Characterization of airborne microbial aerosols during a long-range transported dust event in Eastern China: bacterial community, influencing factors, and potential health effects

Ying Rao1,2,3#, Heyang Li2,4*, Mingxia Chen5, Qingyan Fu6, Guoshun Zhuang1*, Kan Huang1,7,8*

1 Center for Atmospheric Chemistry Study, Shanghai Key Laboratory of Atmospheric Particle Pollution and Prevention (LAP3), 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
#Co-first author
*Corresponding author
The atmospheric microbial aerosols samples during the invasion of dust into Shanghai were analyzed using the 16s rRNA high throughput sequencing technique. The characteristics of bacterial community structure in the mixed polluted aerosols with dust and the influencing environmental factors were revealed. On the phylum level, the dominant groups of atmospheric bacteria were Proteobacteria, Actinomycetes, and Firmicutes, and the relative abundance of Acidobacteria increased significantly during the invasion of dust. At the genus level, the dominant ones were Rubellimicrobium and Paracoccus before the dust arrival, while they changed to Deinococcus and Chroococcidiopsis during the dust invasion, and Clostridium and Deinococcus after the passing of dust. In addition, significant differences of the relative abundances were found in 22 genus among the samples before, during, and after the dust. Based on the analysis of bacterial groups and key environmental factors, it was found that the seven main environmental factors related to bacterial groups were wind speed, SO2, \( \text{SO}_4^{2-}, \text{NO}_3^-, \text{PM}_{10}, \text{NH}_4^+, \) and \( \text{Ca}^{2+} \). It should be emphasized that the relative abundance of Cyanobacteria increased significantly during dust invasion as Cyanobacteria is known to have hepatotoxicity and tumor promotion effect for humans.

Key words: airborne bacteria, bacteria community structure, influencing factors, long-range transport dust
1. 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; Fröhlich-Nowoisky et al., 2016; Peccia et al., 2010). Bioaerosols was unique as circulation of the materials, ecological balance and so many biological phenomena were all relevant to it (Mancinelli et al., 1978). More than 25% of the particles over the Earth's surface was composed of bioaerosols (Jones et al., 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 yr$^{-1}$, while the portion of bacterial aerosols was about 0.74 - 28.1 Tg yr$^{-1}$ (Després et al., 2012). The concentration of bacterial aerosols near the surface of land was usually higher than $1 \times 10^4$ 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 et al., 2013). 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 (Andrew et al., 2006; Ravi et al.,...
Some studies found that bacterial aerosols from deserts or arid areas could be transported intercontinentally (Barberán et al., 2014; Hara et al., 2012; Lim et al., 2011; Polymenakou et al., 2008) 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 bacterium* sp., and *Prevotellaceae bacterium* sp. 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 et al., 2007; Goudie et al., 2014; Yamaguchi et al., 2012). The diffusion of bioaerosols in atmosphere may harm human health, including infectious diseases, allergy, and occupational hazard (Den Boer et al., 2002; Douwes et al., 2000). 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.

2. Methodology

2.1 Aerosols sampling

2.1.1 Microbial aerosols sampling

Microbial aerosols 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 aerosols samples were obtained on the 37-mm sterile filter membrane (FMCE) by using the sampler (XMX-CV, Dycor, Canada) at a flow rate of 530L/min. All the samples obtained were kept under -20 °C until DNA extraction. The sampling information is shown in Table 1.

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

| Group | Time | No. | Date   | Time   | PM\textsubscript{10} (\mu g/m\textsuperscript{3}) * |
|-------|------|-----|--------|--------|-----------------------------------------------|
| 1     | Before dust event (NDS*) | sh1 | 2016.2.18 | 08:00-16:00 | 78 |
|       |      | sh2 | 2016.2.19 | 08:00-16:00 | 84 |
|       |      | sh3 | 2016.2.19 | 18:00-02:00 | 69 |
| 2     | During dust event | sh4 | 2016.2.20 | 06:00-10:00 | 167 |
2.1.2 Airborne aerosols sampling

Atmospheric PM$_{2.5}$ samples were synchronously collected with the microbial aerosols at the same site. Aerosols samples were collected on Whatman 41 filters (Whatman Inc., Maidstone, UK) by a medium-volume sampler (Beijing Geological Instrument-Dickel Co., Ltd., China; model: TSP/PM$_{10}$/PM$_{2.5}$/; flow rate: 77.59 L min$^{-1}$). 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 (Model: Sartorius 2004MP; reading precision: 10 µg) 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).

