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Microbial population structure in near-ground aerosols during fog-haze days in northern China

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Abstract Studies on microbial populations in near-ground aerosols during fog–haze days could help enhance our knowledge concerning the relationship between fog–haze and human health. In this study, microbial populations in aerosols near-ground during a fog–haze event in Beijing were analyzed using clone library methods. Results showed that the bacterial diversity in aerosols during fog–haze days was lower than that after fog–haze days. Proteobacteria alone and Proteobacteria with Firmicutes were respectively detected in 1.5 and 20 m aerosols during fog–haze days. In addition to Proteobacteria and Firmicutes, Bacteroidetes alone and Acidobacteria with Verrucomicrobia were respectively found in aerosols at 20 and 1.5 m after fog–haze days. The fungal species observed during fog–haze days were completely different from those detected after fog–haze days. Ascomycota and Basidiomycota were respectively detected during and after fog–haze days. The distribution of microbial diversity in aerosols exhibited meteorological and site-associated variations. The same potential pathogenic microorganisms were detected at different heights during fog–haze days. This study on the characteristics of microbial population in aerosols could provide a comprehensive understanding of the factors causing the harmful effects of particles on humans during fog–haze days.

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Introduction

Fog–haze events exhibit an episodic tendency in China (Sun et al., 2006; Liu et al., 2008; Zhang et al., 2012; Li et al., 2013a; Zhao et al., 2013; Tao et al., 2012, 2013; Xu et al., 2016; He et al., 2016; Yin et al., 2017). Aerosol particles during fog–haze days can affect human health and cause several diseases, particularly respiratory diseases (Salma et al., 2002; Spindler et al., 2010; Pope et al., 2011; Atkinson et al., 2012; Nirmalkar and Deb, 2016). As such, the properties of aerosols and their microbial composition have been extensively investigated.

Microbial concentration and size distribution in aerosols vary under different weather conditions. Hu et al. (2015) demonstrated that cultured bacterial and fungal concentrations in aerosols are lower on haze days than on non-haze days. Conversely, Gao et al. (2015) observed that cultured bacterial and fungal concentrations in aerosols are higher on haze days than on non-haze days. The total microbial concentration in

aerosols on foggy and hazy days is also higher than that on non-hazy days (Dong et al., 2016). Cultivation methods initially revealed that Gram-positive bacteria were the dominant ones in aerosols (Di Giorgio et al., 1996; Shaffer and Lighthart, 1997). With the development of molecular biological techniques based on DNA analysis, *Proteobacteria*, *Firmicutes*, and *Actinobacteria* are often observed in aerosols (Ranjard et al., 2000; Radosevich et al., 2002; Wilson et al., 2002; Maron et al., 2005). In addition to bacterial populations, fungal populations in aerosols have also been explored (Medrela-Kuder, 2003; Fröhlich-Nowoisky et al., 2009; Dannemiller et al., 2014).

Considering the relationship between microorganisms in aerosols and human health, researchers examined the microbial characteristics of aerosols from near-ground areas. Bowers et al. (2011) observed that bacterial communities in aerosols sampled from different land uses are related to sources but not to local meteorological conditions. They also investigated the seasonal variability of microbial diversity in aerosols and showed that microbial diversity exhibits significant seasonality patterns (Bowers et al., 2013). Microbial populations in aerosols at 2 m near-ground are characterized by a strong seasonal trend related to general climatic variables, but these populations are not significantly affected by urban development, as indicated by anthropogenic pollutants and human population levels (Woo et al., 2013). Gou et al. (2016) sampled Particulate Matter (PM) from 5 m above ground without surrounding barriers and analyzed the microbial populations in PM_1 and PM_{10}. They found that *Proteobacteria*, *Firmicutes*, and *Actinobacteria* are the main communities in PM while *Ascomycota* and *Basidiomycota* are the main fungal groups in PM. Cao et al. (2014) used metagenomic methods and observed that the relative abundance of respiratory microbial allergens and pathogens increases as the concentrations of PM pollution increase during a severe smog event in January in Beijing.

