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Characterization of Airborne Microbial Aerosols during a Long-range Transported Dust Event in Eastern China: Bacterial Community, Influencing Factors, and Potential Health Effects

Ying Rao[1,2,3#], Heyang Li[2,4#], Mingxia Chen[5], Qingyan Fu[6], Guoshun Zhuang[1*], Kan Huang[1,7,8*]

[1] *Center for Atmospheric Chemistry Study, Shanghai Key Laboratory of Atmospheric Particle Pollution and Prevention (LAP[3]), Department of Environmental Science and Engineering, Fudan University, Shanghai 200433, China*
[2] *Third Institute of Oceanography, Ministry of Natural Resources, Xiamen 361005, China*
[3] *Health Center of Minnan Normal University, Zhangzhou 363000, China*
[4] *Fujian Provincial Key Laboratory of Marine Ecological Conservation and Restoration, Xiamen 361005, China*
[5] *Department of Biological technology and Engineering, HuaQiao University, Xiamen 361021, China*
[6] *Shanghai Environmental Monitoring Center, Shanghai, 200030, China*
[7] *Institute of Eco-Chongming (IEC), Shanghai 202162, China*
[8] *Institute of Atmospheric Sciences, Fudan University, Shanghai 200433, China*

ABSTRACT

Samples of atmospheric microbial aerosols were collected before, during, and after a dust invasion in Shanghai and analyzed using 16S rRNA high-throughput sequencing. The bacterial community structures in the mixed pollutive aerosols and dust were characterized, and the key environmental factors were identified. The dominant phyla were Proteobacteria, Actinomycetes, and Firmicutes, and the relative abundance of Acidobacteria increased significantly during the episode. Additionally, marked differences in the relative abundances of the 22 detected genera were observed between the three sampling stages: The dominant genera were *Rubellimicrobium* and *Paracoccus* prior to the arrival of the dust but became *Deinococcus* and *Chroococcidiopsis* during the invasion and then *Clostridium* and *Deinococcus* afterward. Notably, the relative abundance of *Cyanobacteria*, which is known to cause hepatotoxicity and promote tumor growth in humans, grew substantially during the event. Finally, statistical analysis revealed the largest environmental factors affecting the bacterial communities to be wind speed and the SO_2, SO_4^{2-}, NO_3^-, PM_{10}, NH_4^+, and Ca^{2+} concentrations.

Keywords: Airborne bacteria; Bacterial community structure; Influencing factors; Long-range transported dust.

INTRODUCTION

Atmospheric aerosols which contained biological substances such as microorganisms or biomolecules were called *bioaerosols*. Of which, the part that contained microorganisms was called microbial aerosols. Atmospheric microbial aerosols are closely related to air pollution, biosphere, cloud chemistry, climate, and human health (Delort *et al.*, 2010; Peccia *et al.*, 2010; Fröhlich-Nowoisky *et al.*, 2016). Bioaerosols was unique as circulation of the materials, ecological balance and so many biological phenomena were all relevant to it (Mancinelli and Shulls, 1978). More than 25% of the particles over the earth's surface was composed of bioaerosols (Jones and Harrison, 2004). Bioaerosols in $PM_{2.5}$ were estimated in the range of 3–11% by weight (Womiloju *et al.*, 2003; Boreson *et al.*, 2004). The emission intensity of the global microbial aerosols varied from 10–1000 Tg y^{-1}, while the portion of bacterial aerosols was about 0.74–28.1 Tg y^{-1} (Després *et al.*, 2012). The concentration of bacterial aerosols near the surface of land was usually higher than 1×10^4 cells m^{-3} (Bauer *et al.*, 2002), while the concentration of bacteria above the ocean was much less of about 100–1000 cells m^{-3} (Prospero *et al.*, 2005; Griffin *et al.*, 2006).

The diurnal variation of the concentrations of bacteria in the boundary layer was significantly influenced by meteorological factors, e.g., wind speed (Sun *et al.*, 2003). One study conducted in Xiamen, China, suggested the concentrations of bacterial aerosols were associated with the weather conditions, showing the lowest during sunny days while the highest during the haze period (Liao, 2013).

[#] These authors contributed equally to this work.

[*] Corresponding author.
E-mail address: gzhuang@fudan.edu.cn (G. Zhuang); huangkan@fudan.edu.cn (K. Huang)

Similar to the transport of particulate matters, the transport of microbial aerosols in the atmosphere was passive (Sun *et al.*, 2003). The residence time of bacteria in the atmosphere was about one week (Burrows *et al.*, 2009). Dust storm is one of the important natural phenomena in the earth system (Goudie and Middleton, 2006; Ravi *et al.*, 2011; Shao *et al.*, 2011). Some studies found that bacterial aerosols from deserts or arid areas could be transported intercontinentally (Polymenakou *et al.*, 2008; Lim *et al.*, 2011; Hara and Zhang, 2012; Barberán *et al.*, 2014) and the bacterial community of the downwind areas were significantly changed (Maki *et al.*, 2013). During the long-range transport, the aeolian dust mixed with reactive gases and polluted aerosols, and it was common that bacteria adhered on the particles (Yamaguchi *et al.*, 2012). The abundances of bacterial aerosols collected at 1000 km downstream far from the dust source area during the dust episode were 1–10 times more than that during the non-dust period (Jeon *et al.*, 2011). Significant differences of the bacterial community were found between dust samples and non-dust samples. *Aquabacterium sp.*, Flavobacteriales, and Prevotellaceae were dominant in dust samples while *Propionibacterium sp.*, *Bacillus sp.*, and *Acinetobacter sp.* were dominant in non-dust samples (Lee *et al.*, 2009). It was found that dust plumes posed threat on human health not only in the dust source areas but also the downwind areas (Griffin, 2007; Yamaguchi *et al.*, 2012; Goudie, 2014). The diffusion of bioaerosols in atmosphere may harm human health, including infectious diseases, allergy, and occupational hazard (Douwes *et al.*, 2000; Den Boer *et al.*, 2002). Some bacteria carried by dust were pathogenic such as *Neisseria meningitidis* and *Clostridium perfringens*. Meningococcal meningitis caused by *Neisseria meningitidis* was epidemic throughout sub-Saharan Africa as *Neisseria meningitidis* can adhere on dust particles and activate by high iron contents in dust (Thomson *et al.*, 2009). *Clostridium perfringens* has been found as the primary pathogen of food poisoning (Grass *et al.*, 2013) and has also been observed in dust which may cause disease by inhalation (Leski *et al.*, 2011).

