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Seasonal and Short-term Variations of Bacteria and Pathogenic Bacteria on Road Deposited Sediments

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Abstract

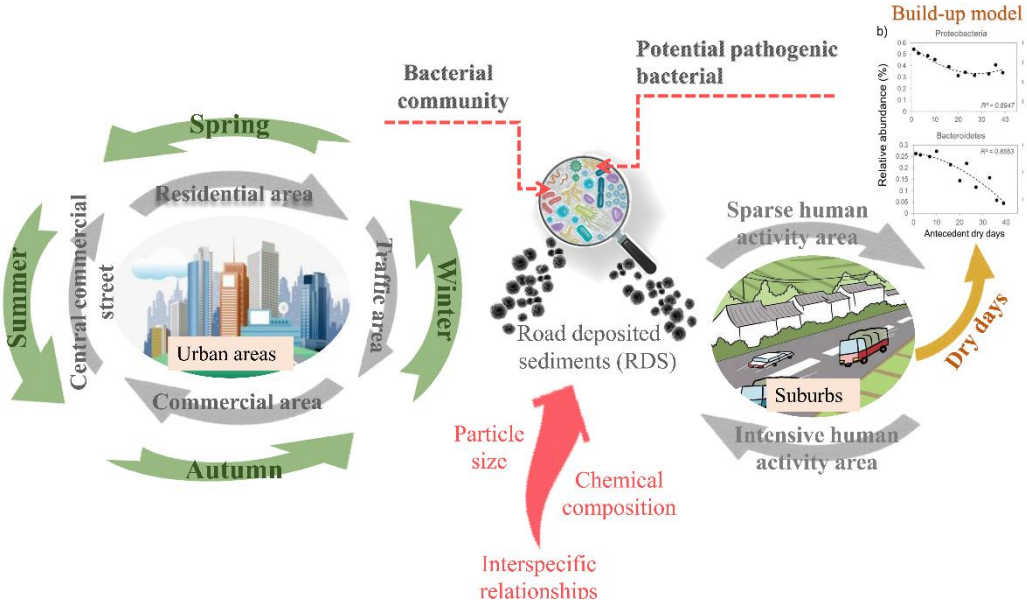
The bacteria (including pathogenic bacteria) attached to road deposited sediments (RDS) may interrelate with the microbe in the atmosphere, soil and water through resuspension and wash-off, and is of great significance to human and ecological health. However, the characteristics of bacterial communities with different time scale on RDS were unknown to dates. Climate change prolonged the dry days between rain events in many areas, making the varied trend of bacterial communities might be more significant in short term. This study revealed the characteristics of bacterial communities on RDS in urban and suburban areas through seasonal and daily scale. The correlations between other factors (land use, particle size, and chemical components) and the bacterial communities were also analyzed. It was found that the season showed a higher association with the bacterial community diversity than land use and particle size in urban areas. The bacterial community diversity increased substantially throughout the short-term study period (41 days) and the variation of dominant bacteria could be fitted by quadratic function in suburbs. In addition, urbanization notably increased the bacterial community diversity, while the potential pathogenic bacteria were more abundant in the suburban areas, coarse RDS (>75 μm), and in spring. The chemical components on RDS showed special correlations with the relative abundance of dominant bacteria. The research findings would fill the knowledge gap on RDS bacterial communities and be helpful for the future research on the assembly process of bacterial communities.

Keywords: Road deposited sediments (RDS), Bacterial communities, Potential

23 pathogenic bacteria, Spatiotemporal variations, Environmental factors.

24

25 **Graphical abstract**



26

1. Introduction

Microorganisms are ubiquitous in nature, with bacteria exceeding 80% of their total abundance[1]. Specially, pathogenic bacteria might increase the probability of disease to human[2]. Road deposited sediments (RDS) arises from tire wear, brake lining dusts, engine exhaust, urban soil, atmospheric deposition, waterbodies (evaporation and settlement), etc. Meanwhile, the RDS with metals, inorganic salts, and organic compounds, was known to affect the air and water environment through resuspension and dispersion by wind, and wash-off by rain, which have received high attention[3-5]. However, the bacteria attached to RDS has been ignored to date, despite its role as both source and sink for bacteria in air and water[6, 7].

The dominant bacteria were highly associated with the specific environment in which they exist[8]. Bacteria in aerosol samples were more diverse than those in water samples at all study sites[9]. Thus, bacterial communities should be also unique on RDS and need to be investigated. Given the disturbance by meteorological factors such as temperature, wind speed and air quality, the surface-bound microbial communities in aerosols and air particles often varied in short time[10]. Therefore, meteorology factors could better explain the community variability of aerosol bacteria than geographical distance in short time reported in previous studies[11, 12]. Airborne bacteria could affect the microbial communities on road surfaces through dry and wet precipitation[2, 13]. Thus, we hypothesized that the bacterial communities on RDS might varied significantly both in seasons (as large temporal scale) and days (as small temporal scale), and a detailed study was conducted. This was vital for designing the sampling method when

comparing the microbial differences between sites.

In addition, environmental factors, like land use, particle size, urbanization, and other chemical elements, may also show a correlation with the abundance of bacteria and pathogenic bacteria[10, 14-16]. Similar studies on the dominant bacteria in the atmosphere showed obvious abundance distinctions in hospital, coastal, or city core areas[17]. *Bacillus, Sphingomonas* and *Staphylococcus* were common bacteria identified in residences[18]. Particle size has also been a crucial factor in microbial diversity and selectivity[19-21]. Airborne bacteria were more likely to attach to fine particles, such as $PM_{2.5}$, and even smaller particles that have a greater impact on the human body[22, 23]. However, the size-distribution patterns were distinct under different circumstances[24, 25]. In the warm season, the bacterial concentration was higher on particles with aerodynamic diameter between 1.1 and 2.1 μm, while bacteria were concentrated on coarse particles (>7 μm) in the cold season[26]. Due to the lower temperature and higher wind speed in winter, especially in coastal cities, it was supposed that compared with fine particles and single bacteria, the continuous agglomeration of fine particles to form larger particles was more conducive to the survival of bacteria in the atmosphere. Researchers found that most bacteria were present in the coarser particles in the atmosphere in Sweden[27], United States[28] and China[29]. Therefore, the land use and particle size may also account for the bacterial community diversity on RDS which are worth studying.