The microbial properties of aerosols from near-ground are of different states under various weather conditions. However, microbial populations in aerosols from human breathing height under special weather conditions, such as fog–haze days, have been rarely examined. The crucial connection between urban atmosphere pollution and human health remains poorly understood because of insufficient intensive investigations on microorganisms. In our study, microbial populations in aerosols from near-ground (1.5 and 20 m) were investigated. Aerosols were sampled during and after fog–haze days from January 29 to February 2, 2013. A cloning library was prepared to analyze the bacterial and fungal diversities and contents in aerosols from different heights under various weather

conditions. The characteristics of the microorganisms in aerosols during and after fog–haze days were compared. Our results could provide valuable data to improve knowledge regarding aerosol properties in different pollution episodes in Beijing.

## Materials and methods

Sample collection

All aerosol samples were consecutively collected at 1.5 m (breathing height) and 20 m (height of no surrounding obstacles) above ground during and after fog-haze days in northern China (116.34° E, 40.01° N) (Fig. 1, Fig. S1 and Table 1). An impingement airborne microorganism sampler (SKC BioSampler, SKC Inc. USA) was used to collect aerosols (Han et al., 2012). The collection vessel of this sampler was filled with 20 ml of sterile phosphate buffered saline as the collection medium. Air samples were transferred into the collection medium by drawing the gas at a flow rate of 12.5 l/min. During fog-haze days, the samples were collected eight hours each days and Continuous three days. After fog-haze days, the samples were collected twelve hours each days and Continuous two days. Several meteorological parameters were observed. Temperature and relative humidity were recorded by using a dewpoint thermohygrometer (WD-35612, OAKTON, Germany). Wind speed and irradiance were measured with a portable anemometer (HD2303, Delta OHM, Italy) and irradiance meter (HD2302.0, Delta OHM, Italy), respectively.

Clone library

The total DNA of aerosols was isolated using a DNA autoplate (TanBead, Taiwan) by Automatic Platform for Magnetic System-16 (TanBead, Taiwan). DNA bands were observed through 1.0% agarose gel electrophoresis.

All of the samples were assayed in duplicate. The universal primers F16S-27/R16S-1492 and ITS1/ITS4 were used to amplify the bacterial 16S rRNA and fungal 18S rRNA segment, respectively (Madsen et al., 2015). PCR amplification was performed using the Gene AmpR PCR System (9700, AB, USA) at a final volume of 50 μl. The reaction mixture contained 2.0 μl of both primers, 2.0 μl of DNA template, 4.0 μl of each dNTPs, 5.0 μl of 10 × PCR buffer (TaKaRa, China), 0.25 μl of Taq DNA polymerase (TaKaRa, China) and 36.75 μl DNA-free water. Temperature cycling conditions of bacterial PCR were 94 °C for 5 min, followed by 25 cycles of 94 °C for 30 s, 56 °C for 30 s, and 72 °C for 90 s. A final extension at 72 °C for 10 min was used. Fungal PCR included 95 °C

Fig. 1 Location of sampling sites in Beijing. ▲: Sampling site
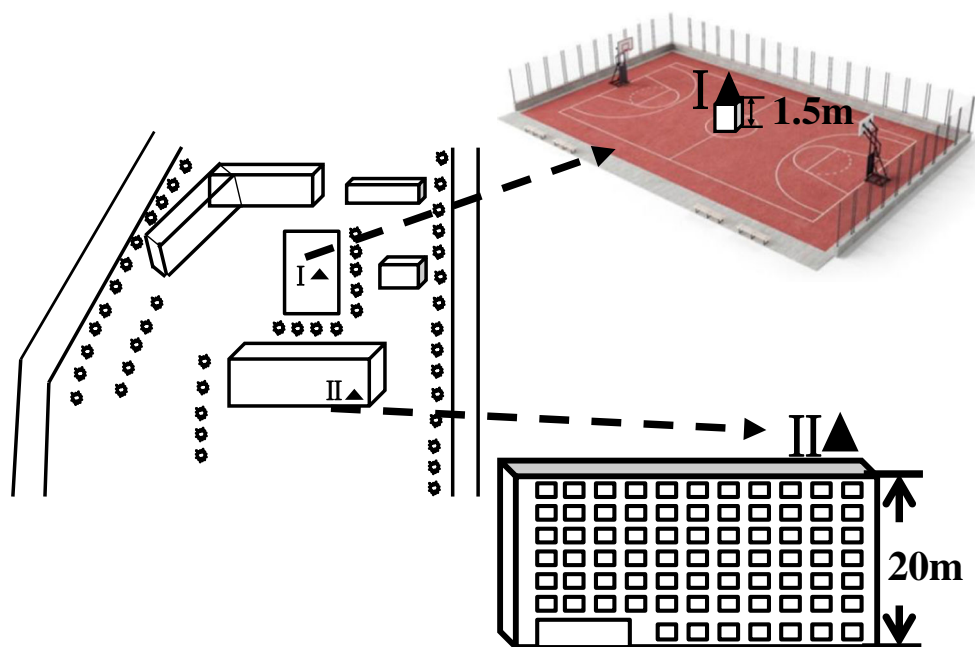
for 5 min, followed by 36 cycles of 95 °C for 30 s, 55 °C for 30 s, and 72 °C for 1 min, followed by 72 °C for 10 min. The PCR products were purified, ligated to the vector, and transformed into competent cells. The positive clones were submitted for sequencing by using an ABI 3730DXL DNA sequencer (AB, USA).

