0.87979
GD-GN	0.972138	0.996219	0.941933	0.987747	0.853723	0.926934
MD-MN	0.890453	0.95094	0.88508	0.949011	0.841441	0.91216
HD-HN	0.716415	0.723817	0.75655	0.730088	0.772534	0.752735

[a] Similarity calculation based on the dominant OTUs (relevant abundance > 1% in every sample).
[b] Similarity calculation based on the major OTUs (relevant abundance > 0.5% in every sample).
[c] Similarity calculation based on the common OTUs (relevant abundance > 0.1% in every sample).

phenomenon indicates that emission control measures and pollution levels slightly affected the community structures of bacteria, and this would implied that the sources of airborne bacteria were relatively stable in a short period of time.

A total of 24 of bacterial phyla were identified in the eight sample groups (SI Fig. S1). The phyla constituting more than 1% in all sequences included *Proteobacteria* (39.55%), *Actinobacteria* (28.56%), *Firmicutes* (15.85%), *Bacteroidetes* (9.19%), and *Cyanobacteria_Chloroplast* (3.60%), with the rest of phyla constituting 3.26% (SI Fig. S2). *Actinobacteria* was the most abundant phylum (36.81%) in the MN sample, whereas, *Proteobacteria* was the most dominant in the other seven sample groups. These dominant bacterial compositions were similar to the taxa identified in other studies (Bowers *et al.*, 2013; Liao *et al.*, 2013; Cao *et al.*, 2014). Furthermore, there were as many as 690 different genera of bacteria identified in the $PM_{2.5}$ samples, although 18224 sequences were sorted as unclassified. The top-ten dominant genera were *Sphingomonas* (6.84%), *Kocuria* (5.77%), *Paracoccus* (5.50%), *Rubellimicrobium* (3.17%), *Curtobacterium* (2.75%), *Streptophyta* (2.45%), *Methylobacterium* (2.26%), *Bacillus* (2.04%), *Clostridium_sensu_stricto* (2.00%), and *Hymenobacter* (2.00%). Nevertheless, 124 genera were identified only once in any sample and 384 genera accounted for less than 0.01% in the total sequences. At the genus level, four genera *Clostridium_XI*, *Deinococcus, Ralstonia*, and *Thermoactinomyces* were more abundant in the $PM_{2.5}$ samples with AQI > 100, than the samples with AQI ≤ 100 (SI Fig. S3). Simultaneously, the bacterial communities in the $PM_{2.5}$ samples collected at AQI ≤ 100 exhibited higher proportions of four genera, which were *Adhaeribacter, Flavisolibacter, Segetibacter*, and *Acidobacteria_Gp4*, compared to samples collected at AQI > 100. The results seemed to show that some bacteria could be responsive to the AQI, but their contribution to the variability of community structures was insufficient. Other studies also indicated that some bacteria showed considerable variation with the pollution levels (Cao *et al.*, 2014; Li *et al.*, 2015), although most airborne bacteria remained stable in the atmospheric environment during the sampling period (Bowers *et al.*, 2011a, b; Wei *et al.*, 2016). So far, characteristics of most airborne bacteria, including cellular activity, pathogenicity, resistance to unfavorable meteorological factors, and biotransformation mechanism are not very clear. Therefore, researches focusing on the relationship between pollution levels and bacterial community structure in $PM_{2.5}$ are restricted by several knowledge and technology gaps which might result in contradictory results from different researches.

Impact of Emission Control and Air Quality Levels on the Fungal Community Structure

