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Bacterial Community Specification in $PM_{2.5}$ in Different Seasons in Xinxiang, Central China

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ABSTRACT

In China, air pollution has become a significant environmental threat to human health in recent years. Airborne bacteria are critical constituents of microbial aerosols, which contain numerous pathogens. However, the effects of seasonal variations, environmental factors such as air pollution, and meteorological factors on microbial diversity are poorly understood. In this study, fine particulate matter ($PM_{2.5}$) samples (n = 12) were collected using a high-volume air sampler over 24-hour periods during all four seasons from April 2017 to January 2018. Concurrently, the average daily concentrations of various air pollutants and the meteorological conditions were monitored. High-throughput sequencing of 16s rRNA was then employed to profile $PM_{2.5}$ bacterial communities. The results showed that the bacterial communities varied significantly by season. Proteobacteria (35.5%), Firmicutes (23.0%), and Actinobacteria (16.2%) were the most abundant bacterial phyla in the $PM_{2.5}$ samples. At the genus level, the diversity of the bacterial communities was significantly correlated with the ozone (O_3) concentration ($r = 0.920$, $p = 0.001$) and air temperature (T) ($r = 0.534$, $p = 0.023$). The results of this study can be used as a reference by other bioaerosol research that focuses on the health effects of atmospheric particulate matter.

Keywords: $PM_{2.5}$; Bacterial biodiversity; Pollutants; Meteorological factors; Ozone.

INTRODUCTION

Air pollution is a significant environmental threat to human health. Numerous epidemiological and clinical studies have documented that exposure to particulate matter (PM) is associated with various adverse health effects (WHO, 2013). The 2015 Global Burden of Disease Study showed that exposure to outdoor $PM_{2.5}$ resulted in 4.241 million deaths in 2015 (GBD 2015 Risk Factors Collaborators, 2016). $PM_{2.5}$ has a diverse range of components, including sulfates, nitrates, metal ions, organic compounds, and microbes (He *et al.*, 2001; Després *et al.*, 2012). Particles of this size are more likely to penetrate and deposit deeper into the tracheobronchial and alveolar regions (Brook *et al.*, 2004). Biological PM, such as bacteria, fungi, viruses, and plant and animal debris, may account for as much as 25% of airborne aerosols (Jaenicke, 2005). $PM_{2.5}$ is hypothesized to have a substantial bacterial component, and these microbial elements may play a critical role in causing adverse health effects; however, the composition and relative abundance of $PM_{2.5}$ bacterial components and the factors that enhance or limit their proliferation and existence are poorly understood.

Airborne microorganisms may be influenced by air pollutants, the season, and other meteorological factors (Fang *et al.*, 2008; Gao *et al.*, 2015; Hu *et al.*, 2015). A temperate climate and/or certain seasons are beneficial for the proliferation and growth of most airborne microorganisms. Li *et al.* (2011) detected higher airborne bacterial concentrations in autumn in the Qingdao terrestrial region compared with the coastal region, and Fang *et al.* (2007) reported a higher bacterial concentration in autumn in Beijing. In addition, meteorological factors, such as temperature (T), relative humidity (RH), and wind speed, influence $PM_{2.5}$ bacteria. Sandstorms have been reported to increase the concentration of culturable bacteria in PM (Li *et al.*, 2011). Moreover, the concentrations of $PM_{2.5}$ microorganisms decrease with

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increasing ambient O_3 concentration (Dong *et al.*, 2016). O_3 can react with olefins and other compounds in the atmosphere, which increases the toxicity of O_3, making it toxic to airborne microorganisms (Dong *et al.*, 2016). Although the concentration of airborne microorganisms may be associated with various environmental factors, few studies have examined this phenomenon.

Culture-based methods can culture only a fraction of the bacteria in PM because the non-culturable microbiome accounts for > 95% of the total microbiome (Li *et al.*, 2011). Culture-independent methods, especially high-throughput sequencing technology, have been shown to be effective analytical techniques for determining the diversity and composition of airborne microorganisms (Bowers *et al.*, 2013; Cao *et al.*, 2014; Du *et al.*, 2018). In this study, we used high-throughput sequencing to study the bacterial communities in $PM_{2.5}$ samples collected during four seasons. Thus, this study aimed to determine the diversity of bacterial communities and their composition in $PM_{2.5}$ in different seasons and their relationship with other atmospheric pollutants and meteorological factors.

