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Bacterial Characterization in Ambient Submicron Particles during Severe Haze Episodes at Ji'nan, China

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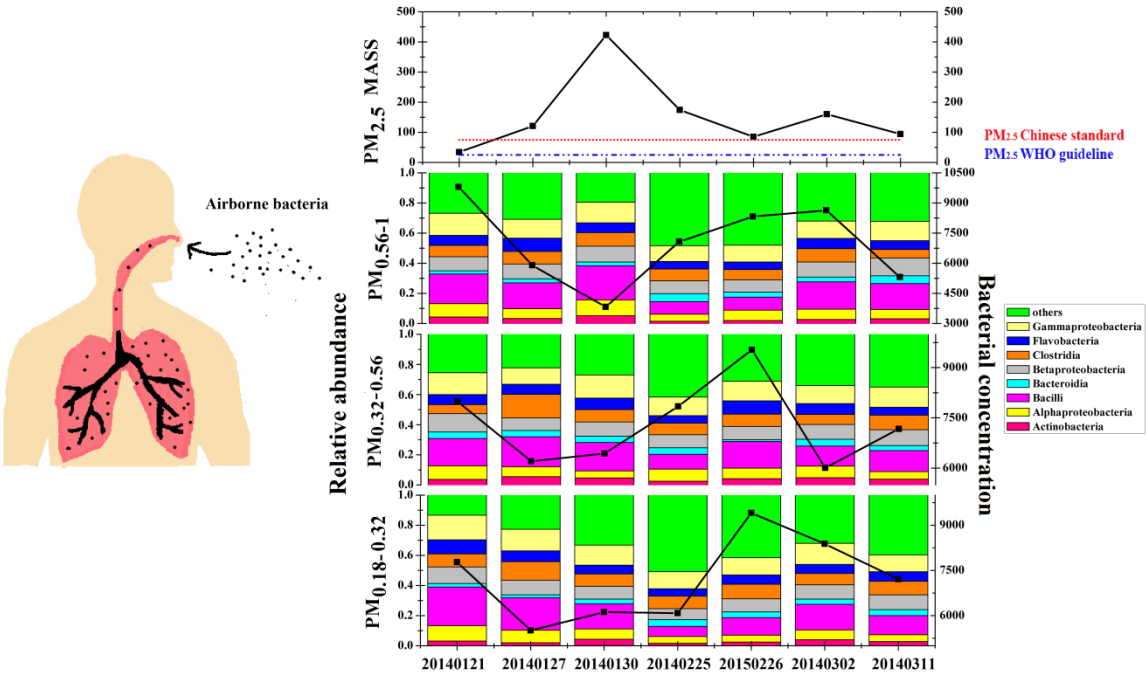
Graphic Abstract



HIGHLIGHTS

1. High bacterial concentration and diverse bacterial community in submicron particles ($PM_{0.18-0.32}$, $PM_{0.32-0.56}$, and $PM_{0.56-1}$) during haze episodes were observed.
2. The bacterial community varied significantly via different size fractions.
3. Source track analysis showed that the ambient bacteria mainly originated from soils, leaf surfaces, and feces.

ABSTRACT

In January 2014, severe haze episodes which sweep across Chinese cities have attracted public concern and interest at home and abroad. In addition to the physicochemical properties of air pollutants, bacteria are thought to be responsible for the spread of respiratory diseases and various allergies. We attempted the bacterial characterization of submicron particles ($PM_{0.18-0.32}$, $PM_{0.32-0.56}$, and $PM_{0.56-1}$) under severe haze episodes using high-throughput sequencing and real-time quantitative PCR detecting system based on 21 samples collected from January to March 2014 at Ji'nan, China. The high bacterial concentration in $PM_{0.32-0.56}$ (7314 cells·m^{-3}), $PM_{0.18-0.3}$ (7212 cells·m^{-3}), and $PM_{0.56-1}$ (6982 cells·m^{-3}) showed significant negative correlations with SO_2, NO_2, and O_3. Under sufficient sequencing depth, 37 phyla, 71 classes, 137 orders, 236 families, and 378 genera were classified, and the bacterial community structure varied significantly in different size fractions. For example, Holophagaceae (Acidobacteria) in $PM_{0.32-0.56}$ showed 6-fold higher abundance than that in $PM_{0.18-0.32}$. Moreover, functional categories and bacterial species (*Lactococcus piscium*, *Pseudomonas fragi*, *Streptococcus agalactiae*, and *Pseudomonas cichorii*) that may potentially be responsible for infections and allergies were also discovered. Source track analysis showed that the ambient bacteria mainly originated from soils, leaf surfaces, and feces. Our results highlighted the importance of airborne microbial communities by understanding the concentration, structure, ecological and health effects, especially those in submicron particles during haze episodes.

Keywords: Bioaerosol; Haze; Bacterial community; Submicron particles

1. Introduction

In the last few decades, the rapid economic growth and energy consumption, along with the lack of measures for protecting atmospheric environments, has resulted in continuous haze episodes in China (Yang et al., 2015; Li et al., 2014). In severe haze episodes, the daily average $PM_{2.5}$ mass concentration of Jing-Jin-Ji regions (Wang et al., 2013; Han et al., 2016) largely exceeded 25 $\mu g \cdot m^{-3}$, which is specified as the limit of the World Health Organization(WHO) $PM_{2.5}$ health guideline, by about 20-fold. Exposure to such high concentrations of airborne particles leads to high morbidity and mortality due to infectious diseases such as cardiovascular diseases, respiratory infections, and lung cancer (Bower et al., 2013; Esposito et al., 2012). Based on the definition of haze by the State Standard of the People's Republic of China (QX/T 113-2010), haze is defined as a complex air pollution process include the following conditions: (1) visibility less than 10 km and relative humidity lower than 80%, and (2) the $PM_{2.5}$ mass concentration higher than 75 $\mu g \cdot m^{-3}$ (Leng et al., 2013; Kong et al., 2014; Jansen et al., 2014; China Meteorological Administration, 2010). In January of 2013 and 2014, severe haze episodes were reported in Beijing (Wei et al., 2016), Hebei (Wang et al., 2013), Nanjing (Kong et al., 2015), and Ji'nan (Wang et al., 2015a) which caused great economic losses and public panic across China. Ji'nan is the capital of Shandong Province and covers an area of 8177 km^2. It is surrounded by hills on three sides which may exacerbate the accumulation of airborne pollutants including atmospheric particles, sulfur dioxide, nitrogen oxide, trace gases, and volatile organic compounds (Liu et al., 2015; Zhang et al., 2014; Li et al., 2011). Majority of existing studies focussed on the bacteria which occupied about 80.8% and 86.1% of the total microbes (Cao et al., 2014) in $PM_{2.5}$ and PM_{10}. Limited studies investigated bacteria in submicron particles (Gou et al. 2016), which can easily penetrate lungs or even the blood stream (Janssen et al., 2011; Visser et al., 2015; Gao et al., 2015b). Hence it is essential to study the bacterial characteristics of such

aforementioned submicron particles in atmosphere.

The near-surface and upper troposphere contain thousands to millions of bacterial cells per cubic meter (Bower et al. 2012). Active bacteria can serve as a medium for the spread of allergens and pathogens in a crowd (Creamean et al., 2013; Husman et al., 1996). There are also increasing evidences indicating that bacteria can act as cloud condensation nuclei, absorbing or reflecting sunlight, or even participating in N-cycling and C-cycling in the ecosystem (Bauer et al., 2003). So far, many investigations on the active bacterial concentration and bacterial community in airborne particles have been conducted (Bertolini et al., 2013; Hospodsky et al., 2015; Prussin et al., 2015). The airborne bacterial concentration in the near-surface ranged from 10^4 to 10^6 cells·m^{-3} (Bowers et al., 2012; Haas et al., 2013; Murata et al., 2014; Goudarzi et al. 2014; Murata et al., 2016). Bower et al. (2013) reported detailed information on the airborne microbial community and sources in $PM_{2.5}$ and $PM_{2.5\text{-}10}$ and found that the bacterial richness and communities structures showed a significant distinction across these two size fractions. In China, Cao et al. (2014) described the microbial communities of $PM_{2.5}$ and PM_{10} using metagenomics during a serious smog event and found that bacteria were the dominant one which was mostly terrestrial-related. Wei et al. (2016) investigated the concentration and size distribution of bioaerosols during haze and sunny days in Beijing. Compared to the sunny day, the fluorescent particle concentrations increased during the haze episodes and decreased with the dissipation of haze occurrences in 3-5 days. Furthermore no obvious difference in the airborne bacterial abundance and community structure were observed between haze and sunny days. Although these studies have illustrated the concentration and community compositions of cultured or uncultured bacteria in atmospheric fine particles, studies on bacterial characterizations in submicron particles are rare, especially during severe haze episodes.

Herein, we first characterized severe haze episodes to reveal the nutrients in submicron

particles from January to March 2014 in Ji'nan. The bacterial concentration and community structure of different particle size fractions was analyzed subsequently. Third, we performed functional analysis of the bacteria in the submicron particles to assess their potential to cause risk to human health. Our study draws a framework of bacterial community in Jinan's submicron particles during haze episodes and emphasizes the health risks of long-term exposure to high concentrations bacteria.