There were 3 dominant OTUs (abundance > 1% in all $PM_{2.5}$ sample groups), 4 major OTUs (abundance > 0.5% in all $PM_{2.5}$ sample groups), and 11 common OTUs (abundance > 0.1% in all $PM_{2.5}$ sample groups) in the fungal communities of $PM_{2.5}$ samples (SI Table S2). Though the similarity of AD-GD was less than 37.5%, AD was similar to the other two $PM_{2.5}$ samples collected during day time, with more than 80% for AD-MD and more than 85% for AD-HD (Table 5, SI Table S5). The similarity between AN and GN was also more than 78.9%. The results showed that emission control measures had little impact on the fungal community structures when ingoring the GD sample. Comparison between paired $PM_{2.5}$ samples collected in daytime and nighttime revealed a high similarity of over 84.1%, except for GD-GN. The average similarity of the three pairs of samples AD-AN, MD-MN, and HD-HN, was 91.5%. Thus, the impact of sampling time was not obvious on the fungal community structure. Oddly, we found that similarities of any random paired comparison were less than 43% when GD sample was included. However, other paired comparisons, excluding GD, showed more than 76% similarity regardless of the pollution level. These results showed that GD was different from the other $PM_{2.5}$ samples because of some unknown factors. When GD was ignored, these high

Table 5. Similarities of fungal communities in $PM_{2.5}$ collected under different air pollution levels.

Pair-wise comparison	Bray-Curtis[a]	Yue & Clayton[a]	Bray-Curtis[b]	Yue & Clayton[b]	Bray-Curtis[c]	Yue & Clayton[c]
AD-GD	0.375024	0.251044	0.34911	0.248425	0.367261	0.248399
AN-GN	0.803206	0.865689	0.796432	0.861222	0.789347	0.857304
GD-MD	0.333305	0.205676	0.256547	0.171263	0.239242	0.151586
GD-HD	0.429283	0.309638	0.347122	0.245168	0.34131	0.21122
MD-HD	0.761001	0.814871	0.770362	0.82289	0.760723	0.829711
GN-MN	0.847834	0.919818	0.842343	0.923918	0.834636	0.928067
GN-HN	0.786562	0.800175	0.785901	0.816972	0.763659	0.836315
MN-HN	0.850206	0.911182	0.855147	0.918957	0.857222	0.932885
AD-AN	0.961054	0.993477	0.958643	0.993158	0.956328	0.993285
GD-GN	0.330195	0.202926	0.272359	0.182548	0.256265	0.165806
MD-MN	0.84083	0.911587	0.846184	0.916539	0.841285	0.92218
HD-HN	0.8609	0.91667	0.85663	0.92227	0.841591	0.931041

[a] Similarity calculation based on the dominant OTUs (relevant abundance > 1% in every sample).
[b] Similarity calculation based on the major OTUs (relevant abundance > 0.5% in every sample).
[c] Similarity calculation based on the common OTUs (relevant abundance > 0.1% in every sample).

similarities of paired comparisons showed that the impacts of emission control measures during the APEC summit, air quality levels, and timing of sampling were very little on the fungal community structures in $PM_{2.5}$. Gao *et al.* (2015a) also indicated that there was no obvious relationship between cultivable fungi and haze levels over the four seasons. However, previous studies showed fungal communities in the atmosphere were influenced by many factors such as wind, sunlight, pollutants, sources and geographical location (Woo *et al.*, 2013; Gao *et al.*, 2016; Yan *et al.*, 2016). Our results implied that the stability of sources of airborne fungi were the most important factor for variation of community structure in a short period of time.

At the phylum level, *Ascomycota* was predominant, comprising 94.728% of the 103810 fungal sequences in $PM_{2.5}$. The other three detected fungal phyla were *Basidiomycota*, *Chytridiomycota*, and *Zygomycota*, which accounted for 1.122%, 0.009%, and 0.013% of the total fungal sequences, respectively. The predominance of *Ascomycota* fungi in airborne particles was identified in many studies (Bowers *et al.*, 2013; Li *et al.*, 2015; Yan *et al.*, 2016), although *Basidiomycota* was more abundant than *Ascomycota* at some times (Fröhlich-Nowoisky *et al.*, 2009). Table S4 lists the 19 detected classes of fungi and indicates that *Dothideomycetes* was the most abundant with more than 49% in all $PM_{2.5}$ sample groups. Several classes of fungi in the GD, including *Sordariomycetes*, *Tremellomycetes*, *Microbotryomycetes*, *Crystobasidiomycetes*, and *Exobasidiomycetes* had higher abundance compared to other sample groups. At the genus level, the fungal sequences could be assigned to 229 genera. *Epicoccum* was the largest fungal genus (11.85%) and other major genera (> 1% of sequence reads) included *Selenophoma* (4.40%), *Cladosporium* (1.38%), and *Mycosphaerella* (1.33%). The proportion of *Selenophoma* in the GD (18.72%) was far more than other sample groups (0.71%–5.73%). The above differences in fungal taxonomy between the GD and other sample groups resulted in different fungal community structure. Several earlier studies (Fröhlich-Nowoisky *et al.*, 2009; Haas *et al.*, 2013; Dannemiller *et al.*, 2014; Oh *et al.*, 2014; Li *et al.*, 2015; Yan *et al.*, 2016) have usually reported less than five fungal genera that were more than 1% abundant, but their categories varied across different studies. These could be related to different emission sources of fungal spores at different research sites.