MATERIALS AND METHODS

Sampling Sites and Sample Collection

$PM_{2.5}$ samples were collected at Xinxiang Medical University from April 2017 to January 2018 in Xinxiang (35°18′13.71°N, 113°55′15.05°E), an industrial city in Central China with a temperate continental climate. The sampling site is nearly 100 m from the nearest major roads. The site is surrounded by trees, greenbelts, and residential and school buildings, with no identified potential industrial pollution sources. To collect $PM_{2.5}$ samples, a high-volume air sampler (TE-6070VFC; Tisch Environmental, Inc., USA) with a flow rate of 1.17 m^3 min^{-1} was used, and the sampler was placed on the rooftop of a seven-story research building (approximately 25 m high). 3 $PM_{2.5}$ samples were collected in 24-hour cycles using a high purity glass fiber filter membrane (8 × 10 in.; Safelab, Beijing) during each of the four seasons (spring [SP], summer [SU], autumn [AU], and winter [WI]; total n = 12). Concurrently, the 24-hour average concentrations of PM_{10} (PM_{10} refers to the particulates that have an aerodynamic diameter smaller than 10 μm), O_3, carbon dioxide (CO), sulfur dioxide (SO_2), and nitrogen dioxide (NO_2), as well as meteorological factors (T and RH), were monitored continuously. The obtained sampling membranes were stored at –20°C until microbiome analysis.

DNA Preparation and Sequencing

The total genomic DNA was extracted from the $PM_{2.5}$ samples using the cetyltrimethylammonium ammonium bromide (CTAB) method. The concentration and purity of DNA were determined through electrophoresis on 1% agarose gel; subsequently, the DNA was diluted with sterile water to a concentration of 1 ng μL^{-1}. For sequencing analyses, the V4 region of the 16s rRNA gene was amplified through polymerase chain reaction (PCR; 98°C for 1 min, followed by 30 cycles at 98°C for 10 s, 50°C for 30 s, and 72°C for 30 s, with a final extension at 72°C for 5 min) that used the primers 515F (5′-GTGCCAGCMGCCGCGGTAA-3′) and 806R (5′-GGACTACHVGGGT WTCTAAT-3′). PCR reactions were performed in a 30-μL reaction solution containing 2X Phusion® High-Fidelity PCR Master Mix (New England Biolabs), 0.2-μM forward and reverse primers, and approximately 10 ng of template DNA (according to the DNA concentration). PCR products were mixed with the same volume of 1× loading buffer and were detected through electrophoresis on 2% agarose gel. A Gene JET™ Gel Extraction Kit (Thermo Scientific) was used to purify PCR amplification products. High-throughput sequencing of 16s rRNA was conducted on the Ion S5™ XL platform.

Bioinformatics Analysis

Single-end reads were assigned to DNA samples by identifying their unique barcode; the raw reads were then truncated by cutting off the barcode and primer sequence. According to the Cutadapt quality control process, filtering of the raw reads was performed under specific filtering conditions to obtain high-quality clean reads (Martin, 2011). Finally, to obtain clean reads, the UCHIME algorithm was used to remove chimeric sequences (Edgar *et al.*, 2011; Haas *et al.*, 2011). Sequence analysis was performed using UPARSE (version 7.0.1001; http://drive5.com/uparse/; Edgar, 2013), and sequences with similarity greater than 97% were assigned to the same operational taxonomic units (OTUs). Representative sequences for each OTU were screened for further annotation. To determine alpha diversity, QIIME (version 1.9.1) was used to calculate the Chao1 and abundance-based coverage estimator (ACE) indices to estimate the species abundance, as well as the Shannon and Simpson indices to estimate community diversity. Principal coordinate analysis (PCoA) based on the unweighted-UniFrac distance matrix was conducted using QIIME to test the difference and variation in various bacterial communities in $PM_{2.5}$ among seasons. R (version 2.15.3) was used to draw PCoA diagrams and analyze the differences between groups.

The R statistical computing environment was used for all community statistics and visualizations. The relationship between environmental factors, and bacterial relative abundance and diversity indices was determined using canonical correspondence analysis (CCA). Furthermore, Spearman's rank correlation analysis was used to examine the correlations between environmental factors (meteorological factors and other pollutants) and the 35 most abundant bacterial genera. The *p* values were corrected for multiple testing with the Holm method by using R *vegan*. A *p* value of < 0.05 was considered statistically significant. Bioinformatics analysis was conducted by Beijing Novogene Bioinformatics Technology Co., Ltd., under the authors' supervision.