All of the sequences from the clone libraries were trimmed to exclude vector sequences and then checked for chimeras with Bellerophon via Greengenes (http://greengenes.lbl.gov/). The aligned sequences (DNAMAN V6) were grouped into universal operational taxonomic units (OTUs) at a threshold of 97% minimum similarity. Individual sequences were also grouped into OTUs to identify microbial distribution in aerosols from different sampling sites. Representative sequence of each OTU in the libraries was aligned to the NCBI database by the BLAST website. Phylogenetic affiliations of all OTUs

were identified by constructing a phylogenetic tree including representative sequences of each OTU and related sequences from the previous NCBI database using the neighbor-joining algorithm by MEGA version 6.1.

Coverage and diversity of clone libraries from different samples

The coverage of each clone library was calculated according to the following equation (Good, 1953):

$$C = 1 - (n/N)$$

n is the number of unique clones
N is the total number of sequences examined

Table 1 Sampling sites location

Date	Meteorological conditions	Continuous sampling times (h)	Height (m)	Sample code of bacterial aerosols	Sample code of fungal aerosols
2013/1/29	During fog-haze days	72	1.5	HYP-FZB1.5	HYP-FZF1.5
2013/1/30 2013/1/31			20	HYP-FZB20	HYP-FZF20
2013/2/1	After fog-haze days	48	1.5	HYP-CB1.5	HYP-CF1.5
2013/2/2			20	HYP-CB20	HYP-CF20

HYP-FZB1.5: bacteria in 1.5 m aerosols during fog-haze days;HYP-FZB20: bacteria in 20 m aerosols during fog-haze days; HYP-FZF1.5: fungi in 1.5 m aerosols duringfog-haze days; HYP-FZF20: fungi in 20 m aerosols during fog-haze days; HYP-CB1.5: bacteria in 1.5 m aerosols after fog-haze days;HYP-CB20: bacteria in 20 m aerosols after fog-haze days; HYP-CF1.5: fungi in 1.5 m aerosols after fog-haze days; HYP-CF20: fungi in 20 m aerosols after fog-haze days

Whether the coverage of a clone library was large enough to represent the diversity of an original community was determined. The diversity of each sample was estimated using the Shannon Wiener index (Ding et al., 2008).

Results

Coverage of clone libraries

A total of 400 sequences for bacteria and 200 sequences for fungi were obtained. After alignment was completed, 7, 2, 22, and 15 OTUs were identified in HYP-FZB20, HYP-FZB1.5, HYP-CB20, and HYP-CB1.5 clone libraries, respectively, and 7, 13, 1, and 4 OTUs were determined in the 50 screened fungal clones from HYP-FZF20, HYP-FZF1.5, HYP-CF20, and HYP-CF1.5 clone libraries, respectively. Coverage analysis indicated that the aerosol libraries represented approximately 83.00%–99.00% of the total number of the examined clones. Thus, a dependable inventory of bacterial 16S rRNA and fungal 18S rRNA gene sequences present in aerosols is provided.

