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ORIGINAL PAPER

Comparison of bacterial community structure in $PM_{2.5}$ during hazy and non-hazy periods in Guilin, South China

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Abstract In recent years, significant efforts have been made to study changes in the levels of air pollutants at regional and urban scales, and changes in bioaerosols during air pollution events have attracted increasing attention. In this study, the bacterial structure of $PM_{2.5}$ was analysed under different environmental conditions during hazy and non-hazy periods in Guilin. A total of 32 $PM_{2.5}$ samples were collected in December 2020 and July 2021, and the microbial community structures were analysed using high-throughput sequencing methods. The results show that air pollution and climate change alter the species distribution and community diversity of bacteria in $PM_{2.5}$, particularly *Sphingomonas* and *Pseudomonas*. The structure of the bacterial community composition is related to diurnal variation, vertical height, and urban area and their interactions with various environmental factors. This is a comprehensive study that characterises the variability of bacteria associated with $PM_{2.5}$ in a variety of environments, highlighting the impacts of environmental effects on the atmospheric microbial community. The results will contribute to our understanding of haze trends in China, particularly the relationship between bioaerosol communities and the urban environment.

Keywords $PM_{2.5}$ · Environmental factors · Bacterial structure · Day-night difference · Haze

1 Introduction

Air pollution is recognised as a global risk factor influencing human health. The presence of a certain number of metabolically active microorganisms in the air and their important roles in the exchange of microorganisms among air, water, soil, plants, animals and humans have been studied (Bai et al., 2021; Šantl-Temkiv et al., 2018). Airborne microbes can affect human health by colonising the skin, mucous membranes, and digestive and respiratory tracts and can subsequently cause a series of illnesses, such as infections, allergies (Severson et al., 2010), acute toxic effects (Thilsing et al., 2015), cardiovascular disease (Xu et al., 2017), infertility (Checa Vizcaíno et al., 2016) and even cancer (Hayleeyesus et al.,

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2015). In China, rapid industrialisation and urbanisation have led to widespread air pollution in recent decades (Zhang, Yang and Li 2020). China's haze pollution is severe, with high concentrations of particulate matter (PM) and a large temporal and spatial coverage of haze, especially $PM_{2.5}$ pollution (Li et al., 2020a, 2020b; Viegas, 2019; Zhang et al., 2020). Particulate matter is small, mobile and contains bacterial aerosols that are potentially hazardous to public health (Ye et al., 2017). A Global Burden of Disease study showed that $PM_{2.5}$ caused 4.1 million premature deaths worldwide in 2016, with over 1 million premature deaths per year due to air pollution and the occurrence of extreme events (Cohen et al., 2017). In 2020, there were global reductions in key pollutants due to the spread of COVID-19 (Berman & Ebisu, 2020; Chauhan & Singh, 2020; Chu et al., 2021; Lei et al., 2020; Zoran et al., 2020). Bioaerosols have been of increasing interest to atmospheric scientists in recent years.

When foggy haze occurs, large amounts of particulate matter and pollutants accumulate, with significant changes in the number and composition of microorganisms carried on particles. As research shows, during hazy periods, the concentration of bacteria increases significantly, and the activity of bacteria decreases significantly, with sustained hazy periods having a more pronounced effect on the activity of bacteria than short hazy periods (Dong et al., 2016; Gong et al., 2020). During periods of haze, the abundance and diversity of bacterial communities decrease, while the relative abundance of pathogenic bacteria increases, and pathogens can survive severe air pollution (Cao et al., 2014; Liu et al., 2018). Li et al. (2015) observed more allergic and infectious genera (e.g. Neisseria) in aerosols on hazy days than on non-hazy days. Accounting for 25% of atmospheric aerosols, $PM_{2.5}$ contains sulphates, nitrates, ammonium salts, organic compounds, metal ions, plant debris and microorganisms and is the main cause of haze formation (Du et al., 2018; Huang et al., 2014). Guilin, a famous city that hosts international tourists, has been focusing on ecological environmental protection for many years, but the number of hazy days has shown a significant increasing trend over the past 35 years. Zhong et al. (2019) investigated the correlation between water-soluble ions, metal elements and the bacterial community composition of $PM_{2.5}$ in hazy and non-hazy weather in Guilin. Their results showed that *Firmicutes, Proteobacteria* and *Bacteroidetes* were the most abundant phyla, accounting for 97.2% of the total bacterial community structure in $PM_{2.5}$ in hazy weather; furthermore, the community structure was significantly and positively correlated with SO_4^{2-}, NO_3^-, NH_4^+, K^+ and Cl^-, and secondary aerosol ions may have led to a change in the composition of $PM_{2.5}$ bacteria.

Nevertheless, airborne microbial communities are mainly affected by meteorological (e.g. temperature (T), relative humidity (RH) and wind speed (WS)) or environmental factors (e.g. air quality index (AQI), $PM_{2.5}$, NO_2, SO_2 and O_3) (Smets et al., 2016; Zhai et al., 2018; Zhen et al., 2017). Recent research on $PM_{2.5}$ has focused on chemical composition, source analysis and effects on human health (Bari & Kindzierski, 2017; Bertolini et al., 2013; Cao et al., 2017; Rogula-Kozłowska, 2016; Tan et al., 2017). Few studies have been conducted to characterise the bacterial community in $PM_{2.5}$ and its pathogenicity, and much is unknown about the factors affecting the structure of the bacterial community in $PM_{2.5}$. The dominant species in $PM_{2.5}$ were previously shown to consist mainly of *Actinobacteria*, *Proteobacteria*, *Firmicutes*, *Cyanobacteria/Chloroplast*, and *Bacteroidetes* (Bowers et al., 2013; Du et al., 2018; Guo et al., 2020; Li et al., 2019). Urban, suburban and rural areas often have different microbial communities due to changes in the relative contributions of microbial sources and disturbances from human activities (Flies et al., 2020; Li et al., 2019; Li, Chen & Yao, 2020a, 2020b; Liu et al., 2019; Mu et al., 2020; Xu et al., 2021). What happens to the airborne microbial community at different air quality levels is also an important scientific question. However, to our knowledge, most previous studies have been conducted by routine 24-h aerosol sampling. Few studies have examined the species composition and community structure of airborne microorganisms at different air quality levels in different seasons. The diurnal variation in $PM_{2.5}$ bacterial diversity and community structure has rarely been studied. Therefore, this study collected $PM_{2.5}$ and used high-throughput sequencing technology to analyse the community composition, distribution characteristics and influencing factors of bacteria in atmospheric $PM_{2.5}$ in Guilin. Our results provide further information on the changes in the community structure of airborne $PM_{2.5}$ bacteria in different urban areas during non-hazy and hazy periods.