2.2 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 Technologies, USA), TBS-380 mini-FLuorometer (Turner Biosystems, USA), and 1% agarose gels. The DNA was stored at -20°C until

| Data source: Shanghai Environmental Monitoring Center (SEMC) |
|---|
| sh5 2016.2.21 14:00-18:00 154 |
| sh6 2016.2.21 18:00-22:00 165 |
| sh7 2016.2.22 08:00-16:00 48 |
| sh8 2016.2.22 18:00-02:00 28 |

*Data source: Shanghai Environmental Monitoring Center (SEMC)
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), 4μL 5×FastPfu Buffer, 2μL dNTPs (2.5 mM), 0.8 μL each primer (5 mM), and 1 μl DNA template (10 ng/μL). 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.

2.3 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 the Trimmomatic (V0.33, http://www.usadellab.org/cms/?page=trimmomatic) quality controlled process. The high-quality sequences were clustered into operational taxonomic units (OTUs) at a 97% nucleic acid similarity using QIIME (Version1.8.0) software (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 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 (version 1.9.1) displayed with R software (V2.15.3).

2.4 Aerosols chemical analysis

Concentrations of ten anions (F\(^{-}\), CH\(_3\)COO\(^{-}\), HCOO\(^{-}\), MSA, Cl\(^{-}\), NO\(_2^{-}\), NO\(_3^{-}\), SO\(_4^{2-}\), C\(_2\)O\(_4^{2-}\), PO\(_4^{3-}\)) and five cations (Na\(^{+}\), NH\(_4^{+}\), K\(^{+}\), Mg\(^{2+}\), Ca\(^{2+}\)) in aqueous extracts of the particle samples were determined by Ion Chromatography (IC, Dionex 3000, 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 ICP-OES (Inductively Coupled Plasma Optical Emission Spectrometer, Model: SPECTRO, Germany).

2.5 Data Analysis

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, Kruskal-Wallis Test was a suitable tool without assuming the data to follow the normal distribution.

Mantel test and Redundancy analysis were used for analyzing the relationship between species and environmental factors. 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 environmental constrained PCA analysis, which is used to analyze the characteristics of species with certain environmental factors.

3. Results and Discussion

3.1 Dust events and transport pathways

On 20 and 21 February 2016, the daily mean PM\textsubscript{10} concentrations in Shanghai reached 114.5 and 113.3 µg/m\textsuperscript{3}, respectively. In the meantime, the mean PM\textsubscript{2.5}/PM\textsubscript{10} ratio was as low as 0.66 and 0.40 compared to that of 0.82 during the previous three days (17 – 19 February), clearly indicating the enhancement of coarse particles. The
Airmass 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, Gansu, Ningxia, and Qinghai provinces. Air quality over these areas were in light to mid 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 aerosols 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 (Huang et al., 2010; Shen et al., 2007; Sun et al., 2004). 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.

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 above.

| 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   | 1.00  | 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          |

### 3.2 Microbial diversity and community structures
Fig. 2. Hierarchical clustering tree of samples during the whole study period. The scale represents the relative distance among samples and is unitless.

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 OUTs in all these three dust samples reached 955, accounting for 55.72% of the total OUTs 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 12 dust samples collected in Beijing were similar, and the dominant phylum were Actinobacteria,
Firmicutes, and Proteobacteria.

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   |

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.