Variation of Microbial Pathogens in $PM_{2.5}$ Samples

According to the directory of pathogenic microorganisms infecting humans promulgated by the Ministry of Health of the People's Republic of China (MOHC), we detected six pathogenic bacterial genera and nine pathogenic fungal genera in the $PM_{2.5}$ samples (SI Fig. S4). The most abundant pathogenic bacteria were *Streptococcus* (0.440%), followed by *Mycobacterium* (0.10%), *Prevotella* (0.082%), *Escherichia* (0.037%), *Rickettsia* (0.015%), and *Erysipelothrix* (0.009%). At the genus level, the directory of human infection by pathogenic microorganisms indicates that *Erysipelothrix*, *Prevotella*, and *Rickettsia* are capable of causing certain diseases. However, the directory also lists only a few species, belonging to the genera *Escherichia*, *Mycobacterium,* and *Streptococcus*, which are known to cause diseases. Therefore, we submitted several representative sequences of the three genera to the National Center for Biotechnology Information (NCBI), and the results indicated that some *Streptococcus* sequences were exactly similar to *Streptococcus gallolyticus,* which was associated with colorectal tumors (Abdulamir *et al.*, 2011). Some *Escherichia* and *Mycobacterium* sequences were homologous with *Escherichia coli* and *Mycobacterium simiae*, respectively. *Mycobacterium simiae* causes respiratory infections (Samra *et al.*, 2005; Ramos *et al.*, 2006) and *Escherichia coli* is a common pathogen that causes gastrointestinal diseases (Paton and Paton, 1998). Compared with pathogenic bacteria, potential pathogenic fungi were more abundant. Genus *Aspergillus* was the most abundant, up to 0.54% in the fungal communities. *Penicillium* was the second richest and some sequences in the genus had a 100% similarity with *Penicillium citrinum*. Other pathogenic genera included *Alternaria* (0.097%), *Arthrinium* (0.004%), *Dactylaria* (0.003%), *Fusarium* (0.186%), *Stachybotrys* (0.035%), *Trichoderma* (0.063%), and *Trichothecium* (0.087%). At the species level, *Fusarium oxysporum*, *Fusarium poae*, *Aspergillus flavus*, and *Aspergillus ochraceus* were identified through sequence comparison in the NCBI at more than 97% similarity, and they were responsible for some plant and human diseases (Musa *et al.*, 2000; Hedayati *et al.*, 2007; Michielse and Martijn, 2009). The total abundance of pathogenic bacteria in the AD and AN sample groups collected during the summit were 0.524% and 0.616%, and pathogenic fungi were 1.406% and 1.143%, respectively. Meanwhile, pathogenic bacteria accounted for 0.4%–1.183% and pathogenic fungi accounted for 0.382%–2.788% in the other sample groups. Hence, the proportion of pathogens in $PM_{2.5}$ was relatively rare and the impact of emission control and pollution level was not apparent. Impacts of airborne particulate matter pollution on human health were clearly recognized (Cheng *et al.*, 2013; Ouyang, 2013), although Cao *et al.* (2014) declared that most of the airborne bacteria and fungi were nonpathogenic to humans. Comparing the abundance of pathogenic bacteria and fungi in different samples collected during the summit and under different air pollution levels, no clear relationship between the emission control measures or air pollution levels and pathogenic microorganisms was demonstrated. Therefore, the pathogenic microorganisms did not seem to be related to the emission control measures and air pollution levels. Our results again indicate that there is no evident association between these pathogens and air quality levels.