2. Materials and methods

2.1. Aerosol Collection.

Aerosol samples were collected from the rooftop of the Lizong building in the central campus of Shandong University located in Ji'nan (36°40'N, 117°3'E). The Lizong building is a six-floored teaching building where classes are conducted from 08:00 to 17:00 from Monday to Friday. To avoid the interference from local anthropogenic emissions on the ground, a Micro-Orifice Uniform Deposit Impactor (MOUDI) and on-line monitoring instruments such as SO_2 analyzer (Model 43C, Thermo, USA), NOx analyzer (Model 42C, Thermo, USA), O_3 analyzer (Model 49C, Thermo, USA) were placed in the rooftop of Lizong building about 20 m from the ground. We sterilized the quartz membrane by baking in a Muffle furnace at 500 °C for 6 h before sampling. After the filter cooled, it was packaged into sterilized aluminum foil and stored in a sealed bag. Before sampling, the inside surfaces of the MOUDI were kept sterile and 75% ethanol was used to sterilize the impactor. Seven sets of aerosol samples were obtained on the 47-mm quartz membrane of the MOUDI for 23h (9:00 am to 8:00 am next day) at a flow rate of 30 lpm during Jan. 20, 2014 to Mar. 31, 2014; these samples were stored at -80 °C until analysis. Each set contained nine samples in nine size-resolved ranges as follows: stage1, ⩾18 μm; stage2, 10–18 μm; stage3, 5.6–10 μm; stage4, 3.2–5.6 μm; stage5, 1.8–3.2 μm; stage6, 1.0–1.8 μm; stage7, 0.56–1.0 μm;

stage8, 0.32–0.56 μm; and stage9, 0.18–0.32 μm. The $PM_{1.0}$ can easily penetrate thoracic and pulmonary airways and plays an important role in haze formation and visibility degradation (Shi et al., 2014). Meanwhile the fact that specific surface area of $PM_{1.0}$ is greater than $PM_{2.5}$ provides evidences that $PM_{1.0}$ containing more health risks. Therefore we used the stage7, stage8, and stage9 samples for the following experiments. An automatic meteorological station (JZYG, PC-4) was employed to measure meteorological factors (wind direction, wind speed, humidity, and temperature) in real time. Meanwhile, a Synchronized Hybrid Ambient Real-Time Particulate monitor (SHARP, Model 5030, Thermo Fisher Scientific, USA) and a Monitor for AeRosols and GAses analyzer (MARGA, ADI20801, Applikon-ECN, Netherlands) were used to analyze the hourly average mass concentration of $PM_{2.5}$, water-soluble ions, and trace gases as described previously. Based on the definition of haze, seven days including six haze days (January 27, 2014; January 30, 2014; February 25, 2014; February 26, 2014; March 2, 2014; and March 11, 2014) and one clear day (January 21, 2014) were selected. Details about sampling time and the chemical characteristics including $PM_{2.5}$, trace gases (SO_2, NO_2, and NH_3), water soluble inorganic ions (NH_4^+, SO_4^{2-}, NH_4^+, K^+, Cl^-, and Na^+), and meteorological factors (wind direction and wind speed) of sampling time are summarized in Figure 1.

2.2. DNA Extraction and PCR Amplification.

DNA was extracted from the quartz membrane fragments (cut into 1.1 cm^2 filter area) using the E.Z.N.A.® Soil DNA Kit (Omega Bio-tek, Norcross, GA, U.S.) according to the manufacturer's instructions. DNA concentration was determined using NanoDrop 2000 (Thermo，Wilmington, Delaware, USA). Extracted DNA samples were stored at −80 °C until further analysis. The V3-V4 region of 16S rRNA was amplified using a bacterial universal PCR primer set 515F (5’-GTGYCAGCMGCCGCGGTAA-3’) and 907R (5’-

CCYCAATTCMTTTRAGTTT-3') (Gallagher et al., 2013). PCR amplification was performed on an ABI GeneAmp® PCR system 9700 (Applied Biosystems, 101 Foster City, CA) using a 20 μL reaction mixture contained 4 μL 5×FastPfu buffer, 2 μL 2.5 mM dNTPs, 0.8 μL 5 μM forward primer, 0.8 μL 5 μM reverse primer, 0.4 μL Fastfu polymerase, 10 ng template DNA, and 11 μL double distilled H_2O. PCR was performed at 95 °C for 3 min; 27 cycles of 95 °C for 10 s, 55 °C for 30 s and 72 °C for 45 s; 72 °C for 10 min; and hold at 10 °C. The final products were separated by 1% agarose gel electrophoresis and purified using an Axygen nucleic acid purification Kit (Axygen, Biosciences, CA, USA). The purified PCR products were prepared for sequencing on the Miseq™ platform (Illumina, San Diego, CA, USA). The nucleotide sequences were deposited in the Sequence Read Archive (SRA) under the accession number SRA385099.

2.3. Real-time Quantitative PCR.

To determine the absolute content of 16S genes in the particles of three sizes, Real-time Quantitative PCR Detecting System (qPCR) was applied in this study. For the qPCR reaction mixtures contained 12.5 μL of the ABI Power SybrGreen qPCR Master Mix (Promega, USA), 0.5 μL of each primer, 2 μL of sample DNA, and 9.5 μL of double distilled H_2O. The PCR program was performed in the ABI 7500 Real-Time PCR system (Applied Biosystems, 101 Foster City, CA) as follows: 50 °C for 2 min; 95 °C for 15 min; 40 cycles of 95 °C for 15 s; and 60 °C for 1 min. The deionized water was employed as the negative controls in this work and was pipetted into the wells in a 96-well microplate with the DNA extracted from the samples. All the samples were tested in triplicate and cycle thresholds worked by ABI 7500 software (Applied Biosystems). Based on the average rRNA gene copy number (3.98), the bacterial cell concentration (cells·m^{-3}) was calculated using the method described by Doorn et al. (2007).

2.4. High-throughput Sequence Analysis.

After sequencing, the primers and barcodes were trimmed from the end of the raw sequences, and low-quality reads (length less than 200 bp and average quality score less than 20) were removed using FASTX-ToolKit. The sequences that passed quality control were processed using QIIME (version 1.17). Alpha-diversity using the Chao1, Coverage, Simpson, and Shannon indexes was also calculated at 97% similarity level. Operational taxonomic units (OTU) were clustered at 97% similarity level using Uclust Soft. The ribosomal database project was employed for taxonomic classification (phylum, class, order, family, and genus) (Amato et al., 2013). To predict the potential functions of the bacterial communities, PICRUSt (Phylogenetic Investigation of Communities by Reconstruction of Unobserved States, http://picrust.github.com) was performed using the 16S rRNA gene data. These predictions were rarefied and analyzed in the Clusters of Orthologous Groups of proteins (COGs) database (http://www.ncbi.nlm.nih.gov/COG/).

2.5. Data Analysis

Spearman correlation coefficients were used to visualize the relationships between bacterial concentration and environmental factors (temperature, humidity, visibility, NO, NO_2, SO_2, CO and O_3). The variation in bacterial abundances across three size fractions at phyla, family, class, and genus level was assessed using analysis of variance (ANOVA) test. Spearman correlation analysis and ANOVA test were conducted by SPSS 16.0 software (SPSS Inc., Chicago, IL). Results were considered statistically significant at P value < 0.05 and P value <0.01. To describe the origin bacteria habitats, we aligned the Hiseq sequences of top 21 OTUs (relative abundance more than 94.5%) in NCBI database. The habitants of top five highest similarities bacteria were linked to the source of airborne bacteria.

3. Results

3.1. General Characteristics of the Haze Episodes in Ji'nan.

The environmental factors including $PM_{2.5}$, water soluble inorganic ions, trace gases, wind speed and wind direction were determined during sampling period. The $PM_{2.5}$ daily average mass concentration ranged from 34.3 to 422.4 μg·m^{-3} and the highest value was about 17-fold higher than the daily average specified by the WHO guideline for $PM_{2.5}$. The mass concentration of water-soluble inorganic ions and trace gases in $PM_{2.5}$ were in the order of SO_4^{2-} > NO_3^- > NH_4^+ > Cl^- > Ca^{2+} > K^+> Mg^{2+} > Na^+, and SO_2 > NO_2 > NH_3, respectively (Figure 1). The total water-soluble inorganic ions accounted for 64% of the $PM_{2.5}$ mass concentration and sulfate, nitrate, and ammonia were the most abundant compositions, which were consistent with the previous report by Wang et al. (2014) who showed that SO_4^{2-}, NH_4^+ and NO_3^- accounted for majority of the total water-soluble ions in $PM_{2.5}$ during the winter haze in Ji'nan. Cl^- (8.9 μg·m^{-3}) and K^+ (1.5 μg·m^{-3}) derived from the biomass burning cannot be ignored in the heating season. Du et al. (2011) determined that it was the higher concentration of K^+ from the biomass burning induced pollution events. They also implied that K^+ was the main existing form of KCl format in Shanghai. Meanwhile the use of firecrackers and fireworks on the eve of Chinese New Year was also identified as the cause for the same phenomenon (Zhang et al., 2010). The mass concentration of K^+ in the eve of 2014 Chinese New Year in Jinan was 18-folds higher than that in February 26, 2014. In addition to these three major ions, Ca^{2+} and Mg^{2+} from continental crust sources were also important during haze episodes in Ji'nan.