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             |

Fig. 3 shows the composition of bacterial community structure of all samples at the phylum level, showing some similarity among the three groups. Sequences in all samples were affiliated with five known bacterial phyla, with confidence score thresholds of 94-97%. The most abundant bacterial phylum found from Group1 –
Group 3 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 $10^4 \sim 10^5$ 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 Group 2 was the highest among all three groups, indicating the dust tended to bring relatively 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., 2003; Griffin et al., 2001; Yamaguchi et al., 2012) and was confirmed with the study on a long-range transport dust event in Japan (Maki et al., 2015).

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

A total of 598, 600, and 491 genus 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 five dominant genus in Group1. *Deinococcus*, *Chroococcidiopsis*, *Cyanobacteria*, *Sphingomonas*, and *Rubellimicrobium* were the top five dominant genus in Group2. As for Group3, *Clostridium*, *Deinococcus*, *Paracoccus*, *Rubellimicrobium*, and *Sphingomonas* were the most abundant genus. 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 *Cyanobacteria*. 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 Group 3(after dust). This hysteresis effect might be due to the strong
adaptability of the bacteria to the harsh environment or the local dust resuspended by the strong wind. In addition, the results of 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.

### 3.3 Relationship between environmental factors and airborne bacteria

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 aerosols chemical components. All data are listed in Table 5.

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

| No. | sh1 | sh2 | sh3 | sh4 | sh5 | sh6 | sh7 | sh8 |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| PM$_{2.5}$ | 53 | 63 | 42 | 77 | 50 | 50 | 35 | 18 |
| (μg/m$^3$) | Wind speed | 4.8 | 4.2 | 3.4 | 3.6 | 3.1 | 3.8 | 6.5 | 6.1 |
| (m/s) | Relative humidity | 43 | 69 | 75 | 57 | 38 | 53 | 92 | 88 |
| (%) | Temperature | 11 | 9 | 8 | 6 | 10 | 8 | 6 | 6 |
| (°C) | Atmospheric pressure | 1023 | 1023 | 1027 | 1028 | 1029 | 1029 | 1027 | 1028 |
| (hPa) | SO$_2$ | 23 | 23 | 17 | 28 | 17 | 15 | 10 | 10 |
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.

Table 6. 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 |
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 7. Partial-Mantel Test of factors on bacterial community

| Wind speed | PM10 | SO2 | NO3⁻ | SO₄²⁻ | Ca²⁺ | NH₄⁺ |
|------------|------|-----|------|-------|------|------|
| *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 (RDA) was applied to investigate the relationship between the environmental factors above and the bacterial community structure. As shown in Fig. 5, during the dust period, the sh6 sample was affected mostly by PM₁₀ while the sh4 and sh5 samples were more affected by wind speed. This was consistent with the results above 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 7 environmental factors above included *Deinococcus, Rubellimicrobium, Sphingomonas, Paracoccus, Chroococcidiopsis, Cyanobacteria, Clostridium, Kocuria, Blastococcus,* and *Methylobacterium.* The relative importance of the 7 factors followed the order of wind speed, SO₂, SO₄²⁻, NO₃⁻,
PM$_{10}$, NH$_4^+$, and Ca$^{2+}$.  

As for the identified top 10 bacteria above, 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; Tian *et al*., 2010; Rajpurohit, 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 (Rajeshwar *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).
3.4 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 (Khalife *et al*., 2019; Franco *et al*., 2017; Lee *et al*., 2017; Duployez *et al*., 2017; Barash *et al*., 2017).

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 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* 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.
4. Conclusions

In this study, aerosols samples were collected in Shanghai before, during, and after a long-range transported dust event. Airborne bacterial community was analyzed by using the high-throughput sequencing. The major conclusions are summarized as below.

1. Bacterial community structures in the three different sampling periods (i.e. before, during, and after dust) were significantly different on the OTU level ($p<0.05$).

