Zhang, Y., Guo, C., Ma, K., Tanga, A., Goulding, K. W. T. and Liu, X. 2022. Characteristics of airborne bacterial communities across different PM2.5 levels in Beijing during winter and spring. *Atmospheric Research.* 273 (1 Aug), p. 106179. https://doi.org/10.1016/j.atmosres.2022.106179

Characteristics of airborne bacterial communities across different $PM_{2.5}$ levels in Beijing during winter and spring

Yangyang Zhang[1], Chongjing Guo[1], Ke Ma[1], Aohan Tang[1], and Xuejun Liu[1]*

[1]College of Resources and Environmental Sciences; Beijing Key Laboratory of Farmland Soil Pollution Prevention and Remediation, China Agricultural University, 100193 Beijing, China

[*]*Corresponding authors* (liu310@cau.edu.cn)

Abstract

Airborne bacteria are important components of fine particulate matter ($PM_{2.5}$), and have received increasing attention because of their impacts on public health and ecological systems. However, the relationships between the bacterial community and $PM_{2.5}$ pollution are poorly understood. The properties of bacterial communities in Beijing at low, medium and high $PM_{2.5}$ levels were analyzed during winter (December 2015-January 2016; January 2017) and spring (March 2016-May 2016; April 2017-May 2017). Variations in bacterial concentrations, Shannon and Simpson indices and relative abundance were significantly related to the seasons. In winter, there were no significant differences in bacterial communities at the three $PM_{2.5}$ pollution levels. In contrast, significant correlations between bacterial abundance and $PM_{2.5}$ levels was observed in spring, and the bacterial concentrations, community richness and diversity indices were significantly higher on heavily polluted days compared to other pollution levels. Correlation results showed that relative humidity (RH), wind speed (WS), O_3, NO_2, and SO_2 were most closely associated with microbial community structure in winter, but temperature (T), NO_2 and SO_2 in spring. Chemical composition, especially that of secondary aerosol particles, which were mainly produced from anthropogenic sources (e.g. fossil fuel combustion, road traffic and industrial emissions), exerted the most control of bacterial community structure of $PM_{2.5}$. The effects of $PM_{2.5}$ levels on

the bacterial community were modified by environmental conditions, heavy metal and nutrient contents of the $PM_{2.5}$. The relationships described provide a foundation for further research into the environmental and $PM_{2.5}$ chemical composition controls on the bacterial community and related health risk from air pollution.

Keywords: Bioaerosol, bacteria, $PM_{2.5}$, air pollution, environmental factors

1. Introduction

With the acceleration of urbanization and industrialization, severe haze pollution has become an urgent challenge to China, causing extensive public concern with regard to sustainable development and human well-being (Li et al., 2017a; Wang et al., 2016a; Zheng et al., 2016). Airborne fine particulate matter ($PM_{2.5}$) plays an important role in the formation of haze (Guo et al., 2014).

$PM_{2.5}$ is a complex mixture, including secondary inorganic aerosols (SIA, i.e. sulfate, nitrate, and ammonium), organic compounds, metal ions, plant debris, and microorganisms. Research has provided a good understanding of the non-biological components of $PM_{2.5}$ and its chemical characteristics, and evaluated the effectiveness of measures to reduce particulate matter (Geng et al., 2019; Wang et al., 2019; Zhang et al., 2019). However, airborne microorganisms are key components of particulate matter (Jaenicke, 2005), affecting human health, air quality, ecosystem interactions, and contributing to the nucleation of particles (Delort et al., 2010; Fuzzi et al., 2015; Steiner et al., 2015; Walser et al., 2015). Air quality is closely related to microbial communities (Du et al., 2018; Gao et al., 2015).

Bacteria are ubiquitous in the atmosphere (Bauer et al., 2003; Yan et al., 2018), acting as pathogens that affect public health and ecological systems (Balloy and Chignard, 2009; Barberán et al., 2014; Prospero et al., 2005).

Pathogenic bacteria can induce several human diseases and trigger allergic reactions (Bowers et al., 2011; Liang et al., 2013). Some bacteria affect cloud development and atmospheric chemistry (Andreae and Rosenfeld, 2008; Burrows et al., 2009), impacting the photochemical and chemical reactions of aerosols (such as increasing the formation of atmospheric ammonium and sulfate) with increasing $PM_{2.5}$ concentrations (Liu et al., 2020).

Bacterial communities vary temporally and spatially, depending on many factors such as the weather, season, human activity and environmental conditions (Dong et al., 2016; Haas et al., 2013; Li et al., 2015; Li et al., 2013). Recent studies have indicated that the relative abundances of microbial allergens and pathogens increase with increasing concentrations of particulate matter (Alghamdi et al., 2014; Liu et al., 2018; Wei et al., 2016; Yan et al., 2018), together with a strong link between airborne bacterial structure and environmental factors (Lee et al., 2017; Xu et al., 2017). Meteorological conditions (relative humidity, wind speed, and temperature) and gaseous pollutants (NO_2, SO_2, O_3, and CO) were shown to be the main factors affecting airborne bacterial communities (Li et al., 2018; Pan et al., 2019; Yan et al., 2018). In summary, the community composition and structure of atmospheric bacteria is determined by the chemical components of particulate matter (e.g. organic substances, transition metals, nitrates, and sulfates) and the interactions between them

such as nutrient supply and heavy metal toxicity (Ariya et al., 2002; Wei et al., 2017; Xu et al., 2017) and, of course, pollution sources (Romano et al., 2019; Romano et al., 2020).

However, the detailed relationships between airborne microbes, air pollutants, and meteorological factors is still poorly understood. The interactions between the biological properties of particulate matter and air quality requires research. We hypothesized that the characteristics of the airborne bacterial community were strongly related to air pollution levels, especially in winter when air pollution is more serious than in spring. To test this hypothesis, airborne bacterial concentrations, richness, diversity and relative abundance were analyzed at a range of $PM_{2.5}$ levels during the winter and spring in Beijing. Redundancy analysis (RDA) of the bacterial community was used to analyze the relationship between it and environmental factors and to suggest measures to mitigate exposure to air pollution in China.

2. Materials and methods

2.1. Sample site and $PM_{2.5}$ collection

$PM_{2.5}$ samples were collected from the roof of an office building of China Agricultural University's (CAU) west campus (40.02° N, 116.28° E) at ~15 m above the ground, in the northwestern urban area of Beijing. The sample site is surrounded by residential areas and is approximately 1.4 km north of Beijing's fifth Ring Road, a major transport artery encircling

Beijing.

$PM_{2.5}$ samples were collected on quartz filters (1851-090, Whatman, UK) using a medium-volume air particulate matter sampler (model: 2034, flow rate: 100 L min^{-1}, Qingdao Laoshan Application, China) for approximately 24 hours per sample (8:00 a.m. to 7:50 a.m. on the next day). In total 100 $PM_{2.5}$ samples were collected, including 39 winter samples (December 2015-January 2016; January 2017) and 61 spring samples (March 2016-May 2016; April 2017- May 2017). Further sampling details are given in Table S1. Quartz filters were heated at 550 °C for 6 h to clean them prior to exposure. After collection, samples were immediately frozen at −80 °C until subsequent analysis. Blank filters were collected using the methods described above, but not exposed in the sampler.