Microbial diversity in aerosols during and after fog–haze days

Shannon–Wiener indexes (Fig. S2a) of bacterial species were 1.32, 0.23, 2.47, and 2.23 for HYP-FZB20, HYP-FZB1.5, HYP-CB20, and HYP-CB1.5, respectively. The species richness of bacteria in aerosols collected from clear days (after fog–haze days in our study) was higher than that in aerosols sampled from fog–haze days. The bacterial diversity in 20 m aerosols was also richer than in 1.5 m aerosols during and after fog–haze days. The change in the bacterial diversity in 1.5 m to 20 m aerosols during fog–haze days was more apparent than that after fog–haze days. Shannon–Wiener indexes (Fig. S2b) of the fungal species were 1.83, 2.15, 0, and 0.42 for HYP-FZF20, HYP-FZF1.5, HYP-CF20, and HYP-CF1.5, respectively. The species richness of the fungal diversity in aerosols during fog–haze days at 1.5 m height was the highest among the four fungal clone libraries. The fungal diversity in aerosols was different from bacterial diversity. The fungal diversity in aerosols during fog–haze days was richer than that after fog–haze days, and the fungal diversity in aerosols at 1.5 m height was richer than that at 20 m height.

Microbial population structure in aerosols during and after fog–haze days

Sequences were assigned to bacterial and fungal affiliations according to their position in the phylogenetic tree (Figs. S3 and S4 and Tables S1 and S2).

During fog–haze days, most of the bacteria in 20 m aerosols were assigned to uncultured *Burkholderia* (51.92%) and uncultured bacterium (28.85%); the minor groups were *Enhydrobacter*, *Achromobacter*, *Anaerovorax*, and *Sporosarcina* (Fig. S3a and Table S1). Under the same weather conditions and in the same sampling sites, six fungal genera, namely, *Stemphylium* (25.93%), *Penicillium* (22.22%), *Curvularia* (18.52%), *Chalara* (14.81%), *Aureobasidium* (14.81%), and *Aspergillus* (3.71%),were detected (Fig. S4a and Table S2). During fog–haze days, only two bacterial genera were observed at human breathing height (1.5 m). As shown in Fig. S3b and Table S1, *Burkholderia* was the most represented bacterial group (94.00%), and the other OTUs were related to uncultured *Achromobacter* (6.00%). The dominant fungal groups at 1.5 m were *Chalara* (35.48%), *Penicillium* (19.37%), *Curvularia* (12.90%), *Aspergillus* (9.68%), and *Stemphylium* (9.68%). The other OTUs were related to uncultured *Talaromyces*, *Cadophora*, *Geomyces*, or*Aureobasidium* (Fig. S4b and Table S2).

After fog–haze days, the represented bacterial groups in HYP-CB20 were *Achromobacter* (47.17%), *Stenotrophomonas* (16.98%), uncultured bacterium (9.45%), and *Sporosarcina* (5.66%). The other minor OTUs were *Idiomarina*, *Nevskia*, *Brevundimonas*, *Tsukamurella*, *Carnobacterium*, *Chryseomicrobium*, *Bacillus*, *Halomonas*, *Sphingomonas*, *Microvirga*, and *Legionellaceae* (Fig. S3c and Table S1). The fungal group in HYP-CF20 was *Cryptococcus* (100%). The bacterial groups in HYP-CB1.5 were uncultured bacterium (37.74%), *Pseudomonas* (22.64%), *Psychrobacter* (11.32%), and *Acinetobacter* (9.43%). The other minor OTUs were *Clostridium*, *Acidobacteria*, *Rhodocyclus*, *Hyphomiceobiaceae*, *Carnobacterium*, and *Verrucomicrobiales* (Fig. S3d and Table S1). The represented fungal group in HYP-CF1.5 was uncultured *Cryptococcus* (96.77%). The other minor OTU was *Clavulina*.