In this study, one dust event in Shanghai during February 20–21, 2016, was monitored based on the air mass backward trajectory modeling and the chemical tracer of aerosols. The 16S rRNA gene analysis method was applied to investigate the structure of bacterial community in atmospheric aerosols before, during, and after the dust. The differences of the bacterial community structure during different periods were revealed. Factors influencing the bacterial community and potential threat on human health were discussed. It should be noted that the assessment of the magnitude of dust impact on human health was beyond the scope of this study.

METHODOLOGY

Aerosol Sampling

Microbial Aerosol Sampling

Microbial aerosol samples were collected on the roof of the 4th Teaching Building of Fudan University in Shanghai, China (121°29′E, 31°14′N; 20 m above ground level). No strong point sources were located around this site. This site has been regarded as a representative of the urban environment of Shanghai, standing for the mixing of traffic, residential, and industrial sources. Microbial aerosol samples were obtained on the 37-mm sterile filter membrane (FMCE) by using the sampler (XMX-CV; Dycor, Canada) at a flow rate of 530 L min^{-1}. All the samples obtained were kept under –20°C until DNA extraction. The sampling information is shown in Table 1.

Airborne Aerosol Sampling

Atmospheric $PM_{2.5}$ samples were synchronously collected with the microbial aerosols at the same site. Aerosol samples were collected on Whatman Grade 41 filters (Whatman Inc., Maidstone, UK) by a medium-volume sampler (flow rate: 77.59 L min^{-1}; TSP/PM_{10}/$PM_{2.5-2}$; Beijing Geological Instrument-Dickel Co., Ltd., China). All the samples were put in polyethylene plastic bags immediately after sampling and then reserved in a refrigerator. The filters were weighed before and after sampling using an analytical balance (reading precision: 10 μg; 2004 MP; Sartorius) after stabilizing in constant temperature (20 ± 1°C) and humidity (40 ± 2%) for 48 hours. All the procedures were strictly quality controlled to avoid any possible contamination of the samples. Meteorological parameters were measured by an automated weather station (Vaisala).

DNA Extraction and PCR Amplification

The frozen filter membrane was cut into pieces and put into a Lysing Matrix E tube, and then the total environmental genomic DNA was extracted using a FastDNA® Spin Kit for Soil (MP Biomedical, USA) according to the standard protocol. The purity and concentration of the DNA was examined by NanoDrop® ND-2000 UV-Vis Spectrophotometer (NanoDrop

Table 1. Sampling information during February 18–22, 2016, in Shanghai.

Group	Time	No.	Date	Time	PM_{10} (μg m^{-3})*
1	Before dust event (NDS*)	sh1	Feb 18	08:00–16:00	78
		sh2	Feb 19	08:00–16:00	84
		sh3	Feb 19	18:00–02:00	69
2	During dust event (DS*)	sh4	Feb 20	06:00–10:00	167
		sh5	Feb 21	14:00–18:00	154
		sh6	Feb 21	18:00–22:00	165
3	After dust event (NDS*)	sh7	Feb 22	08:00–16:00	48
		sh8	Feb 22	18:00–02:00	28

* Data source: Shanghai Environmental Monitoring Center (SEMC).

Technologies, USA), TBS-380 Mini-Fluorometer (Turner Biosystems, USA), and 1% agarose gels. The DNA was stored at –20°C until target-gene amplification. The V3–V4 region of the 16S rRNA gene was PCR amplified using the universal primers 338F (5′-ACTCCTACGGGAGGCAGCAG-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′) (Srinivasan *et al.*, 2012). The 16S rRNA gene amplification was performed in 20-μL reaction volumes containing 1U TransStart FastPfu DNA Polymerase (TransGen Biotech Co., Ltd., China), 4 μL 5× FastPfu Buffer, 2 μL dNTPs (2.5 mM), 0.8 μL each primer (5 mM), and 1 μL DNA template (10 ng μL^{-1}). The PCR reaction was conducted with a temperature program of 27 cycles of 30 s at 95°C, 30 s at 55°C, 45 s at 72°C. The PCR products with length of approximately 450 bp were excised from a 2% agarose gel and purified using the DNA Gel Purification Kit (Axygen Biosciences, USA). Purified amplicons were quantified using QuantiFluor™ ST (Promega, USA) according to the manufacturer's protocol.