RESULTS AND DISCUSSION

Comparison of Bacterial Compositions among the Four Seasons

We acquired a total of 916,229 raw reads and 787,923 high-quality clean reads after specific filtering conditions. Sequences with ≥ 97% similarity were clustered into the

same OTUs; thus, 744,044 effective reads were clustered into the same OTUs. The representative sequence for each OTU was screened for further annotation. Approximately 50 bacterial phyla and 900 bacterial genera were detected from the $PM_{2.5}$ samples. The top 10 phyla and genera in the four seasons are listed in Tables 1 and 2, respectively. Proteobacteria, Firmicutes, and Actinobacteria were the most abundant bacterial phyla in all the samples. Their ranking in spring was as follows: Proteobacteria, Actinobacteria, and Firmicutes, with relative abundances of 37.35%, 24.26%, and 23.90%, respectively. This ranking differs from that in the other seasons (summer, autumn, and winter), in which the ranking was as follows: Proteobacteria, Firmicutes, and Actinobacteria. At the genus level, the most abundant genera were *Chloroplast* and *Lactobacillus*, which belong to the phyla Cyanobacteria and Firmicutes, respectively, followed by *Pseudomonas* and *unidentified_Mitochondria*, which belong to the phylum Proteobacteria. The 3 dominant bacterial phyla were Proteobacteria, Firmicutes, and Actinobacteria, which is consistent with a relevant study conducted in Beijing (Du *et al.*, 2018). In order to make a valid comparison with Du's study, the same sampling and analytical methods as Du's were used in our study. However, at the genus level, a significant difference was observed in bacterial compositions between our samples and the samples from Beijing (Fig. S1). In our study, we identified *Lactobacillus* in spring and summer, *unidentified_Mitochondria* in autumn, and *unidentified_Chloroplast* in winter at the genus level. By contrast, the study in Beijing identified *Kocuria* as the dominant genus in all four seasons. This finding indicates a significant geographical variation of $PM_{2.5}$ bacteria because the capital city of Beijing is 600 km from Xinxiang. Consistent with the results of other studies, this study mainly identified plant-, soil-, and fecal-associated bacteria. For example, *unidentified_Chloroplast* is a plant-associated and soil-inhabiting bacterium (Gao *et al.*, 2017), *Pseudomonas* widely inhabits soil and water (Qu *et al.*, 2016), and *Escherichia* is a fecal-associated bacterium (Hu *et al.*, 2007). These findings indicate that plants, soil, and feces are likely the main sources of bacteria in PM (Sun *et al.*, 2018).

The seasonal differences in the bacterial community of $PM_{2.5}$ were evaluated further through analysis of molecular variance. The bacterial community structure was markedly different between spring and summer ($p = 0.016$). In addition, we used *t*-tests to determine the differential species between the two seasons for each phylogenetic level. Tables S1–S6 show the significant difference in abundant $PM_{2.5}$ bacteria at the phylum and genus levels. In brief, our study demonstrated that considerable seasonal variations existed in $PM_{2.5}$ bacterial profiles.

Relevant studies have demonstrated that bacterial richness is considerably higher in winter than in other seasons (Kumari and Choi, 2014; Du *et al.*, 2018). However, in our study, we found that the bacterial community in $PM_{2.5}$ showed the highest species richness in spring and the highest diversity in summer (Table 3). The discrepancy

Table 1. Relative abundance (%) of top 10 $PM_{2.5}$ bacterial phyla among the four seasons (mean ± SD).

Phylum	Spring	Summer	Autumn	Winter
Proteobacteria	37.35 ± 4.69	34.23 ± 4.81	41.81 ± 6.19	28.65 ± 2.38
Actinobacteria	24.26 ± 5.34	16.90 ± 1.49	13.88 ± 7.97	9.86 ± 4.32
Firmicutes	23.90 ± 2.45	17.76 ± 3.12	23.79 ± 4.47	26.63 ± 1.38
Bacteroidetes	5.24 ± 0.78	7.49 ± 2.66	12.01 ± 7.80	15.84 ± 10.14
Cyanobacteria	3.44 ± 3.35	0.48 ± 0.17	2.96 ± 4.69	9.49 ± 14.80
Acidobacteria	1.71 ± 0.71	6.90 ± 1.77	0.66 ± 0.72	1.66 ± 1.62
Verrucomicrobia	0.47 ± 0.20	3.86 ± 3.42	0.43 ± 0.42	2.46 ± 2.64
Gemmatimonadetes	0.82 ± 0.27	2.46 ± 1.92	0.24 ± 0.15	0.85 ± 0.81
Chloroflexi	0.89 ± 0.34	2.29 ± 1.15	0.35 ± 0.33	0.94 ± 1.00
Planctomycetes	0.39 ± 0.06	1.25 ± 0.39	0.22 ± 0.20	1.08 ± 1.32
Others	1.53 ± 0.34	6.37 ± 0.94	3.65 ± 3.54	2.55 ± 1.73

Table 2. Relative abundance (%) of top 10 $PM_{2.5}$ bacterial genera among the four seasons (mean ± SD).