2.2. Chemical components analysis

Water-Soluble Ions

One quarter of each filter was extracted with 10 mL deionized water (resistivity: 18.2 MΩ cm^{-1}) in a 50 mL centrifuge tube. After a 30-min dissolution in a microwave, the extract was filtered into a 10 mL centrifuge tube though a 0.22 μm filter. Water-soluble ions (Na^+, NH_4^+, K^+, Mg^{2+}, Ca^{2+}, Cl^-, NO_3^-, and SO_4^{2-}) were measured by ion chromatography (ICS-2100 and DX-600 ion chromatograph).

Elemental Analysis

One quarter of each filter was used to measure trace elements after acid

digestion using a microwave accelerated reaction system (MARS6, CEM, USA). 24 trace elements (Al, As, Ba, Ca, Cd, Co, Cr, Cu, Fe, Li, Mg, Mn, Na, Ni, P, Pb, S, Sb, Sc, Se, Sr, Ti, V, Zn) were analyzed by inductively coupled plasma atomic emission spectrometry (ICP-AES, Aurora M90, Bruker Dalton, USA).

2.3. DNA extraction and PCR amplification

One quarter of each filter was cut into pieces and genomic DNA extracted using a FastDNA SPIN Kit (MoBio Laboratories, Carlsbad, CA, USA), according to the manufacturer's protocol. Blank filters were analysed in the same way; no target fragments were observed.

Bacterial 16S rRNA genes in the $PM_{2.5}$ samples were amplified using bacterial universal PCR primers 515 F (5'-GTGTGCCAGCMGCCGCCGTAA-3') and 806 R (5'-GGACTACHVGGGTWTCTAAT-3') targeting the V4 hyper-variable region (Caporaso et al., 2012). PCR amplification was performed in a 25 μL reaction mixture containing 10×Pyrobest buffer (2.5 μL), dNTPs (2 μL, 0.2 mM), BSA (0.15 μL, 10 μg/μL), polymerase (0.15 μL, 5 U/μL), primer (2 μL, 10 μM), and template (2.5 μL) as follows: 94 °C for 5 min; 30 cycles of denaturation at 94 °C for 30 s, annealing at 50 °C for 30 s, extension at 72 °C for 60 s, with a final extension at 72 °C for 5 min. DNA quality was detected by NanoDrop 2000 (Thermo Fisher Scientific, USA). After purification and quantification control, PCR products were tested through

high-throughput sequencing by the Chengdu Institute of Biology using the Illumina MiSeq 2×250 platform.

2.4. Sequence analyses

The open-source software package Qiime was used to process and analyze 16S gene sequences (Caporaso et al., 2010). Subsequent processing was conducted in accordance with the analysis described by Fierer et al. (2008). Low quality fragments (sequences shorter than 200 bp) were removed using the Chimer Uchime command (Edgar et al., 2011). Sequences were clustered into operational taxonomic units (OTUs) by setting a distance measure of 0.03. A representative sequence was annotated with a BLAST analysis against the Silva database. Subsequent analysis of alpha diversity, including Chao 1, Shannon, and Simpson indices, was performed using Qiime with the normalized OTU data. Redundancy analysis (RDA) was conducted to check the differences and variations between bacterial communities across different $PM_{2.5}$ levels and seasons using Canoco 5.0 (Šmilauer and Lepš, 2014).

2.5. Air quality and meteorological conditions

During the sampling period, daily air quality data, i.e. $PM_{2.5}$, NO_2, SO_2, O_3 and CO, was obtained from the National Urban Air Quality Real-time Publishing Platform (http://106.37.208.233:20035/). Daily meteorological factors (relative humidity (RH), temperature (T), wind speed (WS)) were obtained from Weather Underground (http://www.wunderground.com).

3. Results and discussion

3.1 Airborne bacterial concentrations across different seasons and $PM_{2.5}$ pollution levels

Variation in the concentrations of airborne bacteria is shown in Fig. 1. The mean bacterial concentration in spring ($1.2\pm0.3\times10^5$ cells m^{-3}) was twice that in winter ($0.6\pm0.1\times10^5$ cells m^{-3}) ($P<0.001$) (Fig. S1). A similar result was observed in the urban region of Qingdao (Li et al., 2011). This is probably due to the smaller bacterial emissions from environmental sources such as plants, soil and water under the less favorable conditions in winter, especially the lower temperatures (Li et al., 2011; Maier et al., 2009), and the cold and dry climate in winter in Beijing, which inhibits bacterial growth. However, the reverse has been reported at times of very high particulate matter concentrations and frequent hazy days in winter, with bacteria adhering to the particles (Dong et al., 2016; Haas et al., 2013). We observed no significant differences in $PM_{2.5}$ concentrations between winter (94.3 ± 82.9 ug m^{-3}) and spring (67.3 ± 65.9 ug m^{-3}).

To further analyze the relationship between $PM_{2.5}$ and bacterial concentrations, bacterial aerosol samples were divided into three groups corresponding to different $PM_{2.5}$ levels (Fig. S2): non-polluted days ($PM_{2.5}<75$ μg m^{-3}), slightly to moderately polluted days ($75\leq PM_{2.5}<150$ μg m^{-3}), and heavily polluted days ($PM_{2.5}\geq150$ μg m^{-3}). In winter, the mean airborne microbial concentrations were $0.3\pm0.3\times10^5$ cells m^{-3}, $0.1\pm0.2\times10^5$

cells m^{-3}, and $0.1\pm0.1\times10^5$ cells m^{-3} for non-polluted, slightly to moderately polluted, and heavily polluted days, respectively, with no significant differences between them, suggesting a weak impact of air pollution on the concentrations of airborne microorganisms in winter. However, the mean bacterial concentration on heavily polluted days ($3.3\pm2.2\times10^5$ cells m^{-3}) was significantly higher than that on non-polluted and slightly to moderately polluted days in spring, in agreement with previous studies in Xi'an, Beijing and Qingdao (Dong et al., 2016; Li et al., 2017b; Wei et al., 2016). Bacterial concentrations were correlated with the $PM_{2.5}$-based air quality index (AQI) and increased when the air quality level changed from 'Good' to 'Medium' and then stabilized as air quality declined further (Xie et al., 2018). Particulate matter has been widely regarded as a carrier and supplier of the nutrition for bacterial growth (Wei et al., 2015). For each $PM_{2.5}$ level, lower airborne bacterial concentrations were observed in winter, showing that bacterial concentrations respond to $PM_{2.5}$ levels, modified by seasonal variation, especially meteorological conditions.

3.2. Airborne bacteria diversity across seasons and $PM_{2.5}$ pollution levels

Seasonal variations in airborne bacterial diversity are presented in Fig. 2a. Community richness index, Chao 1, showed no significant difference between spring and winter. Shannon and Simpson indices were significantly higher in winter (Shannon=10.6±0.3, Simpson=1.0±0.1) than

those in spring (Shannon=8.1±1.1, Simpson=1.0±0.1), suggesting winter was more conductive to the higher bacterial diversity than spring. Possibly very rapid growth of dominant bacterial communities in the warmer spring season could inhibit the growth of other bacterial communities, while more species survived in the colder winter season due to limited inhibition or competition among them (Liu et al., 2018). In addition, meteorological factors, especially wind speed and relative humidity, contributed through the release and diffusion of airborne bacteria (Kembel et al., 2012). The dry air and strong winds in winter are favorable to the spread of airborne bacteria.

