



Bacterial characterization in ambient submicron particles during severe haze episodes at Ji'nan, China



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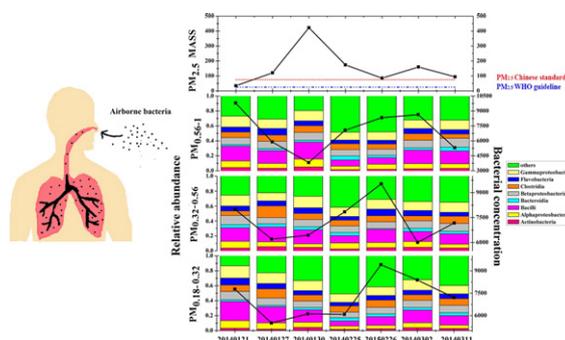
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HIGHLIGHTS

- High bacterial concentration and diverse bacterial community in submicron particles during haze episodes were observed.
- The bacterial community varied significantly via different size fractions.
- Source track analysis showed that the ambient bacteria mainly originated from soils, leaf surfaces, and feces.



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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 $PM_{0.32-0.56}$ ($7314 \text{ cells m}^{-3}$), $PM_{0.18-0.32}$ ($7212 \text{ cells m}^{-3}$), and $PM_{0.56-1}$ ($6982 \text{ cells m}^{-3}$) 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.

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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 and Zhang, 2014). In severe haze episodes, the

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daily average PM_{2.5} mass concentration of Jing-Jin-Ji regions (Wang et al., 2013; Han et al., 2016) largely exceeded 25 µg 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 (Bowers 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 < 10 km and relative humidity lower than 80%, and (2) the PM_{2.5} mass concentration higher than 75 µg 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 (L.W. Wang et al., 2015) which caused great economic losses and public panic across China. Ji'nan is the capital of Shandong Province and covers an area of 8177 km². 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 PM₁₀. 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 (Bowers 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, 1996). There are also increasing evidence 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 10⁴ to 10⁶ cells m⁻³ (Bowers et al., 2012; Haas et al., 2013; Murata and Zhang, 2014; Goudarzi et al., 2014; Murata and Zhang, 2016). Bowers et al. (2013) reported detailed information on the airborne microbial community and sources in PM_{2.5} and PM_{2.5-10} 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 PM₁₀ 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 SO₂ analyzer (Model 43C, Thermo, USA), NO_x analyzer (Model 42C, Thermo, USA), O₃ analyzer (Model 49C, Thermo, USA), and CO analyzer (Model 300E, Teledyne API, 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 23 h (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 evidence 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 (SO₂, NO₂, and NH₃), water soluble inorganic ions (NH₄⁺, SO₄²⁻, NH₄⁺, K⁺, Cl⁻, and Na⁺), and meteorological factors (wind direction and wind speed) of sampling time are summarized in Fig. 1.

2.2. DNA extraction and PCR amplification

DNA was extracted from the quartz membrane fragments (cut into 1.1 cm² 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'-GTGYCAGCMGCCGCGTAA-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

