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Structural Variation in the Bacterial Community Associated with Airborne Particulate Matter in Beijing, China, during Hazy and Nonhazy Days

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ABSTRACT The structural variation of the bacterial community associated with particulate matter (PM) was assessed in an urban area of Beijing during hazy and nonhazy days. Sampling for different PM fractions ($PM_{2.5}$ [<2.5 μm], PM_{10} [<10 μm], and total suspended particulate) was conducted using three portable air samplers from September 2014 to February 2015. The airborne bacterial community in these samples was analyzed using the Illumina MiSeq platform with bacterium-specific primers targeting the 16S rRNA gene. A total of 1,707,072 reads belonging to 6,009 operational taxonomic units were observed. The airborne bacterial community composition was significantly affected by PM fractions ($R = 0.157$, $P < 0.01$). In addition, the relative abundances of several genera significantly differed between samples with various haze levels; for example, *Methylobacillus*, *Tumebacillus*, and *Desulfurispora* spp. increased in heavy-haze days. Canonical correspondence analysis and permutation tests showed that temperature, SO_2 concentration, relative humidity, PM_{10} concentration, and CO concentration were significant factors that associated with airborne bacterial community composition. Only six genera increased across PM_{10} samples (*Dokdonella*, *Caenimonas*, *Geminicoccus*, and *Sphingopyxis*) and $PM_{2.5}$ samples (*Cellulomonas* and *Rhizobacter*), while a large number of taxa significantly increased in total suspended particulate samples, such as *Paracoccus*, *Kocuria*, and *Sphingomonas*. Network analysis indicated that *Paracoccus*, *Rubellimicrobium*, *Kocuria*, and *Arthrobacter* were the key genera in the airborne PM samples. Overall, the findings presented here suggest that diverse airborne bacterial communities are associated with PM and provide further understanding of bacterial community structure in the atmosphere during hazy and nonhazy days.

IMPORTANCE The results presented here represent an analysis of the airborne bacterial community associated with particulate matter (PM) and advance our understanding of the structural variation of these communities. We observed a shift in bacterial community composition with PM fractions but no significant difference with haze levels. This may be because the bacterial differences are obscured by high bacterial diversity in the atmosphere. However, we also observed that a few genera (such as *Methylobacillus*, *Tumebacillus*, and *Desulfurispora*) increased significantly on heavy-haze days. In addition, *Paracoccus*, *Rubellimicrobium*, *Kocuria*, and *Arthrobacter* were the key genera in the airborne PM samples. Accurate and real-time techniques, such as metagenomics and metatranscriptomics, should be developed for a future survey of the relationship of airborne bacteria and haze.

KEYWORDS airborne bacterial community, $PM_{2.5}$, PM_{10}, TSP, haze, high-throughput sequencing

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Particulate matter (PM) is a complicated mixture composed of inorganic substances, organic compounds, and biological components (1). Numerous studies have demonstrated that PM plays an important role in air pollution, visibility reduction, and climate change (2–6). PM varies considerably in size from 0.02 to 100 μm (7). It is necessary to study the characteristics of total suspended particulates (TSP), PM_{10} (particulate size, $<$10 μm), and $PM_{2.5}$ (particulate size, $<$2.5 μm), as the various PM fractions show significantly different compositions (8). Previous evidence suggests that exposure to high levels of PM pollutants, particularly $PM_{2.5}$, is linked to increased human morbidity and mortality, as well as a shortened life span (9, 10).

Bacteria associated with PM are present in the atmosphere in the form of spores, vegetative cells, or dividing cells (11, 12). Before removal from the atmosphere by precipitation or direct deposition onto surfaces, small bacteria can reside in the air for several days and be transported over long distances (13). Because of their important role in the atmosphere, there are many studies on the bacterial communities associated with PM. However, most previous studies have focused on the bacterial diversity associated with PM using traditional methods of identification of culturable isolates (14–24). However, the culture method cannot reflect the actual diversity of airborne bacteria because of its selectivity.

With recent advances in high-throughput sequencing, more accurate diversity and community composition of PM-associated airborne bacteria have been revealed in different regions, including Beijing (China) (25, 26); Hong Kong (China) (27); Chicago (IL), Cleveland (OH), and Detroit (MI) (28); Denver and Greely (CO) (29); Boulder (CO) (30); Washington, DC (31); Colorado Front Range (USA) (32); Milan (Italy) (33, 34); the McMurdo Dry Valleys (Antarctica) (12); and the Pyrenees (Spain) (35). However, to our knowledge, there has been no study on the bacterial communities in various PM fractions (TSP, PM_{10}, and $PM_{2.5}$) during hazy and nonhazy days.

As a metropolis with a population of over 20 million, Beijing has long been suffering from serious haze due to the accumulation of PM (25, 36–40). Under stable weather conditions, PM can accumulate over a long period to form heavy haze (41). High relative humidity (rH) and low temperatures accelerate the chemical transformation to aerosols (42). Regional transport has also been found to play an important role during haze episodes. Ge et al. (43) indicated that the contribution of regional transport to haze was higher than that of local emissions in the Beijing-Tianjin-Hebei region during haze episodes. Secondary organic aerosols and secondary inorganic aerosols, which are the major contributors to PM, have multiple environmental effects, especially visibility degradation, in Beijing (44–46). Huang et al. (44) found that the major sources of haze were coal burning, dust, traffic, biomass burning, and cooking. Airborne bacterial communities associated with PM in Beijing have been characterized (25, 26). However, these studies were based on a limited number of samples or lacked long-term monitoring.