In winter, Chao1, Shannon, and Simpson indexes were not significantly different at the three $PM_{2.5}$ levels (Fig. 2b), as observed in Beijing in the 2014 winter (Yan et al., 2018). However, significant differences were observed in spring (P<0.05), where Chao1 (14255.1±2686.9), Shannon (9.8±0.4), and Simpson (1.0±0.1) indices on heavy polluted days ($PM_{2.5}$ ≥150) were all significantly higher than at other $PM_{2.5}$ levels. These different responses of bacterial diversity to $PM_{2.5}$ levels between winter and spring could be caused by meteorological factors. Wind speed was found to play a vital role in bacterial diversity within one season (Du et al., 2018), being negatively correlated with $PM_{2.5}$ pollution. In spring, wind speed was always relatively high when $PM_{2.5}$ concentrations increased (Table. S2), which would increase the transport of airborne

microorganisms from sources. Compared to spring, slower wind speeds as air pollution increased in winter would not have increased the release and diffusion of airborne bacteria but reduced the dispersion of air pollutants.

3.3. Airborne bacterial communities

A total of 42 bacterial phyla were identified in $PM_{2.5}$ samples. Of these, 14 phyla with relatively high abundances (generally > 1%) were selected as the major bacterial phyla for further analysis. The remainder had an abundance <1% and were grouped as 'Others'. Although the categories and structure of the bacterial community were roughly the same throughout the sampling period, the relative abundance of each category varied. Primary phyla were Proteobacteria, Cyanobacteria, Actinobacteria, Firmicutes and Bacteroidetes, together accounting for over 90% of the total phyla (Fig. 3). Comparing bacterial community structures in different regions of China at the phylum level, Proteobacteria, Actinobacteria and Firmicutes were consistently dominant (Gou et al., 2016; Du et al., 2018; Liu et al., 2018). Proteobacteria (24.3% ± 11%) was the primary phyla in our analyses, and was also identified the most abundant phyla in Qingdao (78.9%) and Xiamen (44.7%) (Liao et al., 2013; Wang et al., 2015), while Actinobacteria were most abundant in Beijing (62.3%) (Wang et al., 2015), and Firmicutes the most abundant in Ji'nan (74.1%) (Xu et al., 2017). The order of dominant bacteria differed in winter and spring (Fig. S3a). Proteobacteria was the most abundant phylum in winter (35.5% ± 1.9%),

followed by Actinobacteria (28.9% ± 1.7%), Firmicutes (13.5% ± 3.9%), Bacteroidetes (7.1% ± 0.9%), and Cyanobacteria (3.1% ± 0.6%). The top five dominant phyla in spring were Cyanobacteria (32.3% ± 13.5%), Actinobacteria (21.3% ± 7.8%), Proteobacteria (19.8% ± 7.2%), Firmicutes (13.8% ± 6.5%), and Bacteroidetes (5.6% ± 1.1%). The abundances of Actinobacteria, Proteobacteria, Firmicutes, and Bacteroidetes were generally higher in winter than spring, but Cyanobacteria abundance was higher in spring.

At the genus level, nearly 25 genera (relative abundance □ 0.4%) were detected in the $PM_{2.5}$ samples. The heatmap of community structure is presented in Fig. 4. Arthrobacter was the most abundant genus (1.9% ± 0.8%), followed by Rubellimicrobium (1.5% ± 0.6%), Psychrobacter (1.2% ± 0.8%), Skermanella (1.1% ± 0.4%), and Hymenobacter (0.9% ± 0.5%). Dominant genera vary across China: Sphingomonas was reported to be dominant in Urumqi (Gou et al., 2016), but Lactococcus, Acinetobacter, and Pseudomonas were the most abundant genera in Jinan, Qingdao and Xi'an, respectively (Wang et al., 2015; Wang et al., 2016b; Xu et al., 2017), suggesting that geographical factors play an important role in determining atmospheric microbial communities at the genus level. Overall genera abundances were clearly correlated with seasons. The abundances of most genera (e.g. Methylobacterium, Hymenobacter, Rubellimicrobium, Sphingomonas, Arthrobacter, Skermanella, and Psychrobacter) were

higher in winter than spring. In contrast, Roseomonas abundance was higher in spring than in winter, while the abundances of Streptococcus, Clostridium, Lactobacillus, Pontibacter, Balneimonas, Actinotalea, and Janthinobacterium were approximately the same in winter and spring (Fig. S3b).

There were no significant differences in the relative abundances of five dominant bacteria across the three $PM_{2.5}$ levels in winter at both phylum and genus levels (Fig. 5), in agreement with some previous studies (Du et al., 2018; Wei et al., 2016). However, some bacteria were positively correlated with $PM_{2.5}$ pollution (Gemmatimonadetes and Nitrospirae) and some negatively (Planctomycetes and TM7) (Fig. S4). Compared to winter, the dominant bacterial community structures varied significantly with increasing $PM_{2.5}$ levels in spring. At the phylum level, the predominant phyla (Proteobacteria, Actinobacteria, and Bacteroidetes) abundances significantly increased with increasing $PM_{2.5}$ levels, whereas the relative abundance of Cyanobacteria was lowest in heavy polluted days. Proteobacteria are widely found in soil, water and air, and also have been found in extreme environments (e.g. at low temperatures, in droughts and in irradiated areas) (Garrity, 2006). Bacteroidetes commonly inhabit aquatic environments and are especially adapted to harsh environments, gradually increasing with pollution levels (Soares et al., 2012). Cyanobacteria have been found to be extensively present on clean or

slightly polluted days, especially during dust storms in spring (Lu et al., 2018; Park et al., 2018; Wei et al., 2020). Accordingly, at the genus level, Hymenobacter from Bacteroidetes, Rubellimicrobium and Skermanella from Proteobacteria, and Arthrobacter from Actinobacteria were most abundant on heavily polluted days. In particular, no phylum or genus significantly varied with increasing $PM_{2.5}$ levels in both winter and spring, suggesting a complex effect of multiple factors on bacterial communities, including sources, meteorological conditions and emission mechanisms of aerosols.