2. During both dust and non-dust periods, the dominant bacteria were similar to a certain extent, i.e. Proteobacteria, Actinomycetes, and Firmicutes. However, the bacterial community structures among samples in different sampling periods were significantly different at the genus level ($P<0.05$). *Rubellimicrobium* and *Paracoccus* were the dominant genus in samples before the arrival of dust. *Chroococcidiopsis* and *Deinococcus* were the dominant genus in samples during the dust period. After the passage of dust, *Clostridium* and *Deinococcus* became the dominant genus.

3. The increased relative abundance of *Moraxella* and *Cyanobacteria* during the long-range transported dust event may cause adverse effects on human health.

4. Statistical analysis based on the Mantel Test showed that bacterial community structure in atmospheric aerosols was related to both meteorological conditions and aerosols masses as well as their chemical components. Of which, wind speed, SO$_2$, SO$_4^{2-}$, NO$_3^-$, PM$_{10}$, NH$_4^+$, and Ca$^{2+}$ played a decreasing role by using the Redundancy Analysis.

Acknowledgements

The authors acknowledge support of National Programme on Global Change and Air-Sea Interaction (grant no. GASI-03-01-03-01), the National Science Foundation of
China (No. 91644105, 41506179), the National Key R&D Program of China (2018YFC0213105), and the Natural Science Foundation of Shanghai (18230722600, 19ZR1421100). 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.

References:

Andrew, S., Goudie, Nicholas, J., Middleton (2006). Desert Dust in the Global System. Springer Berlin Heidelberg. 287. doi: 10.1007/3-540-32355-4.

Barash, A., Chou, T. Y ., (2017). Moraxella atlantae keratitis presenting with an infectious ring ulcer. American Journal of Ophthalmology Case Reports. 7, 62-65. doi: 10.1016/j.ajoc.2017.06.003.

Barberán, A., Henley, J., Fierer, N., Casamayor, E. O., (2014). Structure, inter-annual recurrence, and global-scale connectivity of airborne microbial communities. Sci. Total Environ. 487, 187-195. doi: 10.1016/j.scitotenv.2014.04.030.

Battista, J. R., Earl, A. M., Park, M.J., (1999). Why is Deinococcus radiodurans so resistant to ionizing radiation? Trends in Microbiology. 7 : 362-365. https://doi.org/10.1016/S0966-842X(99)01566-8

Bauer, H., Kasper-Giebl, A., Löflund, M., Giebl, H., Hitzenberger, R., Zibuschka, F., Puxbaum, H., (2002). The contribution of bacteria and fungal spores to the organic carbon content of cloud water, precipitation and aerosols. Atmospheric Research. 64, 109-119. doi: 10.1016/S0169-8095(02)00084-4.

Billi, D., Friedmann, E.I., Hofer, K.G., Caiolal, M.G., (2000). Ionizing-radiation
resistance in the desiccation-tolerant cyanobacterium Chroococcidiopsis. *Appl. Environ. Microbiology.* 66: 1489—1492. DOI:10.1128/aem.66.4.1489-1492.2000.

Boreson, J., Dillner, A. M., Peccia, J., (2004). Correlating bioaerosol load with PM2.5 and PM10 cf concentrations: a comparison between natural desert and urban-fringe aerosols. *Atmos. Environ.* 38, 6029-6041. doi: 10.1016/j.atmosenv.2004.06.040.

Burrows, S. M., Butler, T., Jöckel, P., Tost, H., Kerkweg, A., Pöschl, U., Lawrence, M. G., (2009). Bacteria in the global atmosphere – Part 2: Modeling of emissions and transport between different ecosystems. *Atmos.Chem.Phys.* 9, 9281-9297. doi: 10.5194/acp-9-9281-2009.

Cao, K., Jing, R.Y., Wang, G.H., et al., (2015). Molecular biological research progress in genetic diversity of cyanobacteria. *Soil and Crop.* 4(4): 183-189.

Delort, A., Vaïtilingom, M., Amato, P., Sancelme, M., Parazols, M., Mailhot, G., Laj, P., Deguillaume, L., (2010). A short overview of the microbial population in clouds: Potential roles in atmospheric chemistry and nucleation processes. *Atmos. Res.* 98, 249 – 260.