Bacterial community functions involving with human diseases caused by bacteria were predicted based on 16S rRNA gene sequences using the PICRUSt algorithm. A total of 8 KEGG pathways were identified. However, their proportions in total KEGG pathways were all less than 0.28%. Table S6 listed the details of pathways in every sample group. Wei *et al.* (2017) and Xu *et al.* (2017) predicted the bacterial community function in cloud water and airborne particles. However, they did not indicate how much community functions were associated with human diseases. Our results showed that most of community

functions of bacteria harbored in $PM_{2.5}$ were irrelevant with human diseases.

CONCLUSION

This work demonstrated the distribution characteristics of bacteria and fungi in $PM_{2.5}$ at different air pollution levels. Our results showed that APEC emission control measures had no significant influence on the bacterial and fungal community structures. Additionally, high similarities in bacterial and fungal community structures in the $PM_{2.5}$ samples at different pollution levels were observed, suggesting that there was no significant relationship between air pollution levels and the variations of community structures of $PM_{2.5}$ bioaerosols. We also found that most bacteria and fungi were nonpathogenic to humans and the proportion of pathogens in the total sequences was scarce. Our finding would serve as an important reference for researches working on the characteristics of bioaerosols. Considering the possible reactions between bioaerosols and air pollutants might affect human health, further research should focus on understanding the interaction of pollutants and microbes, and their combined effect on human health.

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SUPPLEMENTARY MATERIAL

Supplementary data associated with this article can be found in the online version at http://www.aaqr.org.

REFERENCE

Abarenkov, K., Henrik Nilsson, R., Larsson, K.H., Alexander, I.J., Eberhardt, U., Erland, S., Høiland, K., Kjøller, R., Larsson, E., Pennanen, T., Sen, R., Taylor, A.F.S., Tedersoo, L., Ursing, B.M., Vrålstad, T., Liimatainen, K., Peintner, U. and Kõljalg, U. (2010). The UNITE database for molecular identification of fungi – Recent updates and future perspectives. *New Phytol.* 186: 281–285.

Abdulamir, A.S., Hafidh, R.R. and Bakar, F.A. (2011). The association of Streptococcus bovis/gallolyticus with colorectal tumors: The nature and the underlying mechanisms of its etiological role. *J. Exp. Clin. Cancer Res.* 30: 11.

Adhikari, A., Reponen, T., Grinshpun, S.A., Martuzevicius, D. and Lemasters, G. (2006). Correlation of ambient inhalable bioaerosols with particulate matter and ozone: A two-year study. *Environ. Pollut.* 140: 16–28.

Amann, R.I., Ludwig, W. and Schleifer, K.H. (1995). Phylogenetic identification and in situ detection of individual microbial cells without cultivation. *Microbiol. Rev.* 59: 143–169.

Arteaga, V.E., Mitchell, D.C., Matt, G.E., Quintana, P.J.E., Schaeffer, J., Reynolds, S.J., Schenker, M.B. and Mitloehner, F.M. (2015). Occupational exposure to endotoxin in $PM_{2.5}$ and pre- and post-shift lung function in California dairy workers. *J. Environ. Prot.* 6: 552–565.

Becker, S., Dailey, L.A., Soukup, J.M., Grambow, S.C., Devlin, R.B. and Huang, Y.C. (2005). Seasonal variations in air pollution particle-induced inflammatory mediator release and oxidative stress. *Environ. Health Perspect.* 113: 1032–1038.

Bertolini, V., Gandolfi, I., Ambrosini, R., Bestetti, G., Innocente, E., Rampazzo, G. and Franzetti, A. (2013). Temporal variability and effect of environmental variables on airborne bacterial communities in an urban area of Northern Italy. *Appl. Microbiol. Biotechnol.* 97: 6561–6570.