3.4. The effects of environmental factors on the bacterial community

RDA analysis was conducted to investigate the relationship between airborne bacterial communities and main predictor variables. The results of RDA analysis on bacterial communities based on phyla abundances, environmental factors (i.e. meteorology, air pollutants, water-soluble nutrients and heavy metals in $PM_{2.5}$) in winter and spring are shown in Fig. 6. In winter, RH, WS, O_3, NO_2, and SO_2 were most closely associated with the microbial community structure (Fig. 6a). RH was strongly positively correlated with some phyla such as Nitrospirae, Verrucomicrobia, Gemmatimonadetes, and [Thermi]. Bacteroidetes, Actinobacteria, TM7, and Crenarchaeota were mostly positively correlated with WS and O_3. Similarly, the bacterial community compositions of the least polluted samples positively correlated with WS. Firmicutes, Actinobacteria, and

Bacteroidetes within the most abundant phylum had different degrees of positive correlation with NO_2 and SO_2. Fig. 6d shows that T, NO_2, and SO_2 were the most important drivers of bacterial communities in spring. Cyanobacteria and [Thermi] were positively correlated with T; Bacteroidetes and Nitrospirae were positively correlated with SO_2; Firmicutes and Bacteroidetes were positively correlated with NO_2. The results were similar to other studies (Lu et al., 2018; Sun et al., 2014), where T, NO_2, O_3, and SO_2 were found to be the important drivers in bacterial community structures. In particular, as a key factor, increased T would facilitate bacterial growth and dispersal through the accelerating movement of convective air (Sun et al., 2014).

To investigate how the chemical composition of particulate matter was correlated with the community structure of bacteria, we further analyzed the correlation between the main bacterial phyla and water-soluble ions (Fig. 6b and 6e), as well as with metal elements (Fig. 6c and 6f). Secondary inorganic ions (NO_3^-, NH_4^+, and SO_4^{2-}) were mainly positively correlated with Firmicutes, but had negative correlations with most phyla (such as Bacteroidetes, Actinobacteria, Proteobacteria) in winter. Zhong et al., (2019) also found that water-soluble components, especially secondary inorganic ions, played an important role in the variability of bacterial community structures in $PM_{2.5}$ during winter haze episodes. In spring, NO_3^- and NH_4^+ were also important factors (Fig. 6e), being positively

correlated with Firmicutes, Bacteroidetes, and Cyanobacteria. Additionally, secondary inorganic ions were strongly correlated with polluted samples in winter, while the overall correlations between secondary inorganic ions and polluted samples or the least polluted samples were found to be irregular in spring. Ca^{2+} is mainly associated with soil dust emissions and was positively correlated with most phyla (such as Actinobacteria, Proteobacteria, TM7, Gemmatimonadetes) in winter, but mainly negatively correlated with Firmicutes and Cyanobacteria. Proteobacteria were also found to be correlated with Ca^{2+} in winter in Italy (Innocente et al., 2017; Romano et al., 2020). However, Ca^{2+} showed different correlations with bacterial community structures in spring, exhibiting a positive correlation with Cyanobacteria but negative correlations with other abundant phyla (such as Firmicutes, Bacteroidetes, Actinobacteria). Cl^- is usually derived from sea salt, and was mainly positively associated with Firmicutes in winter, but negatively correlated with most phyla. In spring, Cl^- was mainly positively correlated with Bacteroidetes and Firmicutes.

As shown in Fig. 6c, Cd, Zn, Se, Ti, and Mo were strongly correlated with bacterial phyla in winter, with an especially positive correlation with Firmicutes. Cd, Zn, and Se mainly arise from anthropogenic sources, such as fossil fuel combustion, road traffic and industrial emissions; Ti and Mo are commonly regarded as crustal elements, such as soil and construction

materials (Gao et al., 2015; Zhang et al., 2017), which suggested that Firmicutes were likely associated with aerosols from anthropogenic pollution/soil dust sources. In spring, Cu (from an anthropogenic source) and Bi (from natural sources such as soil and construction materials) had a significant impact on bacterial community structure, especially for Firmicutes, Cyanobacteria, and Bacteroidetes (Fig. 6f). The relative abundance of Cyanobacteria was 10.6 times higher in spring than in winter (Fig. S2), and was also strongly related to NO_3^- and NH_4^+, indicating that Cyanobacteria were likely associated with mixed soil dust and/or anthropogenic species in spring.

Although the interactions between chemical species and bacterial phyla were different in winter and spring, overall anthropogenic sources (fossil fuel combustion, traffic and industrial emissions) and soil/dust look to be key contributors to bacterial community structure.

4. Conclusions

We analyzed the properties of bacterial communities in $PM_{2.5}$ samples collected in winter and spring in Beijing and identified their main potential drivers. The diversity and relative abundance of airborne bacterial concentrations at the phyla and genera levels differed between winter and spring in response to $PM_{2.5}$ pollution levels. Noticeably, there was no significant association of bacterial community structures with $PM_{2.5}$ concentrations in winter, while bacterial concentrations, community

richness and diversity indices, and predominant phyla (e.g. Proteobacteria, Actinobacteria, and Bacteroidetes) abundances were all significantly higher on heavily polluted days compared to relatively lightly polluted days in spring.

Environmental factors, especially RH, WS, O_3, NO_2, and SO_2, were most closely associated with the microbial community structures in winter, while T, NO_2, and SO_2 had the strongest influence in spring. Chemical species from anthropogenic sources (fossil fuel combustion, road traffic and industrial emissions) and soil/dust also contributed. Clearly the complex and mixed effects of meteorological conditions and aerosol pollution on bacterial community structures, and the underlying mechanisms of interactions between them, should be the focus of future research.

ACKNOWLEDGMENTS

This work was supported by the National Key Research and Development Project of China (2018YFC0213301), the National Research Program for Key Issues in Air Pollution Control (DQGG0208), and China Postdoctoral Science Foundation (2019M660863).

REFERENCES

Alghamdi MA, Shamy M, Redal MA, Khoder M, Awad AH, Elserougy S.

Microorganisms associated particulate matter: a preliminary study. Science of the Total Environment 2014; 479: 109-116.

Andreae M, Rosenfeld D. Aerosol–cloud–precipitation interactions. Part 1. The nature and sources of cloud-active aerosols. Earth-Science Reviews 2008; 89: 13-41.

Ariya PA, Nepotchatykh O, Ignatova O, Amyot M. Microbiological degradation of atmospheric organic compounds. Geophysical Research Letters 2002; 29: 34-1-34-4.

Balloy V, Chignard M. The innate immune response to Aspergillus fumigatus. Microbes and Infection 2009; 11: 919-927.

Barberán A, Henley J, Fierer N, Casamayor EO. Structure, inter-annual recurrence, and global-scale connectivity of airborne microbial communities. Science of the Total Environment 2014; 487: 187-195.

Bauer H, Giebl H, Hitzenberger R, Kasper □ Giebl A, Reischl G, Zibuschka F, et al. Airborne bacteria as cloud condensation nuclei. Journal of Geophysical Research: Atmospheres 2003; 108.

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

Burrows SM, Elbert W, Lawrence M, Pöschl U. Bacteria in the global atmosphere–Part 1: Review and synthesis of literature data for

different ecosystems. Atmospheric Chemistry and Physics 2009; 9: 9263-9280.

Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD, Costello EK, et al. QIIME allows analysis of high-throughput community sequencing data. Nature methods 2010; 7: 335-336.

Delort A-M, Vaïtilingom M, Amato P, Sancelme M, Parazols M, Mailhot G, et al. A short overview of the microbial population in clouds: Potential roles in atmospheric chemistry and nucleation processes. Atmospheric Research 2010; 98: 249-260.

Dong L, Qi J, Shao C, Zhong X, Gao D, Cao W, et al. Concentration and size distribution of total airborne microbes in hazy and foggy weather. Science of the Total Environment 2016; 541: 1011-1018.