Urbanization was characterized with the variations of land use, population, green area and demographic shift[30]. Study has found that urban, accompanied by high

anthropogenic pressures, less green areas and intensive industrial activity, could shape urban microbiomes[30]. And the relative abundance of airborne pathogenic bacteria also increased with urbanization[30]. In addition, it has been demonstrated that the specific bacteria on tree leaf in urban environments differed from those in non-urban environments, and the feedback between urbanization pressures and plant microbiomes might also affect urban microbiomes[31]. Certainly, the bacterial communities on road surfaces should also belong to urban microorganisms. Therefore, we supposed that urbanization would also associate with the bacterial communities on the road surface. Moreover, whether it was in near-surface groundwater[32], surface water[4], drinking water[33], soil[34], or atmospheric particles[17, 30], chemical components have been shown different correlations with the microbial communities. Studies also found that chemical components in the road particles would continue to accumulate, and the concentration was high[14, 35, 36]. The maintenance of the green belts on both sides of the road requires periodic fertilization. And the accumulation of nitrogen and phosphorus on the road surface have been proven[35, 37]. Traffic vehicles (brake lining dusts, tire wear, etc.) and business-related activities continue to emit major heavy metals (Cu, Zn, Pb, Cr, Mn, Fe) and organics to the road[38, 39]. Therefore, it is necessary to analyze the correlations between the chemical components and the dominant bacteria on road surface in depth.

In this study, a detailed and comprehensive investigation of bacterial community diversity and abundance on RDS was carried for the first time considering both seasonal scale and daily scale. The primary research included: 1) assess the variation characteristics of dominant bacteria in different seasons and days; 2) identify the

93 importance of season, land use, urbanization, and particle size on the diversity and
94 abundance of bacteria including pathogenic bacteria; 3) elucidate the correlations
95 among the dominant bacteria, and the correlations between the chemical components
96 and the dominant bacteria. We expect that the study would fill the current knowledge
97 gap in bacterial communities on RDS, and enhance the risk control of potential
98 pathogenic bacteria. This will also help for future research on the interaction and
99 migration process of bacteria among different environments (source and sink).

100 ## 2. Materials and Methods

101 ### 2.1 Sampling Methods

102 RDS were collected in urban and suburban areas of Tianjin, China, a typical
103 megacity in Northern China with more than 15.6 million people. The sampling schedule
104 was divided into two portions according to the study objective. At first, for the purpose
105 of investigating the variation of bacterial communities in season scale, four sites with
106 different land use types in urban area were selected, namely residence area (JM), traffic
107 area (JT), commercial area (SY) and central commercial street (BJ). RDS were
108 collected over an annual cycle from winter 2016 to autumn 2017 (C: spring, S: summer,
109 A: autumn, W: winter). The specific sampling sites are shown in Figure 1 and the
110 detailed characteristics of each sampling site are shown in Table S1. The detailed
111 sampling times are shown in Table S2. Due to the laboratory restrictions, valid samples
112 in the central commercial street were only available in winter and spring. Thus, a total
113 of seventy samples were collected. The exhaustive method for sampling and
114 pretreatment can be seen in the supplementary information (SI).

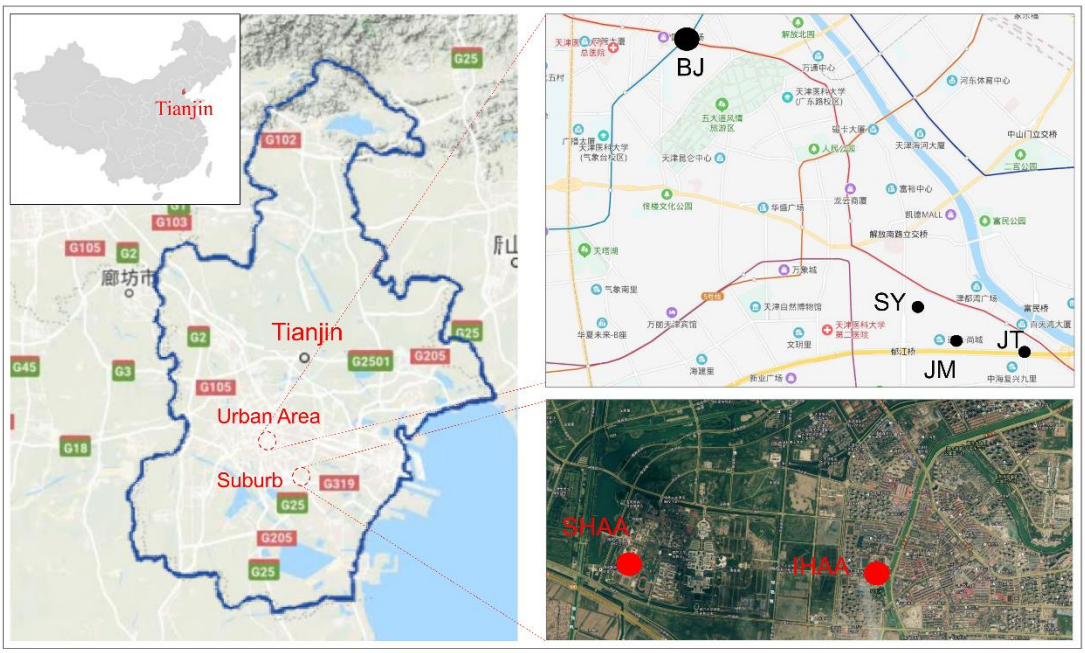


Fig. 1. A vertical view of the studied regions and sampling sites at different spatial resolutions. Four urban sites and two suburban sites were displayed in the map.