This study used high-throughput sequencing to investigate the PM-associated airborne bacterial community in Beijing during the autumn and winter. The aims of the research were to (i) examine how airborne bacterial diversity and community composition are associated with PM, (ii) determine whether the bacterial community composition differs between various types of PM or between samples from different haze levels, (iii) identify the genera associated with PM, and (iv) determine which environmental factors influence the bacterial community composition.

RESULTS

Richness and diversity of airborne bacteria. We obtained 3,616,411 raw reads and 2,911,649 valid reads after demultiplexing and quality filtering (see Table S1 in the supplemental material). After subsampling, a total of 1,707,072 reads (17,782 reads per sample) were clustered into 6,009 unique operational taxonomic units (OTUs) (97% similarity). Alpha diversity was estimated by the Chao1 index, Good's coverage estimator, and Shannon index (Table S1). The rarefaction curves indicated that high-throughput sequencing captured the dominant phylotypes, and Shannon index curves

showed high diversity in the airborne bacterial community (Fig. S1). On average, there were 370 (range, 111 to 904), 903 (range, 216 to 1,786), and 1,146 (range, 255 to 1,970) OTU in $PM_{2.5}$ samples ($PM_{2.5}S$), PM_{10} samples ($PM_{10}S$), and TSP samples (TSPS), respectively, which suggests that the number of OTUs increases as the size fraction increases. The highest values of Chao1 and Shannon index were observed in TSPS, while the lowest ones were found in $PM_{2.5}S$. Moreover, one-way analysis of variance (ANOVA) showed significant differences between the various PM fractions ($P < 0.05$), which indicated significantly higher richness and diversity in bigger-PM-fraction samples. However, no significant differences in Chao1 and Shannon index values were found between the hazy and nonhazy samples (Fig. S2).

Airborne bacterial community composition. We found that the airborne bacterial community was dominated by the *Proteobacteria* (38.5%), including *Gammaproteobacteria* (15.0%), *Alphaproteobacteria* (14.3%), and *Betaproteobacteria* (8.5%), followed by the *Firmicutes* (26.8%), *Actinobacteria* (17.2%), *Bacteroidetes* (7.7%), and *Deinococcus-Thermus* (5.2%) (Table S3 and Fig. 1A). The dominant orders were the *Bacillales* (16.1%) and *Clostridiales* (6.7%), which belong to *Firmicutes*; *Pseudomonadales* (7.4%), *Burkholderiales* (5.6%), and *Rhizobiales* (5%), which belong to *Proteobacteria*; and *Micrococcales* (7.9%), which belongs to *Actinobacteria* (Table S3). *Anoxybacillus* (6.6%) was the most abundant genus, followed by *Acinetobacter* (3.8%), *Pseudomonas* (2.7%), *Sphingomonas* (2.5%), *Staphylococcus* (2.5%), *Thermus* (2.5%), and *Paracoccus* (2.5%) (Table S3 and Fig. 1B).

Airborne bacterial communities with different PM fractions. The Venn diagram presented in Fig. 2A shows that there were 2,530 OTU shared among $PM_{2.5}S$, $PM_{10}S$, and TSPS. Most genera were distributed differently in three PM fractions, except for *Methylobacterium* and *Streptococcus*, which had a similar relative abundances in PM samples. *Anoxybacillus* and *Acinetobacter* were the most abundant genera in both $PM_{2.5}S$ and $PM_{10}S$ but not in TSPS. Moreover, *Pseudomonas* and *Thermus* were the third most abundant genera in $PM_{2.5}S$ and $PM_{10}S$, respectively. In TSPS, *Paracoccus*, *Sphingomonas*, and *Anoxybacillus* were the most abundant genera (Table S4). In addition, some genera were more abundant in certain PM fractions, for example, *Paracoccus*, *Sphingomonas*, and *Kocuria* were richer in $PM_{10}S$ and TSPS (Fig. S3).

The analysis of similarity (ANOSIM; $R = 0.157$, $P < 0.01$) and principal-component analysis (PCoA) indicated that there was a significant effect of PM fractions on airborne bacterial community composition, especially between $PM_{2.5}S$ and TSPS (Fig. S4A). The linear discriminant analysis effect size (LEfSe) analysis demonstrated significant differences in the various bacterial taxa associated with PM fractions. However, only six genera increased in total across $PM_{10}S$ (*Dokdonella*, *Caenimonas*, *Geminicoccus*, and *Sphingopyxis*) and $PM_{2.5}S$ (*Cellulomonas* and *Rhizobacter*). Using LEfSe, we identified a large number of taxa that were significantly increased in TSPS, such as *Paracoccus*, *Kocuria*, and *Sphingomonas*, indicating that more richness and differences existed in TSPS (Fig. S5).

Airborne bacterial communities with different haze levels. The Venn diagram presented in Fig. 2B shows that there were 3,565 OTU shared among non-, light-, and heavy-haze days. More unique OTUs were observed in light-haze samples than in non- and heavy-haze samples. Several genera were abundant in samples with all three levels of haze. For example, *Anoxybacillus* was the most abundant genus in three fractions of samples. *Acinetobacter* was the second most abundant in light- and heavy-haze samples, while it was the fourth most abundant in nonhazy samples. However, some genera showed higher abundance in specific samples. For instance, *Methylophilus* was a relatively abundant genus (>3%) in nonhazy samples but showed low abundance (<1%) in both light- and heavy-haze samples (Table S5).

A heatmap diagram (Fig. S3) was used to illustrate the distributions of dominant bacterial genera in PMs collected from various-haze-level days. For example, *Streptococcus* increased as the haze became heavier; whereas *Sphingomonas* spp. decreased during heavy-haze days. *Methylophilus* spp. increased during nonhazy days.