Du P, Du R, Ren W, Lu Z, Fu P. Seasonal variation characteristic of inhalable microbial communities in $PM_{2.5}$ in Beijing city, China. Science of the Total Environment 2018; 610: 308-315.

Fierer N, Liu Z, Rodríguez-Hernández M, Knight R, Henn M, Hernandez MT. Short-term temporal variability in airborne bacterial and fungal populations. Applied and Environmental Microbiology 2008; 74: 200-207.

Fuzzi S, Baltensperger U, Carslaw K, Decesari S, Denier van der Gon H, Facchini MC, et al. Particulate matter, air quality and climate: lessons learned and future needs. Atmospheric Chemistry and

Physics 2015; 15: 8217-8299.

Gao M, Qiu T, Jia R, Han M, Song Y, Wang X. Concentration and size distribution of viable bioaerosols during non-haze and haze days in Beijing. Environmental Science and Pollution Research 2015; 22: 4359-4368.

Garrity G. Bergey's Manual® of Systematic Bacteriology: Volume Two: The Proteobacteria, Part A Introductory Essays (Vol. 2). Springer Science & Business Media, 2006.

Geng G, Xiao Q, Zheng Y, Tong D, Zhang Y, Zhang X, et al. Impact of China's air pollution prevention and control action plan on $PM_{2.5}$ chemical composition over eastern China. Science China Earth Sciences 2019; 62: 1872-1884.

Gou H, Lu J, Li S, Tong Y, Xie C, Zheng X. Assessment of microbial communities in PM_1 and PM_{10} of Urumqi during winter. Environmental Pollution 2016; 214: 202-210.

Guo S, Hu M, Zamora ML, Peng J, Shang D, Zheng J, et al. Elucidating severe urban haze formation in China. Proceedings of the National Academy of Sciences 2014; 111: 17373-17378.

Haas D, Galler H, Luxner J, Zarfel G, Buzina W, Friedl H, et al. The concentrations of culturable microorganisms in relation to particulate matter in urban air. Atmospheric Environment 2013; 65: 215-222.

Innocente E, Squizzato S, Visin F, Facca C, Rampazzo G, Bertolini V, et al. Influence of seasonality, air mass origin and particulate matter chemical composition on airborne bacterial community structure in the Po Valley, Italy. Science of the Total Environment 2017; 593: 677-687.

Jaenicke R. Abundance of cellular material and proteins in the atmosphere. Science 2005; 308: 73-73.

Kembel SW, Jones E, Kline J, Northcutt D, Stenson J, Womack AM, et al. Architectural design influences the diversity and structure of the built environment microbiome. The ISME journal 2012; 6: 1469-1479.

Lee JY, Park EH, Lee S, Ko G, Honda Y, Hashizume M, et al. Airborne bacterial communities in three east asian cities of China, South Korea, and Japan. Scientific Reports 2017; 7: 1-8.

Li M, Qi J, Zhang H, Huang S, Li L, Gao D. Concentration and size distribution of bioaerosols in an outdoor environment in the Qingdao coastal region. Science of the Total Environment 2011; 409: 3812-3819.

Li W, Yang J, Zhang D, Li B, Wang E, Yuan H. Concentration and community of airborne bacteria in response to cyclical haze events during the fall and midwinter in Beijing, China. Frontiers in Microbiology 2018; 9: 1741.

Li X, Zhang Q, Zhang Y, Zhang L, Wang Y, Zhang Q, et al. Attribution of $PM_{2.5}$ exposure in Beijing–Tianjin–Hebei region to emissions: implication to control strategies. Science Bulletin 2017a; 62: 957-964.

Li Y, Lu R, Li W, Xie Z, Song Y. Concentrations and size distributions of viable bioaerosols under various weather conditions in a typical semi-arid city of Northwest China. Journal of Aerosol Science 2017b; 106: 83-92.

Li Y, Wang W, Guo X, Wang T, Fu H, Zhao Y, et al. Assessment of airborne bacteria and fungi in various university indoor environments: a case study in Chang'an University, China. Environmental Engineering Science 2015; 32: 273-283.

Li Y, Yang L, Meng Q, Qiu X, Feng Y. Emission characteristics of microbial aerosols in a municipal sewage treatment plant in Xi’an, China. Aerosol and Air Quality Research 2013; 13: 343-349.

Liang R, Xiao P, She R, Han S, Chang L, Zheng L. Culturable airborne bacteria in outdoor poultry-slaughtering facility. Microbes and environments 2013: ME12178.

Liao X, Anyi H, Yang X, Chen J, Changping Y, Lin J. Community composition and environmental sources of bacteria and eukaryotic microorganisms in fine air particulate ($PM_{2.5}$) of Xiamen in winter. Ecol. Environ. Sci 2013; 22: 1395-1400.

Liu H, Hu Z, Zhou M, Zhang H, Li Z, Zhang H, et al. Airborne microorganisms exacerbate the formation of atmospheric ammonium and sulfate. Environmental Pollution 2020; 263: 114293.

Liu H, Zhang X, Zhang H, Yao X, Zhou M, Wang J, et al. Effect of air pollution on the total bacteria and pathogenic bacteria in different sizes of particulate matter. Environmental Pollution 2018; 233: 483-493.

Lu R, Li Y, Li W, Xie Z, Fan C, Liu P, et al. Bacterial community structure in atmospheric particulate matters of different sizes during the haze days in Xi'an, China. Science of the Total Environment 2018; 637: 244-252.

Maier RM, Pepper IL, Gerba CP. Environmental Microbiology. Vol 397: Academic Press, 2009.

Pan Y, Pan X, Xiao H, Xiao H. Structural characteristics and functional implications of $PM_{2.5}$ bacterial communities during fall in Beijing and Shanghai, China. Frontiers in Microbiology 2019; 10: 2369.

Park J, Li P-F, Ichijo T, Nasu M, Yamaguchi N. Effects of Asian dust events on atmospheric bacterial communities at different distances downwind of the source region. Journal of Environmental Sciences 2018; 72: 133-139.

Prospero JM, Blades E, Mathison G, Naidu R. Interhemispheric transport of viable fungi and bacteria from Africa to the Caribbean with soil

dust. Aerobiologia 2005; 21: 1-19.

Romano S, Di Salvo M, Rispoli G, Alifano P, Perrone MR, Tala A. Airborne bacteria in the Central Mediterranean: Structure and role of meteorology and air mass transport. Science of The Total Environment 2019; 697: 134020.

Romano S, Perrone M, Becagli S, Pietrogrande M, Russo M, Caricato R, et al. Ecotoxicity, genotoxicity, and oxidative potential tests of atmospheric PM_{10} particles. Atmospheric Environment 2020; 221: 117085.

Šmilauer P, Lepš J. Multivariate analysis of ecological data using CANOCO 5: Cambridge University Press, 2014.

Soares FL, Melo IS, Dias ACF, Andreote FD. Cellulolytic bacteria from soils in harsh environments. World Journal of Microbiology and Biotechnology 2012; 28: 2195-2203.

Steiner AL, Brooks SD, Deng C, Thornton DC, Pendleton MW, Bryant V. Pollen as atmospheric cloud condensation nuclei. Geophysical Research Letters 2015; 42: 3596-3602.