Secondly, another two study sites with obvious regional differences were selected in the suburbs, namely sparse human activity area (SHAA: campus area) and intensive human activity area (IHAA: mixed commercial and residential areas). The regional characteristics were also described in Table S1. After comparing the differences of bacterial community diversity and abundance between urban and suburban areas, suburban areas with less urbanization and external disturbance were selected for further investigating the variation of dominant bacteria in daily scale. The study was carried out in October and November (Autumn) and started immediately at the end of a rain event. The sampling was lasted for 41 days with an interval of 2-3 days, and there was no effective precipitation during the study period. Finally, a total of 24 samples in two sites were obtained.

2.2 DNA Extraction, PCR Amplification and 16S rRNA Gene Amplicon

130 **Sequencing**

131 PowerSoil® DNA Isolation Kits were used to extract DNA from RDS following
132 the manufacturer's instruction, using a Vortex-Genie 2. The DNA extracts were kept at
133 −80 °C, and used for the amplification and subsequent sequencing of a region of
134 bacterial 16S rRNA genes. The detailed methods for PCR Amplification and 16S rRNA
135 Gene Amplicon Sequencing were shown in SI. The bacterial communities were
136 analyzed at the phylum level and order level, and the distribution characteristics of
137 potential pathogenic bacteria at the genus level and species level were studied.

138 **2.3 Chemical Analysis**

139 The heavy metals (Fe, Mn, Pb, Zn, Cu, Cr), inorganic salts (NH_3^+-N, NO_3^-N, TP)
140 and total organic carbon (TOC) on RDS (unit: mg/kg) were tested to determine the
141 correlations between the chemical components and the dominant bacteria. The testing
142 process was described in the SI.

143 **2.4 Statistical Analysis**

144 Beta diversity analysis, alpha diversity analysis (Shannon index, Simpson index,
145 chao 1), AMOVA (analysis of molecular variance), ANOSIM (analysis of similarities),
146 Mann-Whitney U test, T-tests and Principal Coordinate Analysis (PCoA) was used to
147 evaluate the differences of community structure. The statistical description was used to
148 analyze the variation of the OUTs number in study areas. The quadratic function was
149 selected to verify whether the variation trend of relative abundance of dominant bacteria
150 at phylum level and order level in short term could be predicted. Canonical Correlation
151 Analysis (CCA) was performed to explore the correlations between the dominant

bacteria and the studied environmental factors (season, land use, chemical elements, etc.). Spearman correlation analysis was used to investigate the correlations among dominant bacteria. The detailed methods for the statistical analysis were shown in SI.

3. Results and Discussion

3.1 The Variation Characteristics of Bacterial Communities on RDS at Seasonal Scale

The microbial data about relative abundance from urban sites was divided into four groups based on the season, then divided into four groups based on the land use, and two groups based on the particle size (1: > 75 μm and 2: < 75 μm). According to the ANOSIM and AMOVA analysis (Tables. S3-S6), the season showed the most remarkable association with the variation of bacterial abundance, followed by the land use and particle size. The significant difference ($p < 0.001$*) of bacterial abundance among seasons also indicated that the season played a critical role (Table S4).

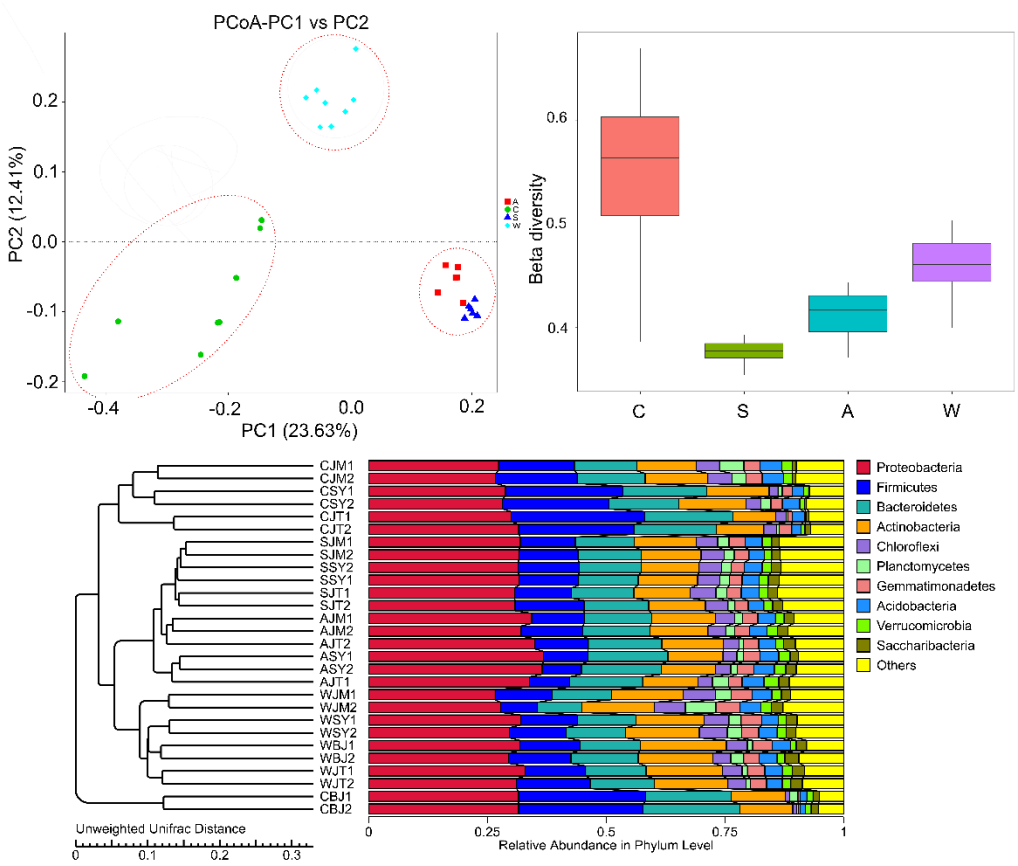


Fig. 2. Seasonal differences in bacterial community among samples by Principal Co-ordinates Analysis (PCoA) based on Unweighted Unifrac (a), boxplot of beta diversity differences based on Unweighted Unifrac (b), and the Unweighted Pair-group Method with Arithmetic Mean (UPGMA) for visualization of the similarity among samples (c).