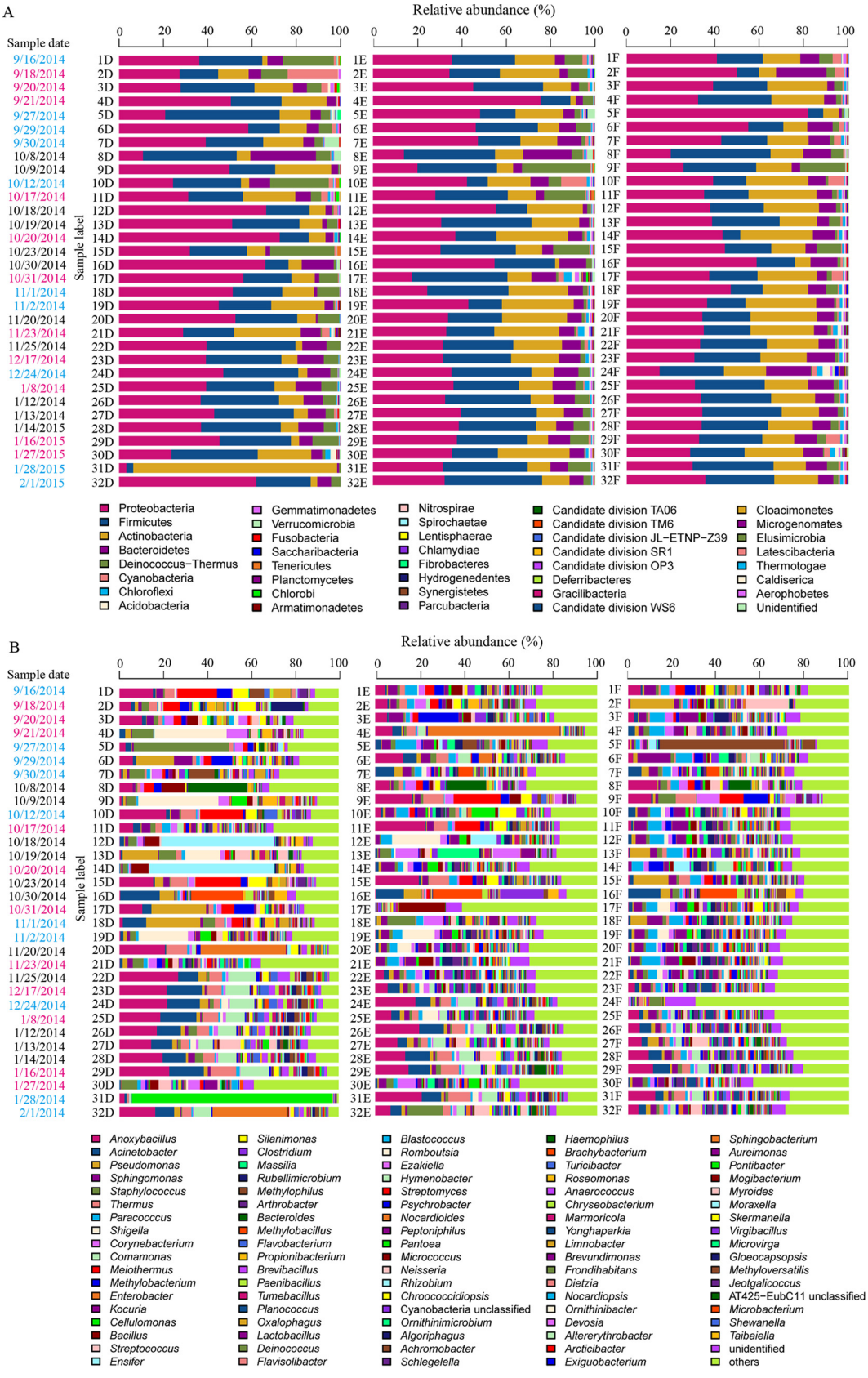

FIG 1 Dominant bacterial groups identified at the phylum level (A) and at the genus level (B). Blue text, pink text, and black text for the sample dates refer to non-, light-, and heavy-haze sample days, respectively. D, E, and F represent $PM_{2.5}$, PM_{10}, and TSP, respectively. The category "others" includes all genera for which relative abundances are lower than 2%.

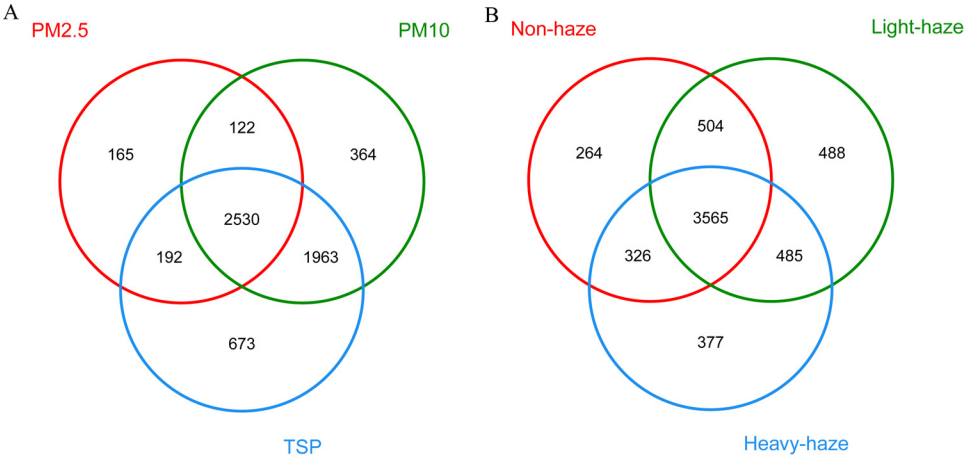

FIG 2 Venn diagrams illustrating the number of unique and shared OTUs among the three PM fractions ($PM_{2.5}S$, $PM_{10}S$, and TSPS) (A) and the three haze levels (non-, light, and heavy haze) (B).