Den Boer, J. W., Yzerman, E. P. F., Schellekens, J., Lettinga, K. D., Boshuizen, H. C., Van Steenbergen, J. E., Bosman, A., Hof, S. V., Van Vliet, H. A., Peeters, M. F., Van Ketel, R. J., Speelman, P., Kool, J. L., Conyn Van Spaendonck, M. A. E., (2002). A Large Outbreak of Legionnaires’ Disease at a Flower Show, the Netherlands, 1999. *Emerg. Infect. Dis.* 8, 37-43. doi: 10.3201/eid0801.010176.

Després, V. R., Huffman, J. A., Burrows, S., Hoose, C., (2012). Primary biological aerosols in the atmosphere: A review of observations and relevance. *Tellus B.* 1-64. doi: 10.3402/tellusb.v64i0.15598.

Douwes, J., Wouters, I., Dubbeld, H., van Zwieten, L, Steerenberg, P., Doekes, G., Heederik, D., (2000). Upper airway inflammation assessed by nasal lavage in
compost workers: A relation with bio-aerosol exposure. *American Journal of Industrial Medicine*. 37, 459-468.

Duployez, C., Loïez, C., Ledoux, G., Armand, S., Jaillette, E., Wallet, F., (2017). A fatal endocarditis case due to an emerging acteriun: Moraxella nonliquefaciens. *ID Cases*. 8,12-13. doi: 10.1016/j.idcr.2017.02.006.

Eguchi, M., Nishikawa, T., Macdonald, K., Cavicchioli, R., Gitschal, J.C., Kjelleberg S., (1996). Responses to Stress and Nutrient Availability by the Marine Ultramicrobacterium Sphingomonas sp. Strain RB2256. *Appl environ microbiology*. 62: 1287-1294. DOI:10.1128/AEM.62.4.1287-1294.

El-Bestawy, E.A., El-Salam, A. Z.A., Mansy, A. E. H., (2007). Potential use of environmental cyanobacterial species in bioremediation of lindane contaminated effluents. *Int Biodeterior Biodegrad*. 59: 180-192. doi:10.1016/j.ibiod.2006.12.005

Franco, J. Ossenkopp, J., Peñarroja, G., (2017). Moraxella catarrhalis meningitis during certolizumab pegol treatment. *Medicina Clínica (English Edition)*. 149,46. doi: 10.1016/j.medcli.2017.02.001.

Fröhlich-Nowoisky et al., (2016). Bioaerosol in the Earth system: Climate, health, and ecosystem interactions. *Atmos. Res*. 15, 346-376.

Goudie,A.S., (2014). Desert dust and human health disorders. *Environment International*. 63, 101-113. doi: 10.1016/j.envint.2013.10.011.

Grass, J.E., Gould, L.H., Mahon, B.E., (2013). Epidemiology of Foodborne Disease Outbreaks Caused by Clostridium perfringens, United States, 1998–2010 *Foodborne Pathogens and Disease*. 10( 2) : 131-136.

Griffin, D. W., (2007). Atmospheric Movement of Microorganisms in Clouds of Desert Dust and Implications for Human Health. *Clin. Microbiol. Rev.* 20, 459-477. doi:
Griffin, D. W., Garrison, V. H., Herman, J. R., Shinn, E. A., (2001). African desert dust in the Caribbean atmosphere: Microbiology and public health. *Aerobiologia*. 17, 203-213. doi: 10.1023/a:1011868218901.

Griffin, D. W., Kellogg, C. A., Garrison, V. H., Lisle, J. T., Borden, T. C., Shinn, E. A., (2003). Atmospheric microbiology in the northern Caribbean during African dust events. *Aerobiologia*. 19, 143-157. doi:10.1023/b:aero.0000006530.32845.8d.

Griffin, D. W., Westphal, D. L., Gray, M. A., (2006). Airborne microorganisms in the African desert dust corridor over the mid-Atlantic ridge, Ocean Drilling Program, Leg 209. *Aerobiologia*. 22, 211-226. doi: 10.1007/s10453-006-9033-z.