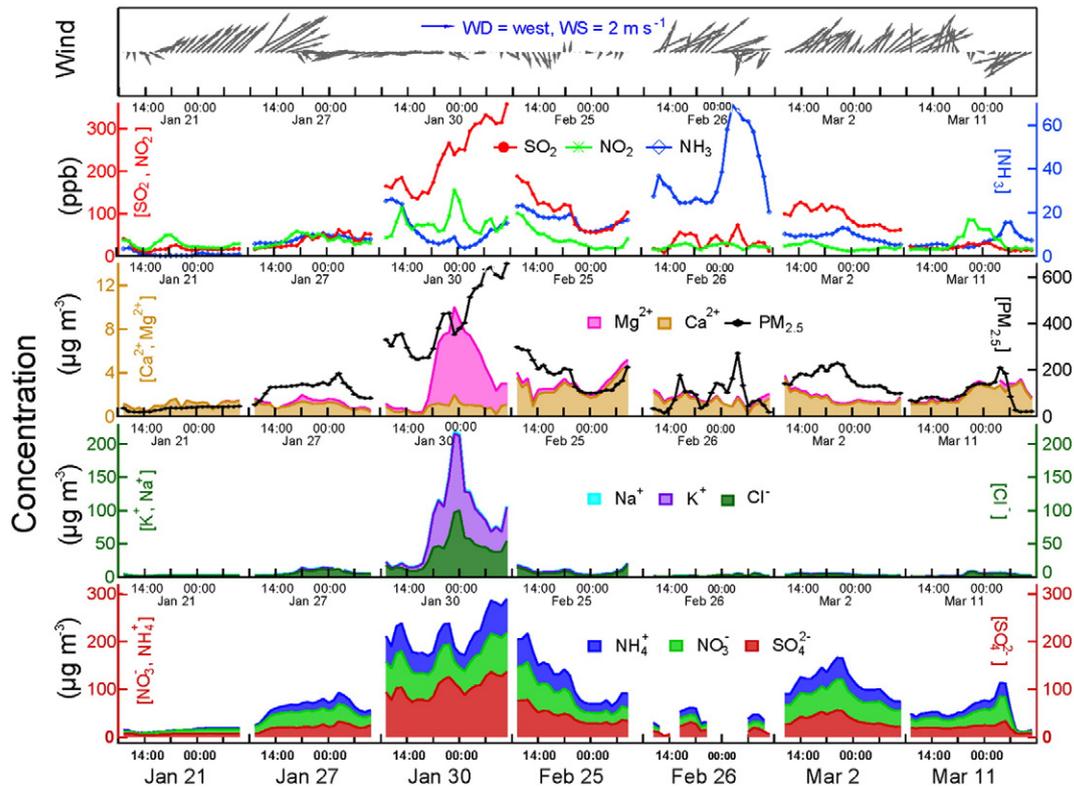


Fig. 1. Time series of the daily average ionic concentration and meteorological parameters during sampling days.

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 de-ionized 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 <200 bp and average quality score <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 >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 $\text{PM}_{2.5}$, water soluble inorganic ions, trace gases, wind speed and wind direction were determined during sampling period. The $\text{PM}_{2.5}$ daily average mass concentration ranged from 34.3 to 422.4 $\mu\text{g m}^{-3}$ 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 SO₄²⁻ > NO₃⁻ > NH₄⁺ > Cl⁻ > Ca²⁺ > K⁺ > Mg²⁺ > Na⁺, and SO₂ > NO₂ > NH₃, respectively (Fig. 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 SO₄²⁻, NH₄⁺ and NO₃⁻ 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, Ca²⁺ and Mg²⁺ 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 17,624–25,573 cells m⁻³, with an average of 21,509 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) (Fig. 2). After sequencing and homogenization, 27,094 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 minimum bacterial species which also confirmed by the lowest Chao1 index (Table 1). The coverage of >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}) (Bowers 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 >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%) (Fig. 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 (Fig. 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 (Fig. 5, ANOVA, P = 0.05, F = 2.3). Holophagaceae (Acidobacteria) were abundant in PM_{0.32–0.56}, displaying 6-fold times higher abundance than in PM_{0.18–0.32}. *Acetobacter* (Proteobacteria) showed a higher relative abundance in PM_{0.32–0.56}, which was 5-fold higher than that in PM_{0.18–0.32}. TA06, an uncultivated candidate phylum, was found only in PM_{0.18–0.32}. And some taxa could be found only in PM_{0.18–0.32} and PM_{0.56–1} 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 (Fig. 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