Illumina Sequencing and Processing of Sequencing Data

Purified amplicons were pooled in equimolar and paired-end sequenced (2 × 300) on an Illumina MiSeq platform (Illumina, USA) according to the standard protocols by Majorbio Bio-Pharm Technology Co., Ltd. (Shanghai, China). Sequences of each sample were separated according to barcodes (exactly matching) and primers (allowing 2 nucleotide mismatching). Paired-end clean reads were merged using FLASH (v1.2.11; https://ccb.jhu.edu/software/FLASH/). Low-quality sequences (< 300 bp in length, < 20 in quality score, containing ambiguous characters and mismatch primer) were removed from raw sequences according to Trimmomatic quality controlled process (v0.33; http://www.usadellab.org/cms/?page=trimmomatic). The high-quality sequences were clustered into operational taxonomic units (OTUs) at a 97% nucleic acid similarity using QIIME software (v1.8.0; http://qiime.org/scripts/assign_taxonomy.html). The singleton OTU were removed using USEARCH (http://www.drive5.com/usearch/manual/chimera_formation.html) after OTU cluster, and then the chimera sequences were detected and removed using the UCHIME *de novo* algorithm (http://www.drive5.com/usearch/manual/uchime_algo.html). The taxonomy of each 16S rRNA gene sequence was analyzed by the RDP classifier algorithm (http://rdp.cme.msu.edu/) against the Silva (SSU123) 16S rRNA database using confidence threshold of 70%. The paired-end raw Illumina sequencing data were deposited into NCBI Sequence Read Archive (SRA; http://www.ncbi.nlm.nih.gov/bioproject?LinkName=biosample_bioproject&from_uid=3273385) under accession number of SRP219061.

Alpha diversity is applied in analyzing complexity of species diversity for each sample. The indices of Chao1, Shannon, and Simpson were selected to identify the community richness and diversity. All indices in this study were calculated using QIIME (v1.9.1) displayed with R software (v2.15.3).

Aerosol Chemical Analysis

Concentrations of 10 anions (F^-, CH_3COO^-, $HCOO^-$, MSA, Cl^-, NO_2^-, NO_3^-, SO_4^{2-}, $C_2O_4^{2-}$, PO_4^{3-}) and 5 cations (Na^+, NH_4^+, K^+, Mg^{2+}, Ca^{2+}) in aqueous extracts of the particle samples were determined by ion chromatography (IC; ICS-3000; Dionex, USA). The recovery of ions was in the range of 80–120% by adding standard reference material of each ion component into the filtrates for ion chromatography analysis. Reproducibility test showed that relative standard deviation was less than 5%. The ion concentrations of the sample blanks were below detection limits or under 0.02 μg m^{-3} and had been deducted from the observation values.

A quarter of the sample filters were digested at 190°C for 1 h with 8 mL HNO_3 and 2 mL HF. After cooling, the solutions were diluted to 30 mL with distilled-deionized water. Blank filters were in parallel processing in order to reduce the error. A total of 21 elements (Al, As, Ba, Ca, Cd, Co, Cr, Cu, Fe, K, Mg, Mn, Na, Ni, P, Pb, S, Sn, Sr, Ti and Zn) were determined by inductively coupled plasma optical emission spectrometry (ICP-OES; SPECTRO, Germany).

Data Analysis

The Kruskal-Wallis test was used to reveal the difference of community structure among the collected samples. As the number of samples was small in this study, the Kruskal-Wallis test was a suitable tool without assuming the data to follow the normal distribution.

The Mantel test and redundancy analysis were used for analyzing the relationship between species and environmental factors. The Mantel test is a non-parametric method which is often used to test the relationship between the distance matrix of community and the distance matrix of environmental factors in ecology. Redundancy analysis is environmentally constrained PCA analysis, which is used to analyze the characteristics of species with certain environmental factors.

RESULTS AND DISCUSSION

Dust Events and Transport Pathways

On February 20 and 21, 2016, the daily mean PM_{10} concentrations in Shanghai reached 114.5 and 113.3 μg m^{-3}, respectively. In the meantime, the mean $PM_{2.5}/PM_{10}$ ratio was as low as 0.66 and 0.40 compared to that of 0.82 during the previous three days (February 17–19), clearly indicating the enhancement of coarse particles. The air mass backward trajectory analysis on these two days showed that Shanghai was mainly affected by the mixed air masses from northwest China (Fig. 1). According to the report from China Meteorological Administration, dust originated in southern Xinjiang, central and western Inner Mongolia, and Gansu, Ningxia, and Qinghai Provinces. Air quality over these areas were in light to medium pollution levels and some were even encountered heavy pollution during this period. Hence, it was clearly that air quality in Shanghai on February 20–21 was mainly affected by the long-range transported dust from northwestern China.

Chemical analysis of aerosol components was further used to confirm this long-range transport dust event. The elemental Ca/Al ratio in atmospheric particulate matters had been proven to be useful for distinguishing the source regions of dust (Sun *et al.*, 2004; Shen *et al.*, 2007; Huang *et al.*, 2010). Table 2 shows the typical Ca/Al ratios of the dust source regions in China and the measured Ca/Al ratios of dust samples collected in this study.