Sun Z, Li G, Wang C, Jing Y, Zhu Y, Zhang S, et al. Community dynamics of prokaryotic and eukaryotic microbes in an estuary reservoir. Scientific Reports 2014; 4: 1-8.

Walser SM, Gerstner DG, Brenner B, Bünger J, Eikmann T, Janssen B, et al. Evaluation of exposure–response relationships for health effects

of microbial bioaerosols–a systematic review. International Journal of Hygiene and Environmental Health 2015; 218: 577-589.

Wang B, Lang J, Zhang L, Fang J, Cao C, Hao J, et al. 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 2015; 36: 2727-2734.

Wang G, Zhang R, Gomez ME, Yang L, Zamora ML, Hu M, et al. Persistent sulfate formation from London Fog to Chinese haze. Proceedings of the National Academy of Sciences 2016a; 113: 13630-13635.

Wang W, Fu H-l, Wang T-l, Song Y, Fu J, Xiang M, et al. Characteristics of microbial aerosols on haze days in autumn in Xi'an, China. Acta Scientiae Circumstantiae 2016b; 36: 279-288.

Wang Y, Li W, Gao W, Liu Z, Tian S, Shen R, et al. Trends in particulate matter and its chemical compositions in China from 2013–2017. Science China Earth Sciences 2019; 62: 1857-1871.

Wei K, Zheng Y, Li J, Shen F, Zou Z, Fan H, et al. Microbial aerosol characteristics in highly polluted and near-pristine environments featuring different climatic conditions. Science Bulletin 2015; 60: 1439-1447.

Wei K, Zou Z, Zheng Y, Li J, Shen F, Wu C-y, et al. Ambient bioaerosol particle dynamics observed during haze and sunny days in Beijing.

Science of the Total Environment 2016; 550: 751-759.

Wei M, Liu H, Chen J, Xu C, Li J, Xu P, et al. Effects of aerosol pollution on $PM_{2.5}$-associated bacteria in typical inland and coastal cities of northern China during the winter heating season. Environmental Pollution 2020; 262: 114188.

Wei M, Xu C, Chen J, Zhu C, Li J, Lv G. Characteristics of bacterial community in cloud water at Mt Tai: similarity and disparity under polluted and non-polluted cloud episodes. Atmospheric Chemistry and Physics 2017; 17: 5253-5270.

Xie Z, Li Y, Lu R, Li W, Fan C, Liu P, et al. Characteristics of total airborne microbes at various air quality levels. Journal of Aerosol Science 2018; 116: 57-65.

Xu C, Wei M, Chen J, Wang X, Zhu C, Li J, et al. Bacterial characterization in ambient submicron particles during severe haze episodes at Ji'nan, China. Science of the Total Environment 2017; 580: 188-196.

Yan D, Zhang T, Su J, Zhao L-L, Wang H, Fang X-M, et al. Structural variation in the bacterial community associated with airborne particulate matter in Beijing, China, during hazy and nonhazy days. Applied and Environmental Microbiology 2018; 84: e00004-18.

Zhang X, Xu X, Ding Y, Liu Y, Zhang H, Wang Y, et al. The impact of meteorological changes from 2013 to 2017 on $PM_{2.5}$ mass reduction in key regions in China. Science China Earth Sciences 2019; 62:

1885-1902.

Zhang Y, Wei J, Tang A, Zheng A, Shao Z, Liu X. Chemical characteristics of $PM_{2.5}$ during 2015 spring festival in Beijing, China. Aerosol and Air Quality Research 2017; 17: 1169-1180.

Zheng G, Duan F, Ma Y, Zhang Q, Huang T, Kimoto T, et al. Episode-based evolution pattern analysis of haze pollution: method development and results from Beijing, China. Environmental Science & Technology 2016; 50: 4632-4641.

Zhong S, Zhang L, Jiang X, Gao P. Comparison of chemical composition and airborne bacterial community structure in $PM_{2.5}$ during haze and non-haze days in the winter in Guilin, China. Science of the Total Environment 2019; 655: 202-210.

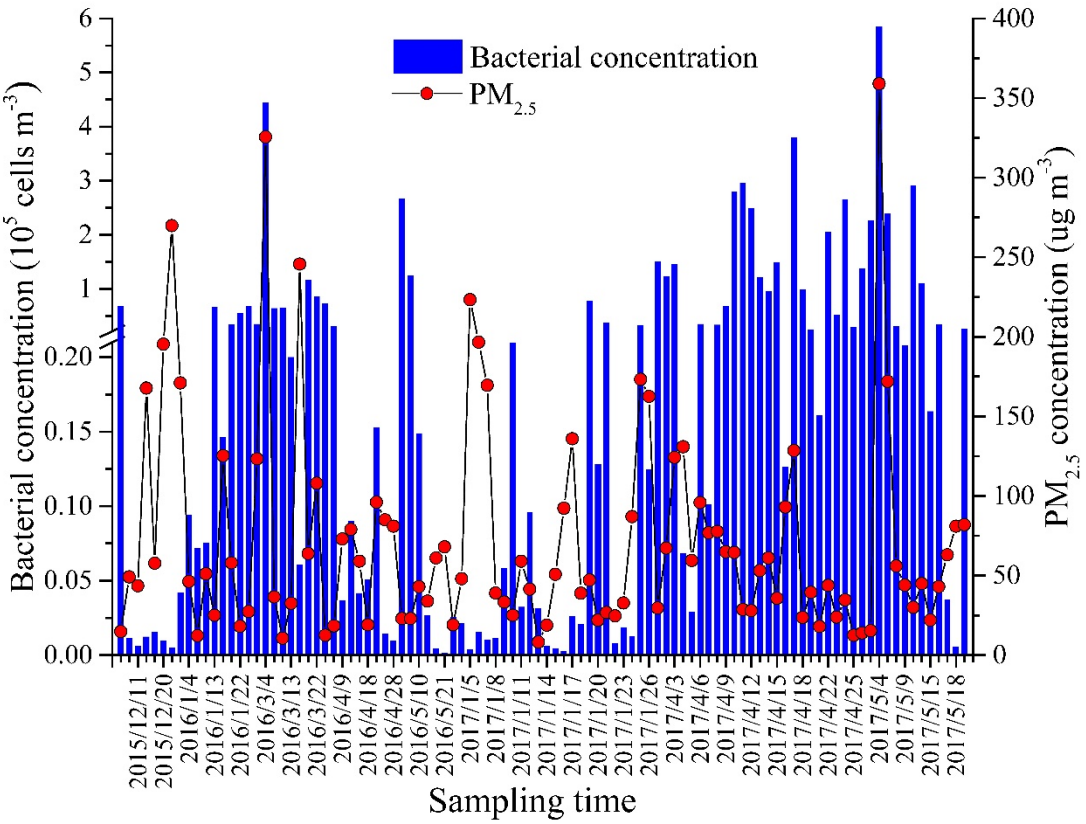

Figure. 1. Concentrations of total airborne microbes in $PM_{2.5}$ samples.