2 Materials and methods

2.1 Sampling conditions

The urban sampling site was located in a hotel in the centre of Guilin (25.27°N, 110.28°W), where 28 $PM_{2.5}$ samples were collected at ground level (1.5 m) and on the roof of the building (18 m); the hotel is surrounded by residential buildings, markets, schools, train stations and streets with heavy traffic. The sampling periods were December 2020 and July 2021, representing periods of severe haze and periods of good air quality. The other sampling site was in a suburban area 20 km from the other site in an open environment with no surrounding blocks; four samples were taken at this site. The total number of samples was 32 (Table S1). Quartz fibre filter membranes with a diameter of 90 mm (Whatman, UK) were used as the sampling medium, and the membranes were baked in a muffle furnace at 800 °C for 2 h to remove impurities. An integrated atmospheric sampler (KC-6120, Laoshan, China) with an airflow rate of 100 $L{\cdot}min^{-1}$ was used to collect $PM_{2.5}$ in the air, and the membranes were replaced every 11.5 h (at 07:00 and 19:00 daily, corresponding to day and night, respectively). After collecting $PM_{2.5}$, the filter membranes were transported back to a laboratory be stored in a refrigerator (−20 °C). Meteorological data including temperature, relative humidity and wind speed were obtained from a portable automatic meteorological station (FY-Q4, Wuhan, China) installed near each sampling site, and data on atmospheric pollutants (including PM, NO_2, SO_2, O_3 and CO) were obtained from nearby meteorological stations. The mean values of the environmental factors for the different sampling periods are shown in Table S2.

2.2 High-throughput sequencing

PCR amplification of the bacterial 16S rRNA gene V3–V4 region was performed using the forward primer 338F (5′-ACTCCTACGGGAGGCAGCA-3′) and the reverse primer 806R (5′-GGACTACHVGGG TWTCTAAT-3′). Sample-specific 7-bp barcodes were incorporated into the primers for multiplex sequencing. The PCR components contained 5 µl of buffer (5×), 0.25 µl of Fast pfu DNA Polymerase (5 U/µl), 2 µl (2.5 mM) of dNTPs, 1 µl (10 µM) of each forward and reverse primer, 1 µl of DNA template, and 14.75 µl of ddH2O. Thermal cycling consisted of initial denaturation at 98 ℃ for 5 min, followed by 25 cycles consisting of denaturation at 98 ℃ for 30 s, annealing at 53 ℃ for 30 s, and extension at 72 ℃ for 45 s, with a final extension of 5 min at 72 °C. PCR amplicons were purified with Vazyme VAHTSTM DNA Clean Beads (Vazyme, Nanjing, China) and quantified using the Quant-iT PicoGreen dsDNA Assay Kit (Invitrogen, Carlsbad, CA, USA). After the individual quantification step, amplicons were pooled in equal amounts and paired-end 2×250 bp sequencing was performed using the Illumina NovaSeq platform with NovaSeq 6000 SP Reagent Kit (500 cycles) at Shanghai Personal Biotechnology Co., Ltd. (Shanghai, China).

2.3 Sequence assembly and preprocessing

Microbiome bioinformatics was mainly performed with QIIME2 2019.4 (Bolyen et al., 2018), with slight modification according to the official tutorials (https://docs.qiime2.org/2019.4/tutorials/). Briefly, raw sequence data were demultiplexed using the demux plugin, followed by primer cutting with the cutadapt plugin (Martin, 2011). Sequences were then quality filtered, denoised, and merged, and chimaeras were removed using the DADA2 plugin (Callahan et al., 2016). Nonsingleton amplicon sequence variants (ASVs) were aligned with mafft (Katoh et al., 2002) and used to construct a phylogeny with fasttree2 (Price et al., 2009). Alpha-diversity metrics (Chao1 (Chao, 1984), Shannon (Shannon C. E. 1948, 1949) and Pielou's evenness (Pielou, 1966; Smets et al., 2016)), beta diversity metrics (unweighted UniFrac (Lozupone & Knight, 2005)) were estimated using the diversity plugin with samples rarefied to 48,027 sequences per sample. Taxonomy was assigned to ASVs using the classify-sklearn naïve Bayes taxonomy classifier in the feature-classifier plugin (Bokulich et al., 2018) against the SILVA Release 132 Database (Kõljalg et al., 2013).

2.4 Bioinformatics and statistical analysis

Sequence data analyses and visualisations were performed and generated using R packages (v3.2.0) and OriginPro 8.5 software (OriginLab Corporation, USA). ASV-level alpha diversity indices, such as the Chao1 richness estimator, Shannon diversity index and

Pielou's evenness, were calculated using the ASV table in QIIME2 and visualised as box plots. Beta diversity analysis was performed to investigate the structural variation of microbial communities across samples using UniFrac distance metrics (Lozupone & Knight, 2005) and visualised via principal coordinate analysis (PCoA) and unweighted pair-group method with arithmetic means (UPGMA) hierarchical clustering (Ramette, 2007). A Venn diagram was generated to visualise the shared and unique ASVs among samples or groups using the R package "VennDiagram", based on the occurrence of ASVs across samples/groups regardless of their relative abundance (Zaura et al., 2009). Linear discriminant analysis effect size (LEfSe) was performed to detect differentially abundant taxa across groups using the default parameters (Segata et al., 2011). Redundancy analysis (RDA) was applied to assess the relationships between the bacterial community structure and environmental parameters. Averages and standard deviations of the data were calculated by Microsoft Excel 2010. Statistical comparisons of Shannon, Chao1 and Pielou's evenness indices among different samples were made by one-way analysis of variance (ANOVA). Differences were considered significant at $p < 0.05$.

2.5 Back trajectory analysis

The origins and atmospheric paths of different air masses were tracked using the National Oceanic and Atmospheric Administration Hybrid Single Particle Lagrangian Integrated Trajectory (HYSPLIT) trajectory model (http://www.arl.noaa.gov/hysplitarc-bin/traj1arc) (Stein et al., 2015). Backwards trajectories were calculated for 48 h at an altitude of 500 m for both sampling periods. Isentropic vertical motion was chosen, and trajectories were calculated for each day at 11:00 and 23:00 (UTC) using an archived meteorological dataset (REANALYSIS). Consistent with the sampling time, there were six entries for the hazy period (20 December 2020 to 23 December 2020) and eight entries for the non-hazy period (19 July 2021 to 23 July 2021).