3.2. Bacterial concentration and community structure.

The bacterial concentration in $PM_{0.18-1}$ was in the range of 17624-25573 cells·m^{-3}, with an

average of 21509 cells·m^{-3}. The maximum of bacterial concentration occurred in $PM_{0.32–0.56}$, with a value of 7314 cells·m^{-3}, followed by $PM_{0.18-0.32}$ (7212 cells·m^{-3}) and $PM_{0.56-1}$ (6982 cells·m^{-3}) (Figure 2). After sequencing and homogenization, 27094 reads were gained for each sample and a total of 236, 188, and 222 OTUs were obtained in $PM_{0.18-0.32}$, $PM_{0.32-0.56}$, and $PM_{0.56-1}$, respectively. The $PM_{0.32-0.56}$ had the the minimum bacterial species which also confirmed by the lowest Chao1 index (Table 1). The coverage of more than 99% indicates the reliability of the experimental data. Meanwhile the S_{obs}/S_{Chao1} reaches 78% saturation, indicating a sufficient sampling (Toti et al., 2000). In addition, $PM_{0.32-0.56}$ and $PM_{0.56-1}$ had the same Shannon index, suggesting the similar bacterial diversity for these two particle sizes. This finding was different from a previous report, where bacterial richness in fine particulate matter ($PM_{2.5}$) was lower than that in coarse particulate matter ($PM_{2.5-10}$) (Bower et al., 2013).

In addition to bacterial concentration and alpha diversity estimators, the species comprising the bacterial community also play an essential role in the evaluation of health risks. In this study, 37 phyla, 71 classes, 137 orders, 236 families, and 378 genera were observed during the haze episodes. The predominant bacterial phyla (relative abundance more than 1%) were Firmicutes (78.9%), Proteobacteria (16.5%), Bacteroidetes (2.4%), and Actinobacteria (1.7%), which corresponded to the following bacterial classes: Bacilli (78.6%), Gammaproteobacteria (15.1%), Flavobacteria (2.0%), and Actinobacteria (1.7%) (Figure 3). With regard to the bacteria identified at the order level, Lactobacillales, Bacillales, and Pseudomonadales were found to be dominant with the relative abundances of 46.1%, 32.5%, and 10.5%, respectively. At a higher taxonomic level, the most abundant genera were found to be *Lactococcus*, *Bacillus*, *Pseudomonas*, and *Psychrobacter*, with relative abundances of 43.4%, 28.4%, 7.6%, and 2.3%, respectively (Figure 4). The dominant taxa in the bacterial community in $PM_{0.18-0.32}$, $PM_{0.32-0.56}$, and $PM_{0.56-1}$ present a similar distribution at the phyla, order, and genus levels. It was consistent with those of previous studies and indicated that

the bacterial community structure was similar within the same seasons (Bertolini et al., 2013). However, the structure of non-dominant bacteria community at different levels disclose a remarkable variation (Figure 5, ANOVA, P=0.05, F=2.3). Holophagaceae (Acidobacteria) were abundant in $PM_{0.32\text{-}0.56}$, displaying 6-fold times higher abundance than in $PM_{0.18\text{-}0.32}$. *Acetobacter* (Proteobacteria) showed a higher relative abundance in $PM_{0.32\text{-}0.56}$, which was 5-fold higher than that in $PM_{0.18\text{-}0.32}$. TA06, an uncultivated candidate phylum, was found only in $PM_{0.18\text{-}0.32}$. And some taxa could be found only in $PM_{0.18\text{-}0.32}$ and $PM_{0.56\text{-}1}$ such as Candidate division WS3, *Rheinheimera*, and *Fastidiosipila*.

3.3. Implications on Human health Risks and COG Analysis.

Given the high diversity of inhalable bacteria, the potential influence of these bacteria on humans and nature is worth noticing. 43.7% of the identified bacteria, including *Lactococcus piscium*, *Pseudomonas fragi*, *Streptococcus agalactiae*, and *Pseudomonas cichorii*, identified at the species level appeared to have interactive effects on human, animal, and plant health with an average coverage of 35.4%, 7.1%, 0.7%, and 0.5%, respectively. In addition to these pathogens, we also determined functional gene distribution in the airborne bacterial community in this study. The COGs were considered to be related to four functional groups and twenty-five function descriptions (Figure 6). Almost 80% of multi-copied genes with known functions were assigned with COG codes; for example, 9.3% of the genes were assigned a COG code of ‘E’, which represents amino acid transport and metabolism, and 7.1% of the genes were found to be functionally involved in carbohydrate transport and metabolism. Other codes such as K (transcription), J (translation, ribosomal structure and biogenesis), P (inorganic ion transport and metabolism), M (cell wall/membrane/envelope biogenesis), and C (energy production and conversion) also occupied a large proportion of the multi-copied genes (more than 5%). In addition, some of the genes detected in the airborne particulate

matter were poorly characterized with unknown function.

3. Discussion

Air pollution has been studied extensively by aerosol chemistry and physics. However the correlation between haze episodes and bacterial community has not been fully understood. The high concentration of airborne pollutants in haze episodes may provide nutrients (sulfur, nitrogen, and ammonia) and thus affect the bacterial community structure in submicron particles. For example, SO_4^{2-} has a distinct ability to influence the existence and growth of microbes and thus affect on their relative abundance (Scherer et al., 1981). Ca^{2+} is recognized to be associated with cellular processes such as the cell cycle and cell division in bacteria and can affect protein stability, enzymatic activity, and signal transduction, thus controlling protein functions (Michiels et al., 2002). Cl^-, employed in chemical agents, is related to water disinfection processes and thus causes bacterial stress injury. The high concentration of K^+ and Na^+ may disturb the structure and function of bacteria and then cause the death of bacteria. Previous studies have also showed that certain bacteria were related to atmospheric dynamics. For example, *Pseudomonas* species were likely to be involved in atmospheric processes such as desulfuration and denitration (Robinson et al., 2012). *Bacillus* species could participate in the nitrogen and carbon cycling in ecosystems (Ulrich et al., 2008). Therefore, under conditions of severe air pollution, the increased concentrations of ambient pollutants in submicron particles could be associated with the variation in bacterial concentration and community structure.

In the present study, the bacterial concentration in submicron particles were much higher than the China Scientific Ecology Center guideline (1000 cells·m^{-3}) and those reported in other haze studies in China (Beijing: 224 ± 186 CFU·m^{-3}, and Xi'an: 1102–1736 CFU·m^{-3}) (Gao et al., 2015a; Li et al., 2015), but slightly lower or similar to those reported in some

previous studies in other countries (Italy: 4.6×10^5 ribosomal operons per cubic meter, about 1.19×10^5 cells·m^{-3}, and USA: 2.72×10^4 cells·m^{-3}) (Bertolini et al., 2013; Bowers et al., 2012). The difference was possibly because most of the previous studies on airborne bacterial concentrations were performed by the culture method, while the qPCR method was used in this study. The culture method aims at the cultured bacteria which occupied only 1% of the total bacteria, e.g. Gao et al. (2016) calculated the bacterial concentration (705 ± 474 CFU m^{-3}) during summer based on the count method. It is therefore not surprising to find that the bacterial concentration reported here was much higher than that reported in the other studies. This high concentration indicated that the residents in Ji'nan face higher health risks. The size distribution of these airborne bacteria in submicron particles were similar to the trend observed in coarse particles that Dong et al. reported, with two peaks at 1.1–2.1 μm and 4.7–7.0 μm, which occupied 18.6% and 19.2% of the total airborne microbes collected from October 2013 to August 2014 in Qingdao (Dong et al.,2015). Dong et al. (2015) believed that 71.5% of the microbes existed in the coarse particles (>2.1 μm) and confirmed a distinct unimodal distribution with one peak at 2.1–3.2 μm during the heating period in winter, which may be caused by a combined effect of coal combustion, higher PM, and dust from the ground. Undoubtedly, in this study, all the samples were collected during the heating seasons in Ji'nan; therefore, we hypothesized that the single peak observed at 0.32–0.56 μm may have been due to the same reason and the additional emission caused by the fireworks. However, it is not clear whether airborne bacterial concentration varied with changes in environmental factors. Previous study showed that $PM_{2.5}$ and visibility showed a positive and negative correlation with airborne bacterial concentration during haze episodes (Li et al., 2015; Alghamdi et al.,2014; Gao et al.,2016). Goffau et al. (2009) reported that gram-positive bacteria grow faster under lower relative humidity in the atmosphere. Cao et al. (2014) found that the relative abundance of microbial pathogens in $PM_{2.5}$ increased with

increase of air pollution level. While in this study, no obvious correlation with visibility, relative humidity and $PM_{2.5}$ were observed. The bacterial concentration exhibited a significant negative correlation with NO_2 and SO_2, and O_3 (Table 2). It is likely that SO_2, NO_2, and O_3 have a toxic effect on microorganisms (Abdel Hameed et al., 2012). High concentration of SO_2, NO_2, and O_3 will inhibit the growth and breeding of the bacteria.