Hara, K., Zhang, D. Z., (2012). Bacterial abundance and viability in long-range transported dust. *Atmos. Environ*. 47, 20-25. doi: 10.1016/j.atmosenv.2011.11.050.

Huang, K., Zhuang, G. S., Li, J., Wang, Q., Sun, Y., Lin, Y., Fu, J., (2010). Mixing of Asian dust with pollution aerosol and the transformation of aerosol components during the dust storm over China in spring 2007. *Journal of Geophysical Research Atmospheres*. 15, 1307-1314. doi: 10.1029/2009JD013145.

Jeon, E. M., Kim, H. J., Jung, K., Kim, J. H., Kim, M. Y., Kim, Y. P., Ka, J. O., (2011). Impact of Asian dust events on airborne bacterial community assessed by molecular analyses. *Atmos. Environ*. 45, 4313-4321. doi: 10.1016/j.atmosenv.2010.11.054.

Jones, A. M., Harrison, R. M., (2004). The effects of meteorological factors on atmospheric bioaerosol concentrations—a review. *Sci. Total Environ*. 326, 151-180. doi: 10.1016/j.scitotenv.2003.11.021.

Joux, F., Jeffrey, W.H., Lebaron, P., Mitchell, D.L.(1999). Marine bacterial isolates display diverse responses to UV-Bradiation. *Appl environ microbiology*. 65: 3820 –
Khalife, M., Merashli, M., KanJ, S.S., (2019). Moraxella nonliquefaciens septic arthritis in a hematopoietic stem cell transplant patient a case report and review of the literature. *Journal of Infection and Public Health*. 12, 309-312. doi: 10.1016/j.jiph.2019.01.059.

Leski, T.A., Malanoski, A.P., Gregory, M.J., Lin, B., Stenger, D.A., (2011) Application of a broad-range resequencing array for detection of pathogens in desert dust samples from Kuwait and Iraq. *Appl Environ Microbiol.* 77:4285 – 92. doi:10.1128/AEM.00021-11.

Lee, S., Choi, B., Yi, S. M., Ko, G., (2009). Characterization of microbial community during Asian dust events in Korea. *Sci. Total Environ.* 407, 5308-5314. doi: 10.1016/j.scitotenv.2009.06.052.

Lee, W.S., Shueh, P.R., Yu, F.L., Chen, F.L., Hsieh, t.H., Qu, T.Y., (2017). Moraxella osloensis bacteremia complicating with severe pneumonia in a patient with lung cancer. *Journal of Microbiology, Immunology and Infection.* 50, 395-396. doi: 10.1016 /j.jmii.2015.03.005.

Liao, X., (2013). Study on the Community Composition and Distribution Characteristics of Microorganisms in the Air of Xiamen.Xiamen: Jimei University.

Lim, N., Munday, C. L., Allison, G. E., O'Loingsigh, T., Deckker, P. D., Tapper N. J., (2011). Microbiological and meteorological analysis of two Australian dust storms in April 2009. *Sci. Total Environ.* s412-413:223-231. doi: 10.1016/j.scitotenv.2011.10.030.

Maki, T., Hara, K., Kobayashi, F., Kurosaki, Y., Kakikawa, M., Matsuki, A., Chen, B., Shi, G., Hasegawa, H., Iwasaka, Y., (2015). Vertical distribution of airborne
bacterial communities in an Asiandust downwind area, Noto Peninsula. *Atmos. Environ.* 119, 282-293.

Maki, T., Kakikawa, M., Kobayashi, F., Yamada, M., Matsuki, A., Hasegawa, H., Iwasaka, Y. (2013). Assessment of composition and origin of airborne bacteria in the free troposphere over Japan. *Atmos. Environ.* 74, 73-82. doi: 10.1016/j.atmosenv.2013.03.029. https://s100.copyright.com/AppDispatchServlet?publisherName=ELS&contentID=S1352231013002057&orderBeanReset=true

Mancinelli, R. L., Shulls, W. A., (1978). Airborne Bacteria in an Urban Environment. *Appl. Environ. Microbiol.* 35, 1095-1101.