3 Results

3.1 Air mass flow and meteorological conditions during hazy and non-hazy periods

The backwards trajectory of airflow consistent with the sampling moment shows that 1/3 of the airflow during the hazy period came from the north and 1/3 came from nearby areas; the air masses were slow moving and tended to carry pollutants at an average height below 1000 m, representing a typical winter airflow trajectory, with an average AQI value of 98.67 ± 8.8 and $PM_{2.5}$ concentration of 73.87 ± 7 $\mu g \cdot m^{-3}$. Airflow in the non-hazy period originates from the eastern and southwestern sea, with an altitudinal range of 500–2500 m, representing a typical summer airflow trajectory, during which the mean AQI value in Guilin was 40.06 ± 8.33 and the $PM_{2.5}$ concentration was 25.34 ± 5.01 $\mu g \cdot m^{-3}$, with pollutant concentrations accounting for 1/3 of the hazy days. The hazy period was in the colder winter months (9.85 ± 1.83 °C), and the non-hazy period was in the hotter summer months (29.26 ± 9.94 °C). Because of the rainy weather, the humidity was higher in the non-hazy period ($76.03 \pm 9.94\%$) than in the hazy period ($48.08 \pm 19.01\%$) (Fig. 1).

3.2 Abundance and diversity of microorganisms in different samples

After sequencing, 1505 genera in 123 orders belonging to 48 phyla were identified based on species annotations. The Shannon, Chao1 and Pielou's evenness indices of the α diversity index varied within each subgroup, as shown in Fig. 2. The Shannon index is usually used to estimate microbial diversity; the Chao1 index is usually used to estimate abundance, and Pielou's evenness index is used to characterise the evenness of the community. In general, the larger the values of these indices are, the higher the community diversity and community abundance. In terms of pollution levels, species diversity and richness were higher in the samples from the hazy period than in the samples from the non-hazy period (average values of the Shannon, Pielou's evenness and Chao1 indices during the hazy period were 3.16, 953 and 1038, respectively, compared with 1.72, 342 and 377 during the non-hazy period), with greater variation in bacterial α diversity indices during the day and less

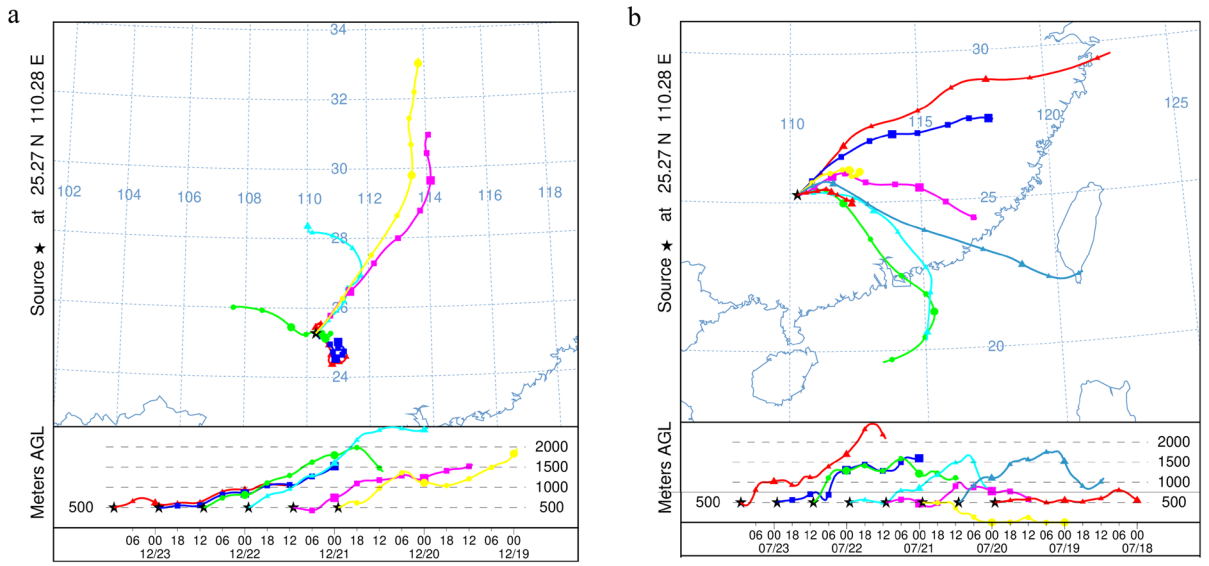

Fig. 1 Backwards trajectories calculated with the NOAA HYSPLIT model for air masses arriving at Guilin at heights of 500 m during **a** hazy and **b** non-hazy conditions

variation at night (the values of the Shannon index ranged from 2.55–5.41 during the hazy period), with a daytime IQR of 2.5 and a night-time IQR of 0.48; the values of the Shannon index ranged from 1.46–2.78 during the non-hazy period, with a daytime IQR of 0.85 and a night-time IQR of 0.26), and storey height had little effect on bacterial diversity. Statistical analysis showed that there was a significant difference in bacterial diversity between the hazy and non-hazy periods ($p = 1.83e{-}5 < 0.01$, $F = 31.17$), simultaneous significant differences in bacterial diversity between daytime and night-time ($P = 0.0497 < 0.05$, $F = 4.36$ for the Shannon index), and storey height did not significantly affect the diversity of the bacterial community ($P = 0.08 > 0.05$, $F = 3.32$ for the Shannon index).

Principal coordinate analysis (PCOA) is based on the unweighted UniFrac algorithm and is a beta diversity analysis. Similarities and differences in the community compositions of different samples can be visualised in the distance matrix. If the positions of samples are relatively in the coordinate system, the results show a high degree of similarity in community structure between the samples. The results for samples collected from different times of day or at different heights are shown in Fig. 3. Figure 3(a) shows that samples from the hazy and non-hazy periods have clear clustering characteristics, with the vast majority of bacterial communities in the non-hazy period overlapping each other (red and blue), while most points in the hazy period overlap each other (purple and green); as the daytime samples are more dispersed, the samples within the group cannot be clustered, indicating a greater variability in the communities during the daytime. There was also a high degree of similarity between the different groups of samples from the top and bottom of the building (Fig. 3(b)), with no significant differences between samples collected from different floors over the same period and greater variability in the communities at the bottom of the building.

Venn diagrams can be used to count the number of co-occurring and unique species in multiple groups or samples, visualising the similarity and overlap in species numbers. A Venn diagram of the ASVs is shown in Fig. 4. The number of ASVs associated with $PM_{2.5}$ was higher in the hazy period than in the non-hazy period. The number of ASVs in the H_DL group during the hazy period was higher than that in the other groups, indicating the greater specificity of the samples in this group, a result that is consistent with that obtained earlier.