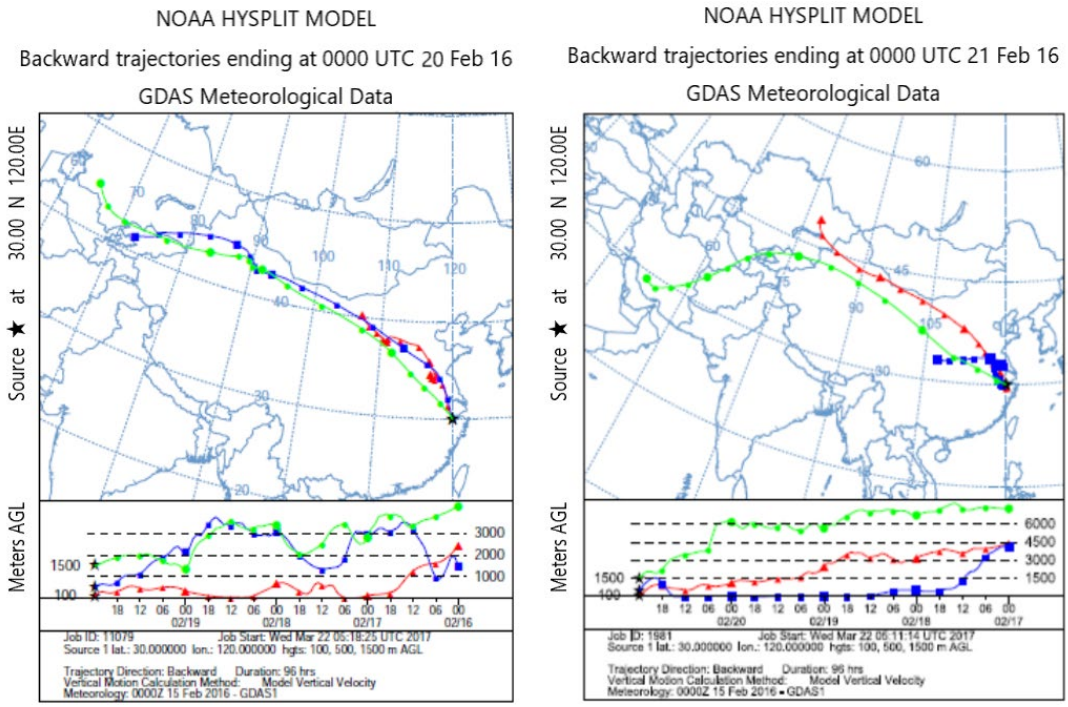

Fig. 1. Three days' air mass backward trajectory starting at Shanghai on February 20 and 21, 2016.

Table 2. Elemental ratio of Ca/Al in the major Chinese dust source regions and dust samples in Shanghai.

Type	Ca/Al	References
Earth crust	0.45	Taylor and McLennan (1985)
Taklamakan Desert	1.99	Zhang *et al.* (1996)
Loess Plateau	0.87	Nishikawa *et al.* (1991)
Northern dust source area	1	Zhang *et al.* (2003)
Western dust source area	1.3	Zhang *et al.* (2003)
Sample sh4	1.15	This study
Sample sh5	1.23	This study
Sample sh6	1.19	This study

The Ca/Al ratios in the dust samples, i.e., sh4, sh5, and sh6 (see sampling information in Table 1), ranged from 1.15 to 1.23, which were close to the ratio of Ca/Al from the dust source area in northwest China and corroborated the backward trajectory analysis.

Microbial Diversity and Community Structures

The high-throughput sequencing analysis results showed that the eight samples collected during different sampling periods showed good clustering effect at the OTU level (Fig. 2). The clustering results were in good agreement with the sample groups in Table 1. Due to the influence of dust, the samples in the three sampling groups had their respective similar bacterial structures, while the bacterial structures among the three sampling groups were significantly different. Compared to the samples during the non-dust periods (Group1 and Group3), the dust samples (Group2) had the shortest clustering distance among all three groups, indicating that the bacterial structure of the three samples in Group2 were the most similar. The number of mutual OTUs in all these three dust samples reached 955, accounting for 55.72% of the total OTUs of the three dust samples. The mutual OTU accounted for 69.96%, 73.24%, and 72.18% of the three dust samples, respectively, indicating the dominant OTU of the dust samples were similar. Yamaguchi *et al.* (2016) also found the dominant bacteria in twelve dust samples collected in Beijing were similar, and the dominant phylum were Actinobacteria, Firmicutes, and Proteobacteria.

Alpha diversity index was further used to analyze the diversity of bacterial structure. The values of *Coverage*, *Ace*, *Chao*, *Shannon*, and *Simpson* in each group are listed in Table 3. It is shown that the gene coverage (*Coverage*) in each group was more than 99%, indicating the majority of the sample sequences were detected. Index of richness (*Chaos* and *Ace*) of each group were at high levels, as well as for the index of diversity (*Shannon* and *Simpson*). As shown in Table 4, the significance test suggested there were significant differences of diversity between the dust samples (Group2) and the other two groups of non-dust samples (Group1 and Group3). As a comparison, no significant differences were found between the non-dust samples.

Fig. 3 shows the composition of bacterial community

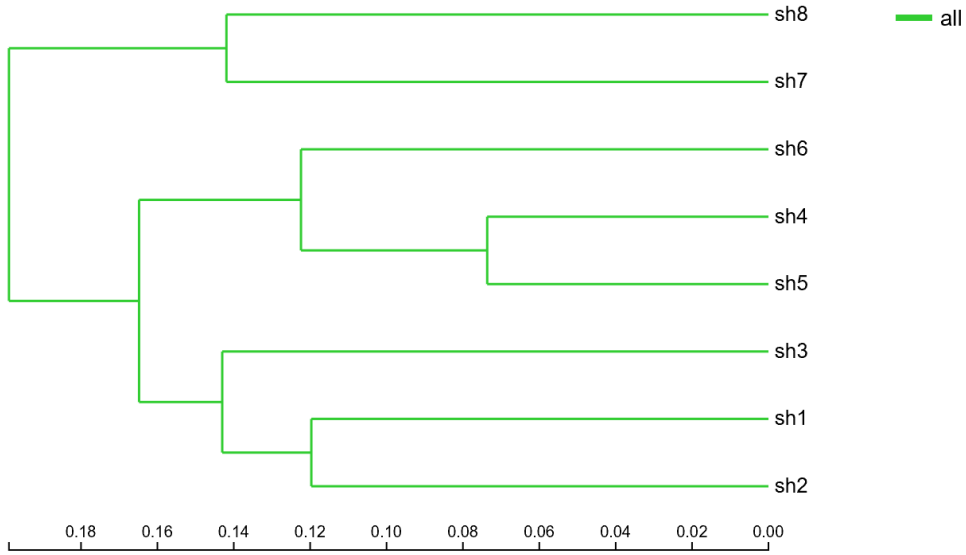

Fig. 2. Hierarchical clustering tree of samples during the whole study period. The scale represents the relative distance among samples and is unitless.