The similarity of the community structure in different season was displayed according to the Principal Component Analysis (PCoA) and the cluster analysis (UPGMA) (Figure 2). Obviously, the bacterial communities were unique in winter and spring, respectively, which differed from those in summer and autumn at all study sites (Figure 2a and 2c). The number of OTUs (the operational taxonomic units with 97% sequence identity) (Figure S1) and alpha diversity index (Shannon index, Simpson index, chao 1) (Table S7) in summer and autumn was significantly larger than that in

177 winter and spring, which indicated a higher bacterial community diversity ($p < 0.001$*,
178 Table S4)[15, 40]. Moreover, the beta diversity differences (Mann-Whitney U test, $p <$
179 0.001*) among seasons were shown in Figure 2b, and the boxplot could intuitively
180 display the community similarity within the groups. And a significant community
181 discrepancy within different land uses was observed in spring[41]. This was also
182 confirmed by the high difference coefficient between spring and other seasons, as
183 shown in the β diversity index heatmap (Figure S2). It was inferred that the temperature
184 in summer and autumn was much fitter for the microbes, and varied gently during the
185 season transition period, so that a more abundant bacterial community diversity and
186 similar community structure were presented in summer and autumn[40]. This was not
187 consistent with the findings from Li et al. [30] who observed that the highest spatial
188 variations of bacterial communities were in summer in Xiamen, China. One of the
189 possible reasons was that the temperature variation in different study areas was not
190 accordant. Xiamen has a high temperature throughout the year, but Tianjin has a large
191 temperature fluctuation in different seasons. Certainly, other anthropogenic activities
192 might also be important influencing factors.

193 In addition, the OTUs number (Figure S1) and alpha diversity index (Shannon
194 index, Simpson index, chao 1) (Table S8) also showed significant distinctions among
195 different land uses. The largest OTUs number and alpha diversity index in spring,
196 autumn and winter were all in the residential area, while the traffic area had the largest
197 OTUs number and alpha diversity index in summer. The highest bacterial community
198 diversity in the residential area indicated that the bacterial communities were strongly

associated with higher human density and living habits. Moreover, the bacterial community diversity in the commercial area and the central commercial street, with high business activities, was higher in winter than in spring. This might relate to more frequent hand sweeping and effective cleaning practices (i.e., sweeper-washer vehicle) in spring in study sites. Therefore, the accumulated particles on road surface were reduced in spring, consequently reducing the accumulation and diversity of bacteria.

In general, the dominant bacteria at phylum level included Proteobacteria (30.3%-62.5%), Bacteroidetes (3.2%-51.5%), Firmicutes (2.6%-35%), Actinobacteria (2.7%-22%), Acidobacteria (9%), Fusobacteria (8.7%), Cyanobacteria (6.5%) and Thermomicrobia (6.1%) (Figure S3). No obvious differences were observed in dominant bacteria (top 10) at phylum level among different land uses and seasons, while the relative abundance of Firmicutes and Bacteroidetes were significantly increased in spring (Figure S3). This was consistent with previous study that the dominant bacteria at the phylum level were roughly similar across an urban environment, although the relative abundance varied[12, 17]. However, the significant differences of the dominant bacteria were observed at the order level (T test, $p < 0.05$) (Figures S4 and S5). Rhodocyclales in summer, Burkholderiales in autumn, and Rhodobacterales in winter exhibited higher abundance than other bacteria, and the differences between different seasons ($p < 0.05$) are also displayed in Figure S6. And all of them were classified as Proteobacteria (phylum level). Proteobacteria are Gram-negative bacteria, and their resistance to most antibiotics makes them resilient in the environment[42]. Burkholderiales, including many potential pathogenic bacteria, bring a great threat to

221 livestock and human health in autumn. Besides, Bacteroidales in spring which belongs
222 to Bacteroidetes (phylum level) showed higher abundance than other bacteria.
223 Lactobacillales as Firmicutes (phylum level) mainly appeared on RDS in winter. It has
224 been suggested that high temperature was detrimental to the growth of Firmicutes[16].

225 Furthermore, an interesting phenomenon was that the number of OTUs on coarse
226 particles was greater than that on fine particles. This was contrary to metal and other
227 pollutants where the higher contents were accumulated on fine particles[4, 35]. The
228 pollutant concentration was defined as the pollutant contents in unit mass of particles.
229 The smaller the particle size, the larger the specific surface area, which was more
230 conducive to ion adsorption[43]. Therefore, the concentration of pollutants in fine
231 particles was relatively higher. However, the size of bacteria was larger than ion and
232 molecule, and coarse particles were more beneficial to the enrichment of different types
233 and sizes of bacteria[25, 44]. Although less previous studies have been conducted on
234 bacterial community diversity on RDS, results in this study were similar to the bacterial
235 community diversity observed in air and water particles. The bacterial community
236 diversity on coarse particles (PM_{10}-$PM_{2.5}$) was approximately 80% higher than that
237 observed in fine particles ($PM_{2.5}$)[40]. Particle-attached bacterial communities in rivers,
238 coastal areas, and open seas were usually more diverse than free-living bacterial
239 community diversity[19, 45]. On the basis of the discussion above, season as a factor
240 explained most variability of the bacterial community diversity and relative abundance
241 on RDS, though significant variation also displayed among different study sites and
242 different size of particles. In addition, this study mainly discussed the impacts of part

deterministic factors on the bacteria communities. However, the bacteria assembly was affected by both deterministic and stochastic processes[32, 46-48]. This will be further investigated in our future research.