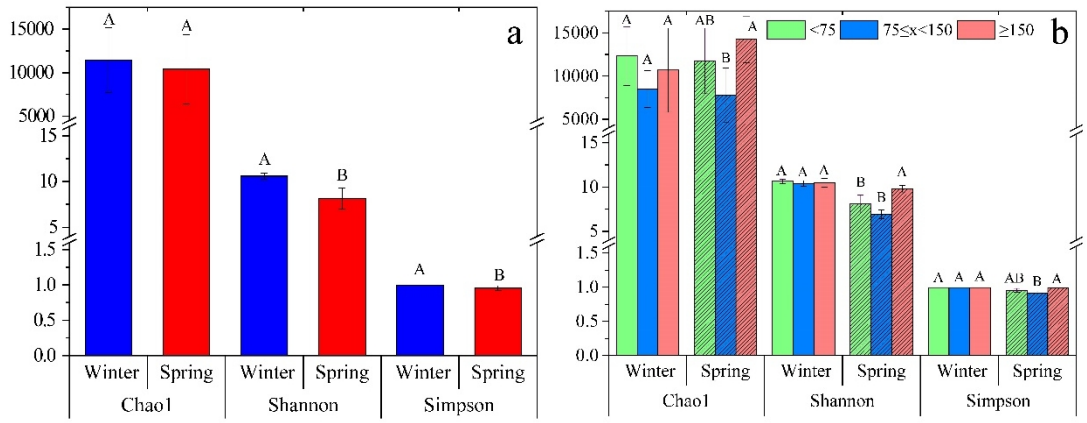

Figure. 2. Chao1 and alpha diversity (Shannon and Simpson) indices of bacteria in $PM_{2.5}$ samples during winter and spring (a), and in three levels of $PM_{2.5}$ concentrations (b).

*Error bars denote standard deviation. Uppercase letters denote statistical differences in Chao1 and alpha diversity indices of bacteria between winter and spring or between the three levels of $PM_{2.5}$ concentrations ($P<0.05$).

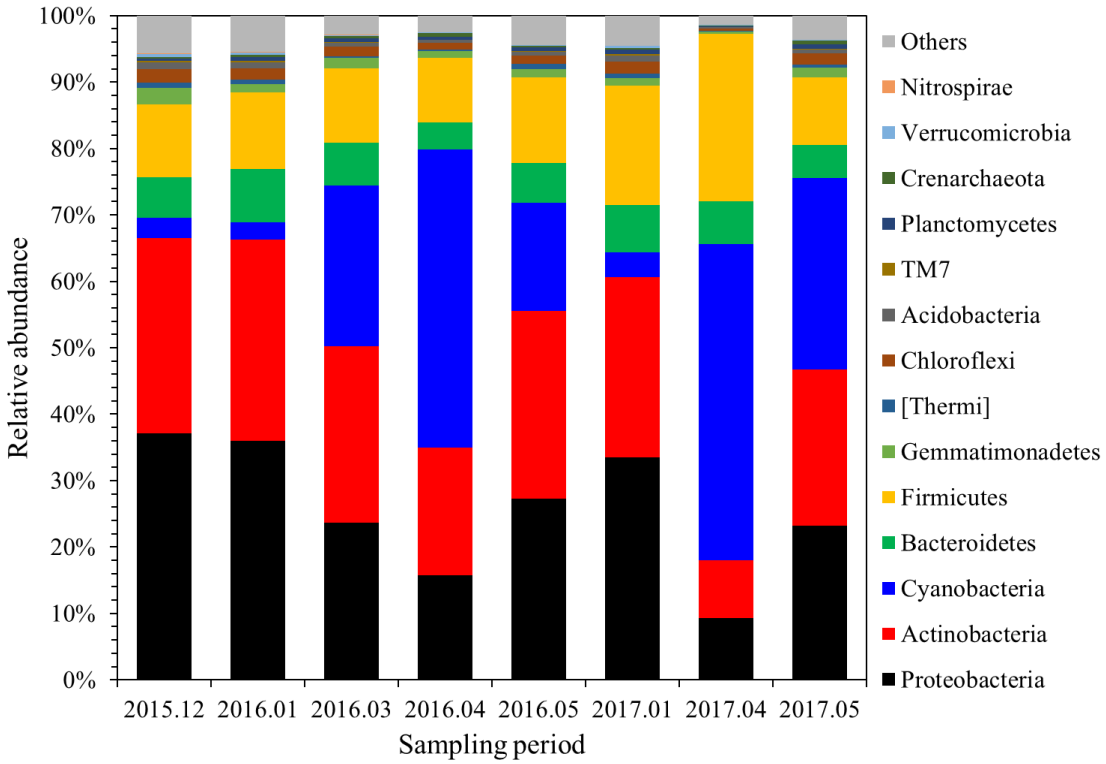

Figure. 3. Bacterial community structures of $PM_{2.5}$ samples at the phylum level.

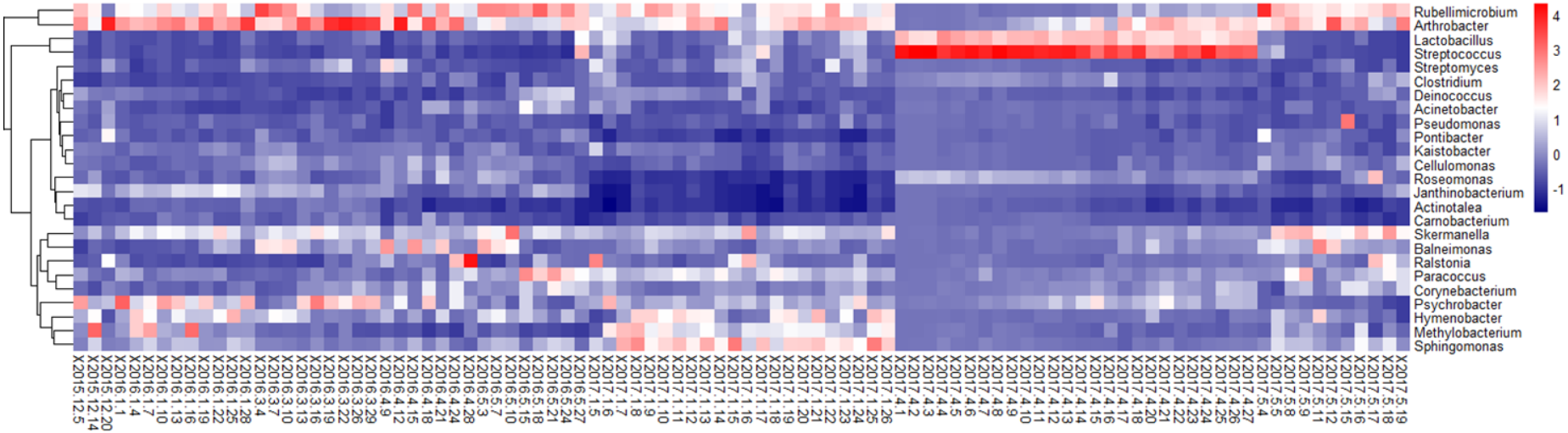

Figure. 4. Heatmap of the dominant genuses (relative abundance higher than 0.4%) during the sampling period at the genus level