Genus	Spring	Summer	Autumn	Winter
unidentified_Chloroplast	3.38 ± 3.33	0.33 ± 0.16	2.90 ± 4.63	9.27 ± 14.89
Enhydrobacter	0.02 ± 0.03	0.46 ± 0.70	4.13 ± 7.14	0.02 ± 0.01
Pseudomonas	0.49 ± 0.16	1.10 ± 0.32	2.23 ± 2.82	5.30 ± 5.88
Parabacteroides	0.03 ± 0.02	0.10 ± 0.11	4.00 ± 6.79	0.20 ± 0.12
unidentified_Mitochondria	1.18 ± 1.04	0.22 ± 0.38	4.57 ± 6.33	1.53 ± 2.19
Escherichia-Shigella	0.16 ± 0.10	0.97 ± 0.45	3.41 ± 4.73	2.01 ± 1.10
Bifidobacterium	0.05 ± 0.02	0.40 ± 0.15	3.11 ± 4.91	0.99 ± 0.37
Lactobacillus	7.01 ± 0.55	3.58 ± 1.96	2.74 ± 2.16	1.29 ± 0.79
Paracoccus	2.89 ± 3.13	0.16 ± 0.13	0.73 ± 1.03	0.44 ± 0.29
Akkermansia	0.01 ± 0.01	0.25 ± 0.21	0.29 ± 0.49	2.22 ± 2.70
Others	84.80 ± 7.49	92.43 ± 2.99	71.88 ± 2.30	76.73 ± 16.83

between our results and the results of previous studies may be explained by the different geographical locations and their corresponding climatic conditions. Xinxiang has a temperate continental climate characterized by four seasons. Northwesterly winds prevail in winter because of the influence of the Siberian monsoon, whereas no dominant wind direction exists in spring. As previously reported, the wind speed reduces air pollution levels and strongly influences airborne bacterial diversity (Kumari and Choi, 2014). A previous study showed that wind speed is correlated with the diversity and composition of bacterial communities (Kembel *et al.*, 2012). A strong wind is beneficial for the diffusion of PM and its microorganisms (Du *et al.*, 2018). Even within the same season, variations in microbial community richness and diversity as well as variations in the total concentration of PM caused by wind are apparent (Du *et al.*, 2018).

Bacterial Diversity in $PM_{2.5}$

Table 3 summarizes the number of observed OTUs and diversity of bacterial communities in $PM_{2.5}$ (mean ± standard deviation). To determine alpha diversity, the Shannon and Simpson indices were calculated to compare community diversity, and the ACE and Chao1 indices were estimated to compare community richness (Table 3). The spring samples showed the highest species richness, with ACE and Chao1 indices of 2310 ± 189 and 2257 ± 165, respectively, whereas the autumn samples had the lowest species richness, with ACE and Chao1 indices of 1330 ± 634 and 1320 ± 629, respectively. The Wilcoxon rank sum test showed that bacterial community richness was significantly different between autumn and spring (p = 0.039 for ACE). Furthermore, the highest Shannon and Simpson indices, community diversity indices, were observed in summer (9.17 ± 0.57 and 1.00 ± 0.001), followed by spring (8.63 ± 0.35 and 0.99 ± 0.002), winter (7.91 ± 1.27 and 0.97 ± 0.042), and autumn (7.07 ± 2.14 and 0.94 ± 0.080). The Wilcoxon rank sum test showed that the diversity of the bacterial community was significantly different between the summer and autumn (p = 0.049 for the Shannon indices). The number of OTUs in spring was higher than that in other seasons. These results indicated that the abundance and diversity of the bacterial community differed between seasons. Furthermore, we detected a relationship between environmental factors and alpha diversity indices. The Spearman's rank test showed that both T (r = 0.66, p = 0.02) and O_3 (r = 0.75, p = 0.005) were correlated with the Simpson index.

The variation in bacterial communities in $PM_{2.5}$ collected during different seasons was analyzed using PCoA; the closer the samples were, the more similar the species compositions were. PCoA analysis of bacterial communities showed that $PM_{2.5}$ samples collected in spring and winter were clustered, whereas those collected in summer and autumn were relatively scattered (Fig. 1). Therefore, the PCoA results demonstrated that microbial community variation was significantly correlated with the season.