Due to the limit reports on the bacterial composition in $PM_{1.0}$, we compared the results with several other studies emphasized on the bacterial composition in $PM_{2.5}$, PM_{10}, and TSP. At the phylum level, Cao et al. (2014) showed that Actinobacteria, Proteobacteria, Chloroflexi, Firmicutes, Bacteroidetes, and Euryarchaeota were the most abundant phyla in $PM_{2.5}$ and PM_{10} during severe haze episodes. At the order level, lower abundance of Bacillales (2.0% and 3.0%) and higher Actinomycetales (80.0% and 60.0%) were reported in $PM_{2.5}$ and PM_{10} samples obtained in Milan, Italy, during winter (Franzetti et al., 2011). However, the abundance of Actinomycetales in the submicron particles in Ji'nan was very low in this study (less than 1%, about 0.001%). This result was also markedly different from that reported by Bertolini et al. (2013) who showed that Actinobacteridae, Clostridiales, and Sphingobacteriales were the major taxa in the airborne bacterial community in an urban area of Northern Italy. The results may be caused by the different analysis method and sequencing platform. Bertolini et al. (2013) target on the V5-V6 region based on the primer 783F-1046R using the Illumina GA-IIx sequencing, while our target area were V3-V4 based on the universal primer (515F-907R) by Illumina Miseq platform. The different sampling and analysis method produce a difference in these two studies. At the genus level, distinct different bacterial community were found compared to Wang et al. (2015b) that *Arthrobacter* and *Frankia* from the phylum Actinobacteria were the dominant genus in $PM_{2.5}$ during haze episodes in Beijing. From another perspective, the abundant genera (*Lactococcus*, *Bacillus*, *Arthrobacter*, *Streptococcus*, *Leuconostoc*, and *Lactobacillus*) implying that about 76.4% of

the bacteria were recognized to be gram-positive, which is consistent with the report of Fang et al. (2007) who illustrated that 80–86% of the total airborne bacteria were gram-positive in outdoor environments in Beijing. The authors stated that the reason for this was that gram-positive bacteria had stronger resistance and survival ability than gram-negative bacteria under adverse conditions (aerosolized chemical pollutants, strong sunlight, and lower humidity). While in this study, no obvious difference was observed in the abundances of most abundant genus, no matter whether gram-positive or gram-negative bacteria (Figure 4). The results were consistent with previous investigation by Wei et al. (2016), in that the dominant bacterial community showed no significant difference between haze and clear days during Jan. 2014 in Beijing. The possible explanation was the short-time sampling (less than one year). Bower et al. (2012) found that the local terrestrial source environments influence is more in shifting the bacterial communities, than the atmospheric condition. In future studies, the quarterly and annual sampling were essential to analyze the variation between haze and non-haze days.

Among these identified taxa, some specific bacteria were identified to be linked to human health risks. *Lactococcus piscium*, a well-known pathogen to affect salmonid fish (Williams et al., 1990), *Pseudomonas fragi* is responsible for bacteriological spoilage in dairy products and causes great economic losses in the dairy industry (Pereira et al.,1957). *Streptococcus agalactiae* can result in invasive infections such as skin and skin structure infections, urinary tract infections, osteomyelitis, endocarditis, and meningitis in adults (Farley et al., 2001). *Pseudomonas cichorii*, which is usually isolated from the soil, shows pathogenicity in plants including eggplant, lettuce, celery, and chrysanthemum crops and has important economic effects (Hojo et al., 2008; Pauwelyn et al., 2010). Our results show that long-term exposure to high concentrations of these ambient bacteria would pose a risk to people living in such hostile environments. On the other hand, the well-known beneficial bacterium S*treptococcus*

thermophiles, which has the ability to reduce the risks of antibiotic-associated diarrhea and lung cancer in mice, was also detected and showed an average abundance of 0.7% (Beniwal et al., 2003). In addition, bacteria with the aforementioned functions during the haze episodes may have an important role in the degradation of high concentrations of pollutants. For example, some species belonging to the *Streptococcus* genus have been shown to be able to degrade organic acids (Amato et al., 2007); certain strains of *Sphingomonas* can degrade organic matter such as polynuclear aromatic hydrocarbons (Ye et al., 1995). *Bacillus badius* (0.5%), a well-known alkaliphilic bacterium, can degrade organic matter such as aniline and anthracene (Ahmed et al., 2012).

The impact of airborne bacterial communities on bio-ecosystems and human health needs to be investigated in future studies using bacterial cultures and metagenomics analysis.

Apart from the bacterial community structure in submicron particles, the source of the bacteria should be identified in order to understand the inhalable microbes further. The natural biosphere provides various natural environment sources for primary biological aerosol particles. Generally, the primary biological aerosol reside mainly in soil, plant, rock surface, leaf surface, animal secreta, skin or hair, human activity, and ocean (Bower et al. 2012). The microorganisms aerosolized into atmosphere and rapidly deposited rather than suspended due to the high settling velocities (Despres et al. 2011). Since unique taxa may exist in specific environments, the potential source of the bacteria can be identified by identification of the unique taxa. In other words, the bacteria, which are derived from a specific habitat can be linked to the source in the environments. During the cold season, soil was an important source for the atmospheric bacteria, which was indicated by the high abundance of soil-inhabiting bacteria such as *Lactococcus*, *Bacillus*, and *Arthrobacter* during this season (Bowers et al., 2011). Bacteria originating from the surfaces of leaves (*Pseudomonas*) have been found to be abundant in warm temperate regions (Yashiro et al.,

2011). Furthermore, bacteria linked to feces have been observed such as members of *Escherichia* and *Streptococcus* (DeLeon-Rodriguez et al., 2013). Nevertheless, the high similarities between the bacterial genera detected in the diverse seasons and locations suggest that part of the airborne bacterial community may change by the spread of bacteria by long-term transport (air flow from ocean, dust events, or precipitation). Jeon et al. (2011) showed that airborne bacterial concentration increased significantly and the ambient bacterial community structure changed markedly during dust events in Asia. However, this did not seem to be the case in this study. Our results indicated that the sources of the airborne bacterial community in particulate matter may be environments such as soils, leaf surfaces, and feces.

4. Conclusion

Bacteria, including their concentration, community characteristics, correlation with environmental factors, and role in infectious process of diseases and ecological process may have been underestimated. In the present study, high bacterial concentration and significant negative correlation with the NO_2, SO_2, and O_3 in atmosphere were detected. The diverse bacteria and pathogens in submicron particles during haze episodes was observed for the first time by the high-throughput sequencing, yet no significant difference for the dominant bacterial genus between haze and non-haze days were observed. The results also indicate that the most abundant genera show highly similarity across three size fraction, while bacteria with low abundance show a significant difference such as *Acetobacter* and *Fastidiosipila*. We also acknowledge that the ambient bacteria mainly originated from soils, leaf surfaces, and feces. This knowledge helps for the comprehensive understanding of bacterial community biodiversity in submicron particles particularly those potential pathogens during haze episodes.

Conflict of Interest

The authors declare no conflict of interest.

Acknowledgments

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References

Abdel Hameed AA, Khoder MI, Ibrahim YH, Osman ME, Ghanem S. Study on some factors affecting survivability of airborne fungi. Sci Total Environ 2012; 414: 696-700.

Ahmed AT, Othman MA, Sarwade VD, Kachru GR. Degradation of anthracene by alkaliphilic bacteria bacillus badius. Environ Pollut 2012; 1: 97-104.

Alghamdi MA, Shamy M, Redal MA, Khoder M, Awad AH, Elserougy S. Microorganisms associated particulate matter: A preliminary study. Sci Total Environ 2014; 479–480: 109-116.

Amato P, Demeer F, Melaouhi A, Fontanella S, Martin-Biesse AS, Sancelme M, Laj P, Delort AM. A fate for organic acids, formaldehyde and methanol in cloud water: their biotransformation by microorganisms. Atmos Chem Phys 2007; 7: 4159-4169.

Amato KR, Yeoman CJ, Kent A, Righini N, Carbonero F, Estrada A, Gaskins HR, Stumpf RM, Yildirim S, Torralba M, Gillis M, Wilson BA, Nelson KE, White BA, Leigh SR. Habitat degradation impacts black howler monkey (Alouatta pigra) gastrointestinal microbiomes. ISME J 2013; 7: 1344-1353.

Bauer H, Giebl H, Hitzenberger R, Kasper-Giebl A, Reischl G, Zibuschka F, Puxbaum H.

Airborne bacteria as cloud condensation nuclei. J Geophys Res 2003; 108: 1919-1964.

Beniwal RS, Arena VC, Thomas L, Narla S, Imperiale TF, Chaudhry RA, Ahmad UA. A randomized trial of yogurt for prevention of antibiotic-associated diarrhea. Digest Dis Sci 2003; 48: 2077-2082.

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

Bowers RM, McLetchie S, Knight R, Fierer NS. patial variability in airborne bacterial communities across land-use types and their relationship to the bacterial communities of potential source environments. ISME J 2011; 5: 601-612.

Bowers RM, McCubbin IB, Hallar AG, Fierer N. Seasonal variability in airborne bacterial communities at a high-elevation site. Atmos Environ 2012; 50: 41-49.