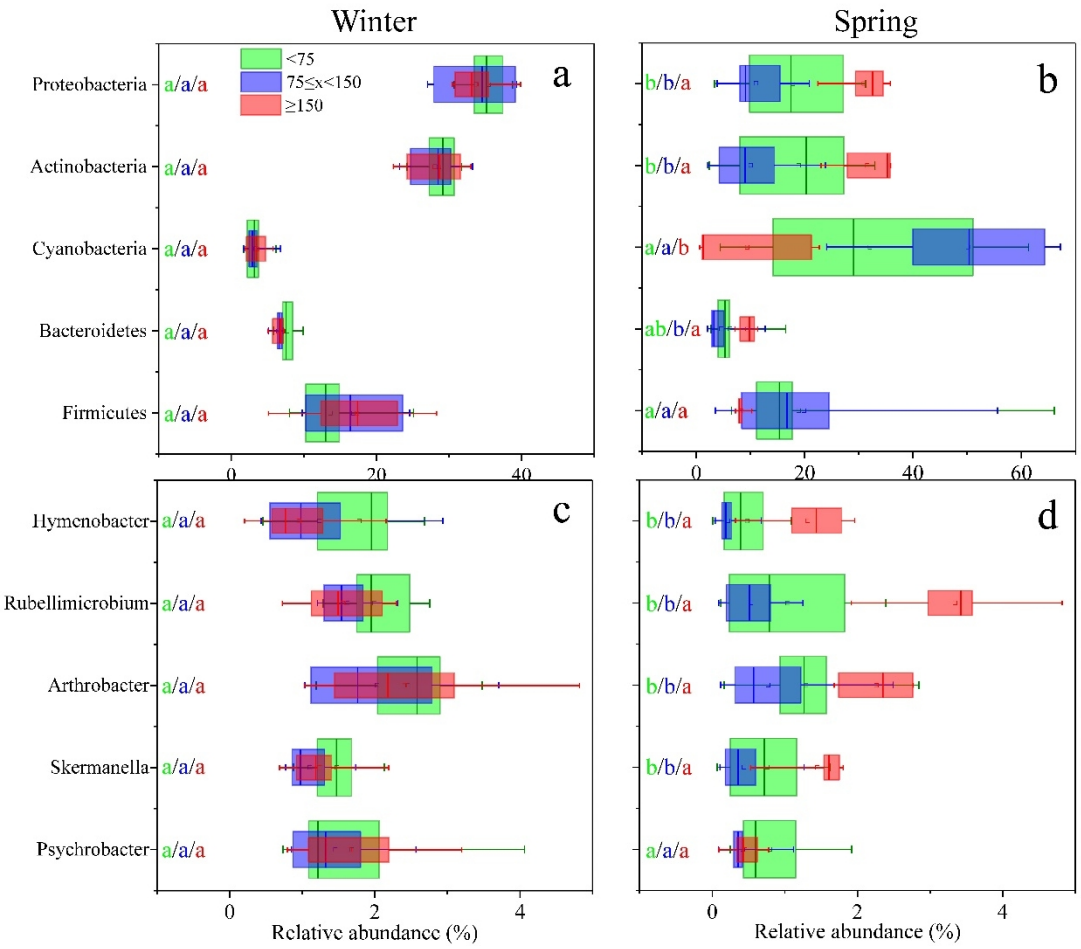

Figure. 5. Variation in bacterial communities in the three $PM_{2.5}$ pollution levels at the phylum level (a: winter, b: spring) and genus level (c: winter, b: spring)

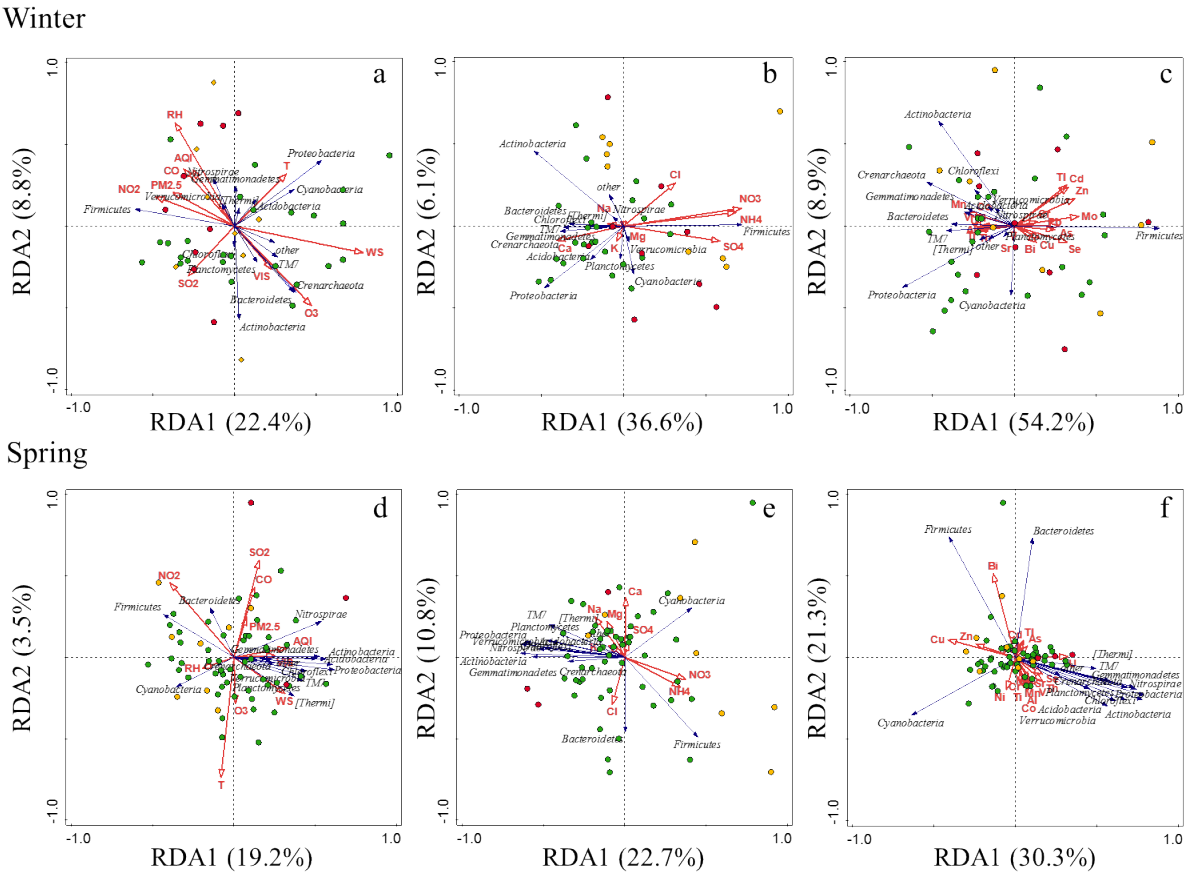

Figure. 6. Ordination plots from a Redundancy Analysis (RDA) of bacterial communities based on phyla abundances as determined by: environmental factors (a, d), water-soluble nutrients (b, e), and heavy metals (c, f) in $PM_{2.5}$ at the three $PM_{2.5}$ levels in winter (a-c) and spring (d-f).