Potential Bacterial Pathogens in $PM_{2.5}$

Pathogenic bacteria have previously been found in airborne PM (Fröhlich-Nowoisky *et al.*, 2009; Gou *et al.*, 2016). Using the directory of pathogenic microorganisms infecting humans promulgated by the National Health Commission of the People's Republic of China to detect pathogens, in our study, we found 18 pathogenic bacteria in the $PM_{2.5}$ samples that belonged to 9 genera. *Escherichia coli* (19.66%), *Acinetobacter lwoffii* (11.73%), and *Pseudomonas aeruginosa* (5.27%) were the major pathogenic bacteria in all $PM_{2.5}$ samples collected in Xinxiang. *E. coli* was the most abundant bacteria in summer (2.9%), autumn (10.24%), and winter (6.03%). In spring, *A. lwoffii* was the most abundant bacteria, accounting for 8.18%. *A. lwoffii* is a Gram-negative aerobic bacillus present in the normal oropharynx and skin, and it is an opportunistic pathogen that can cause infections in humans with impaired immune systems (Ku *et al.*, 2000; Regalado *et al.*, 2009; Singh *et al.*, 2016). *P. aeruginosa* is a Gram-negative bacillus found in warm, moist environments and can be identified from soil, water, and normal human skin; ozone has high germicidal effectiveness against *P. aeruginosa* (Zuma *et al.*, 2009). In our study, ozone levels were the highest in summer while *P. aeruginosa* was at the lowest relative abundance of 0.09%. In this study, *Clostridium novyi* and *Campylobacter jejuni* were detected in autumn. *C. novyi* is an opportunistic pathogen that can cause infection in devitalized/hypoxic tissues; it also can foster spore germination and proliferation with the concomitant expression of virulence factors and toxins (Aronoff and Kazanjian, 2018). *C. jejuni*, a Gram-negative bacterium, is commonly associated with gastroenteritis and infects humans mainly through the food chain or other routes from the environment (Giallourou *et al.*, 2018; Oh *et al.*, 2018). $PM_{2.5}$ can be deposited in the respiratory bronchioles and alveoli through the upper airways, and it induces various inflammatory responses and cellular immune impairment (Guan *et al.*, 2016). The high concentration of $PM_{2.5}$ as well as its pathogenic bacteria may play a role in the development of respiratory diseases.

Relationship between Environmental Factors and $PM_{2.5}$ Bacterial Community

Table S7 lists the air pollutant concentrations and

Table 3. Operational taxonomic units (OTUs) and alpha diversity indices for each season.

Season	OTUs	ACE	Chao1	Shannon	Simpson
Spring	2343 ± 150	2310 ± 189	2257 ± 165	8.63 ± 0.35	0.99 ± 0.002
Summer	1608 ± 825	1619 ± 781	1625 ± 808	9.17 ± 0.57	1.00 ± 0.001
Autumn	1312 ± 626	1330 ± 634	1320 ± 629	7.07 ± 2.14	0.94 ± 0.080
Winter	1993 ± 255	2081 ± 264	2090 ± 297	7.91 ± 1.27	0.97 ± 0.042

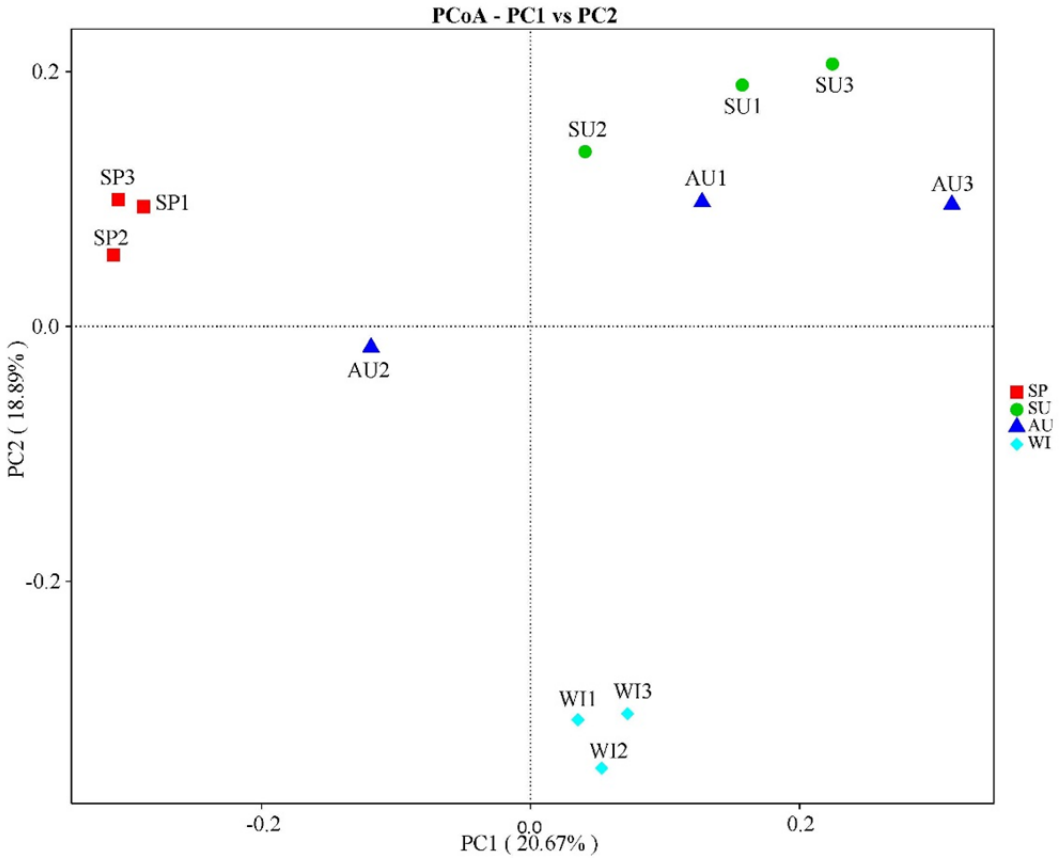

Fig. 1. Principal coordinate analysis of the samples using the unweighted-UniFrac distance matrix: The colored shape labels of red squares, green circles, upper blue triangles, and sky blue diamonds correspond to spring (SP), summer (SU), autumn (AU), and winter (WI) samples, respectively. The numbers next to PC 1 and PC 2 explain the percentages of community variations that are attributed to each PC.