Boldo, E., Medina, S., Tertre, A.L., Hurley, F., Mücke, H.G., Ballester, F. and Aguilera, I. (2006). Apheis: Health impact assessment of long-term exposure to $PM_{2.5}$ in 23 European cities. *Eur. J. Epidemiol.* 21: 449–458.

Bowers, R.M., Mcletchie, S., Knight, R. and Fierer, N. (2011a). Spatial variability in airborne bacterial communities across land-use types and their relationship to the bacterial communities of potential source environments. *ISME J.* 5: 601–612.

Bowers, R.M., Sullivan, A.P., Costello, E.K., Collett, J.L., Knight, R. and Fierer, N. (2011b). Sources of bacteria in outdoor air across cities in the midwestern United States. *Appl. Environ. Microbiol.* 77: 6350–6356.

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

Brook, R.D., Barry, F., Wayne, C., Yuling, H., George, H., Michael, L., Russell, L., Murray, M., Jonathan, S. and Smith, S.C. (2004). Air pollution and cardiovascular disease: A statement for healthcare professionals from the Expert Panel on Population and Prevention Science of the American Heart Association. *Circulation* 109: 2655–2671.

Bush, R.K. and Portnoy, J.M. (2001). The role and abatement of fungal allergens in allergic disease. *J. Allergy Clin. Immunol.* 107: 430–440.

Cao, C., Jiang, W.J., Wang, B.Y., Fang, J.H., Lang, J.D., Tian, G., Jiang, J.K. and 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: 1499–1507.

Chen, C., Sun, Y.L., Xu, W.Q., Du, W., Zhou, L.B., Han, T.T., Wang, Q.Q., Fu, P.Q., Wang, Z.F. and Gao, Z.Q. (2015). Characteristics and sources of submicron aerosols above the urban canopy (260 m) in Beijing, China, during the 2014 APEC summit. *Atmos. Chem. Phys.* 15: 22889–22934.

Cheng, Z., Jiang, J., Fajardo, O., Wang, S. and Hao, J. (2013). Characteristics and health impacts of particulate

matter pollution in China (2001–2011). *Atmos. Environ.* 65:186–194.

Dannemiller, K.C., Lang-Yona, N., Yamamoto, N., Rudich, Y. and 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.

Degobbi, C., Lopes, F.D.T.Q.S., Carvalho-Oliveira, R., Muñoz, J.E. and Saldiva, P.H.N. (2011). Correlation of fungi and endotoxin with $PM_{2.5}$ and meteorological parameters in atmosphere of Sao Paulo, Brazil. *Atmos. Environ.* 45: 2277–2283.

Deng, X., Zhang, F., Rui, W., Long, F., Wang, L., Feng, Z., Chen, D. and Ding, W. (2013). $PM_{2.5}$-induced oxidative stress triggers autophagy in human lung epithelial A549 cells. *Toxicol. in Vitro* 27: 1762–1770.

Dockery, D.W., Pope, C.A., Xu, X., Spengler, J.D., Ware, J.H., Fay, M.E., Ferris, B.G. and Speizer, F.E. (1993). An association between air pollution and mortality in six US cities. *N. Engl. J. Med.* 329: 1753–1759.

Dong, L., Qi, J., Shao, C., Zhong, X., Gao, D., Cao, W., Gao, J., Bai, R., Long, G. and Chu, C. (2016). Concentration and size distribution of total airborne microbes in hazy and foggy weather. *Sci. Total Environ.* 541: 1011–1018.

Englert, N. (2004). Fine particles and human health—A review of epidemiological studies. Toxicol. Lett. 149: 235–242.

Fahlgren, C., Bratbak, G., Sandaa, R.A., Thyrhaug, R. and Zweifel, U.L. (2010). Diversity of airborne bacteria in samples collected using different devices for aerosol collection. *Aerobiologia* 27: 107–120.

Feng, Y., Chen, Y., Guo, H., Zhi, G., Xiong, S., Li, J., Sheng, G. and Fu, J. (2009). Characteristics of organic and elemental carbon in $PM_{2.5}$ samples in Shanghai, China. *Atmos. Res.* 92: 434–442.