Pathogenic property in aerosols during and after fog–haze days

Among these identified bacterial and fungal species, some microorganisms are potential pathogens (Table S3). During fog–haze days, *Burkholderia fungorum* (Peeters et al., 2016) and *Achromobacter* sp. (Coward et al., 2016) were also detected at 20 and 1.5 m height. The respective relative percentages of *Burkholderia fungorum* were 1.92% and 94% at 20 and 1.5 m height, and the respective relative percentages of *Achromobacter* sp. were 5.77% and 6% at 20 and 1.5 m height. After fog–haze days, several species, including 47.17% *Achromobacter* sp., 1.89% *Sphingomonas aurantiaca* (Babic et al., 2015), 1.89% *Nevskia* sp. (Low et al., 2016), 1.89% *Idiomarina* sp. (Cosseau et al., 2016), 3.78% *Legionella* bacterium (Alum and Isaacs, 2016), and 1.89% *Tsukamurella tyrosinosolvens* (Romano et al., 2011),
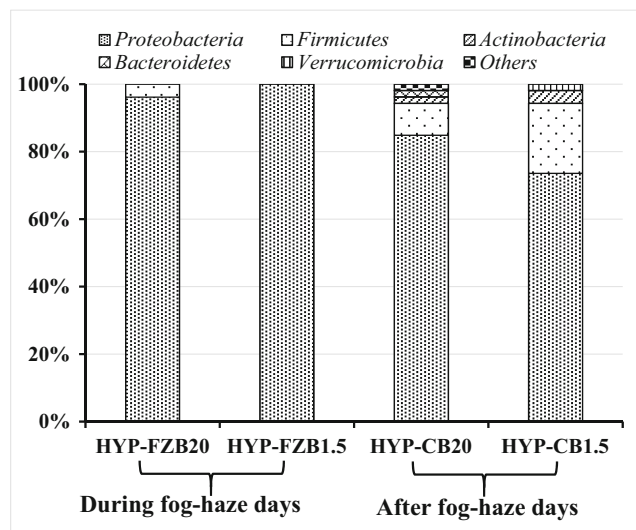
were detected at 20 m height. Two potential pathogens, namely, 3.77% *Acinetobacter lwoffii* (Baldeo et al., 2015) and 11.32% *Psychrobacter* sp. (Benhamed et al., 2014) were detected at 1.5 m height. For fungi, 14.81% and 3.23% *Aureobasidium* sp. (Oliveira et al., 2013) were respectively found at 20 and 1.5 m height during fog–haze days, and 100% and 90.32% *Cryptococcus albidus* (Liu et al., 2014) were respectively observed at 20 and 1.5 m height after fog–haze days.

Discussion

Studies on microbial populations in aerosols have indicated that bacteria affiliated with *Proteobacteria*, *Firmicutes*, *Actinobacteria*, and *Bacteroidetes* and fungi associated with *Ascomycota* and *Basidiomycota* are detected in near-ground aerosols (Bowers et al., 2013). However, the precise characteristics of microorganisms in aerosols on human breathing height have yet to be identified under unique weather conditions, such as fog–haze days. In our work, aerosols were sampled from human breathing height (1.5 m) and at a height at which no surrounding barriers were observed (20 m) during and after fog-haze days. At the phylum level, the microbial populations were different between during and after fog-haze days.

In Fig. 2, only *Proteobacteria* and *Firmicutes* were detected in aerosols during fog–haze days. Five bacterial phyla, namely, *Proteobacteria*, *Firmicutes*, *Actinobacteria*, *Bacteroidetes*, and *Verrucomicrobia*, were detected in aerosols after fog–haze days. As the dominant bacterial phylum, *Proteobacteria* also varied under different weather conditions
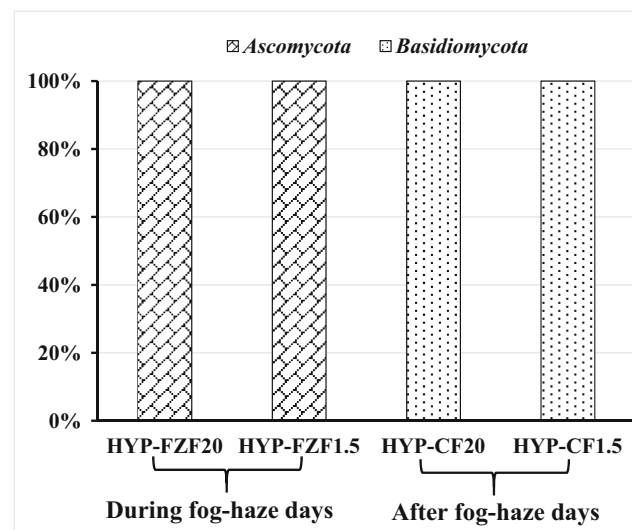
with deepening classification (Fig. 4). During fog–haze days, only *Betaproteobacteria* were detected. *Gammaproteobacteria*, *Alphaproteobacteria*, and *Betaproteobacteria* were found in aerosols after fog–haze days. The difference in fungal groups was more evident. All of the fungi detected during fog–haze days were affiliated with *Ascomycota*, and all of the fungi found after fog–haze days were associated with *Basidiomycota* (Fig. 3).