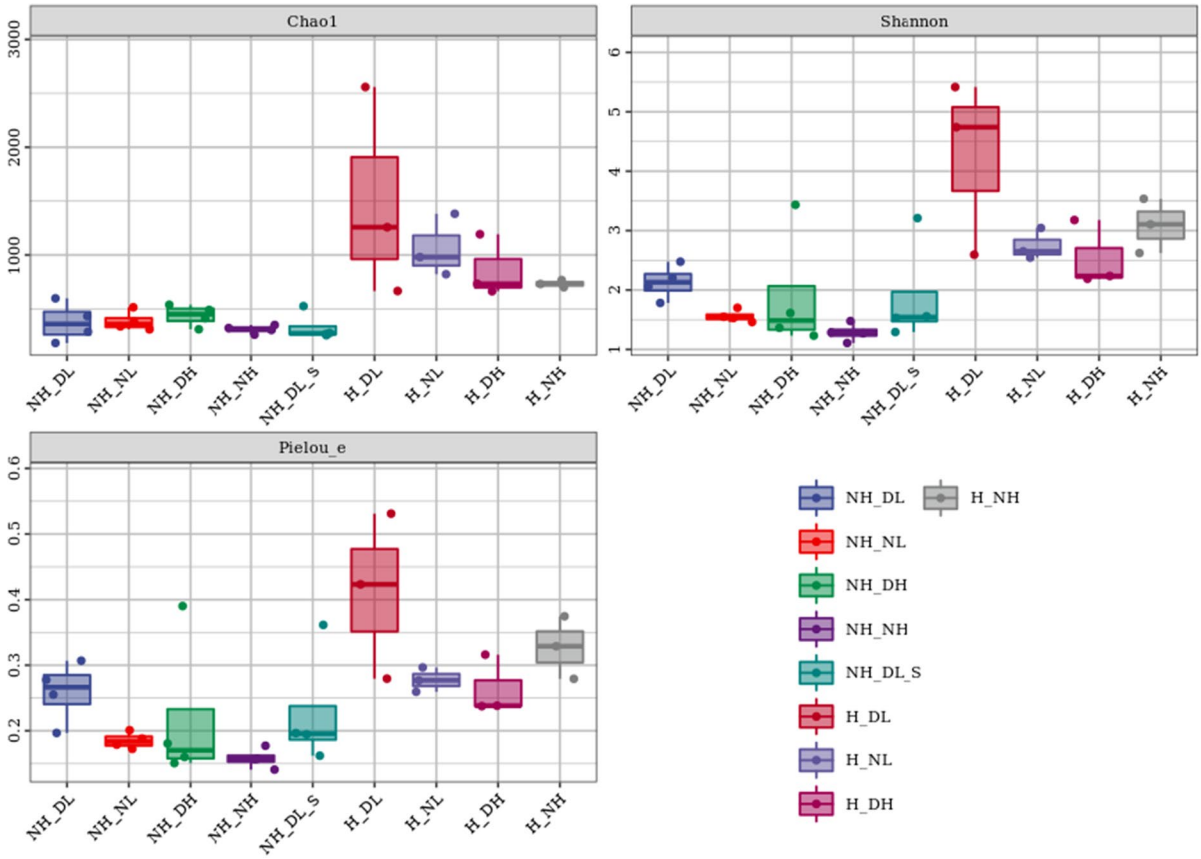

Fig. 2 α Diversity index (H, hazy; NH, non-hazy; D, day; N, night; H, high ground; L, lowland; S, suburb)

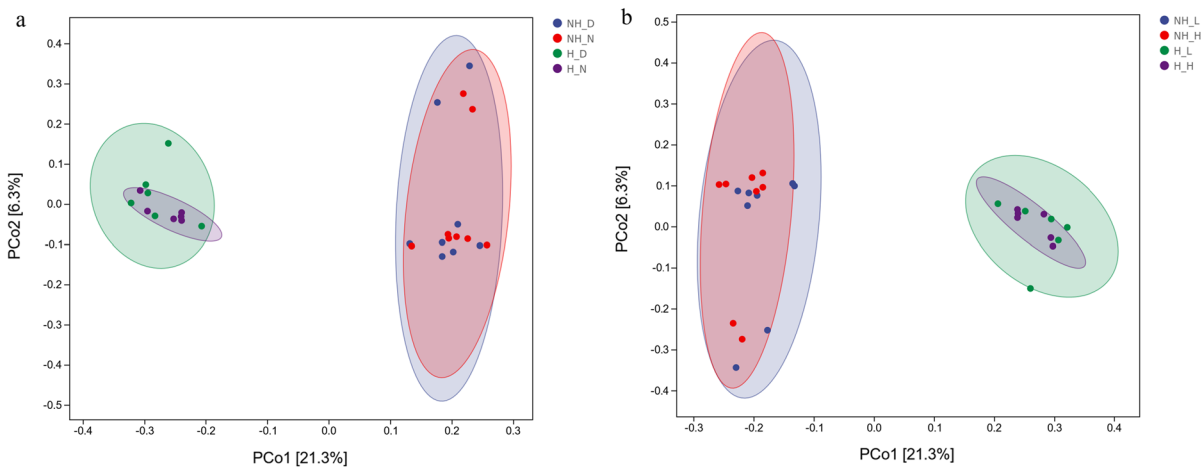

Fig. 3 Principal coordinate analysis of $PM_{2.5}$ bacterial communities: **a** daytime and night-time communities and **b** rooftop and ground floor communities