Table 3. Alpha diversity index of samples.

Group	Ace	Chao	Coverage	Shannon	Simpson
1	1805	1802	0.998	5.779	0.007
2	1337	1358	0.993	5.720	0.008
3	1897	1911	0.996	5.981	0.005

Table 4. Comparison of diversity index between groups.

	P-value (Group1–2)	*P*-value (Group2–3)	*P*-value (Group1–3)
Ace	0.0107	0.0124	0.0432
Chao	0.0194	0.0123	0.0437
Shannon	0.0267	0.0088	0.5554
Simpson	0.0544	0.0544	0.7201

structure of all samples at the phylum level, showing some similarity among the three groups. Sequences in all samples were affiliated with 5 known bacterial phyla, with confidence score thresholds of 94–97%. The most abundant bacterial phylum found from Group1–Group3 was Proteobacteria (39.28%, 30.40%, and 38.22%), followed by Actinobacteria (25.02%, 28.38%, and 21.76%) and Firmicutes (12.39%, 12.36%, and 21.47%). This was similar with a study in Japan (Yamaguchi *et al.*, 2014).

The results showed that the dominant phylum were similar during the three different sampling periods. One study on a dust event observed in Japan showed that although dust acted as good carriers for abundant bacteria (around 10^4–10^5 cells m^{-3}), the dominant bacteria at the phylum level did not change significantly in the downwind areas (Yamaguchi *et al.*, 2012). The abundance of Actinobacteria in Group2 was the highest among all three groups, indicating the dust tended to bring higher amounts of Actinobacteria cells to the downwind areas (Yamaguchi *et al.*, 2016). Previous studies also found that Actinobacteria was the dominant type in both Asian and African dust (Griffin *et al.*, 2001, 2003; Yamaguchi *et al.*, 2012) and was confirmed with the study on a long-range transported dust event in Japan (Maki *et al.*, 2015).

A total of 598, 600, and 491 genera were detected in Group1, Group2, and Group3, respectively. The dominant genera with relatively high abundances in three groups are shown in Fig. 4 and showed changes to some extent as below. *Rubellimicrobium*, *Paracoccus*, *Sphingomonas*, *Deinococcus*, and *Chroococcidiopsis* were the top 5 dominant genus in Group1. *Deinococcus*, *Chroococcidiopsis*, *Cyanobacteria*, *Sphingomonas*, and *Rubellimicrobium* were the top 5 dominant genera in Group2. As for Group3, *Clostridium*, *Deinococcus*, *Paracoccus*, *Rubellimicrobium*, and *Sphingomonas* were the most abundant genera. It should be noted the relative abundance of *Cyanobacteria* increased obviously during dust (Group 2) and then decreased after dust (Group 3), which indicated that dust acted as an efficient medium for the accumulation of C*yanobacteria.* Some bacteria such as *Clostridium* were found increased dramatically after the dust (5.71% in Group3) compared to the samples during the dust (2.40% in Group2). This was consistent with a survey about heavy dust storm in Beijing (Yamaguchi *et al.*, 2016). The highest abundance of *Clostridium* was found of the samples in Group3 (after dust). This hysteresis effect might be due to

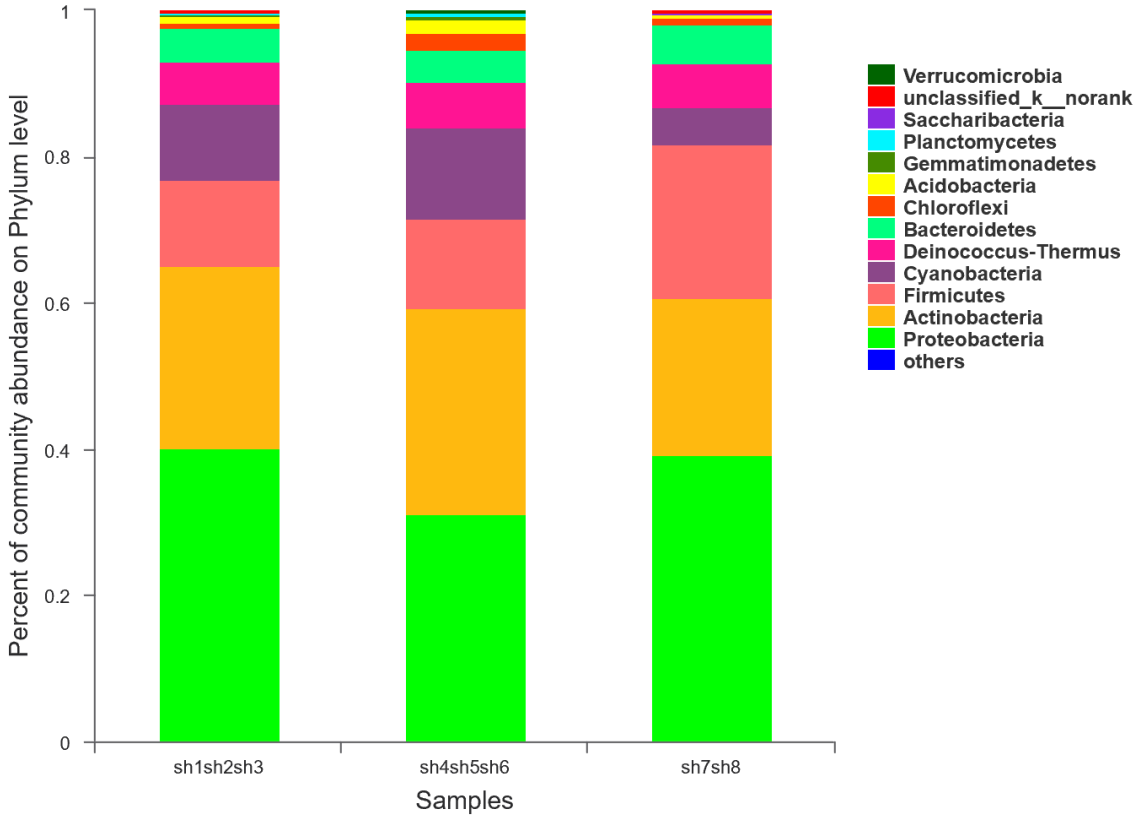

Fig. 3. Composition of bacterial community structure at the phylum level before (sh1sh2sh3), during (sh4sh5sh6), and after (sh7sh8) the dust.