3.2 Effects of Urbanization on Bacterial Communities on RDS

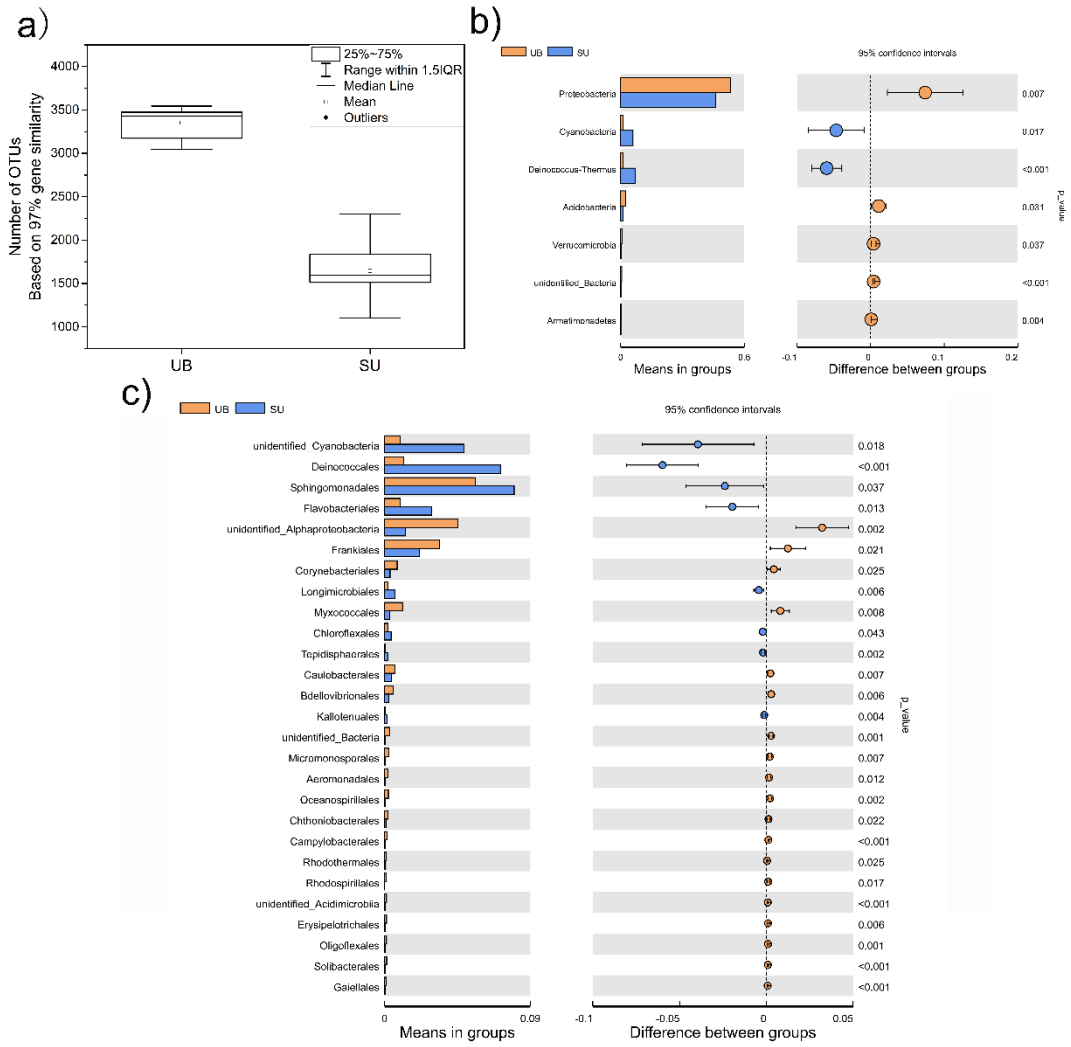


Fig. 3. a) Box-plot of the number of OTUs determined on RDS collected from urban (UB) and suburban (SU) areas; Differences analysis of bacterial species between groups based on T-test at phylum level b), and order level c).

The effects of urbanization on bacterial communities in urban and suburban areas were further investigated in autumn. It was observed that the number of OTUs (Figure

253 3a) and alpha diversity index (Table S9) in urban areas was remarkably higher than that
254 in the suburbs which meant that urbanization notably increased the bacterial community
255 diversity on RDS. Human activities, plants, and industrial activities related to
256 urbanization have been reported to have a prominent association with microbial
257 communities[30, 34]. Liddicoat et al.[49] found that the OTUs showed noteworthy
258 differences in human-altered and natural soil environments. In addition, the bacterial
259 abundance also showed significant differences between the urban and suburban areas
260 according to the ANOSIM analysis (R = 0.3609), the AMOVA analysis (p < 0.001*)
261 and the PCoA analysis (Figure S7).