Bowers RM. Clements N, Emerson JB., Wiedinmyer C, Hannigan MP, Fierer N. Seasonal variability in bacterial and fungal diversity of the near-surfaces atmosphere. Environ Sci Technol 2013; 47: 12097-12106.

Cao C, Jiang WJ, Wang BY, Fang JH, Lang JD, Tian G, Jiang JK, Zhu TF. Inhalable microorganisms in Beijing's $PM_{2.5}$ and PM_{10} pollutants during a severe smog event. Environ Sci Technol 2014; 48: 1499-1507.

China Meteorological Administration. Observation and forecasting levels of haze. The State Standard of the People’s Republic of China QX/T 113-2010 (in Chinese), Beijing: China Meteorological Press; 2010.

Creamean JM, Suski KJ, Rosenfeld D, Cazorla A, DeMott PJ, Sullivan RC, White AB, Ralph FM, Minnis P, Comstock JM, Tomlinson JM, Prather KA. Dust and biological aerosols from the Sahara and Asia influence precipitation in the Western U.S. Science

2013; 339: 1572-1578.

DeLeon-Rodriguez N, Lathem TL, Rodriguez-R LM, Barazesh JM, Anderson BE, Beyersdorf AJ, Ziemba LD, Bergin M, Nenes A, Konstantinidis KT. Microbiome of the upper troposphere: species composition and prevalence, effects of tropical storms, and atmospheric implications. P Natl Acad Sci the USA 2013; 110: 2575-80.

Després VR, Huffman JA, Burrows SM, Hoose C, Safatov AS, Buryak G, Frohlich-Nowoisky J, Elbert W, Andrae M, Poschl U, and Jaenicke R. Primary biological aerosol particles in the atmosphere: a review. Tellus B 2012; 64.

Dong LJ, Qi JH, Shao CC, Zhong X, Gao DM., Cao WW, Gao JW, Bai R, Long GY, Chu CC. Concentration and size distribution of total airborne microbes in hazy and foggy weather. Sci Total Environ 2015; 541: 1011-1018.

Doorn RV, Szemes M, Bonants P, Kowalchuk GA, Salles JF, Ortenberg E. Quantitative multiplex detection of plant pathogens using a novel ligation probe-based system coupled with universal, high-throughput real-time PCR on OpenArrays™. BMC Genomics 2007; 8: 276.

Du HH, Kong LD, Cheng TT, Chen JM, Du JF, Li L, Xia XG, Leng CP, Huang GH. Insights into summertime haze pollution events over Shanghai based on online water-soluble ionic composition of aerosols. Atmos Environ 2011; 45: 5131-5137.

Esposito V, Lucariello A, Savarese L, Cinelli MP, Ferraraccio F, Bianco A, De Luca A, Mazzarella G. Morphology changes in human lung epithelial cells after exposure to diesel exhaust micron sub particles ($PM_{1.0}$) and pollen allergens. Environ Pollut 2012; 171: 162-167.

Fang ZG, Ouyang ZY, Zheng H, Wang XK, Hu LF. Culturable airborne bacteria in outdoor environments in Beijing, China Microb Ecol 2007; 54: 487-496.

Farley M M. Group B streptococcal disease in nonpregnant adults. Clin Infect Dis 2001;

33:556-561.

Franzetti A, Gandolfi I, Gaspari E, Ambrosini R, Bestetti G. Seasonal variability of bacteria in fine and coarse urban air particulate matter. Appl Microbiol Biotechnol 2011; 90: 745-753.

Gallagher LK, Glossner AW, Landkamer LL, Figueroa LA, Mandernack KW, Munakata-Marr J. The effect of coal oxidation on methane production and microbial community structure in Powder River Basin coal. Int J Coal Geol 2013; 115: 71-78.

Goffau MC, Yang XM, Van Dijl JM, Harmsen HM. Bacterial pleomorphism and competition in a relative humidity gradient. Environ Microbiol 2009; 11: 809–822.

Gou HG, Lu JJ, Li SM, Tong YB, Xie CB, Zheng XW. Assessment of microbial communities in PM1 and PM10 of Urumqi during winter. Environ Poll 2016; 214: 202-210.

Gao M, Jia RZ, Qiu TL, Han ML, Song Y, Wang XM.. Seasonal size distribution of airborne culturable bacteria and fungi and preliminary estimation of their deposition in human lungs during non-haze and haze days. Atmos Environ 2015a; 118: 203-210.

Gao M, Guttikunda SK, Carmichael GR, Wang WS, Liu ZR, Stanier CO, Saide PE, Yu M. Health impacts and economic losses assessment of the 2013 severe haze event in Beijing area. Sci Total Environ 2015b; 511: 553-561.

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

Goudarzi G, Shirmardi M, Khodarahmi F, Hashemi-Shahraki A, Alavi N, Ankali KA, Babaei AA, Soleimani Z, Marzouni MB. Particulate matter and bacteria characteristics of the Middle East Dust (MED) storms over Ahvaz, Iran. Aerobiologia 2014; 30: 345-356.

Haas D, Galler H, Luxner J, Zarfel G, Buzina W, Friedl H, Marth E, Habib J, Reinthaler FF. The concentrations of culturable microorganisms in relation to particulate matter in urban air. Atmos Environ 2013; 65: 215-222.

Han B, Zhang R,Yang W, Bai ZP, Ma ZQ, Zhang WJ. Heavy haze episodes in Beijing during January 2013: Inorganic ion chemistry and source analysis using highly time-resolved measurements from an urban site. Sci Total Environ 2016; 544: 319-329.

Handley BA, Webster AJF. Some factors affecting the airborne survival of bacteria outdoors. J Appl Bacteriol 1995; 79: 368-378.

Hojo H, Koyanagi M, Tanaka M, Kajihara S, Ohnishi K, Kiba A, Hikichi Y. The hrp genes of Pseudomonas cichorii are essential for pathogenicity on eggplant but not on lettuce. Microbiology 2008;154: 2920-2928.

Hospodsky D, Yamamoto N, Nazaroff WW, Miller D, Gorthala S, Peccia J. Characterizing airborne fungal and bacterial concentrations and emission rates in six occupied children's classrooms. Indoor Air 2015; 25: 641-652.

Husman T. Health effects of indoor-air microorganisms. Scand. J Work Env Hea 1996; 22: 5-13.

Jansen RC, Shi Y, Chen JM, Hu YJ, Xu C, Hong SM, Li J, Zhang M. Using hourly measurements to explore the role of secondary inorganic aerosol in PM2.5 during haze and fog in Hangzhou, China. Adv Atmos Sci 2014; 31: 1427. doi:10.1007/s00376-014-4042-2

Janssen NAH, Hoek G, Simic-Lawson M, Fischer P, Bree L, Brink H, Keuken M, Atkinson, RW, Anderson HR, Brunekreef B, Cassee FR. Black carbon as an additional indicator of the adverse health effects of airborne particles compared with PM_{10} and $PM_{2.5}$. Environ Health Persp 2011; 119: 1691-1699.

Jeon EM, Kim HJ, Jung K, Kim JH, Kim MY, Kim YP, Ka J. Impact of Asian dust events on airborne bacterial community assessed by molecular analyses. Atmos Environ 2011; 45: 4313-4321.

Kong LD, Yang YW, Zhang SQ, Zhao X, Du HH, Fu HB, Zhang SC, Cheng TT, Yang X,

Chen JM, Wu D, Shen JD, Hong SM, Jiao L. Observations of linear dependence between sulfate and nitrate in atmospheric particles. J Geophys Res 2014, 119: 341-361.

Kong S, Li XX, Li L, Yin Y, Chen K, Yuan L, Zhang YJ, Shan YP, Ji YQ. Variation of polycyclic aromatic hydrocarbons in atmospheric $PM_{2.5}$ during winter haze period around 2014 Chinese Spring Festival at Nanjing: Insights of source changes, air mass direction and firework particle injection. Sci Total Environ 2015; 520: 59-72.

Leng CP, Cheng TT, Chen JM, Zhang RY, Tao J,Huang GH, Zha SP, Zhang MG, Fang W, Li X, Li L. Measurements of surface cloud condensation nuclei and aerosol activity in downtown Shanghai. Atmos Environ 2013; 69: 354-361.

Li WJ, Zhou SZ, Wang XF, Xu Z, Yuan C, Yu YC, Zhang QZ, Wang WX. Integrated evaluation of aerosols from regional brown hazes over northern China in winter: Concentrations, sources, transformation, and mixing states. J Geophys Res 2011; 116(D9):1-11.

Li M, Zhang L. Haze in China: current and future challenges. Environ Pollut 2014; 189: 85-86.

Li YP, Fu HL, Wang W, Liu J, Meng QL, Wang WK. Characteristics of bacterial and fungal aerosols during the autumn haze days in Xi'an, China. Atmos Environ 2015; 122: 439-447.

Liu ZC, Li N, Wang N. Characterization and source identification of ambient VOCs in Jinan, China. Air Qual Atmos Hlth 2015; 9: 1-7.