Though there was no significant differences in bacterial communities among non-, light-, and heavy-haze samples ($R = 0.017$, $P > 0.05$; Fig. S4B), several genera with significant different relative abundances were observed using LEfSe analysis. We found that *Methylobacillus*, *Tumebacillus*, and *Desulfurispora* increased in heavy-haze samples. Conversely, *Sphingomonas*, *Massilia*, and *Oxalophagus* increased in nonhaze samples (Fig. 3).

The effects of environmental factors on the airborne bacterial community. The canonical correspondence analysis (CCA) (Fig. 4A) and permutation test (Table S6) were performed to examine the relationships between the environmental factors and bacterial community composition. Five environmental factors, including temperature ($r^2 = 0.72$, $P < 0.01$), SO_2 concentration ($r^2 = 0.29$, $P < 0.01$), rH ($r^2 = 0.12$, $P < 0.01$), PM_{10} ($r^2 = 0.1$, $P < 0.01$), and CO concentration ($r^2 = 0.09$, $P < 0.05$) showed significant correlations with airborne bacterial community composition.

Figure 4B shows the distributions of bacterial genera in relation to environmental factors. Genera, such as *Methylophilus*, *Meiothermus*, *Oxalophagus*, *Ensifer*, and *Propionibacterium*, were positively correlated with temperature and rH at the lower concentrations of SO_2 and CO. In contrast, *Algoriphagus*, *Comamonas*, *Achromobacter*, *Brevibacillus*, *Planococcus*, and the two most abundant genera (*Acinetobacter* and *Anoxybacillus*) were negatively correlated with temperature and rH at the higher concentrations of SO_2 and CO.

Airborne bacterial cooccurrences within the networks. To study the possible interactions among airborne bacteria in the PM samples, the 122 dominant genera (relative abundance, >0.1%) were used to construct a network. In the resulting network, each node is a bacterial genus (Fig. 5), and edges indicate significant cooccurrent (green) or mutual exclusion (red) interactions. The network included 48 nodes and 155 edges. However, all the networks generated had exclusively cooccurrent interactions and never exhibited mutual exclusions. Topological properties are always used in network analysis to describe the complex pattern of interrelationships. The average network distance between all pairs of nodes (average path length) was 1.97. The clustering coefficient (the degree to which they tend to cluster together) was 0.46. The heterogeneity value (the possibility of hubs) of this network was 0.85. *Paracoccus* (21 edges), *Rubellimicrobium* (19 edges), and *Kocuria* (17 edges), which increased in bigger-PM samples, were the key genera in the airborne PM samples. *Arthrobacter* (17 edges), which increased in light-haze samples, was the key genus.

DISCUSSION

As a result of our chosen sampling and sequencing method, we observed higher diversity and richness than most previous culture-independent studies in airborne