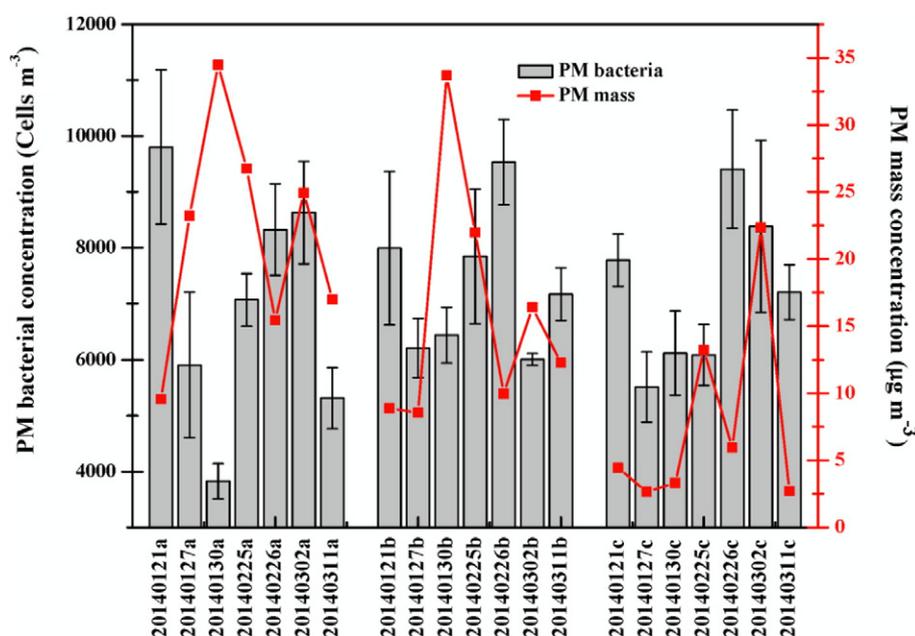


Fig. 2. Daily average bacterial concentration and PM mass concentration in PM_{0.56–1}, PM_{0.32–0.56}, and PM_{0.18–0.32}. (a-PM_{0.56–1}, b-PM_{0.32–0.56}, c-PM_{0.18–0.32}).

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}	27,094	236	302	289	0.99	2.48
PM _{0.32–0.56}	27,094	188	293	249	0.99	2.40
PM _{0.56–1}	27,094	222	292	272	0.99	2.44

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 (>5%). In addition, some of the genes detected in the airborne particulate matter were poorly characterized with unknown function.

4. 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, SO₄²⁻ has a distinct ability to influence the existence and growth of microbes and thus effect on their relative abundance (Scherer and Sahm, 1981). Ca²⁺ 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 × 10⁵ ribosomal operons per cubic meter, about 1.19 × 10⁵ cells m⁻³, and USA: 2.72 × 10⁴ 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}

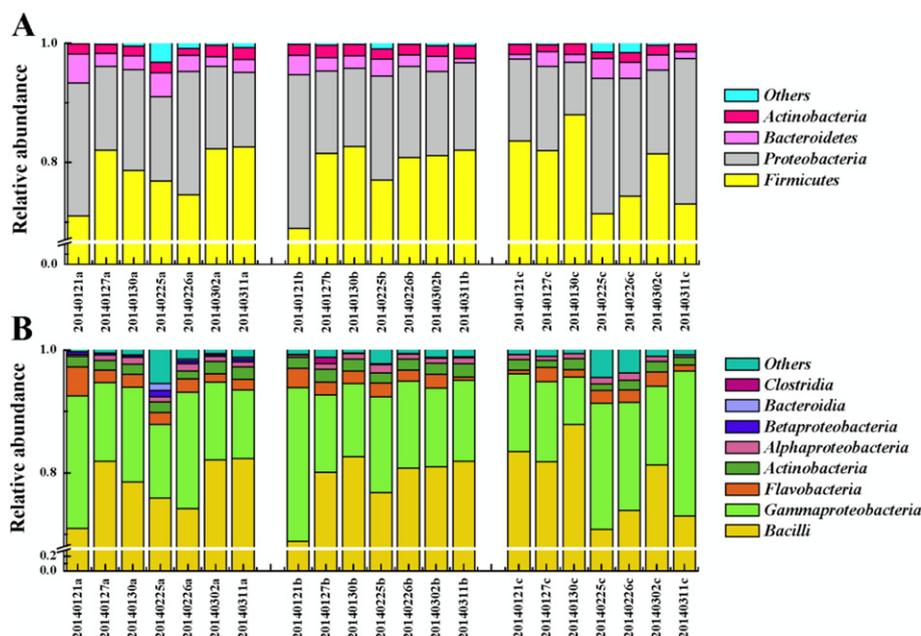


Fig. 3. (A–B) Relative abundance of bacteria at the phylum and class level in PM_{0.56–1}, PM_{0.32–0.56}, and PM_{0.18–0.32}. (a-PM_{0.56–1}, b-PM_{0.32–0.56}, c-PM_{0.18–0.32}).