262 Proteobacteria, Actinobacteria and Bacteroidetes were the dominant bacteria at the
263 phylum level in both urban and suburban areas (Figure S8a). The relative abundance of
264 Proteobacteria were greater in urban areas than in suburbs, with significant differences
265 calculated by T-test (p<0.01) (Figure 3b). The relative abundance of Cyanobacteria and
266 Deinococcus-Thermus were higher in suburbs. Cyanobacteria was widely distributed,
267 mainly in freshwater and seawater, and also found in soil, tree trunks and leaves[50, 51].
268 The higher relative abundance of Cyanobacteria in the suburban areas might be related
269 to the larger green areas around the study sites in the suburbs. Furthermore,
270 Gammaproteobacteria, Pseudomonadales, Micrococcales, Rhodobacterales,
271 Sphingomonadales, and Cytophagales were the dominant bacteria at order level in both
272 study areas (Figure S8b). However, the relative abundance of Cyanobacteria,
273 Deinococcales, Sphingomonadales, Flavobacteriales and Longimicrobiales were
274 greater in suburban areas, while the relative abundance of Alphaproteobacteria,

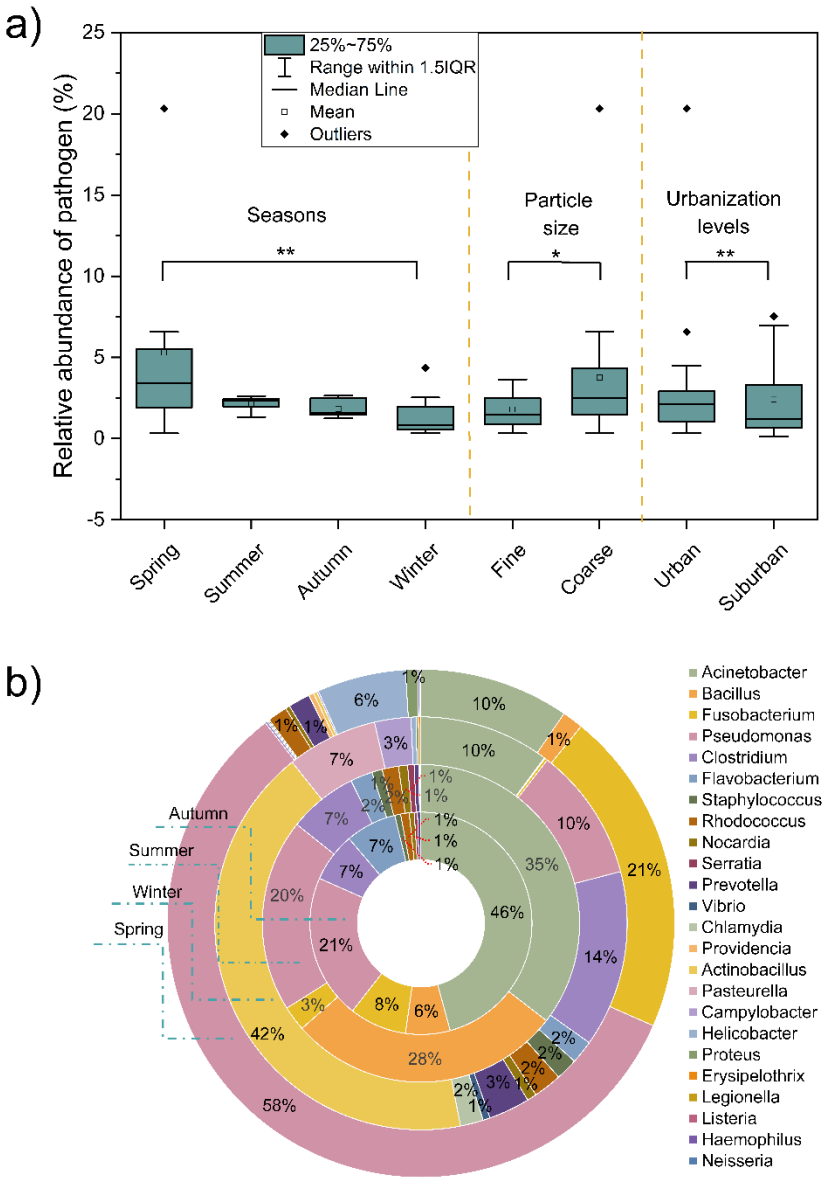
Frankiales, Corynebacteriales and Myxococcales were higher in urban areas, with significant differences (T-test, $p<0.01$). Therefore, urbanization showed a noteworthy association with the bacterial community diversity and abundance on RDS.

A plausible speculation for the variation of bacterial community diversity and abundance affected by urbanization included human activity, plants, air quality, etc. The size of green area on both sides of the road at the sampling sites in the urban area was small, and the plants were mainly holly and sycamore. While the size of green area at the sampling sites in the suburbs was large (Table S1), and there were many types of plants, including Chinese locust tree, ash tree, phoenix tree, armeniaca mume, and willow trees. Different species of plants and soil environments might have different correlations with the bacterial community diversity on RDS[15, 17]. The intensive human activity and large traffic flow in urban areas, such as hospitals and shopping malls, might generate a lot of different kinds of garbage and sewage. Thus, the bacterial community diversity and abundance might increase significantly, even it has a higher management level[52, 53]. The air quality might also play an important role. Studies found that the concentrations of total bacteria and the abundance of bacterial genera increased when air pollution became severe, then might indirectly interfere with the microbial communities on the road surface through wet and dry precipitation[42, 54-56]. Our previous study found significant differences in bacterial abundance between upper and lower snow samples on road surfaces, confirming that precipitation was an effective route for air-bound bacteria to affect microbes on road surface[57]. In addition, the distinction in road cleaning and the use of reclaimed water in regions might become key sources for

297 the bacterial communities on RDS[9, 53]. We supported the idea that the synergistic effects
298 caused by urbanization (i.e., population, traffic volume, human activity, green area, etc.)
299 promoted the transformation of bacterial communities. The contributions of
300 urbanization on the microbial community deserve further study.