Metcalf, J. S., Richer, R., Cox, P. A., Codd, G. A., (2012). Cyanotoxins in desert environments may present a risk to human health. *Sci. Total Environ.* s421-422, 118-123. doi: 10.1016/j.scitotenv.2012.01.053. https://s100.copyright.com/AppDispatchServlet?publisherName=ELS&contentID=S0048969712001349&orderBeanReset=true.

Nishikawa, M., S. Kanamori, N. Kanamori, and T. Mizoguchi (1991), Kosa aerosol as eolian carrier of anthropogenic material, *Sci. Total Environ.*, 107, 13 – 27, doi:10.1016/0048-9697(91)90247-C.

Peccia, J., Hospodsky, D., Bibby, K., (2010). New Directions: A revolution in DNA sequencing now allows for the meaningful integration of biology with aerosol science. *Atmos. Environ.* 45, 1896-1897. doi: 10.1016/j.atmosenv.2010.11.037.

Polymenakou, P. N., Mandalakis, M., Stephanou, E. G., Tselepides, A., (2008). Particle Size Distribution of Airborne Microorganisms and Pathogens during an Intense African Dust Event in the Eastern Mediterranean. *Environ.Health.Perspect.* 116, 292-296. doi: 10.1289/ehp.10684.
Prospero, J. M., Blades, E., Mathison, G., Naidu, R., (2005). Interhemispheric transport of viable fungi and bacteria from Africa to the Caribbean with soil dust. *Aerobiologia*. 21, 1-19. doi: 10.1007/s10453-004-5872-7.

Rajpurohit, Y. S., Misra, H. S., (2010). Characterization of a DNA damage-inducible membrane protein kinase from Deinococcus radiodurans and its role in bacterial radioresistance and DNA strand break repair. *Molecular Microbiology*. 77: 1470-1482.

Rajeshwar, P. S., Manfred, K., Almut, G., Donat-P, H., et al., (2001). Responses of aquatic algae and cyanobacteria to solar UV-B. *Plant Ecology*, 154: 219-236.

Ravi, S., D'Odorico, P., Breshears, D. D., Field, J. P., Goudie, A. S., Huxman, T. E., Li, J., Okin, G. S., Swap, R. J., Thomas, A. D., Van Pelt, S., Whicker, J. J., Zobeck, T. M., (2011). Aeolian processes and the biosphere. *Reviews of Geophysics*. 49, 114-123. doi: 10.1029/2010RG000328.

Shao, Y. P., Wyrwoll, K. H., Chappell, A., Huang, J. P., Lin, Z. H., McTainsh, G. H., Mikami, M., Tanaka, T. Y., Wang, X. L., Yoon, S., (2011). Dust cycle: An emerging core theme in Earth system science. * Aeolian Research*. 2, 181-204. doi: 10.1016/j.aeolia.2011.02.001. https://s100.copyright.com/AppDispatchServlet?publisherName=ELS&contentID=S1875963711000085&orderBeanReset=true

Shen, Z. X., Cao, J. J., Arimoto, R., Zhang, R. J., Jie, D. M., Liu, S. X., Zhu, C. S., (2007). Chemical composition and source characterization of spring aerosol over Horqin sand land in northeastern China. *Journal of Geophysical Research*. 112, 37-42. doi: 10.1029/2006JD007991.

Srinivasan, S., Hoffman, N. G., Morgan, M. T., et al., (2012). Bacterial Communities in Women with Bacterial Vaginosis: High Resolution Phylogenetic Analyses Reveal
Relationships of Microbiota to Clinical Criteria. *PLoS ONE* 7: e37818.