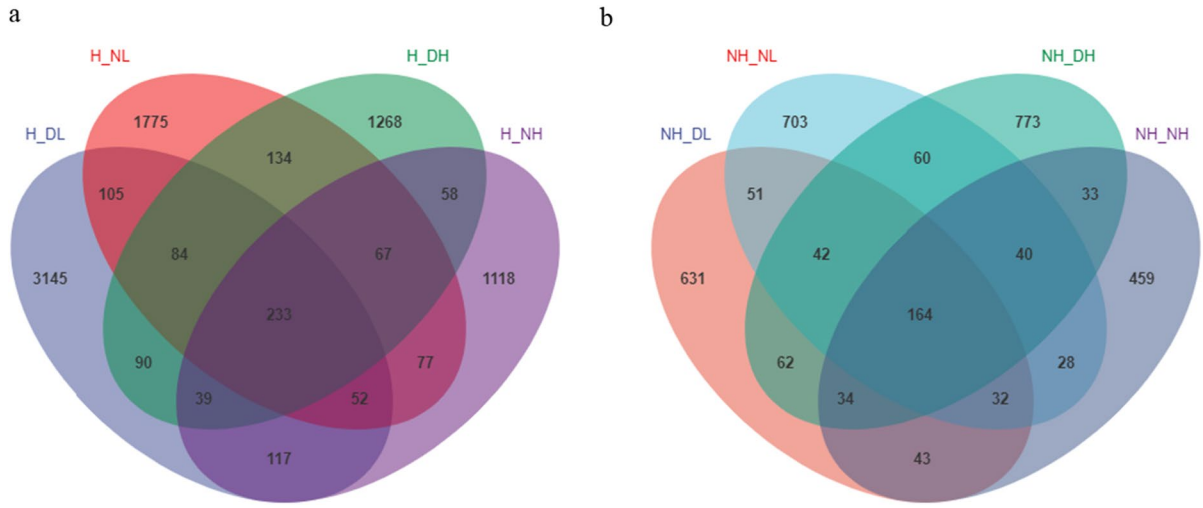

Fig. 4 Venn diagram of the ASVs during **a** hazy and **b** non-hazy periods

3.3 Characteristics of $PM_{2.5}$ bacterial communities during hazy and non-hazy periods

The NH_DL and H_DL groups were compared to reflect the differences in bacterial structure between the hazy and non-hazy periods. Fifteen of the 48 phyla had abundances above 0.05% of the total sample, and most of these common phyla were gram-negative, aerobic and nonpathogenic to humans. *Proteobacteria* (60.8%), *Actinobacteria* (29.1%), *Firmicutes* (5.4%) and *Bacteroidetes* (2%) dominated the samples from the hazy period, with average relative abundances of over 1%. *Proteobacteria* dominated the samples from the non-hazy period (95.68%), followed by *Firmicutes* (2%), *Actinobacteria* (1.1%) and *Bacteroidetes* (0.3%). The proportion of the dominant phylum (*Proteobacteria*) in the samples from the hazy period was lower than that in the samples from the non-hazy period (Fig. 5), suggesting an influx of newly transported microorganisms during periods with severe $PM_{2.5}$ pollution. Among the phyla with low abundances (1%-0.05%), all except *Deinococcus-Thermus* had a much higher abundance of species in the hazy phase than in the non-hazy phase. At the genus level (Fig. 6), the bacteria in the community during the hazy phase were dominated by *Pseudomonas* (48.81%), *Rhodococcus* (17.74%), *Geodermatophilus* (9.81%), and *Schlegelella* (3.61%). *Sphingomonas* (90.96%) was predominant during the non-hazy period. These results indicate a significant difference in the composition of the bacterial community during the hazy and non-hazy periods.

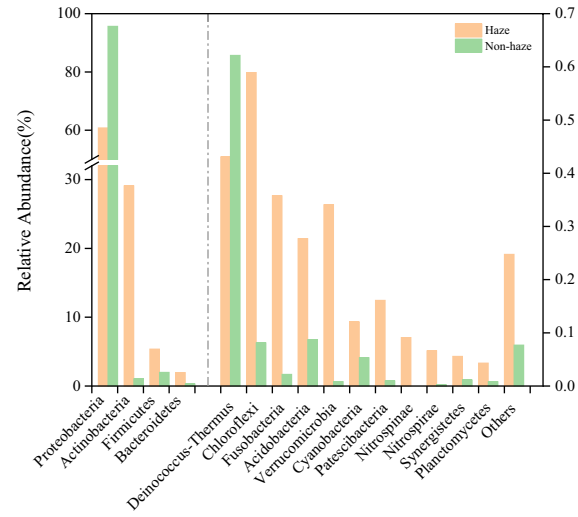

Fig. 5 Relative abundances of phyla in the bacterial communities during the hazy and non-hazy periods. "Others" indicates phyla with a relative abundance of less than 0.05%

3.4 Spatial and temporal characteristics of bacterial communities

To further compare differences in species composition between samples, the abundance data for the 20 genera with the highest mean abundances were used

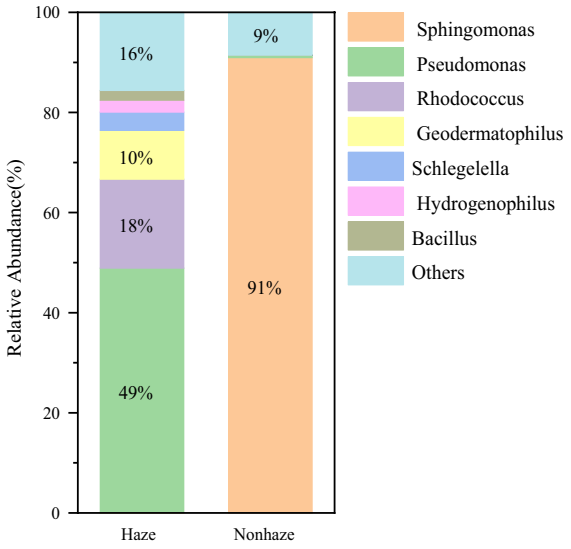

Fig. 6 Relative abundances of genera in the bacterial communities during the hazy and non-hazy periods. "Others" indicates genera with relative abundances below 1%

to create a heatmap (Fig. 7). Judging by the degree of contamination, *Geodermatophilus*, *Pseudomonas*, *Rhodococcus* and *Ralstonia* are more associated with and more abundant in the hazy phase than the non-hazy phase. In addition, the greater divergence of H_DL from the other samples is due to the specific abundance of *Schlegelella*, *Hydrogenophilus*, *Tepidiphilus*, *Bacillus* and *Sphingobacterium* in this sample and similar genera in the NH_DL and NH_DH groups, all of which are diurnal, indicating a more complex diurnal environment.

3.5 Differences in bacterial community structure between urban and suburban areas

To identify the specialised communities in the suburban and urban samples, we set up suburban sampling sites located next to a university experimental teaching building with a small number of pedestrians on the surrounding roads and used the LEfSe tool to perform statistical analyses from the phylum level to the genus level. As shown in Fig. 8, four bacterial taxa were significantly enriched in the suburban sample group, including a taxon at the phylum level (Proteobacteria), a taxon at the order level (Acidithiobacillales), a taxon at the family level (Acidithiobacillaceae), and a taxon at the genus level (KCM_B_112). For the urban samples, 36 bacterial taxa were significantly enriched, eight of which had a relative abundance greater than 3, indicating significant enrichment, including a taxon at the phylum level (Actinobacteria), a taxon at the class level (Clostridia), three taxa at the order level (Clostridiales, Micrococcales, and Deinococcales), two taxa at the family level (Rhizobiaceae and Deinococcaceae), and a taxon at the genus level (Deinococcus). There were more significantly enriched bacterial taxa in the urban sample groups than in suburban sample groups, which is consistent with the results of previous species richness analyses.

3.6 Environmental factors affecting the structure of bacterial communities

Figure 9 depicts the RDA results based on the various influencing factors and bacterial community structures at the genus level. RDA1 accounted for 97.53% of the total community structure. The arrows representing the air pollutants (NO_2, SO_2, O_3, PM_{10}, $PM_{2.5}$ and CO) extend in a single direction, indicating a strong link between the pollutants. The large effect of temperature on community structure is negatively correlated with air pollutants.