301 **3.3 Potential Pathogenic Bacteria at Genus/Species Level on RDS**

302 The enrichment of potential pathogenic bacteria on RDS might induce risks to
303 human health. Potential pathogenic bacteria at genus and species levels were identified
304 according to the "*Directory of Pathogenic Microbes Infecting Humans*", formulated by
305 the ministry of health of the People's Republic of China, which has been referred in
306 many studies[58-60]. The potential pathogenic bacterial communities on RDS exhibited
307 differences between seasons, urbanized areas (p<0.01) and different sized particles
308 (p<0.05) (Figure 4a). Potential pathogenic bacteria were most abundant in spring and
309 approximately 5.3% of all sequences were identified, which was consistent with the
310 conclusion by Yamamoto et al.[61]. While the relative abundance of potential pathogenic
311 bacteria in summer (2.2%), autumn (1.8%), and winter (1.4%) was less varied. Similar
312 to the analysis of OTUs number, the relative abundance of potential pathogenic bacteria
313 in coarse particles was also greater than that in fine particles. Thus, ensuring effective
314 daily road sweeping can reduce the accumulation of coarse particles, thereby reducing
315 the risk of potential pathogenic bacteria to a certain extent. Compared to urban areas,
316 the relative abundance of potential pathogenic bacteria in suburbs was higher.



317

318 Fig. 4. a) Relative abundance of potentially pathogenic bacteria identified in the microbial

319 communities; b) The proportion of each potentially pathogenic bacterial genera in the different

320 seasons. * indicates significant differences between samples at $p < 0.05$, ** indicates significant

321 differences between samples at $p < 0.01$ based on AMOVA analysis.

322 Twenty-four pathogenic bacteria genus were detected on RDS in different seasons

(Figure 4b), and the top 10 predominant pathogenic bacteria at species level were shown in Table S10. Obviously, the relative abundance of potential pathogenic bacteria varied with seasons. The relative abundance of *Pseudomonas* (58%) and *Fusobacterium* (21%) were higher in spring than in summer (20%, 3%), autumn (21%, 8%) and winter (10%, 0.2%). *Actinobacillus* (42%) and *Clostridium* (14%) were the predominant pathogen genera in winter. *Acinetobacter* (35%), *Bacillus* (28%) and *Pseudomonas* (20%) in summer, and *Acinetobacter* (46%) and *Pseudomonas* (21%) in autumn were the predominant pathogenic bacteria genus. *Acinetobacter* and *Pseudomonas* were often detected in atmospheric particles in previous studies[41, 42]. *Acinetobacter*, as an important opportunistic pathogen that causing nosocomial infections, could induce respiratory infections, sepsis, wounds and skin infections. *Pseudomonas* is also one of the main pathogenic bacteria causing nosocomial infections. Besides, the major potential pathogenic bacteria species in spring included *Fusobacterium mortiferum* (20.7%), *Pseudomonas veronii* (36.9%) and *Pseudomonas fragi* (14.8%). *Acinetobacter lwoffii* were the predominant pathogenic species in summer (44.1%) and autumn (31.5%), followed by *Fusobacterium mortiferum* (8.3%) and *Bacillus aryabhattai* (20%), respectively. According to the directory of pathogenic microorganisms, the harmfulness of *Acinetobacter lwoffii* belonged to the third category (total of three categories and the first category is the most pathogenic microorganisms). In winter, the predominant pathogenic bacteria species on RDS were *Pseudomonas stutzeri* (46.8%) and *Actinobacillus minor* (15%). In addition, *Acinetobacter* (44%) and *Flavobacterium* (43%) were the predominant pathogenic

345 bacteria genera at suburban sites (Figure S9). *Flavobacterium* is opportunistic pathogen
346 that could cause pneumonia. *Acinetobacter lwoffii* (42.5%) and *Flavobacterium*
347 *johnsoniae* (18.7%) were detected as the predominant pathogenic bacteria species at
348 suburban sites. In this context, different types of potential pathogenic bacteria could
349 enrich in areas with different urbanization gradients, which deserves further research
350 attention.

351 **3.4 The Variation of Bacterial Communities at Daily Scale**

352 Considering that the bacterial community diversity might alter in a continuous
353 short-term period, the bacterial communities was observed more than 40 consecutive
354 dry weather days in autumn[12]. Two study sites with distinctive characteristics (Figure
355 1) were selected in the suburbs. The bacterial communities exhibited significant
356 differences between the two study areas according to the AMOVA analysis (p <0.001*).
357 After a heavy rain, most of particles accompanied with pollutants and microorganisms
358 were washed away by runoff and only a small part of them remained on the road
359 surface[21, 35, 62]. Thus, the sampling started immediately after a rain event, and with the
360 accumulation of dry days, the number of OTUs at the two study sites increased
361 gradually (Figure 5). Therefore, it could be concluded that the bacterial community
362 diversity raised at daily scale. Especially at the IHAA site with intensive human and
363 commercial activities, the variations were even more prominent. This could be
364 confirmed according to the variation range of OTUs number in the study period (Table
365 S11). This might be driven by different pollution sources from the surrounding
366 environment, including human, plants, animals, vehicles, atmosphere, soil, etc.[21, 42]. In

a previous study, bacterial community diversity was higher in parks due to the large green areas[63], while in this study, the IHAA site with intensive human activities was observed to have higher bacterial community diversity than the SHAA site with large green areas.