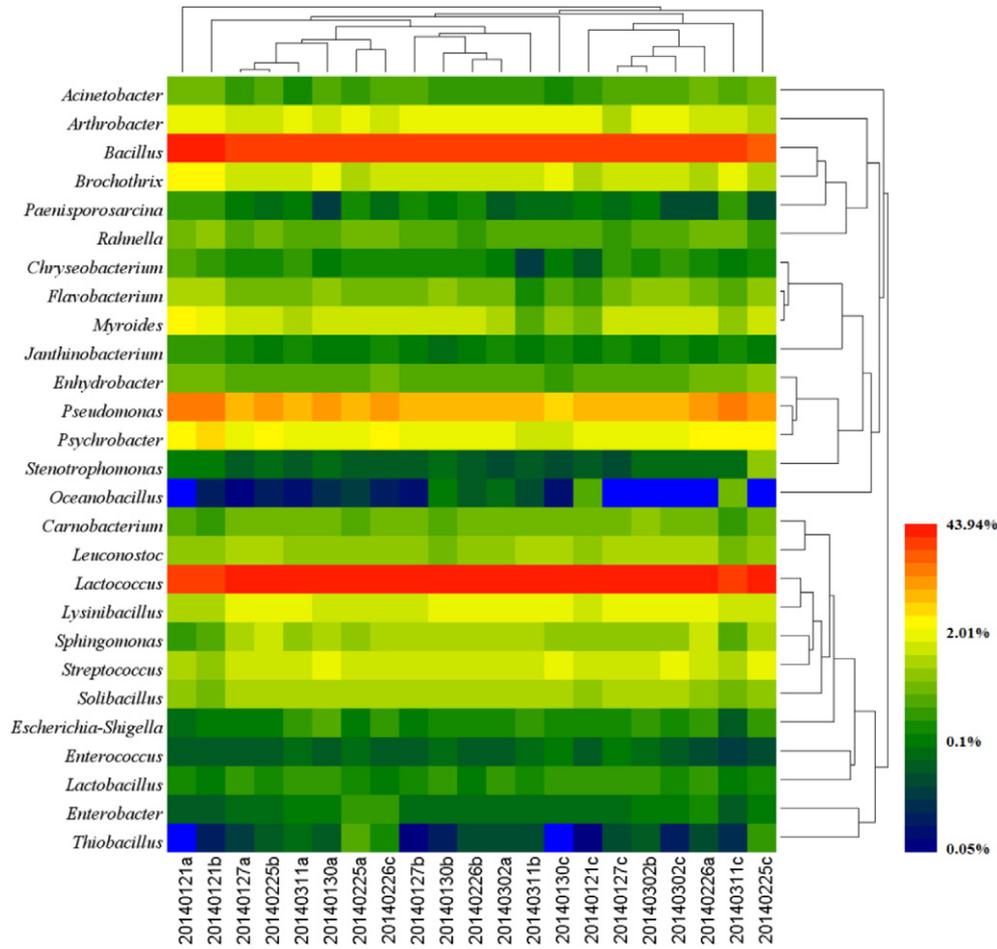


Fig. 4. Heatmap of the dominant genus (relative abundance higher than 0.05%) in PM_{0.56-1}, PM_{0.32-0.56} and PM_{0.18-0.32} at the genus level. (a-PM_{0.56-1}, b-PM_{0.32-0.56}, c-PM_{0.18-0.32}).

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 NO₂ and SO₂, and O₃ (Table 2). It is likely that SO₂, NO₂, and O₃ have a toxic effect on microorganisms (Abdel Hameed et al., 2012). High concentration of SO₂, NO₂, and O₃ 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}, PM₁₀, 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 PM₁₀ 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 PM₁₀ 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 (<1%, about 0.001%). This

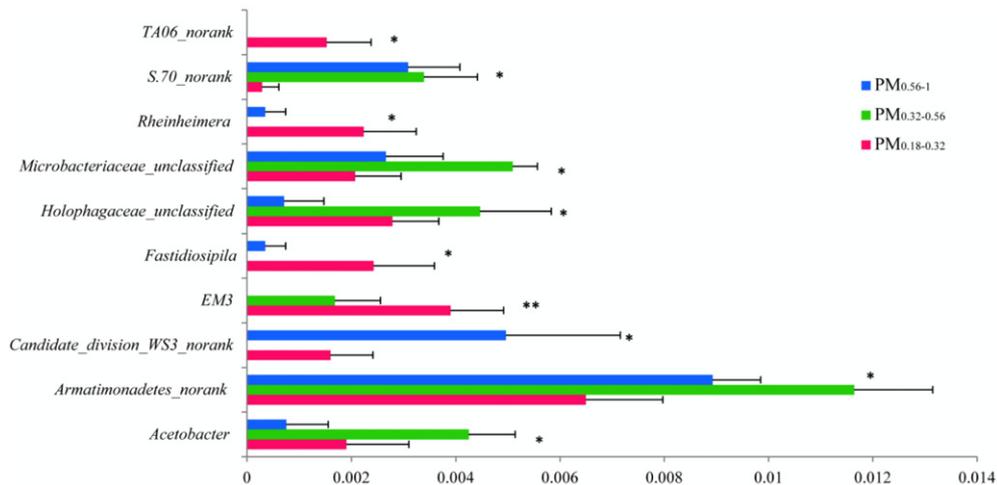


Fig. 5. Relative abundance of the taxa (at the phyla, family, or genus levels) that were found to show significant difference across aerosol size fractions: *P < 0.05, **P < 0.01.