Michiels J, Xi CW, Verhaert J, Vanderleyden J. The functions of Ca^{2+} in bacteria: a role for EF-hand proteins? Trends Microbiol 2002; 10: 87-93.

Murata K, Zhang DZ. Transport of bacterial cells toward the Pacific in Northern Hemisphere westerly winds. Atmos Environ 2014; 87:138-145.

Murata K, Zhang DZ. Concentration of bacterial aerosols in response to synoptic weather and land-sea breeze at a seaside site downwind of the Asian continent. J Geophys Res 2016; 121, doi:10.1002/2016JD025028.

Pauwelyn E, Vanhouteghem K, Cottyn B,Vos PD, Maes M, Bleyaert P, Höfte M. Epidemiology of Pseudomonas cichorii, the cause of lettuce midrib rot. J Phytopathol 2011; 159: 298–305.

Pereira JN, Morgan ME. Nutrition and physiology of Pseudomonas fragi. J Bacteriol 1957;74: 710-713.

Prussin II AJ, Garcia EB, Marr LC. Total virus and bacteria concentrations in indoor and outdoor Air. Environ Sci Technol Lett 2015; 2: 84-88.

Robinson CV, Elkins MR, Bialkowski KM, Thornton DJ, Kertesz MA. Desulfurization of mucin by Pseudomonas aeruginosa: influence of sulfate in the lungs of cystic fibrosis patients. J Med Microbiol 2012; 61: 1644-1653.

Scherer P, Sahm H. Influence of sulphur-containing compounds on the growth of Methanosarcina barkeri in a defined medium. Appl Microbiol Biot 1981; 12: 28-35.

Shi Y, Chen JM, Hu DW, Wang L, Yang X, Wang XM. Airborne submicron particulate (PM_1) pollution in Shanghai, China: Chemical variability, formation/dissociation of associated semi-volatile components and the impacts on visibility. Sci Total Environ 2014; 473: 199-206.

Toti DS, Coyle FA, Miller JA. A structured inventory of Appalachian grass bald and heath bald spider assemblages and a test of species richness estimator performance. J Arachnol 2000; 28: 329-345.

Ulrich A, Klimke G, Wirth S. Diversity and Activity of Cellulose-Decomposing Bacteria, Isolated from a Sandy and a Loamy Soil after Long-Term Manure Application. Microb Ecol 2008; 55: 512-522.

Visser S, Slowik JG, Furger M, Zotter P, Bukowiecki N, Dressler R, Flechsig U, Appel K, Green D, Tremper AH, Young DE, Williams PI, Allan JD, Herndon S, Williams LR, Mohr C, Xu L, Ng N, Detournay A, Barlow J, Halios CH, Fleming ZL, Baltensperger U, Prevot ASH. Kerb and urban increment of highly time-resolved trace elements in PM10, PM2.5 and PM1.0 winter aerosol in London during ClearfLo 2012. Atmos Chem Phys 2015; 14: 2367-2386.

Wang LT,Wei Z, Chen MZ, Zheng Y. The 2013 severe haze over the southern Hebei, China: model evaluation, source apportionment, and policy implications. Atmos Chem Phys 2013; 13: 28395-28451.

Wang XX, Chen JM, Sun JF, Li WJ, Yang LX, Wen L,Wang WX, Wang XM, Collett Jr JL, Shi Y, Zhang QZ, Hu JT, Yao L, Zhu YH, Sui X, Sun XM, Mellouki A. Severe haze episodes and seriously polluted fog water in Ji'nan, China. Sci Total. Environ 2014; 493: 133-137.

Wang LW, Wen L, Xu CH, Chen JM, Wang XF, Yang LX, Wang WX, Yang X, Sui X, Yao L, Zhang QZ. HONO and its potential source particulate nitrite at an urban site in North China during the cold season. Sci Total Environ 2015a; 538: 93-101.

Wang BY, Lang JD, Zhang LN, Fang JH, Cao C, Hao JM, Zhu T, Tian G, Jiang JK. Characterizing Beijing's airborne bacterial communities in $PM_{2.5}$ and PM_1 samples during haze pollution episodes using 16S rRNA gene analysis method. Huan Jing Ke Xue in Chinese 2015b; 36: 2727-2734.

Wei K, Zou ZL, Zheng YH, Li J, Shen FX, Wu CY, Wu YS, Hu M, Yao MS. Ambient bioaerosol particle dynamics observed during haze and sunny days in Beijing. Sci Total Environ 2016; 550:751-759.

Williams AM, Fryer JL, Collins MD. Lactococcus piscium sp. nov. a new Lactococcus species from salmonid fish. FEMS Microbiol Lett 1990; 56: 109-113.

Yang L, Han LZ, Chen ZL, Zhou JB, Wang J. Growing trend of China's contribution to haze research. Scientometrics 2015; 105: 525-535.

Yashiro E, Spear RN, McManus PS. Culture-dependent and culture-independent assessment of bacteria in the apple phyllosphere. J Appl Microbiol 2011; 110: 1284-1296.

Ye DY, Siddiqi MA, Maccubbin AE, Kumar S, Sikka HC. Degradation of polynuclear aromatic hydrocarbons by Sphingomonas paucimobilis. Environ Sci Technol 1995; 30: 136-142.

Zhang M, Wang XM, Chen JM, Cheng TT, Wang T, Yang X, Gong YG, Geng FH, Chen CH. Physical characterization of aerosol particles during the Chinese New Year's firework events. Atmos Environ 2010; 44: 5191–5198.

Zhang JM, Chen JM, Yang LX, Sui X, Yao L, Zheng LF, Wen L, Xu CH, Wang WX. Indoor $PM_{2.5}$ and its chemical composition during a heavy haze–fog episode at Jinan, China. Atmos Environ 2014; 99: 641-649.

Table 1. Alpha-diversity indexes (97%) from $PM_{0.18-0.32}$, $PM_{0.32-0.56}$, and $PM_{0.56-1}$: Sobs (number of OTUs), ACE, Chao1, Coverage, and Shannon.

Sample	$N_{sequences}$	Sobs	ACE	Chao1	Coverage	Shannon
$PM_{0.18-0.32}$	27094	236	302	289	0.99	2.48
$PM_{0.32-0.56}$	27094	188	293	249	0.99	2.40
$PM_{0.56-1}$	27094	222	292	272	0.99	2.44

Table 2. Spearman's correlation coefficients between airborne pollutants and meteorological parameters with bacterial concentration in $PM_{0.18-0.32}$, $PM_{0.32-0.56}$, $PM_{0.18-0.32}$ and $PM_{0.18-1}$ (*P < 0.05).

Bacteria	Temperature	Humidity	Visbility	$PM_{2.5}$	NO	NO_2	SO_2	CO	O_3
$B_{0.56-1}$	0.429	-0.393	0.464	-0.571	-0.071	-0.750	-0.607	0.321	-0.643
$B_{0.32-0.56}$	0.071	0.071	0.464	-0.571	0.429	-0.071	-0.571	-0.214	-0.143
$B_{0.18-0.32}$	0.571	-0.036	0.393	-0.500	-0.214	-0.821*	-0.750	0.321	-0.786*
$B_{0.18-1}$	0.500	-0.143	0.536	-0.679	0.000	-0.786*	-0.821*	0.250	-0.571

List of Figures

Figure 1 Time series of the daily average ionic concentration and meteorological parameters during sampling days.

Figure 2 Daily average bacterial concentration and PM mass concentration in $PM_{0.56-1}$, $PM_{0.32-0.56}$, and $PM_{0.18-0.32}$. (a-$PM_{0.56-1}$, b-$PM_{0.32-0.56}$, c-$PM_{0.18-0.32}$).

Figure 3 (A-B) Relative abundance of bacteria at the phylum and class level in $PM_{0.56-1}$, $PM_{0.32-0.56}$, and $PM_{0.18-0.32}$. (a-$PM_{0.56-1}$, b-$PM_{0.32-0.56}$, c-$PM_{0.18-0.32}$).

Figure 4 Heatmap of the dominant genus (relative abundance higher than 0.05%) in $PM_{0.56-1}$, $PM_{0.32-0.56}$, and $PM_{0.18-0.32}$ at the genus level. (a-$PM_{0.56-1}$, b-$PM_{0.32-0.56}$, c-$PM_{0.18-0.32}$).

Figure 5 Relative abundance of the taxa (at the phyla, family, or genus levels) that were found to show significant difference across aerosol size fractions: *$P < 0.05$, **$P < 0.01$.

Figure 6 Categorization of the microbial community genome contigs according to COGs functional categories.