Fröhlich-Nowoisky, J., Pickersgill, D.A., Després, V.R. and Pöschl, U. (2009). High diversity of fungi in air particulate matter. *Proc. Natl. Acad. Sci. U.S.A.* 106: 12814–12819.

Frøsig, A., Bendixen, H. and Sherson, D. (2001). Pulmonary deposition of particles in welders: On-site measurements. *Arch. Environ. Health* 56: 513–521.

Gao, J.F., Fan, X.Y., Li, H.Y. and 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: 788–798.

Gao, M., Jia, R., Qiu, T., Han, M., Song, Y. and Wang, X. (2015a). Seasonal size distribution of airborne culturable bacteria and fungi and preliminary estimation of their deposition in human lungs during non-haze and haze days. *Atmos. Environ.* 118: 203–210.

Gao, M., Qiu, T., Jia, R., Han, M., Song, Y. and Wang, X. (2015b). Concentration and size distribution of viable bioaerosols during non-haze and haze days in Beijing. *Environ. Sci. Pollut. Res. Int.* 22: 4359–4368.

Gao, M., Yan, X., Qiu, T., Han, M. and Wang, X. (2016). Variation of correlations between factors and culturable airborne bacteria and fungi. *Atmos. Environ.* 128: 10–19.

Gingell, S.M., Campbell, R. and Martin, M.H. (1976). The effect of zinc, lead and cadmium pollution on the leaf surface microflora. *Environ. Pollut.* 11: 25–37.

Haas, D., Galler, H., Luxner, J. and Zarfel, G. (2013). The concentrations of culturable microorganisms in relation to particulate matter in urban air. *Atmos. Environ.* 65: 215–222.

Haas, D., Habib, J., Luxner, J., Galler, H., Zarfel, G., Schlacher, R., Friedl, H. and Reinthaler, F.F. (2014). Comparison of background levels of culturable fungal spore concentrations in indoor and outdoor air in southeastern Austria. *Atmos. Environ.* 98: 640–647.

He, K., Yang, F., Ma, Y., Zhang, Q., Yao, X., Chan, C.K., Cadle, S., Chan, T. and Mulawa, P. (2001). The characteristics of $PM_{2.5}$ in Beijing, China. *Atmos. Environ.* 35: 4959–4970.

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

Huang, H.L., Lee, M.Q. and Shih, H.W. (2017). Assessment of indoor bioaerosols in public spaces by real-time measured airborne particles. *Aerosol Air Qual. Res.* 17: 2276–2288.

Kozich, J.J., Westcott, S.L., Baxter, N.T., Highlander, S.K. and Schloss, P.D. (2013). Development of a dual-index sequencing strategy and curation pipeline for analyzing amplicon sequence data on the MiSeq Illumina sequencing platform. *Appl. Environ. Microbiol.* 79: 5112–5120.

Lang, J., Zhang, Y., Zhou, Y., Cheng, S., Chen, D., Guo, X., Chen, S., Li, X., Xing, X. and Wang, H. (2017). Trends of $PM_{2.5}$ and chemical composition in Beijing, 2000-2015. *Aerosol Air Qual. Res.* 17: 412–425.

Li, Y., Fu, H., Wang, W., Liu, J., Meng, Q. and Wang, W. (2015). Characteristics of bacterial and fungal aerosols during the autumn haze days in Xi'an, China. *Atmos. Environ.* 122: 439–447.

Liao, X., Anyi, H.U., Yang, X.Y., Chen, J.S., Changping Y.U. and Lin, J.Q. (2013). Community composition and environmental sources of bacteria and eukaryotic microorganisms in fine air particulate ($PM_{2.5}$) of Xiamen in winter. *Ecol. Environ. Sci.* 22: 1395–1400 (in Chinese).

Matthias-Maser, S., Gruber, S. and Jaenicke, R. (1995). The size distribution of primary biological aerosol particles with radii > 0.2 μm in an urban/rural influenced region. *Atmos. Res.* 39: 279–286.