3.7 Changes in the abundances of opportunistic pathogenic bacteria

In this study, several common potentially opportunistic pathogens were identified, such as Pseudomonas, Acinetobacter, Streptococcus, Staphylococcus, Serratia and Fusobacterium (Table 1). Pathogens were more abundant during the hazy period, suggesting that air pollution increases the risk of disease exchange in humans. The higher abundance of potential pathogens in suburban areas than in urban areas may be influenced by land use type and vegetation cover (Flies et al., 2020). Another study demonstrated that rural environmental microbiomes and infant gut microbiomes were more diverse than those of their urban peers (Qian et al., 2017). Although natural source-related pathogens may increase due to beneficial conditions, we speculate that the numerous potential pathogens from anthropogenic sources (i.e. human activity, hospitals, and wastewater treatment plants) may be the main players resulting in the increased ratio of pathogens during the summer in urban

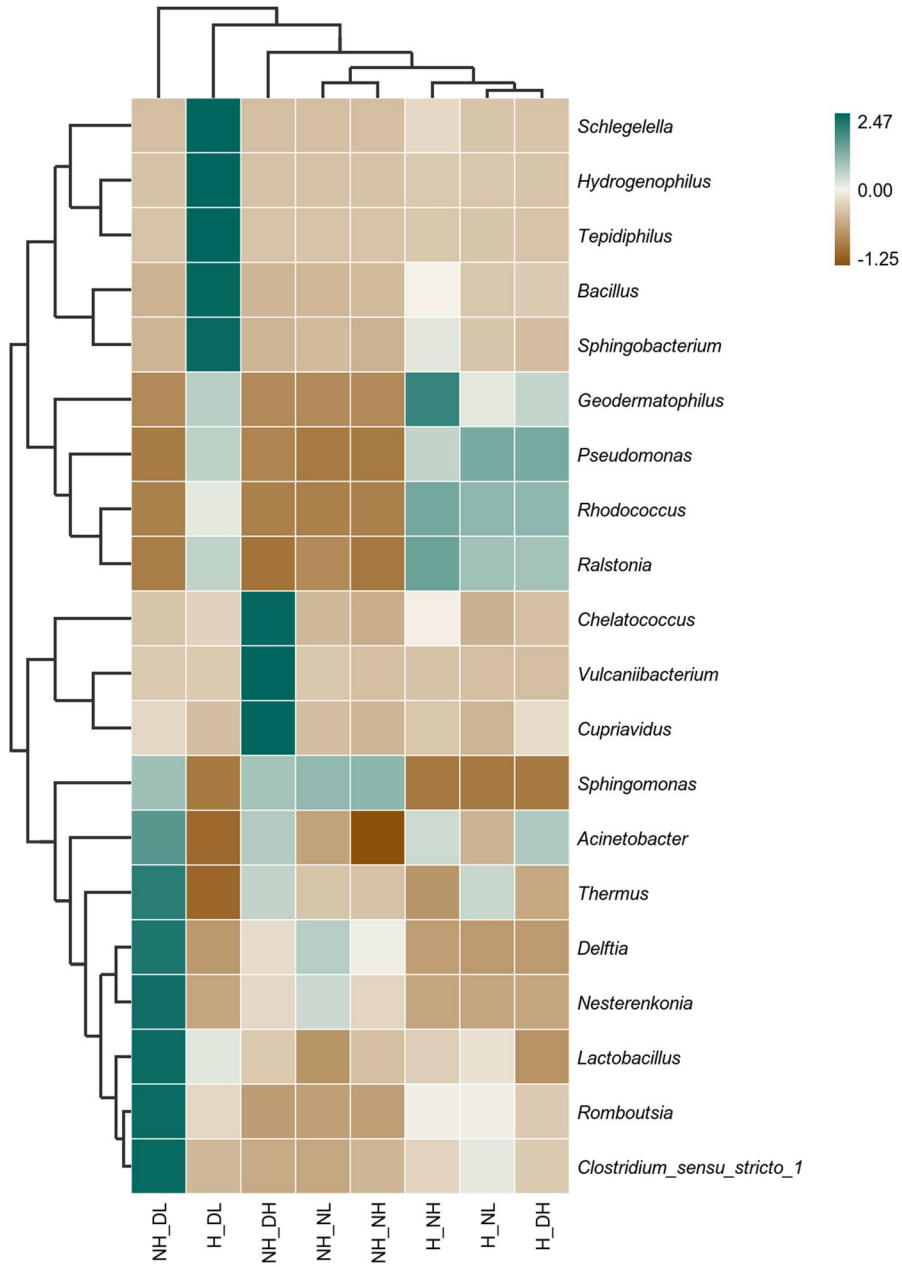

Fig. 7 Heatmap of abundance data for the 20 genera with the highest mean abundances

and suburban regions. Increasing human populations, as a result of urbanization, can lead to higher abundances of pathogens and infection rates due to human activities (Bradley & Altizer, 2007). In particular, *Pseudomonas* is the dominant organism in urban areas. *Pseudomonads* are well known for their striking ability to utilise many different carbon sources, with some species able to utilise more than 100 different carbon sources, allowing them to easily survive in various conditions (Bai et al., 2021). *Pseudomonas* and *Acinetobacter* are abundant in $PM_{2.5}$ pollution, and members of these genera are potential respiratory pathogens (Pan et al., 2019; Yan et al., 2018). *Streptococcus* is a gram-positive bacterium and an important pathogen that can cause various diseases, such as infective endocarditis, meningitis, and pneumonia (Mushtaq et al., 2011). *Acinetobacter* is a gram-negative bacterium