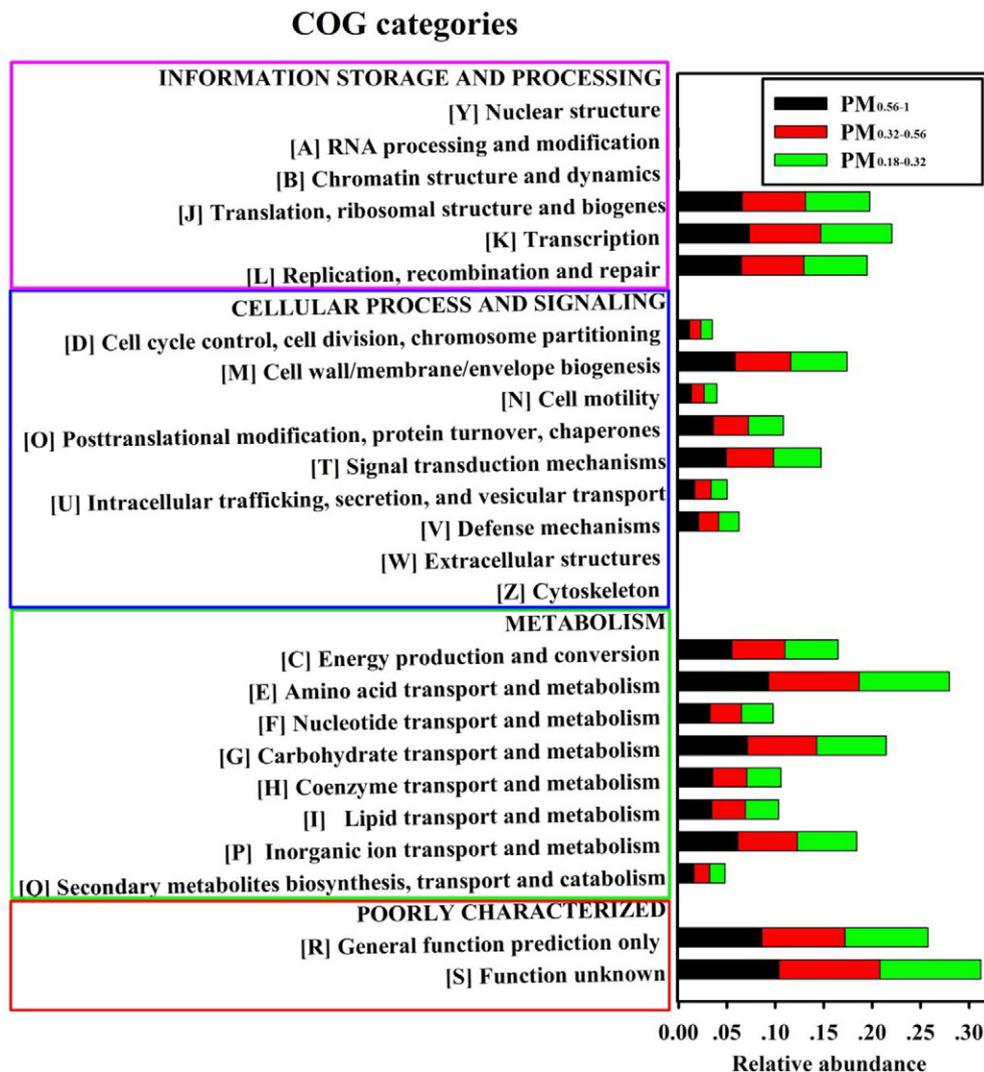


Fig. 6. Categorization of the microbial community genome contigs according to COGs functional categories.

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 B.Y. Wang et al. (2015) 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 (Fig. 4). The results were consistent with previous investigation by Wei et al. (2016), in that the dominant bacterial

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	Visibility	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

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). Bowers 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 and Morgan, 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, 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., 2011). 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 *Streptococcus thermophilus*, 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 secret, skin or hair, human activity, and ocean (Bowers et al., 2012). The microorganisms aerosolized into atmosphere and rapidly deposited rather than suspended due to the high settling velocities (Després et al., 2012). 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.

5. 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 NO₂, SO₂, and O₃ 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.

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