Meng, Z.Y., Jiang, X.M., Yan, P., Lin, W.L., Zhang, H.D. and Wang, Y. (2007). Characteristics and sources of $PM_{2.5}$ and carbonaceous species during winter in Taiyuan, China. *Atmos. Environ.* 41: 6901–6908.

Michielse, C.B. and Martijn, R. (2009). Pathogen profile update: Fusarium oxysporum. *Mol. Plant Pathol.* 10: 311–324.

Mouli, P.C., Mohan, S.V. and Reddy, S.J. (2005). Assessment of microbial (bacteria) concentrations of ambient air at semi-arid urban region: Influence of meteorological factors. *Appl. Ecol. Environ. Res.* 3: 139–149.

Musa, M.O., Al Eisa, A., Halim, M., Sahovic, E., Gyger,

M., Chaudhri, N., Al Mohareb, F., Seth, P., Aslam, M. and Aljurf, M. (2000). The spectrum of Fusarium infection in immunocompromised patients with haematological malignancies and in non-immunocompromised patients: A single institution experience over 10 years. *Br. J. Haematol.* 108: 544–548.

Nocker, A., Burr, M. and Camper, A.K. (2007). Genotypic microbial community profiling: A critical technical review. *Microb. Ecol.* 54:276–289.

Nonnenmann, M.W., Bextine, B., Dowd, S.E., Gilmore, K. and Levin, J.L. (2010). Culture-independent characterization of bacteria and fungi in a poultry bioaerosol using pyrosequencing: A new approach. *J. Occup. Environ. Hyg.* 7: 693–699.

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

Ouyang, Y.D. (2013). China wakes up to the crisis of air pollution. *Lancet Respir. Med.* 1: 12.

Paton, J.C. and Paton, A.W. (1998). Pathogenesis and diagnosis of Shiga toxin-producing Escherichia coli infections. *Clin. Microbiol. Rev.* 11: 450–479.

Pope III, C.A., Burnett, R.T., Thun, M.J., Calle, E.E., Krewski, D., Ito, K. and Thurston, G.D. (2002). Lung cancer, cardiopulmonary mortality, and long-term exposure to fine particulate air pollution. *JAMA* 287: 1132–1141.

Ramos, A., Noblejas, A., Daza, R., Martin, T., Masip, V. and Ortega, A. (2006). Mycobacterium simiae pulmonary infection in an hiv-positive patient. *Infect. Med.* 23: 133–135.

Risom, L., Møller, P. and Loft, S. (2005). Oxidative stress-induced DNA damage by particulate air pollution. *Mutat. Res.* 592: 119–137.

Ruprecht, J. (2005). Abundance of cellular material and proteins in the atmosphere. *Science* 308: 73–73.

Samra, Z., Kaufman, L., Pitlik, S., Shalit, I and Bishara, J. (2005). Emergence of Mycobacterium simiae in respiratory specimens. *Scand. J. Infect. Dis.* 37: 838–841.

Stoeck, T., Zuendorf, A., Breiner, H.W. and Behnke, A. (2007). A molecular approach to identify active microbes in environmental eukaryote clone libraries. *Microb. Ecol.* 53: 328–339.

Sun, Y., Wang, Z., Wild, O., Xu, W., Chen, C., Fu, P., Du, W., Zhou, L., Zhang, Q., Han, T., Wang, Q., Pan, X., Zheng, H., Li, J., Guo, X., Liu, J. and Worsnop, D.R. (2016). "APEC Blue": Secondary aerosol reductions from emission controls in Beijing.*Sci. Rep*. 6: 20668.

Tang, G., Zhu, X., Hu, B., Xin, J., Wang, L., Münkel, C., Mao, G. and Wang, Y. (2015). Impact of emission controls on air quality in Beijing during APEC 2014: Lidar ceilometer observations. *Atmos. Chem. Phys.* 15: 12667–12680.