corresponding meteorological factors for each sample. The highest average concentration of $PM_{2.5}$ was detected in winter; the highest average concentrations of PM_{10}, SO_2, and CO were detected in spring; and the highest average concentration of O_3 was detected in summer. Moreover, the highest average NO_2 concentration was found in autumn.

Assessing the effects of environmental factors on bacteria is necessary because they influence bacterial survival, growth, and diffusion (Hwang *et al.*, 2010; Zhong *et al.*, 2016). In the present study, CCA was used to analyze the relationships between the bacterial community composition and environmental factors (Fig. 2). The CCA biplot shows that certain environmental factors were distinctly correlated to different samples (Fig. 2). The first axis explains 27.31% of the variation in the genus-environment relationship, and the second axis accounts for 16.98%. The order of the degree of influence of these environmental factors was as follows: T > O_3 > RH > PM_{10} > CO > $PM_{2.5}$ > SO_2 > NO_2. In addition, the most critical drivers of the bacterial community structure were T and O_3, which accounted for 82.63% and 75.83% of the variation, respectively. This result is consistent with those reported by Lu *et al.* (2018), who found that T, O_3, and NO_2 had more significant effects on the bacterial community than did other environmental factors. However, in our study, the influence of NO_2 was the lowest.

We hypothesized that analyzing the correlation between environmental factors, and bacterial relative abundance and diversity indices would be a more effective and precise method of distinguishing bacteria that might be significantly related to environmental factors. Spearman's correlation analysis was used to investigate which bacteria are more closely related to environmental factors. The result revealed that bacterial genera presented significant associations with many environmental factors, especially T and O_3. *Bacteroides*, *Subdoligranulum*, and *Clostridium_sensu_stricto_1* were significantly and negatively correlated with both T and O_3 (Table 4 and Fig. 3).

Tropospheric O_3 is a ubiquitous and highly reactive photochemical oxidant gas that results from the chemical reaction of ultraviolet radiation with nitrogen oxides and volatile organic compounds, which mainly originate from the burning of fossil fuels and from industrial sources (Brook *et al.*, 2004; Wu *et al.*, 2011). This photochemical production is stimulated by intensive sunlight and high T, and the tropospheric O_3 concentration is the highest in summer and the lowest in winter (Wang *et al.*, 2018). Exposure to tropospheric O_3 has been shown to induce inflammation, airway hyper-responsiveness, and oxidative DNA damage (Williams *et al.*, 2008; Palli *et al.*, 2009;