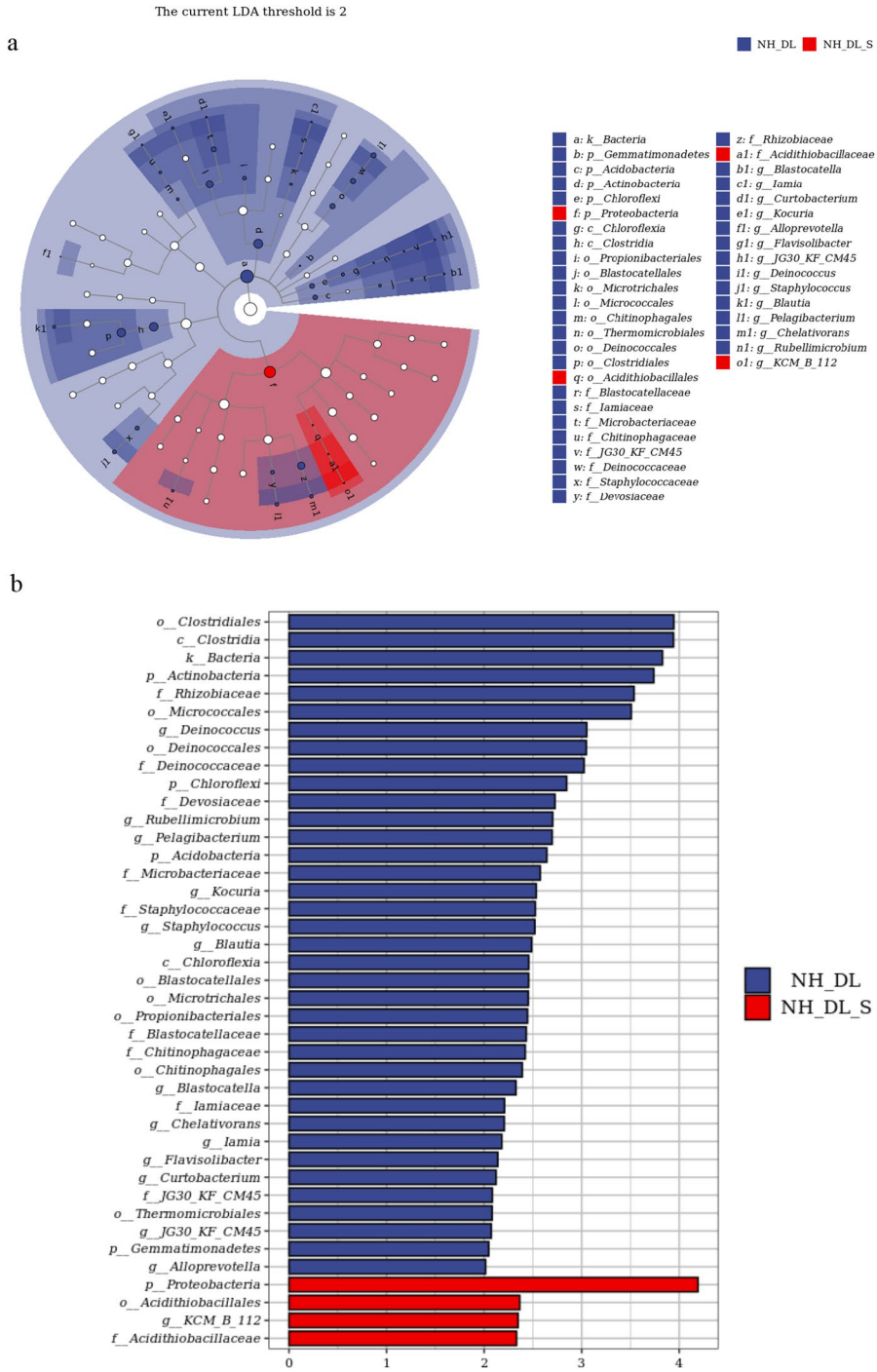

Fig. 8 LEfSe of differences in community composition. **a** The branching species taxonomic diagram (cladogram) shows the taxonomic hierarchy of the major taxonomic units in the sample communities from phylum to genus (from the inner to the outer circles). **b** Histogram of the distributions of LDA values for significantly different species, showing the species that are significantly enriched within each group and their level of importance

that is commonly found in water and soil and can survive in a wide range of temperatures, low pH, and dry environments (Chen et al., 2021). *Staphylococcus aureus* in an aerosol form causes abscesses (Masalha et al., 2001) and may adhere to the skin (Eames et al., 2009). Although these genera may include nonpathogenic species, virulent pathogens can cause low-abundance disease; therefore, chronic exposure to opportunistic pathogens may be harmful to public health (Zhen et al., 2019).

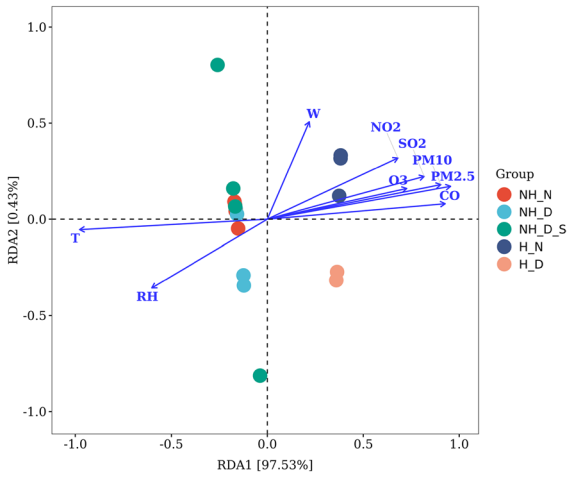

Fig. 9 Redundancy analysis showing the relationships between environmental factors and airborne bacterial community composition

4 Discussion

In this study, we examined the abundances of bacterial species during hazy and non-hazy periods, at the bottom and top of buildings, during the day and at night, and in rural and urban areas by high-throughput sequencing. The results highlighted the changes in the bacterial community characteristics under different meteorological and environmental conditions. However, the factors involved in influencing the diversity and abundance of airborne particulate matter bacteria remain unclear. We collected a higher diversity of bacterial communities in PM2.5 during the hazy period than during the non-hazy period; atmospheric flow plays a crucial role in the occurrence and persistence of haze, and compared to southerly air currents, northerly winds are a favourable meteorological condition for pollutant accumulation (Pan et al., 2018). Haze in the Guilin area is facilitated by inland air transport from the north (Zhang et al., 2017). Stable atmospheric circulation may lead to the accumulation of pollutants near the surface and to the accumulation of microorganisms (Lu et al., 2018; Wang et al., 2019). Spatial variability was also observed during the non-hazy period, with high diversity observed in urban areas and low diversity observed in suburban areas. One reason for this spatial variability may be human activity, with the degrees of plant and soil cover leading to site-specific biological sources and contributions in different locations (Bowers et al., 2011a). Another possible reason is the high concentration of gaseous pollutants in urban areas resulting from urbanisation, as these pollutants can provide large amounts of nutrients for bacteria and promote the survival of microorganisms. The increase in some chemical components may also inhibit the growth of some microorganisms, leading to changes in community structure (Gou et al., 2016; Sun et al. 2018).

Bacterial abundance and community diversity varied considerably between periods, as shown in Figs. 2 and 3. The average temperature during the hazy period was 20 °C lower than that during the non-hazy period (Table S2). In general, low temperatures were detrimental to microbial growth and multiplication, in contrast to suitable temperatures that favoured the growth and multiplication of bacteria and fungi. However, this is contrary to our findings on species richness. We suspect that during the warm season, the overgrowth of relatively dominant microbial populations inhibits the growth of nondominant bacterial communities (Bai et al., 2021; Gao et al., 2017). At low temperatures, inhibition or competition between

Table 1 Relative abundances of pathogenic bacteria

	Acinetobacter	Pseudomonas	Streptococcus	Staphylococcus	Serratia	Fusobacterium
Hazy urban areas	0.55%	48.60%	0.14%	0.07%	0.01%	0.18%
Non-hazy urban areas	0.59%	0.87%	0.02%	0.06%	0.06%	0.01%
Non-hazy suburban areas	1.50%	1.33%	0.04%	0.02%	0.05%	0.01%
Xi'an (Lu et al., 2018)	0.39%	25.59%	0.46%	0.04%	0.02%	–
Xiamen (Li et al., 2019)	11.44%	10.97%	0.34%	8.73%	–	–
Beijing (Chen et al., 2021; Du et al., 2018)	0.10%	8.16%	0.26%	0.13%	–	–