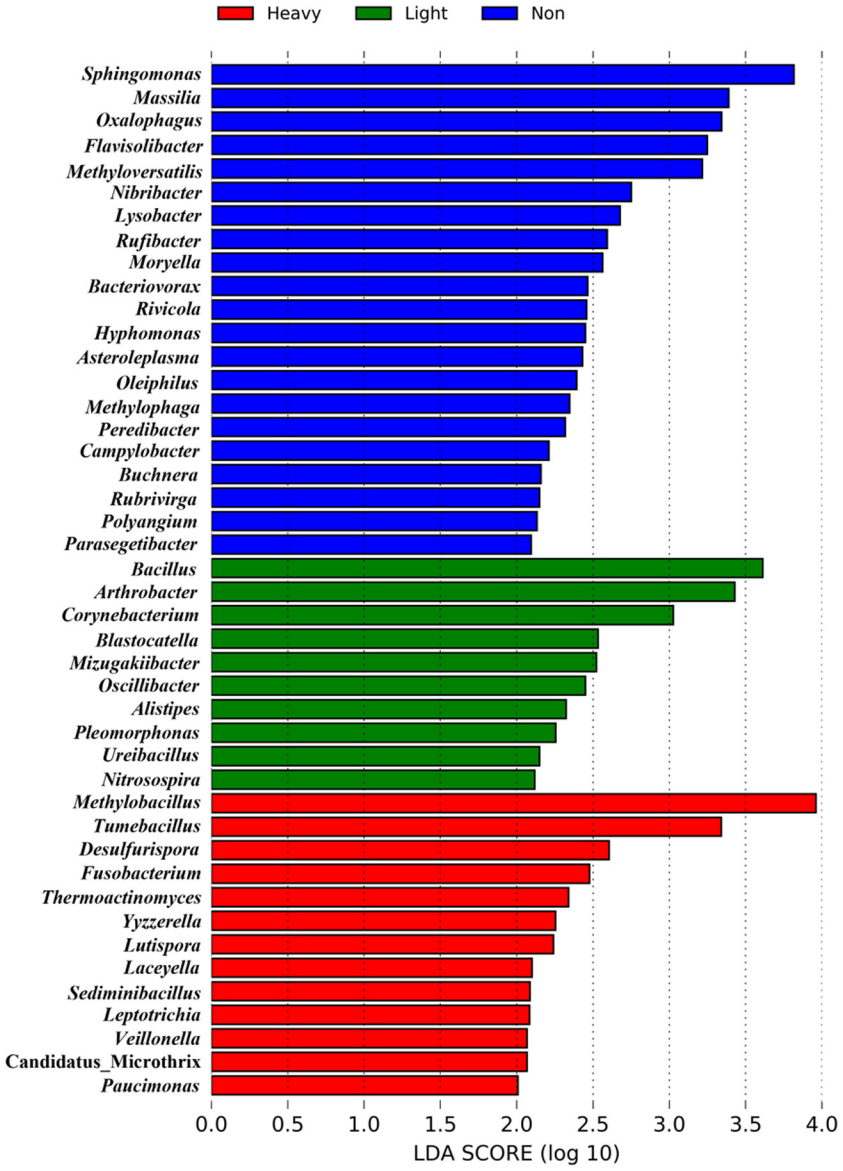

FIG 3 LEfSe analysis illustrating differentially abundant bacterial genera among samples with different haze levels. LDA scores (only those genera that obtain a log LDA score of >2 are ultimately considered) can be interpreted as the degree of consistent difference in relative abundance between genera in non-, light-, and heavy-haze samples.

bacterial communities (26, 28–30, 32, 47); however, similar diversity patterns were observed in the surface atmosphere in the United States (29). The increased size of the PM fraction increased the diversity and richness of airborne bacterial communities. In addition, no significant differences in airborne bacterial diversity and richness were observed between hazy and nonhazy days. To our knowledge, no similar studies of airborne bacterial diversity in various haze levels have been reported, as previous studies have largely focused on viable bacterial concentration. Wei et al. (48) reported that heavily polluted areas had similar concentrations of viable bioaerosol particles with near-pristine environments. The PM can act as carriers and supply the nutrition, which leads to increases to the airborne microbial concentration. However, bacteria could also be inactivated by atmospheric pollutants with the increase of PM (49). Thus, the increase in PM may have a variety of effects on the airborne bacterial diversity and richness.

The PM-associated bacterial community composition in Beijing found in this study

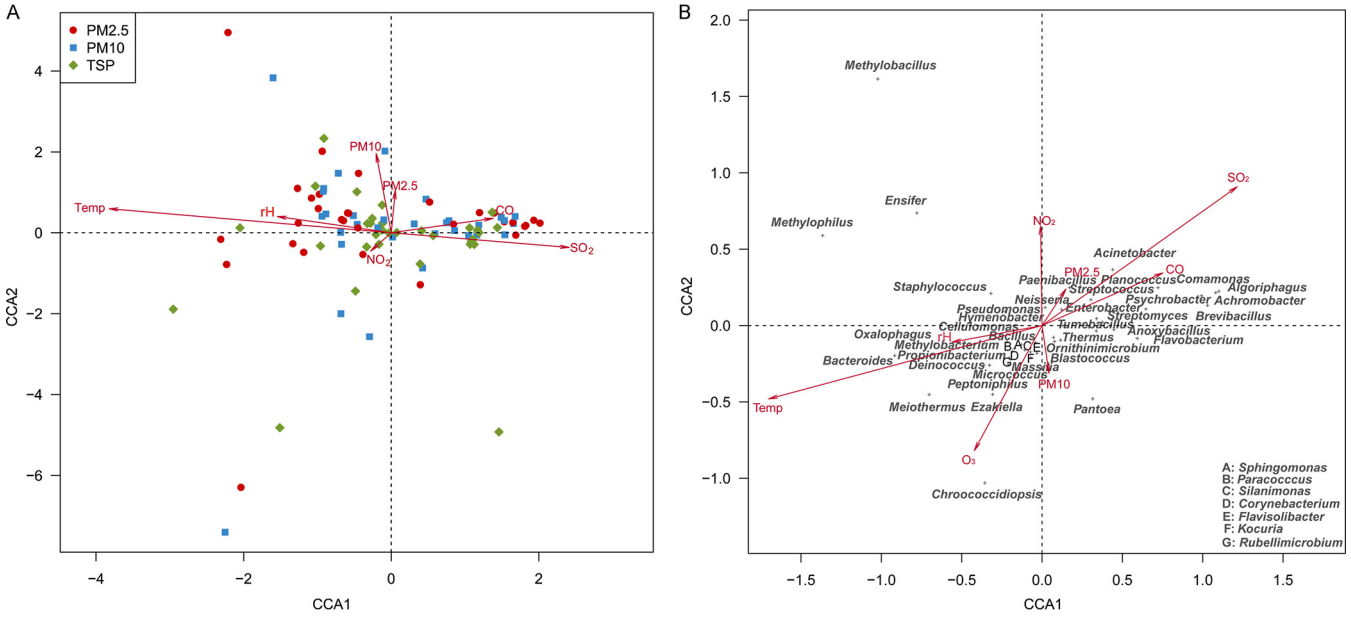

FIG 4 Canonical correspondence analysis showing the relationships between environmental factors and airborne bacterial community composition (A), and the relationships between environmental factors and dominant genera (B).

is qualitatively similar to that identified from other air surveys (28, 34, 35, 50). The high abundance of human-associated *Gammaproteobacteria* (*Acinetobacter*, *Pseudomonas*, etc.) and plant-associated *Alphaproteobacteria* (*Sphingomonas*, *Paracoccus*, etc.) may be because of the features of region in a large city (28, 34). Public places, such as parks,