Sun, Y. L., Zhuang, G. S., Yuan, H., Zhang, X. Y., Guo, J. H., (2004). Characteristics and sources of 2002 super dust storm in Beijing. *Chin. Sci. Bull.* 49, 698-705. doi: 10.1007/BF03184268.

Sun, Z. H., Lu, J. C., Tong, Y. C., Wang, L., Chen, M. L., Shun, R. Q., (2003). Study on laws of ground layer meteorological element variation and bacteria in air concentration distribution in Beijing. *Environmental Monitoring of China*. 4, 11-17. doi: 10.19316/j.issn.1002-6002.2003.04.006.

Taylor, S.R. and McLennan, S.M. (1985) The Continental Crust: Its Composition and Evolution. *Blackwell Scientific Publication, Carlton*, 312 p.

Tian, B., Wang, H., Ma, X. Q., Hu, Y. P., Sun, Z. T., Shen, S. C., Wang, F., Hua, Y. J., (2010). Proteomic analysis of membrane proteins from a radioresistant and moderate thermophilic bacterium *Deinococcus geothermalis*. *Molecular Biosystems*, 6(10): 2068-2077.

Thomson, M.C., Jeanne, I., Djingarey, M., (2009). Dust and epidemic meningitis in the Sahel: a public health and operational research perspective. *IOP Conf Ser Earth Environ Sci.* 7. http://dx.doi.org/10.1088/1755-1307/7/1/012017.

White, O., Eisen, J. A., Heidelberg, J. F., Hickey, E. K., Peterson, J. D., Dodson, R. J., Haft, D. H., Gwinn, M. L., Nelson, W. C., Richardson, D. L., Moffat, K. S., Qin, H., Jiang, L., Pamphile, W., Crosby, M., Shen, M., Vamathevan, J. J., Lam, P., McDonald, L., Utterback, T., Zalewski, C., Makarova, K. S., Aravind, L., Daly, M. J., Minton, K. W., Fleischmann, R. D., Ketchum, K. A., Nelson, K. E., Salzberg, S., Smith, H. O., Venter, J. C., Fraser, C. M., (1999). Genome sequence of the radioresistant bacterium *Deinococcus radiodurans* R1 Science. 286:
Womiloju, T. O., Miller, J. D., Mayer, P. M., Brook, J. R., (2003). Methods to determine the biological composition of particulate matter collected from outdoor air. *Atmos. Environ.* 37, 4335-4344. doi: 10.1016/S1352-2310(03)00577-6.

Yamaguchi, N., Park, J., Kodama, M., Ichijo, T., Baba, T., Nasu, M., (2014). Changes in the Airborne Bacterial Community in Outdoor Environments following Asian Dust Events. *Microbes. Environ.* 29, 82-88. doi: 10.1264/jsme2.me13080.

Yamaguchi, N., Baba, T., Ichijo, T., Himezawa, Y., Enoki, K., Saraya, M., Li, P. F., Nasu, M., (2016). Abundance and Community Structure of Bacteria on Asian Dust Particles Collected in Beijing, China, during the Asian Dust Season. *Biol. Pharm. Bull.* 39, 68-77. doi: 10.1248/bpb.b15-00573.

Yamaguchi, N., Ichijo, T., Sakotani, A., Baba, T., Nasu, M., (2012). Global dispersion of bacterial cells on Asian dust. *Sci. Rep.* 2, 525. doi: 10.1038/srep00525.

Zhang, X., G. Zhang, Z. An, T. Chen, X. Huang, G. Zhu, and D. Zhang (1996), Element tracers for Chinese source dust (in Chinese), *Sci. China Ser. D.*, 26(5), 421–428.

Zhang, X. Y., S. L. Gong, Z. X. Shen, F. M. Mei, X. X. Xi, L. C. Liu, Z. J. Zhou, D. Wang, Y. Q. Wang, and Y. Cheng (2003), Characterization of soil dust aerosol in China and its transport and distribution during 2001 ACE – Asia: 1. Network observations, *J. Geophys. Res.*, 108(D9), 4261, doi:10.1029/2002JD002632.