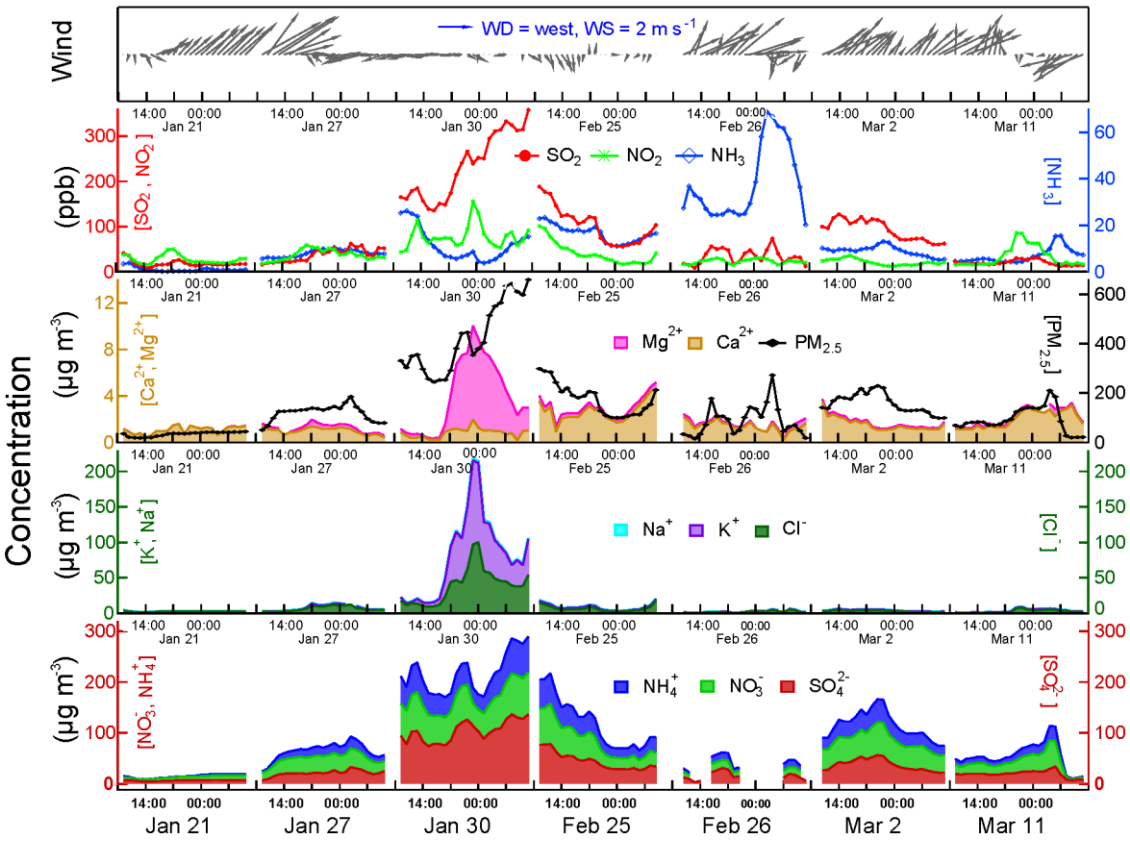
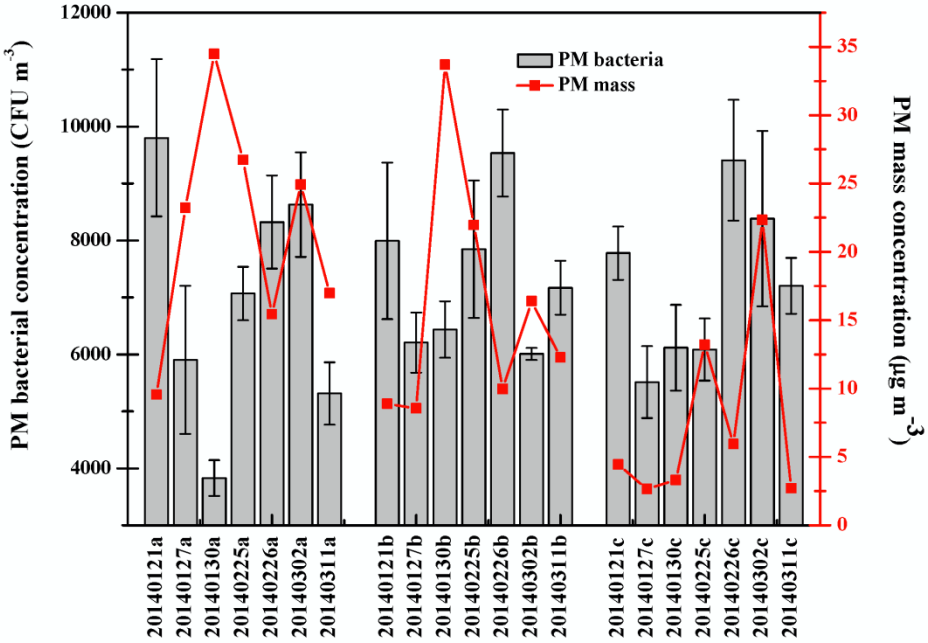
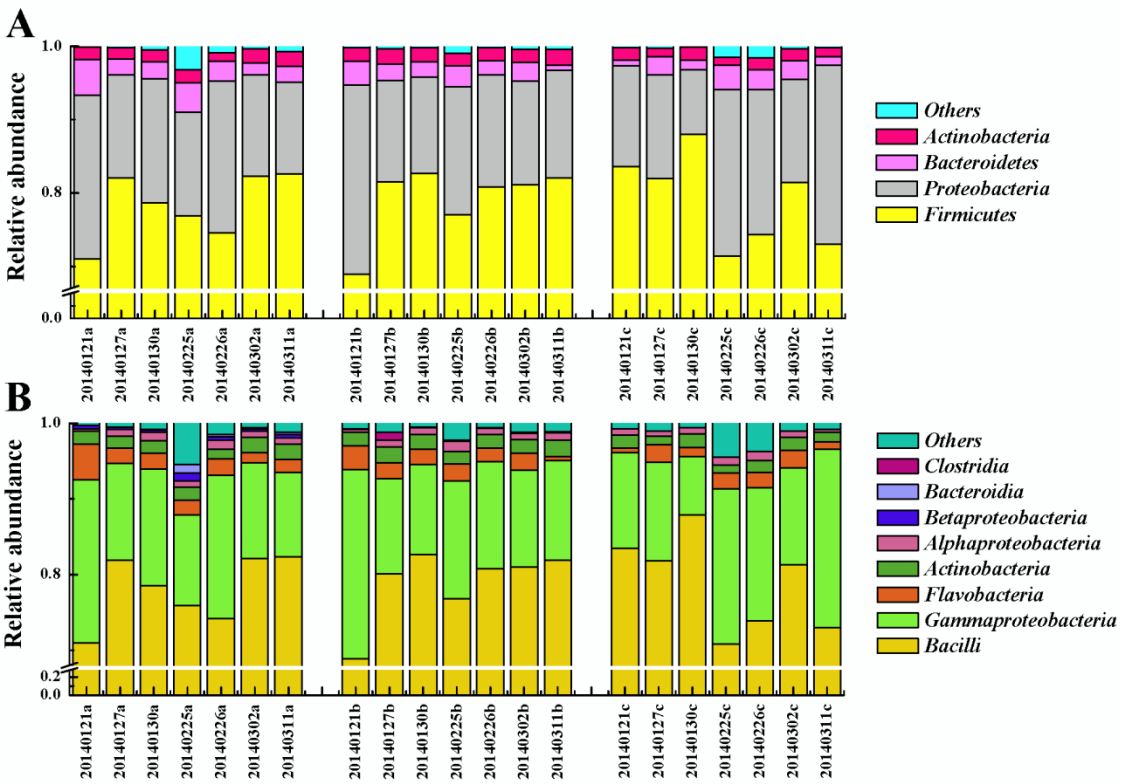

Wind
WD = west, WS = 2 m s-1
Concentration
(ppb)
[SO2, NO2]
[NH3]
SO2
NO2
NH3
(μg m-3)
[Ca2+, Mg2+]
[PM2.5]
Mg2+
Ca2+
PM2.5
[K+, Na+]
[Cl-]
Na+
K+
Cl-
[NO3-, NH4+]
[SO42-]
NH4+
NO3-
SO42-
14:00
00:00
Jan 21
Jan 27
Jan 30
Feb 25
Feb 26
Mar 2
Mar 11

Figure 1



642

643 **Figure 2**


A
Relative abundance
1.0
0.8
0.0
Others
Actinobacteria
Bacteroidetes
Proteobacteria
Firmicutes
20140121a
20140127a
20140130a
20140225a
20140226a
20140302a
20140311a
20140121b
20140127b
20140130b
20140225b
20140226b
20140302b
20140311b
20140121c
20140127c
20140130c
20140225c
20140226c
20140302c
20140311c
B
Relative abundance
1.0
0.8
0.2
0.0
Others
Clostridia
Bacteroidia
Betaproteobacteria
Alphaproteobacteria
Actinobacteria
Flavobacteria
Gammaproteobacteria
Bacilli
20140121a
20140127a
20140130a
20140225a
20140226a
20140302a
20140311a
20140121b
20140127b
20140130b
20140225b
20140226b
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20140225c
20140226c
20140302c
20140311c