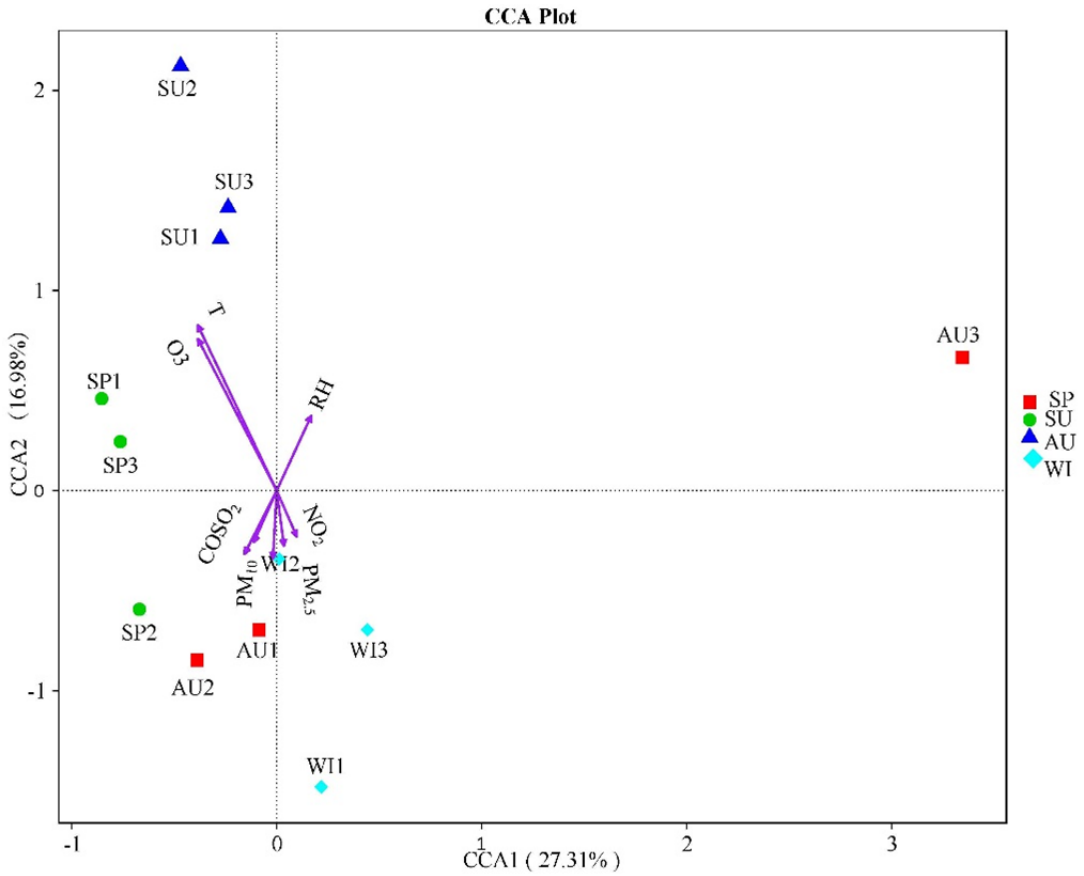

Fig. 2. Canonical correspondence analysis shows the relationships between environmental factors and the $PM_{2.5}$ bacterial community composition; environmental factors are represented by arrows. The length of the arrow represents the correlation between the environmental factor and the distribution of the bacterial community; the longer the arrow is, the greater the correlation. An acute angle between two environmental factors indicates that they are positively correlated, whereas an obtuse angle indicates that they are negatively correlated.

Table 4. Bacterial genera significantly correlated with environmental factors.

Bacterial genus	Environmental factor(s)	*p*-value	Correlation coefficient
unidentified_Chloroplast	RH	0.015	–0.678
Parabacteroides	T	0.039	–0.601
Acinetobacter	PM_{10}	0.021	0.655
	CO	0.007	0.734
	SO_2	0.016	0.674
Bacteroides	T	0.003	–0.769
	O_3	0.001	–0.818
Massilia	RH	0.026	–0.636
Geobacillus	T	0.001	–0.840
Sphingomonas	T	0.039	0.601
Rubrobacter	PM_{10}	0.034	–0.613
Atopostipes	CO	0.028	0.630
Subdoligranulum	T	0.003	–0.771
	O_3	0.016	–0.676
Clostridium_sensu_stricto_1	T	0.018	–0.664
	O_3	0.002	–0.797
Solirubrobacter	O_3	0.027	0.634
RB41	T	0.007	0.734
	O_3	0.003	0.783
Micrococcus	SO_2	0.038	0.604
	CO	0.009	0.713