Our study found that microbial populations in aerosols demonstrated site-related characteristics under different weather conditions. In Fig. 4, the bacteria at 1.5 m height were also found at 20 m height during fog–haze days, but this phenomenon did not appear after fog–haze days. During fog–haze days, *Burkholderiales* was observed at 1.5 m height. *Burkholderiales*, *Rhodocyclales*, and *Pseudomonadales* were also found at 20 m height. After fog–haze days, three bacterial orders, namely, *Rhodocyclales*, *Pseudomonadales*, and *Rhizobiales*, were detected in 1.5 m aerosols, and seven bacterial orders, namely, *Burkholderiales*, *Oceanospirillales*, *Alteromonadales*, *Legionellales*, *Xanthomonadales*, *Caulobacterales*, and *Sphingomonadales*, were observed in 20 m aerosols.

For fungi, the species detected at 20 m height were nearly detected at 1.5 m height during and after fog–haze days (Fig. 5). The classes and genera were also different at various heights during and after days. After fog–haze days, *Tremellomycetes* were detected at 20 m height, whereas *Agaricomycetes* and *Tremellomycetes* were found at 1.5 m height. Class taxonomic levels of *Dothideomycetes*, *Leotiomycetes*, and *Eurotiomycetes* did not differ during fog–haze days at 1.5 and 20 m height. Conversely, these fungi varied in the genus taxonomic level at different heights. The following genera were detected at 20 m height: *Stemphylium*,

Fig. 2 Proportion of bacterial population in aerosols during and after fog-haze days (HYP-FZB1.5: bacteria in 1.5 m aerosols during fog-haze days; HYP-FZB20: bacteria in 20 m aerosols during fog-haze days; HYP-CB1.5: bacteria in 1.5 m aerosols after fog-haze days; HYP-CB20: bacteria in 20 m aerosols after fog-haze days)

Fig. 3 Proportion of fungal population in aerosols during and after fog-haze days (HYP-FZF1.5: fungi in 1.5 m aerosols during fog-haze days; HYP-FZF20: fungi in 20 m aerosols during fog-haze days; HYP-CF1.5: fungi in 1.5 m aerosols after fog-haze days; HYP-CF20: fungi in 20 m aerosols after fog-haze days)
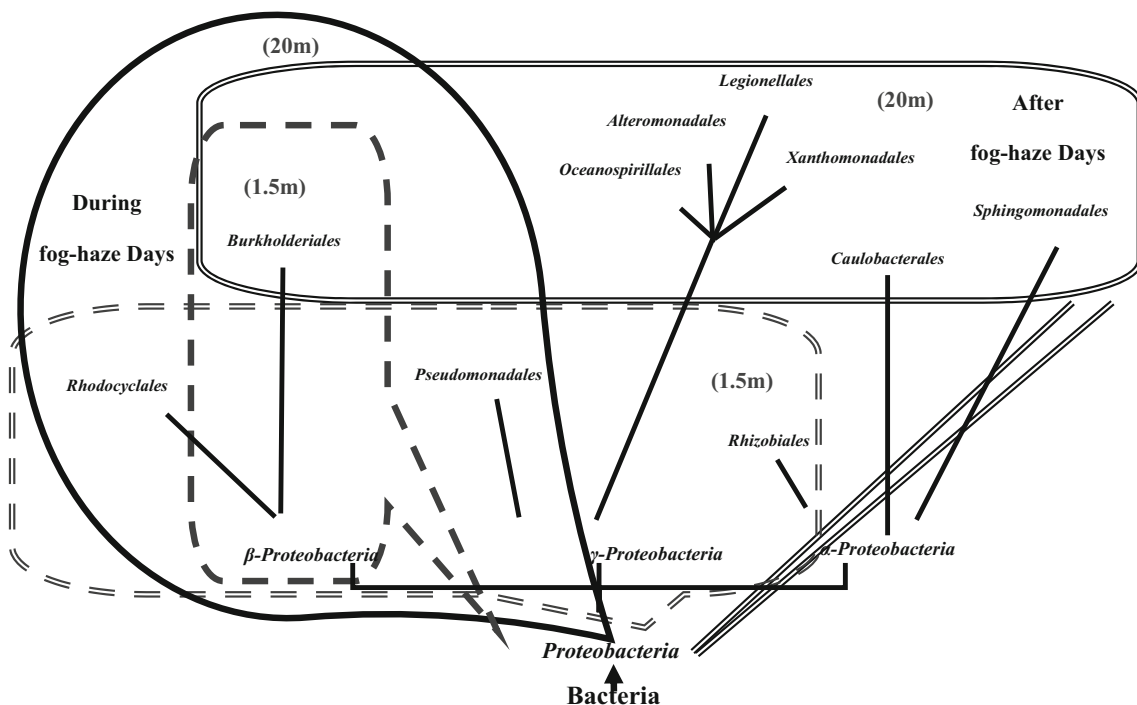
Fig. 4 Bacterial property of affiliation in near-ground aerosols during and after fog-haze days