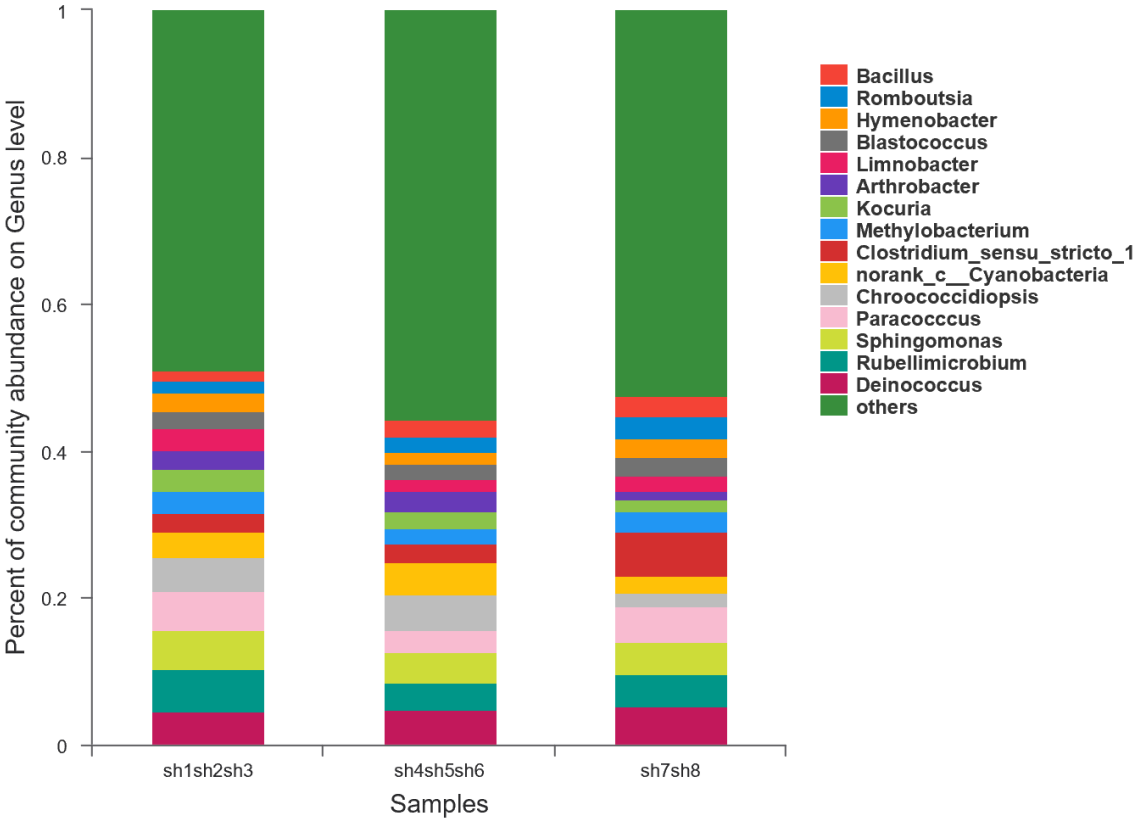

Fig. 4. Composition of bacterial community structure at the genus level.

the strong adaptability of the bacteria to the harsh environment or the local dust resuspended by the strong wind. In addition, the results of the Kruskal-Wallis test showed there were significant differences in 22 bacteria among the three groups at the genus level and some of them have been identified as pathogens or potential pathogens such as *Moraxella* and *Bryocella* (Table S1). *Moraxella* was found increased during the dust and reached the highest abundance after the dust. The health effect of *Moraxella* will be discussed in Section 3.4.

Relationship between Environmental Factors and Airborne Bacteria

The Mantel test, redundancy analysis (RDA), and other statistical methods were applied to study the effect of environmental factors on the airborne bacteria. The considered environmental factors included meteorological parameters such as wind speed, temperature, and relative humidity and atmospheric pollutants such as SO_2, O_3, CO, NO_2, $PM_{2.5}$, PM_{10}, and major aerosol chemical components. All data are listed in Table 5.

The results based on the Mantel test analysis are shown in Table 6. It is shown that wind speed, PM_{10}, and Ca^{2+} correlated moderately with the airborne bacteria, probably suggesting the positive effect of dust on enhancing the bacteria. In addition, SO_2, SO_4^{2-}, and NO_3^- were also moderately correlated with bacteria at the genus level. These species were indicative of anthropogenic emissions, suggesting human activities were also beneficial for the enhancement of airborne bacteria.

In order to eliminate the potential influence of the internal interactions among environmental factors on the bacterial community, the partial Mantel test analysis of each environmental factor control matrix and bacterial community was made (Table 7). The results showed that the correlation between the remaining environmental factors and the bacterial community still existed.

Table 5. The environmental data (particulate ions, gases, and meteorological parameters) for each sample.