Figure 3

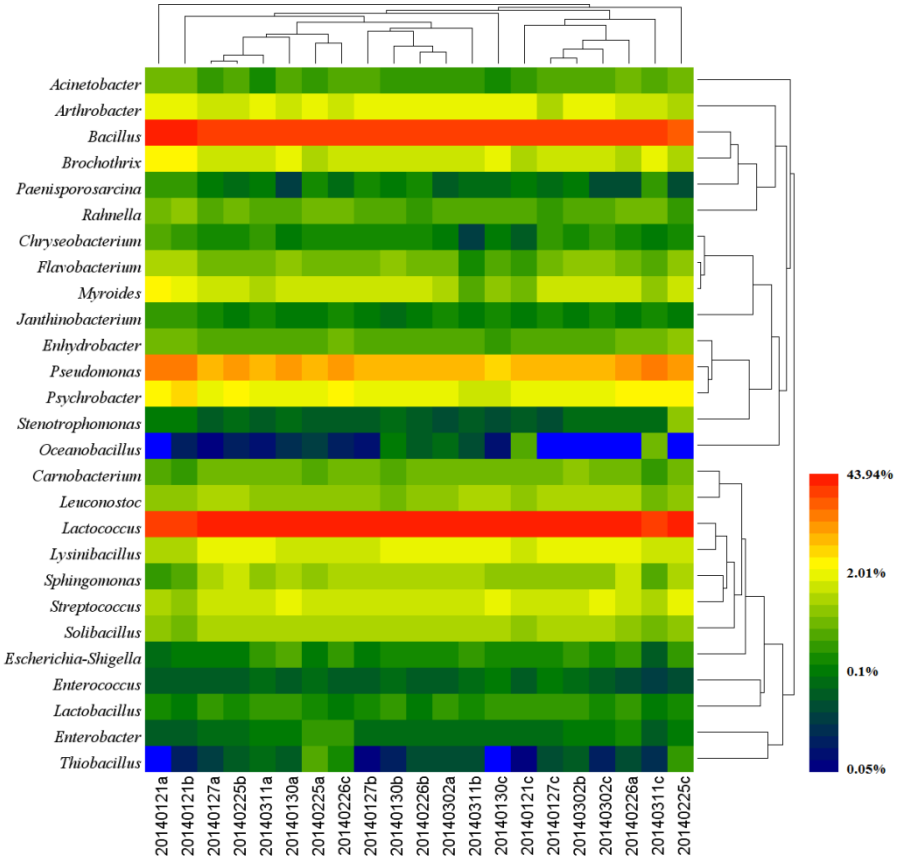
Acinetobacter
Arthrobacter
Bacillus
Brochothrix
Paenisporosarcina
Rahnella
Chryseobacterium
Flavobacterium
Myroides
Janthinobacterium
Enhydrobacter
Pseudomonas
Psychrobacter
Stenotrophomonas
Oceanobacillus
Carnobacterium
Leuconostoc
Lactococcus
Lysinibacillus
Sphingomonas
Streptococcus
Solibacillus
Escherichia-Shigella
Enterococcus
Lactobacillus
Enterobacter
Thiobacillus
20140121a
20140121b
20140127a
20140225b
20140311a
20140130a
20140225a
20140226c
20140127b
20140130b
20140226b
20140302a
20140311b
20140130c
20140121c
20140127c
20140302b
20140302c
20140226a
20140311c
20140225c
43.94%
2.01%
0.1%
0.05%

Figure4

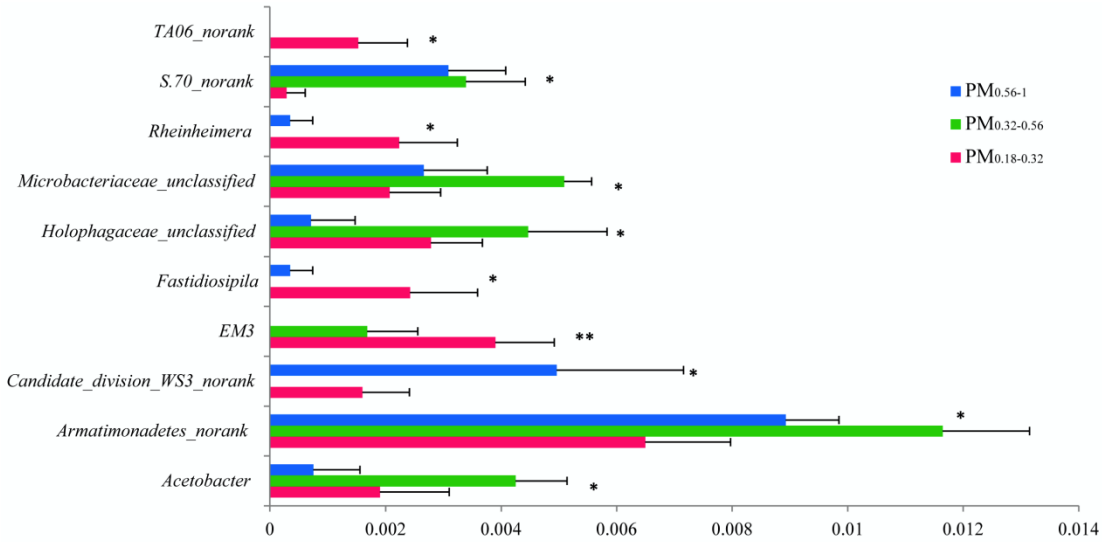
TA06_norank
S.70_norank
Rheinheimera
Microbacteriaceae_unclassified
Holophagaceae_unclassified
Fastidiosipila
EM3
Candidate_division_WS3_norank
Armatimonadetes_norank
Acetobacter
PM0.56-1
PM0.32-0.56
PM0.18-0.32
0
0.002
0.004
0.006
0.008
0.01
0.012
0.014

Figure 5

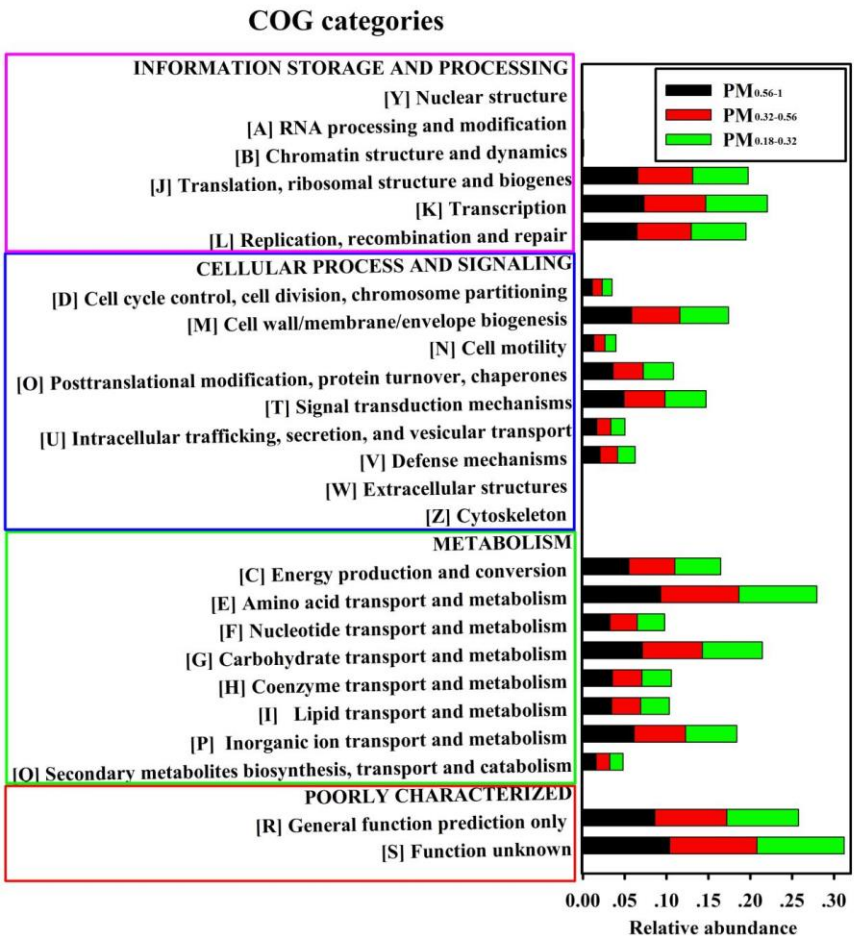
COG categories
INFORMATION STORAGE AND PROCESSING
[Y] Nuclear structure
[A] RNA processing and modification
[B] Chromatin structure and dynamics
[J] Translation, ribosomal structure and biogenes
[K] Transcription
[L] Replication, recombination and repair
CELLULAR PROCESS AND SIGNALING
[D] Cell cycle control, cell division, chromosome partitioning
[M] Cell wall/membrane/envelope biogenesis
[N] Cell motility
[O] Posttranslational modification, protein turnover, chaperones
[T] Signal transduction mechanisms
[U] Intracellular trafficking, secretion, and vesicular transport
[V] Defense mechanisms
[W] Extracellular structures
[Z] Cytoskeleton
METABOLISM
[C] Energy production and conversion
[E] Amino acid transport and metabolism
[F] Nucleotide transport and metabolism
[G] Carbohydrate transport and metabolism
[H] Coenzyme transport and metabolism
[I] Lipid transport and metabolism
[P] Inorganic ion transport and metabolism
[Q] Secondary metabolites biosynthesis, transport and catabolism
POORLY CHARACTERIZED
[R] General function prediction only
[S] Function unknown
PM0.56-1
PM0.32-0.56
PM0.18-0.32
0.00 .05 .10 .15 .20 .25 .30
Relative abundance

Figure 6