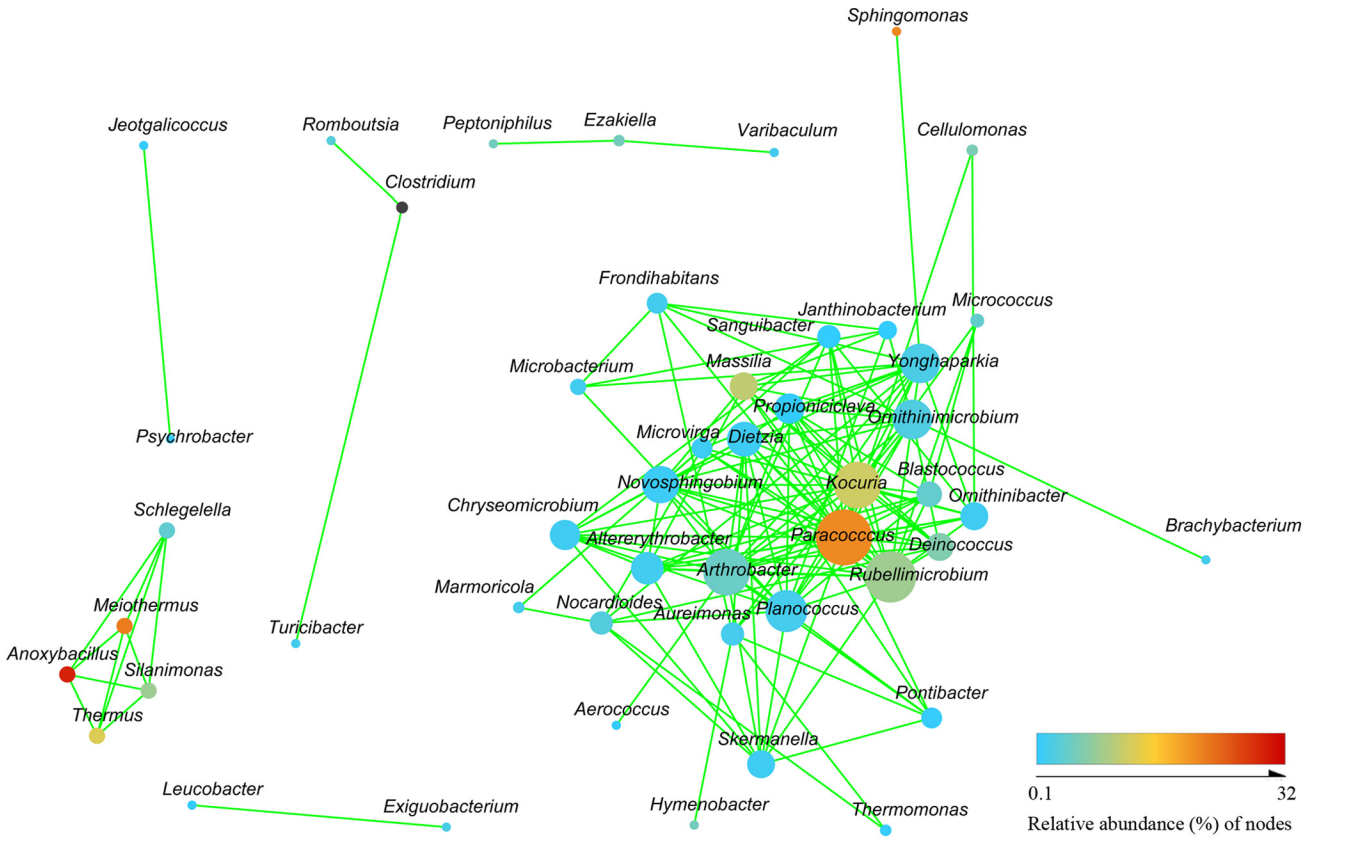

FIG 5 Network analysis indicating interactions among dominant bacteria.

hospitals, and the theater, are not too far from our sampling site, which would strongly contribute to defining the dominant phylum. Spore-forming bacteria, most of which are classified as *Firmicutes* and *Actinobacteria*, were also found to be increased in the atmospheric environments of Texas (51), Colorado (50), Hong Kong (27), Milan (34), Antarctica (12), and the Arctic (52). This suggests that these groups could be the result of the selection for spore-forming bacteria during long-range transport (12). *Anoxybacillus* was the most abundant genus in this study and also dominated the airborne bacterial community in Antarctica (12). Previous studies indicated that *Anoxybacillus* spp., isolated from hot springs, are thermophilic bacteria and might be highly mobile as spores (12, 53). In addition, *Acinetobacter* spp. are ubiquitous in soil and water, resistant to dry conditions, and easily transported by air (54). Hervàs et al. (55) proposed *Acinetobacter* spp. to be indicators of airborne transport from the Sahara. The ability to be transported and retain DNA integrity in the atmosphere may contribute to the dominance of these genera.

The PM fraction influenced the composition of the airborne bacterial community in this study. Various genera were distributed in different PM fractions, probably because of their different sources. In previous studies, road and soil dust, coal combustion, biomass burning, and vehicle exhausts contributed most sources of PM in haze episodes in Beijing (56, 57). Coal combustion and biomass burning were the most important sources of fine particles, and soil dust contributed most to coarse particles (56, 57). For example, one strain of *Cellulomonas* was isolated from coal mine soil (58), and two strains of *Cellulomonas* were isolated from medium-added coal tar as the sole source of carbon and energy (59). Therefore, *Cellulomonas* spp. may be able to live with coal or biomass ashes, which explained the significant increase in *Cellulomonas* spp. in $PM_{2.5}S$ compared with other fractions. In addition, most genera that significantly increased in coarse particles, such as *Dokdonella*, *Caenimonas*, *Paracoccus*, and *Kocuria*, were consistently sourced from soil (53). In addition, the features of each bacterial genus may influence the bacterial community in different sizes of PM. We suggest that spore-forming bacteria, such as *Anoxybacillus* and *Bacillus* spp., decrease in larger-PM fractions because of the small size of spores. Microorganisms that can easily aggregate will form larger PM. For example, certain species of *Sphingomonas* can aggregate by polar fimbriae (53), which may increase *Sphingomonas* spp. in TSPS. Furthermore, pathogens, allergens, and toxins in fine-PM fractions are more likely to penetrate deeper into the human lung, triggering serious diseases (60). In particular, potential opportunistic pathogens were also observed in these samples. *Pseudomonas* and *Acinetobacter* spp. were in great abundance in $PM_{2.5}S$; thus, these genera are known to have members that are potential pathogens of respiratory tract disease (61, 62).