No.	sh1	sh2	sh3	sh4	sh5	sh6	sh7	sh8
$PM_{2.5}$ (μg m^{-3})	53	63	42	77	50	50	35	18
Wind speed (m s^{-1})	4.8	4.2	3.4	3.6	3.1	3.8	6.5	6.1
Relative humidity (%)	43	69	75	57	38	53	92	88
Temperature (°C)	11	9	8	6	10	8	6	6
Atmospheric pressure (hPa)	1023	1023	1027	1028	1029	1029	1027	1028
SO_2 (μg m^{-3})	23	23	17	28	17	15	10	10
NO_2 (μg m^{-3})	46	88	75	48	29	30	40	31
CO (mg m^{-3})	0.83	0.91	0.81	1.1	0.6	0.63	0.75	0.56
O_3 (μg m^{-3})	86	28	40	32	101	98	76	76
PM_{10} (μg m^{-3})	78	84	69	167	154	165	48	28
Cl^- (μg m^{-3})	0.91	0.85	0.93	1.45	1.47	0.47	0.28	0.43
NO_3^- (μg m^{-3})	4.51	5.29	7.27	5.97	7.38	5.62	2.24	4.18
SO_4^{2-} (μg m^{-3})	4.74	5.01	6.97	6.19	7.08	5.64	1.98	3.90
Ca^{2+} (μg m^{-3})	2.68	2.72	2.39	2.87	3.46	2.74	2.32	2.31
Na^+ (μg m^{-3})	0.61	0.41	0.44	0.60	0.72	0.32	0.22	0.52
NH_4^+ (μg m^{-3})	2.75	2.83	2.28	3.62	4.03	3.41	2.17	2.15
K^+ (μg m^{-3})	1.16	0.75	0.85	0.85	1.66	0.64	0.24	0.64

Table 6. The Mantel test of environmental factors on airborne bacteria (*r* and *P* represent correlation coefficient and probability, respectively).

	r	*P*		*r*	*P*
$PM_{2.5}$	0.2110	0.4060	Cl^-	0.2413	0.3570
Wind speed	0.6311	0.0120	PM_{10}	0.4862	0.0160
Relative humidity	0.2999	0.1060	NO_3^-	0.5703	0.0070
Temperature	0.2184	0.2390	SO_4^{2-}	0.6365	0.0060
Atmospheric pressure	0.0242	0.9040	Ca^{2+}	0.5777	0.0010
SO_2	0.5083	0.0320	Na^+	0.1594	0.5320
NO_2	–0.0138	0.9270	NH_4^+	0.3726	0.0430
CO	–0.1650	0.4780	K^+	0.4358	0.0690
O_3	–0.1757	0.3340			

Table 7. The partial Mantel test of factors on bacterial community.

	Wind speed	PM_{10}	SO_2	NO_3^-	SO_4^{2-}	Ca^{2+}	NH_4^+
r	0.8074	0.7272	0.5703	0.6961	0.6641	0.6108	0.5354
P	0.0020	0.0040	0.0040	0.0050	0.0060	0.0060	0.0160

The redundancy analysis was applied to investigate the relationship between the environmental factors and the bacterial community structure. As shown in Fig. 5, during the dust period, the sh6 sample was affected mostly by PM_{10} while the sh4 and sh5 samples were more affected by wind speed. This was consistent with the results that dust particles tended to bring more bacteria. Also, the dusty days were usually accompanied by higher wind speed. At the genus level, the top 10 bacteria that were significantly affected by the seven environmental factors in Table 7 included *Deinococcus*, *Rubellimicrobium*, *Sphingomonas*, *Paracoccus*, *Chroococcidiopsis*, *Cyanobacteria*, *Clostridium*, *Kocuria*, *Blastococcus*, and *Methylobacterium*. The relative importance of the seven factors followed the order of wind speed, SO_2, SO_4^{2-}, NO_3^-, PM_{10}, NH_4^+, and Ca^{2+}.

As for the identified top 10 bacteria, most of them can survive and associate with dust due to their strong adaptability to the environmental. For instance, *Deinococcus* has strong resistance to radiation, ultraviolet radiation, peroxides, and other DNA damage agents (Battista *et al.*, 1999). Studies have shown that this unique characteristic may be related to its special genome structure and special membrane proteins (White *et al.*, 1999; Rajpurohit and Misra, 2010; Tian *et al.*, 2010). *Cyanobacteria* has been found widely in the environment (Cao *et al.*, 2015) and has developed self-protection system during evolution (e.g., screening pigments), which facilitate it exist under extreme conditions such as cold, hot, drought or poor nutrition environment (Sinha *et al.*, 2001; El-Bestawy *et al.*, 2007). *Sphingomonas* is able to tolerate harsh environment especially the nutrition-deficient environment through the effective adjustment of its growth rate (Eguchi *et al.*, 1996; Joux *et al.*, 1999). *Chroococcidiopsis* is also widely distributed in the world as some species of it can survive drought and strong radiation (Billi *et al.*, 2000).

The Risk of Airborne Bacteria on Human Health during the Long-range Transported Dust Events

It should be noted that some opportunistic pathogen was found increasing during and after the dust. For example, the relative abundance of *Moraxella* was found increasing quickly during the dust and continue to increase after the dust. *Moraxella* is one of the normal flora on the skin and mucosal surface of human. Infection of *Moraxella* mainly occurs in people with hypoimmunity such as children, patients with tumor, chemotherapy or radiotherapy. In case of infection, Moraxella may cause arthritis, meningitis, pneumonia, endocarditis, sepsis, and keratitis (Barash and Chou, 2017; Duployez *et al.*, 2017; Franco *et al.*, 2017; Lee *et al.*, 2017; Khalife *et al.*, 2019).