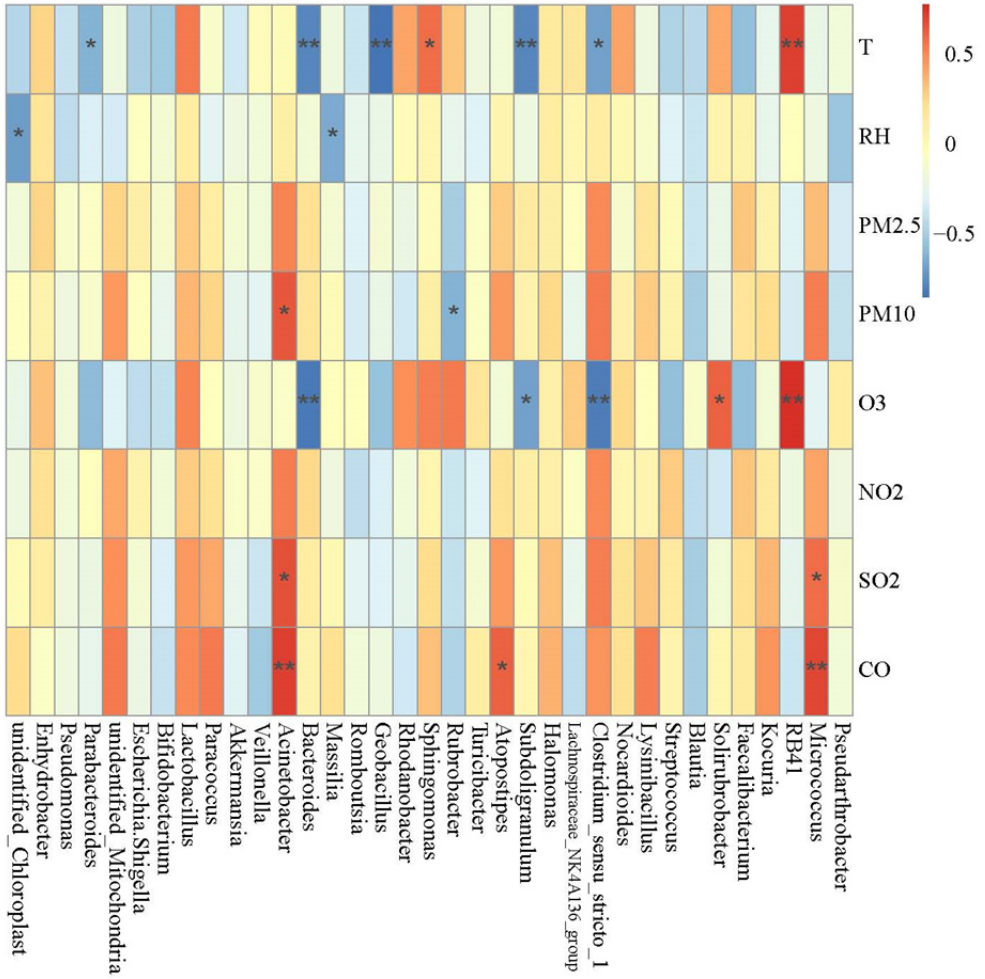

Fig. 3. Spearman's correlation analysis performed between the relative abundance of bacterial genera and environmental factors. The value heat map shows the Spearman correlation coefficient r, which is between –1 and 1. A negative r value signifies a negative correlation, whereas a positive value signifies a positive correlation. * indicates that the significance test was $p < 0.05$, and ** indicates the significance test was $p < 0.001$.

Feng *et al.*, 2016). Epidemiological evidence has shown a strong association between increasing levels of tropospheric O_3 and the occurrence of respiratory diseases, such as asthma and lung inflammation (Zmirou *et al.*, 2004; Wu *et al.*, 2011; Feng *et al.*, 2016). Furthermore, O_3 has a toxic effect on microorganisms in $PM_{2.5}$ (Hameed *et al.*, 2012), and high concentrations of O_3 slow down the growth of the bacteria in $PM_{2.5}$ (Xu *et al.*, 2017). The synergistic effect of O_3 and $PM_{2.5}$ has been reported to cause serious cardiac and systematical injury, with an apparent dose response tendency (Wang, 2013); however, the relative abundance of pathogenic bacteria was found to be negatively correlated with the O_3 concentration, which may be caused by the phototoxic oxidant effect of O_3 (Tiedemann *et al.*, 2000; Dong *et al.*, 2016; Liu *et al.*, 2018). In addition, this study identified that Gram-negative bacteria (e.g., *Bacteroides*, *Subdoligranulum*, and *Parabacteroides*), which possess a thin peptidoglycan lamella that leads to weaker resistance (Zuma *et al.*, 2009), were more susceptible to O_3.

Moreover, we found that T was correlated with $PM_{2.5}$ microorganisms. Increased T may promote bacterial growth and accelerate convective air movements, thereby enhancing bacterial dispersal in the atmosphere (Smets *et al.*, 2016). $PM_{2.5}$ is largely caused by traffic activity and contains hydrocarbons and other chemical compounds. T enhances chemical reactions on the particle surface and may help to form compounds that are more toxic to microorganisms (Alghamdi *et al.*, 2014). Although a relationship was found between airborne bacterial communities and environmental factors, the underlying mechanisms of these interactions remain unclear.

CONCLUSIONS

This study demonstrated that the $PM_{2.5}$ bacterial community in Xinxiang, Central China, possessed the highest species richness during spring and the highest diversity during summer and analyzed the relationship between the microbial community structure and environmental factors. The results showed that the variations in the bacterial community composition and structure were significantly related to the season. Both the O_3 concentration and the air

T showed a significant correlation with the $PM_{2.5}$ bacterial community composition. Our findings serve as a critical reference for studies evaluating the characteristics and effects of bioaerosols as well as those focusing on the effects of atmospheric PM on human health. However, this study has a few limitations. First, it used only a single sample site in an industrial city. Second, a small number (3) of samples per season was collected, and the results were influenced by the specific variations in temperature and humidity on the sampling days. Therefore, these results should be interpreted with caution. Additionally, the present study evaluated only the bacterial diversity and communities in $PM_{2.5}$. Although bacteria account for nearly 80% of atmospheric microorganisms, fungi and viruses in $PM_{2.5}$ should also be investigated, considering their potential adverse effects on human health. Therefore, future studies should examine fungal and viral communities in PM as well as their relationships with the chemical composition of $PM_{2.5}$.

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SUPPLEMENTARY MATERIAL

Supplementary data associated with this article can be found in the online version at http://www.aaqr.org.

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