Aureobasidium, and *Curvularia* with *Dothideomycetes*; *Chalara* with *Leotiomycetes*; and *Penicillium* and *Aspergillus* with *Eurotiomycetes*. In addition to these genera, *Cadophora* and *Geomyces* with *Leotiomycetes* and *Talaromyces* with *Eurotiomycetes* were detected at 1.5 m height.

Potential pathogenic bacteria, such as *Burkholderia fungorum* and *Achromobacter* sp., were also detected in aerosols at 20 and 1.5 m height during fog–haze days, and the percentages of these two species increased from 1.92% to 94% and from 5.77% to 6% at 20 and 1.5 m height, respectively. As fog–haze disappears, completely different
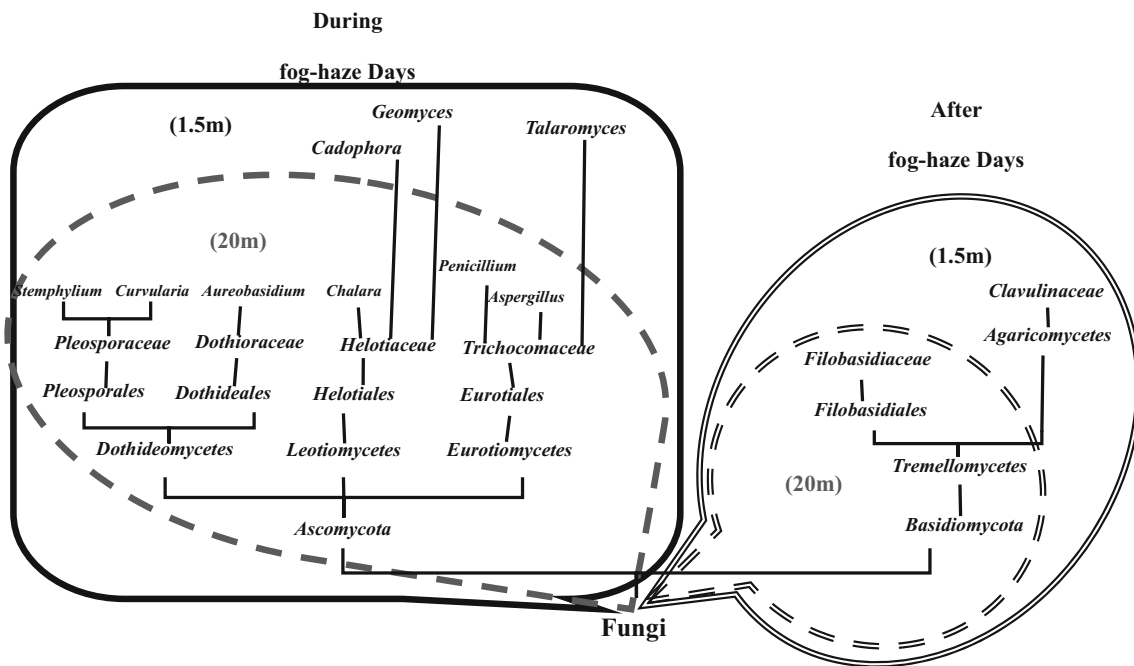