Compared to large bacteria, small bacteria, such as spores, can easily float in the air and increase in concentration during haze events, perhaps because of low wind speed on hazy days. Moreover, spore-forming bacteria with high resistance to pollution can also survive in harsh environments, such as surfaces of soil, rocks, and plants, and are the origin of airborne bacteria. This may explain why *Methylobacillus* and *Tumebacillus* spp. are rich during hazy days. In addition, previous studies have reported that most of the haze pollution in Beijing originates from traffic, coal burning, biomass burning, and cooking (44, 63). Therefore, the thermophilic sulfate-reducing bacterium *Desulfurispora* (64), the numbers of which increased on hazy days, may retain intact DNA in extreme hot experiments, such as coal burning areas. *Sphingomonas* spp., which were common in previous studies on airborne bacterial communities (55, 65–67) and decreased in hazy days in this study, are sensitive to pollution. It is therefore difficult to retain DNA integrity in samples, possibly because these bacteria are non-spore forming. Though several taxa were found to be related to haze levels, no significant difference was observed in the bacterial communities between hazy and nonhazy samples. In contrast, we observed a significant difference in the fungal community between hazy and nonhazy samples in our previous study (68). We speculate that fungi, which are than bacteria, cannot be easily suspended in the air during hazy days because of the weak convection.

Based on our results, temperature had the highest correlation with bacterial community composition, which is similar to the results from previous studies (16, 18, 21, 22). According to Alghamdi et al. (18), temperature may help enhance the chemical reactions of PM to form more toxic compounds. In addition, the effect of temperature could also be interpreted as having an indirect effect on microbial growth on the surfaces of road, soil, and plants, as it has a drying effect and is a factor in favor of aerosolization of settled particles influencing the structure of bacterial community. PM_{10} is significantly correlated with bacterial community composition, which indicates that PM_{10} might influence the colonization of the bacteria. The increase of the PM concentration may heighten the opportunity for the colonization of the bacteria on the surface of rocks, urban roads, and so on. In addition, there are many other factors influencing the bacterial community in the atmosphere, such as wind speed and sunlight duration. Therefore, airborne bacterial communities should be surveyed in the long term, and their relationships with environmental factors require further study.

Cooccurrence patterns identified using network analysis can reflect the interactions between microbes (69). Similar methods have been applied to study bacterium-bacterium (70, 71), bacteriophage-bacterium (65), and bacterium-fungus (66) interactions. Mutual exclusions in the network in the current study were not found, suggesting a positive nature among microbes in the airborne PM samples. The network properties offer the possibility for quick and easy comparisons among complex data sets from different ecosystem types to explore how the general traits of a certain habitat type may influence the composition of microbial communities (69). We calculated the average path length for the network, and the value was 1.97, indicating that approximately two edges separate average nodes. This value was lower than those in environmental interactions in marine networks (bacteria and archaea, 3.05; bacteria, protist, and virus, 3.00; and bacteria and phage, 3.10) (65, 67, 72) and soil networks (bacteria, 5.53) (69). Hubs are thought to describe key nodes that, if removed, would lead to large changes in the community (67). Thus, key genera might be *Paracoccus*, *Rubellimicrobium*, *Kocuria*, and *Arthrobacter*. The four genera that were rich in the samples of larger-PM fractions may have a characteristic in which they gather other bacteria to form bigger PM.

In summary, the results presented here represent an analysis of the airborne bacterial community associated with PM and advance our understanding of the diversity and composition of these communities. We observed the shift in bacterial community composition with PM fraction sizes, but no significant difference was found among haze levels. Our results also indicated that multiple factors influenced the airborne bacterial community composition. More attention should be given to airborne bacteria and their role in the atmosphere. Metagenomics and metatranscriptomics will provide further details on the potential and actual functions of airborne bacteria with higher accuracy; however, analysis of such data remains technically challenging. Consequently, an accurate real-time technique for survey of airborne bacteria needs to be developed in future.

MATERIALS AND METHODS

Sample collection. $PM_{2.5}$ samples ($PM_{2.5}$S; samples containing only PM smaller than 2.5 μm), PM_{10} samples (PM_{10}S; samples containing only PM smaller than 10 μm), and TSP samples (TSPS; samples containing total suspended particulates) were collected from the roof of the Conference Building at Institute of Medicinal Biotechnology, Chinese Academy of Medical Sciences (39°52′43″N, 116°23′21″E, ~8 m above the ground, ~400 m from Temple of Heaven Park), an area without major industrial pollution sources nearby. We collected a total of 96 PM samples over 32 days (collecting only one sample per day for each PM fraction, which represents 32 samples for each PM fraction [i.e., 96 in total for all 3]) within the period of September 2014 to February 2015 during different haze levels (Table S1). Sampling was conducted by portable ambient air samplers (AirMetrics, USA), with one sampler each for $PM_{2.5}$S, PM_{10}S, and TSPS (three samplers in total). The samplers were positioned at a distance of 5 m from each other and operated at the same time. Ambient air was drawn at an average flow rate of 5 liters/min for 24 h per sampling day.