Tarigan, Y.G., Chen, R.Y., Lin, H.C., Jung, C.Y., Kallawicha, K., Chang, T.P., Hung, P.C., Chen, C.Y. and Chao, H.J. (2017). Fungal bioaerosol exposure and its effects on the health of mushroom and vegetable farm workers in Taiwan. *Aerosol Air Qual. Res.* 17: 2064–2075.

Valavanidis, A., Fiotakis, K. and Vlachogianni, T. (2008). Airborne particulate matterand human health: Toxicological assessment and importance of size and composition of particles for oxidative damage and carcinogenic mechanisms. *J. Environ. Sci. Health Part C* 26: 339–362.

Wang, J., Guinot, B., Dong, Z., Li, X., Xu, H., Xiao, S., Ho, S.S.H., Liu, S. and Cao, J. (2017). $PM_{2.5}$-bound polycyclic aromatic hydrocarbons (PAHs), oxygenated-PAHs and phthalate esters (PAEs) inside and outside middle school classrooms in Xi'an, China: Concentration, characteristics and health risk assessment. *Aerosol Air Qual. Res.* 17: 1811–1824.

Wang, Q., Garrity, G.M., Tiedje, J.M. and Cole, J.R. (2007). Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. *Appl. Environ. Microbiol.* 73: 5261–5267.

Wei, K., Zou, Z., Zheng, Y., Li, J., Shen, F., Wu, C.Y., Wu, Y., Hu, M. and Yao, M. (2016). Ambient bioaerosol particle dynamics observed during haze and sunny days in Beijing. *Sci. Total Environ.* 550: 751–759.

Wei, M., Xu, C., Chen, J., Zhu, C., Li, J. and Lv, G. (2017). Characteristics of bacterial community in cloud water at Mt Tai: Similarity and disparity under polluted and non-polluted cloud episodes. *Atmos. Chem. Phys.* 17: 5253–5270.

Woo, A.C., Brar, M.S., Chan, Y., Lau, M.C.Y., Leung, F.C.C., Scott, J.A., Vrijmoed, L.L.P., Zawar-Reza, P. and Pointing, S.B. (2013). Temporal variation in airborne microbial populations and microbially-derived allergens in a tropical urban landscape. *Atmos. Environ.* 74: 291–300.

Xiu, G., Wu, X., Wang, L., Chen, Y., Yu, Y., Xu, F. and Wu, L. (2015). Characterization of Particulate matter, ions and OC/EC in a museum in Shanghai, China. *Aerosol Air Qual. Res.* 15: 1240–1250.

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

Xu, D., Dan, M., Song, Y., Chai, Z. and Zhuang, G. (2005). Concentration characteristics of extractable organohalogens in $PM_{2.5}$ and PM_{10} in Beijing, China. *Atmos. Environ.* 39: 4119–4128.

Yan, D., Zhang, T., Su, J., Zhao, L.L., Wang, H., Fang, X.M., Zhang, Y.Q., Liu, H.Y. and 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.

Zhan, C., Zhang, J., Cao, J., Han, Y., Wang, P., Zheng, J., Yao, R., Liu, H., Li, H. and Xiao, W. (2016). Characteristics and sources of black carbon in atmospheric dustfall particles from Huangshi, China. *Aerosol Air Qual. Res.* 16: 2096–2106.

Zhao, M., Wang, S., Tan, J., Hua, Y., Wu, D. and Hao, J. (2016). Variation of urban atmospheric ammonia pollution and its relation with $PM_{2.5}$ chemical property in winter of Beijing, China. *Aerosol Air Qual. Res.* 16: 1378–1389.

Zheng, M., Salmon, L.G., Schauer, J.J., Zeng, L., Kiang,

C.S., Zhang, Y. and Cass, G.R. (2005). Seasonal trends in $PM_{2.5}$ source contributions in Beijing, China. *Atmos. Environ.* 39: 3967–3976.

Zhou, X., Cao, Z., Ma, Y., Wang, L., Wu, R. and Wang, W. (2016). Concentrations, correlations and chemical species of $PM_{2.5}/PM_{10}$ based on published data in China: Potential implications for the revised particulate standard. *Chemosphere* 144: 518–526.

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