It was also found that the relative abundance of *Cyanobacteria* in dust samples was higher than that of non-dust samples, indicating that certain amounts of *Cyanobacteria* could be brought to the downwind areas via the long-range transport of dust. It has been found out that cyanotoxins in

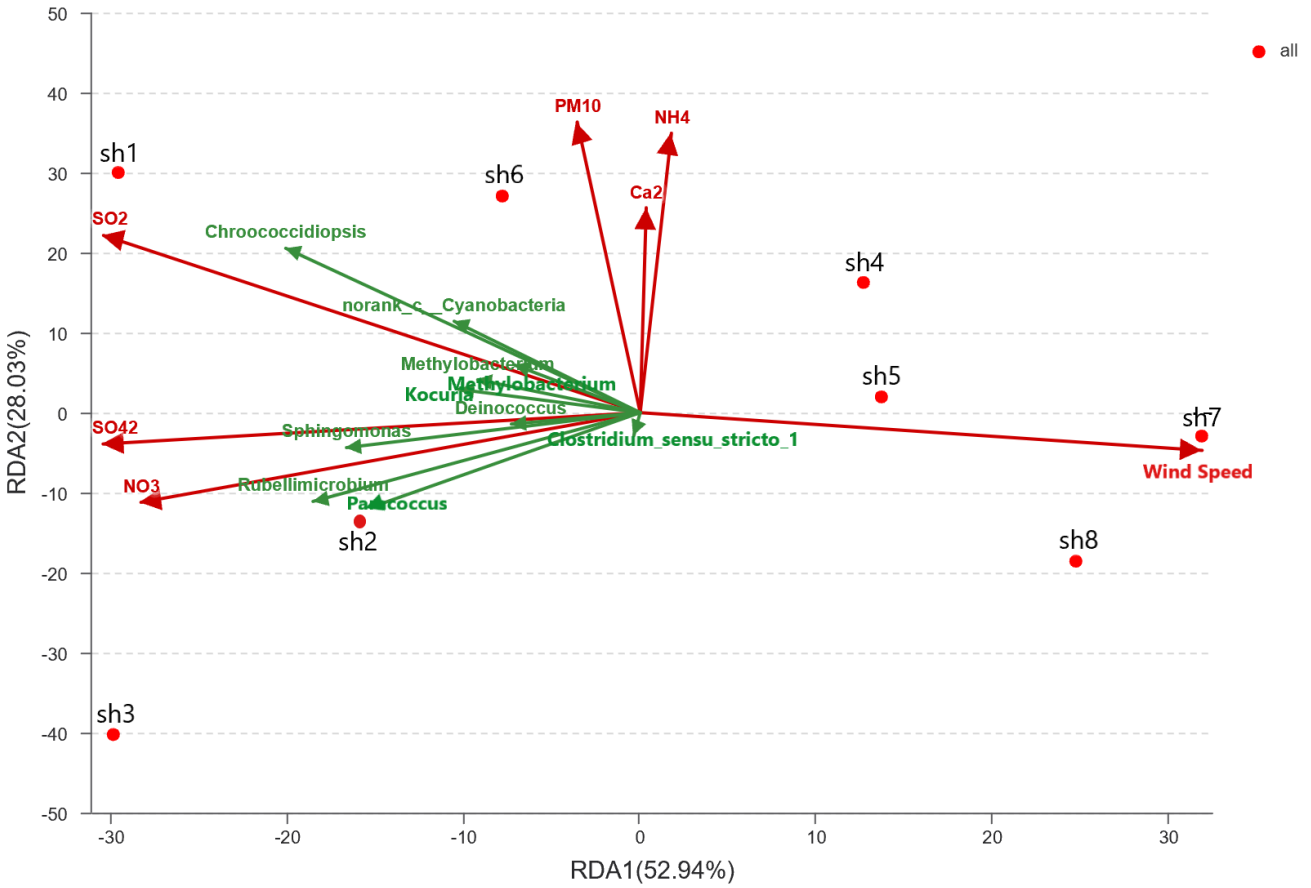

Fig. 5. RDA analysis of environmental factors and airborne bacterial communities.

$PM_{2.5}$ were harmful for humans by inhalation (Metcalf *et al.*, 2012). Cyanobacterial toxins mainly cause extensive necrosis in the epithelial cells of the nasal cavity and respiratory tract by respiratory exposure. In addition, cyanobacterial toxins carried by the dust would eventually be subject to deposit and part of it may enter the water bodies of the downstream areas. Under this circumstance, the sources of drinking waters could be also polluted. Overall, the increased relative abundance of opportunistic pathogen such as *Moraxella* and the toxins producing bacteria such as *Cyanobacteria* during the transported dust may potentially bring threat on human beings.

CONCLUSIONS

In this study, aerosol samples were collected in Shanghai before, during, and after a long-range transported dust event. Then, the airborne bacterial communities were analyzed by using high-throughput sequencing. The major conclusions are summarized below.

1) The bacterial community structures during the three sampling periods (viz., before, during, and after the event) differed significantly in terms of OTUs ($P < 0.05$).
2) The dominant phyla, viz., Proteobacteria, Actinomycetes, and Firmicutes, remained similar to a certain extent throughout all three periods, but the dominant genera differed significantly ($P < 0.05$), being *Rubellimicrobium* and *Paracoccus*, *Chroococcidiopsis* and *Deinococcus*, and *Clostridium* and *Deinococcus* during the first, second, and third stages, respectively.
3) The increased relative abundances of *Moraxella* and *Cyanobacteria* during such dust events may adversely affect human health.
4) Statistical analysis based on the Mantel test indicated that the bacterial community structures were determined by the mass and the chemical composition of the aerosol, as well as key meteorological parameters, which—in decreasing order of influence, according to the redundancy analysis—were the SO_2, SO_4^{2-}, NO_3^-, PM_{10}, NH_4^+, and Ca^{2+} concentrations.

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SUPPLEMENTARY MATERIAL

Supplementary data associated with this article can be found in the online version at https://doi.org/10.4209/aaqr.2020.01.0030

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