The PM samples were collected on 47-mm quartz aerosol collection filters (Pall, USA). Prior to sampling, all the filters were sterilized by autoclaving at 121°C for 20 min. The filter holders of the samplers were cleaned with 75% ethanol, and all the tools used for changing new filters were autoclaved

every day to avoid contamination. After sampling, the filters were kept in a 2-ml centrifuge tube after cutting into small pieces with scissors and stored at −20°C (68).

The air quality index (AQI) (73), was used to indicate the haze level. We defined a day with AQI lower than 100 as a nonhazy day, that with an AQI in the range of 100 to 200 as a light-haze day, and that with an AQI higher than 200 as a heavy-haze day.

Environmental factors, such as concentrations of $PM_{2.5}$, PM_{10}, CO, SO_2, and NO_2, were recorded every 6 h in each sampling day, and the average value was used as the data set for analysis from the monitoring data of Temple of Heaven Park site (~800 m from the sampling site) of the Beijing Municipal Environmental Monitoring Center (http://zx.bjmemc.com.cn/). Temperature (temp) and relative humidity (rH) were recorded according to the reports of the Chinese National Meteorological Center (http://www.nmc.cn/). The environmental factors are listed in Table S2.

DNA extraction and PCR amplification. Sample filters in the 2-ml centrifuge tubes were removed from the fridge, and the sample filters were directly transferred into PowerBead tubes (Mo Bio, USA). The samples were then heated to 65°C for 10 min, followed by vortexing for 10 min. The remaining steps of the DNA extraction were performed according to the PowerSoil DNA isolation protocol. The V3–V4 region of the bacterial rRNA gene was amplified by PCR (95°C for 3 min, followed by 27 cycles at 95°C for 30 s, 55°C for 40 s, and 72°C for 45 s, and a final extension at 72°C for 10 min) using primers 338F (5′-barcode-ACTCCTACGGGAGGCAGCA-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′) (74). PCR amplification was conducted using high-fidelity TransStart FastPfu DNA polymerase (Transgen, China). The PCRs were performed in triplicate in 20-μl mixtures containing 4 μl of 5× FastPfu buffer, 2 μl of 2.5 mM dinucleoside triphosphates (dNTPs), 0.8 μl of each primer (5 μM), 0.4 μl of FastPfu polymerase, and 2 to 4 μl (according to the concentration of DNA) of template DNA.

Illumina MiSeq sequencing. Amplicons were purified using the AxyPrep DNA gel extraction kit (Axygen, USA), according to the manufacturer's instructions, and quantified using QuantiFluor-ST (Promega, USA). Purified amplicons were pooled in equimolar amounts and paired-end sequenced (2 × 250 bp) on an Illumina MiSeq platform, according to the standard protocols.

Processing of sequencing data. Raw read sequences were demultiplexed and quality filtered using QIIME (version 1.8.0) (75), with the following criteria: (i) the 300-bp reads were truncated at any site receiving an average quality score of <20 over a 50-bp sliding window, discarding the truncated reads that were shorter than 50 bp; (ii) exact barcode matching, a two-nucleotide mismatch in primer matching, with reads containing ambiguous characters being removed; and (iii) only paired-end reads with overlap longer than 10 bp were assembled according to their overlap sequence. Reads that could not be assembled were discarded.

Statistical analyses. All samples were subsampled at same sequence depth (17,782 reads). Operational taxonomic units (OTUs) were clustered with 97% similarity cutoff using UPARSE (version 7.1). Chimeric sequences were identified and removed using UCHIME. The OTUs were used as a basis for calculating α-diversity and β-diversity metrics. The taxonomy of each 16S rRNA gene sequence was analyzed using the RDP Classifier (76) against the SILVA (SSU115) 16S rRNA database using a confidence threshold of 70% (77). Statistical analyses of the OTU diversity of each air sample (Chao1 index, Good's coverage estimator, and Shannon's index) were performed using the QIIME 1.8.0 software (75). Statistical comparisons of Chao1 and Shannon indices among different samples were made by one-way analysis of variance (ANOVA) using the Statistical Package for the Social Sciences (SPSS, version 17.0). An analysis of similarity (ANOSIM) was performed using the QIIME 1.8.0 software to determine whether various fractions of PM samples and haze levels had significantly different bacterial community compositions. Bar plots, Venn diagrams, canonical correspondence analysis (CCA), principal-coordinate analysis (PCoA), and heatmaps were created in the R software (version 3.7.0). Linear discriminant analysis effect size (LEfSe; https://huttenhower.sph.harvard.edu/galaxy/) was applied to identify differentially abundant bacterial taxa among samples with different PM fractions and haze levels (78). Only those taxa that obtained a log linear discriminant analysis (LDA) score of >2 were ultimately considered. The network was generated using the CoNet plugin (version 1.0b7) for Cytoscape (version 3.6.0) on the basis of the nonparametric Spearman correlation coefficients, with a minimal cutoff threshold ρ of 0.6 ($P < 0.01$, Bonferroni corrected) (79, 80). We present correlation data for dominant genera (relative abundance, >0.1%) that were detected in airborne bacteria.

Accession number(s). The raw reads were deposited into the NCBI Sequence Read Archive (SRA) database under accession number SRP068265.

SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at https://doi.org/10.1128/AEM.00004-18.

SUPPLEMENTAL FILE 1, PDF file, 1.6 MB.

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