"–" Means no record

different species of microorganisms is weak, allowing more species to coexist in the community. In addition, bacteria can remain active at low temperatures by increasing their membrane fluidity (Kumar et al., 2002). Wind speed and relative humidity are important factors in the release and spread of airborne microorganisms from various sources (Smets et al., 2016). Higher wind speeds carry more microorganisms into the near-surface atmosphere and favour their suspension, which leads to higher concentrations of microorganisms (Savage et al., 2012). However, it has also been shown that strong air currents dilute the concentration of bacteria (Hara & Zhang, 2012; Jeon et al., 2011). In addition, excessive humidity causes moisture to adhere to the particulate matter causing it to increase in weight and volume, accelerating the rate of settling of the particulate matter and thus reducing the suspension of microorganisms in the air. Relative humidity, on the other hand, mainly affects microbial content, with most studies finding that 40–60% humidity promotes the growth and reproduction of microorganisms (Fröhlich-Nowoisky et al., 2014). It has also been noted that high humidity also favours the growth and reproduction of bacteria, but reduces their activity (Mouli et al., 2005). However, precise identification of the environmental factors that actually affect the structure of airborne bacterial communities is challenging, since their variations are often tightly related to seasonality. In this way, the effects of each environmental factor are difficult to disentangle from those of each season considered as a whole. To date, only a few studies have been able to identify a single meteorological factor that affects the structure of microbial communities in the atmosphere (Gandolfi et al., 2015). Consequently, aside from field monitoring, laboratory chamber bioaerosol studies are necessary to determine how a single environmental factor affects bioaerosol (Lu et al., 2018).

Proteobacteria, *Actinobacteria* and *Firmicutes* were the dominant bacteria in the hazy phase; these bacteria have been found to be widespread in soil, water and the atmosphere and can survive in extreme environments, tolerating harsh conditions such as low temperatures, dryness and high levels of ultraviolet radiation (Chen et al., 2021; Wei et al., 2020). Therefore, we speculate that species with good resistance can exist in an atmospheric environment with different pollution levels for a long time and occupy a dominant position. The dominant bacterium in the non-hazy phase was *Sphingomonas*, which belongs to the group of alpha-anamorphic bacteria, known to be associated with plants and possessing a high degree of environmental adaptability, having been shown to survive in nutrient-poor environments (Eguchi et al., 2001). The large abundance of bacteria such as *Sphingomonas* further supports the finding that plant sources have a strong influence on the composition of airborne bacterial communities during the non-hazy period when the air is cleaner and the community structure is homogeneous. *Sphingomonas* includes more than 20 species that are very diverse in terms of phylogenetic, ecological and physiological characteristics (Asaf et al., 2020; Balkwill, Fredrickson and Romine 2003). Such bacteria may come not only from the local environment but also from long-distance air transport from marine or coastal areas. This could partly explain the high levels of *Sphingomonas* during the non-hazy period, and the high presence of this type of bacteria in the atmosphere has been reported in other studies (Franzetti et al., 2011; Gou et al., 2016; Park et al., 2020; Xu et al., 2021).

As the sampling site was located in an old town with no tall buildings nearby, high sampling was conducted on the top floor (seventh floor) of a hotel surrounded by low-rise residential buildings. Bacteria in the air at high levels not only come from a large number of ground-level emission sources but are also greatly influenced by the long-distance transport of air currents (Weger et al., 2016). Within the present study, the variability of samples collected near the ground during the hazy period was high, which was correlated with human activity levels. Variability in bacterial communities lags behind environmental changes to a certain extent (Xie et al., 2021). Human activity is more frequent during the day and has a stronger impact on the bacterial community during the day than at night (Bowers et al., 2013). Studies have shown that the diurnal distribution of different microbial species also varies, for example, *Aspergillus* shows a bimodal distribution at 10:00 and 20:00, and the concentration of *Penicillium* and *Cladosporium* reaches a maximum at 20:00 h (Lin & Li, 2000). There was no significant variability in bacterial community structure during the non-hazy period either in terms of the height difference or time of day, suggesting that clean air makes bacterial communities more stable. Environmental changes associated with urbanization, including land use changes, population

and housing densities, agricultural intensification, and changes in wild and domestic plant and animal populations, may affect aerial microbial communities (Bowers et al., 2011a, 2011b; Lymperopoulou et al., 2016; Mhuireach et al., 2016). *Chryseobacterium*, *Acinetobacter*, and *Devosia* have been reported to be the dominant genera in topsoil on poultry farms (Morens et al., 2004; E. Kaczorek et al., 2017; Wu et al., 2019). *Rubellimicrobium*, *Deinococcus* and *Paracoccus* have been reported to dominate the bazaar. *Massilia* is the dominant species in the garden, readily degrading organic matter as a substrate and increasing reproduction rates when there is an adequate source of carbon in the environment, and its presence has been detected in desert and soils (Gou et al., 2017). Gao et al. (2018) studied air microorganisms in five hospitals and found that the distribution of bacterial species in hospitals was similar to that in outdoor environments. *Acidithiobacillus*, belonging to the phylum *Proteus*, is a differential suburban species, and this group of bacterial species appears to be associated with the nitrogen cycle, carbon cycle, sulphur cycle and human pathogens (González et al., 2016).

5 Conclusion

In this study, we focused on revealing the relationship between bacterial community structure and pollution levels in $PM_{2.5}$ in Guilin, analysing the dominant bacteria at different heights and times of day, and assessing the changing characteristics of bacterial communities in urban and suburban areas. Although the $PM_{2.5}$ bacterial communities in different environments shared some common microbial populations, the hazy and non-hazy periods were different in terms of the abundances and types of major taxa. This variation may be related to the different sources of *Proteobacteria* and *Actinobacteria*, which are the most representative taxa. The results of the current study confirm that $PM_{2.5}$ bacterial community structure is influenced more by pollution levels than by time of day or sampling height. Variations in bacterial community structure may be related to bacterial sources, bacterial adaptations and environmental factors. Furthermore, a comparison of the results from the current study and those from previous studies conducted in the region suggests that different bacterial communities can be found in different $PM_{2.5}$ samples, and therefore, more investigative studies are needed to fully explain the structure, temporal variability and potential sources of bacterial communities and to further reveal the ecology of bioaerosols.

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Author's contribution ZWY, JXS, YCT and CQC sampled and TFL performed the laboratory work and wrote the manuscript. ZWY performed part of the laboratory work and revised the manuscript. QH designed the research and revised the manuscript. JHW analysed the data for the work. All authors contributed significantly to the preparation of the manuscript. All authors approved the submission of this manuscript.

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Data availability All relevant data are provided in the manuscript and supplementary tables, and the raw sequences of 16S rRNA were deposited in the NCBI Sequence Read Archive under accession number PRJNA824984.

Declarations

Conflict of interest The authors declare that they have no conflicts of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

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