Fig. 5 Fungal property of affiliation in near-ground aerosols during and after fog-haze days

pathogenic bacteria were observed in 20 and 1.5 m aerosols. At 20 m, six potential pathogenic bacteria, namely, 47.17% *Achromobacter* sp., 1.89% *S. aurantiaca*, 1.89% *Nevskia* sp., 1.89% *Idiomarina* sp., 1.89% *Legionella*-like amoebal pathogen, and 1.89% *Tsukamurella tyrosinosolvens* and two potential pathogenic bacteria, namely, 11.32% *Psychrobacter* sp. and 3.77% *Acinetobacter lwoffii*, were observed. For potential pathogenic fungi, 14.81% and 3.23% *Aureobasidium* sp. were respectively detected in aerosols at 20 and 1.5 m height during fog–haze days, and 100% and 90.32% *Cryptococcus* sp. were respectively found in aerosols at 20 and 1.5 m height after fog–haze days. These results suggested that the distribution of microbial populations in aerosols presented at different heights near-ground during fog-haze days compared with that after fog-haze days.

The difference in microbial distribution in near-ground aerosols under various weather conditions can be determined on the basis of meteorological conditions. In our work, particulate matter concentration, irradiance, wind speed, temperature, and relative humidity were recorded and monitored. In Table 2, these weather conditions mainly differed in terms of irradiance and wind speed during and after fog–haze days. During fog–haze days, irradiance and wind speed were 2.54–5.60 and 0.02–0.07 m/s, respectively. After fog–haze days, irradiance and wind speed increased to 65.16–438.50 W/m^2 and 1.10–7.83 m/s. A previous study indicated that the formation of fog–haze is closely related to meteorological conditions (Quan et al., 2011), particularly wind speed. Wind speed is a key factor that affects aerosol concentration during fog–haze days. Low wind speeds likely affect the diffusion and transport of aerosols Li et al., 2013b) and therefore accounted for the similarity of microbial populations in aerosols between 20 and 1.5 m during fog–haze days in our study. After fog–haze days, microbial populations, especially bacterial population, differed between 20 and 1.5 m because of particle diffusion as wind speed increased (1.10–7.83 m/s).

As an important microbial carrier, aerosols possibly affect human health (Tie et al., 2009; Xu et al., 2013). In our study, potential pathogenic bacteria and fungi detected at 20 m could be detected at 1.5 m during fog–haze days. The relative contents of potential pathogenic bacteria, such as *Burkholderia fungorum* and *Achromobacter* sp., at 1.5 m were higher than

those at 20 m. However, this phenomenon was not observed after fog–haze days. This result implied that these potential pathogenic microorganisms posed greater harm to humans during fog–haze days than after fog–haze days.

Conclusions

The characteristics of microbial populations in aerosols during fog–haze outbreak periods in Beijing are examined. Although bacterial diversity in aerosols during fog–haze days is lower than that after fog–haze days, the distribution of bacterial populations in aerosols from different sampling sites varies under different weather conditions. The bacterial population in aerosols is similar between 20 and 1.5 m during fog–haze days. After fog–haze days, the bacteria in aerosols detected at 20 and 1.5 m height also differ. Only *Carnobacterium* sp. was also detected in 20 and 1.5 m aerosols. The fungal diversity in aerosols during fog–haze days is higher than that after fog–haze days, and the fungal population in aerosols is similar during and after fog–haze days. This phenomenon is also observed in potential pathogenic microorganisms. Two potential pathogenic bacteria, namely, *Burkholderia fungorum* and *Achromobacter* sp., are detected at 20 and 1.5 m height, and their percentages increase as height decreases during fog–haze days.

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Table 2 The meteorological parameters during sampling dates

	Fog-Haze days	
	During	After
Temperature (°C)	−1.00-3.00	2.00–8.00
Relative Humidity (%)	63.50–86.50	35.00–40.00
Wind speed(m/s)	0.02–0.07	1.10–7.83
Irradiance(W/m^2)	2.54–5.60	65.16–438.50

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