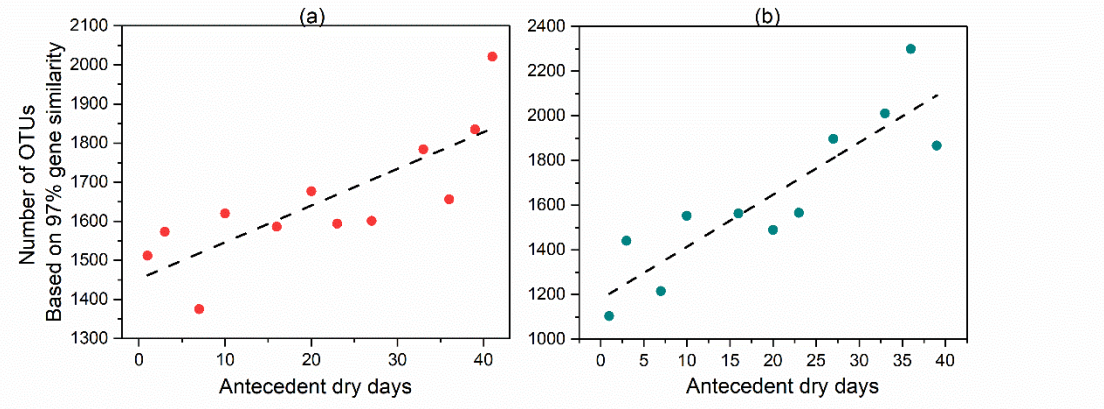


Fig. 5. The variation of the OTUs number on RDS with cumulative time at the sparse human activity area (SHAA) (a) and the intensive human activity area (IHAA) (b) in the suburban areas.

The variations of the dominant bacteria at phylum level and order level were fitted to the quadratic function to determine if the observed abundance patterns varied predictably with time (Figures 6 and S10). The relative abundance of Proteobacteria and Bacteroidetes displayed roughly opposing patterns over time at the two study sites. Proteobacteria decreased slowly at the SHAA site, but decreased rapidly at the beginning and then followed a seemingly rising trend at the IHAA site, which was the opposite inclination as the Bacteroidetes. The decrease of the relative abundances of Proteobacteria and Bacteroidetes might be related to the increase in bacterial community diversity at the two study sites over time. It was also possible that Bacteroidetes were more sensitive to environmental variations (decreasing temperature as autumn progressed). The Actinobacteria and Deinococcus-Thermus first increased

and then decreased over time at the SHAA site. At the IHAA site, the Actinobacteria appreciably increased with time, while the Deinococcus-Thermus fluctuated throughout the study period with no significant trend. The Cyanobacteria and Firmicutes populations enlarged over time at both study sites.

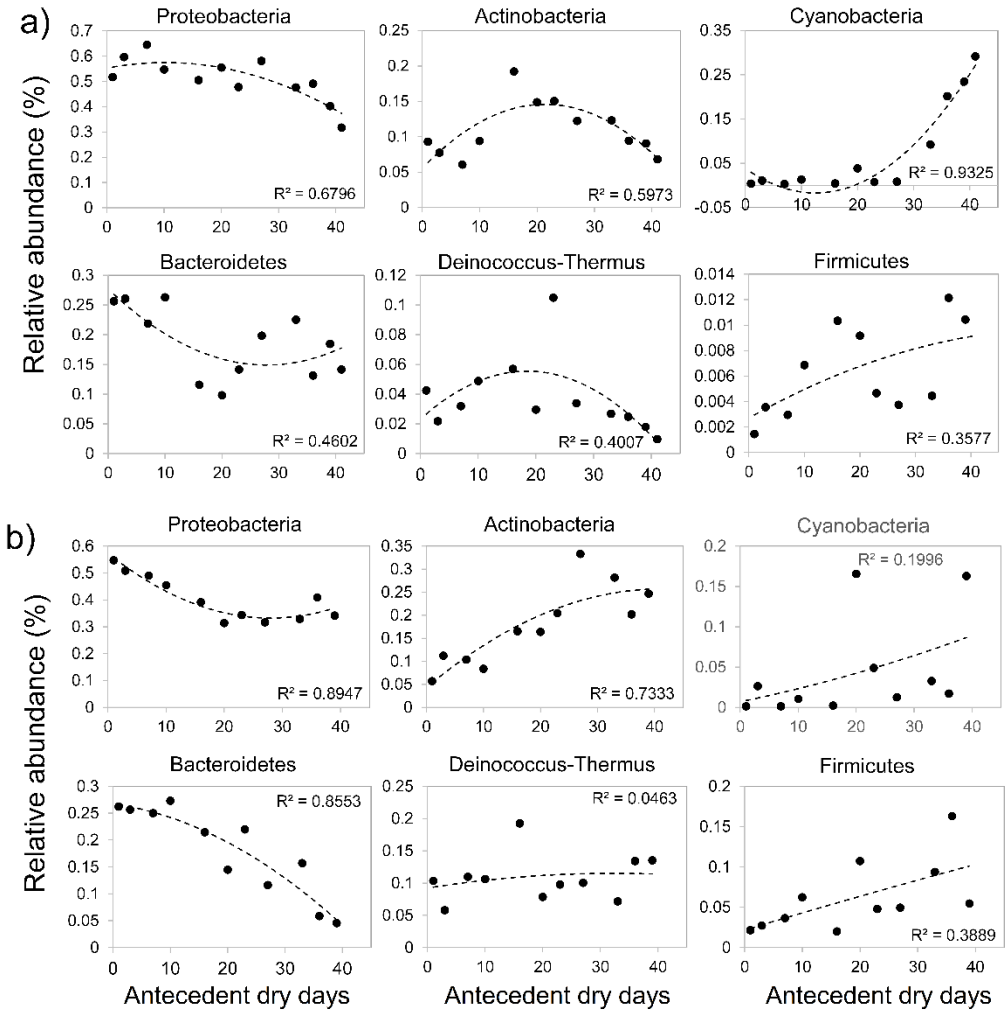


Fig. 6. Temporal variability of the relative abundances of dominant bacteria at phylum level over 41 days where the lines represents the best-fit quadratic function to the data from the SHAA site a) and the IHAA site b).

The quadratic function noted above could also be used to describe the major changes of predominant bacteria at order level over time during the study period (Figure

S10). The dominant bacteria (>1%) at two suburban study areas were significantly different. The relative abundances of Burkholderiales and Cytophagales declined consistently at both study sites. Burkholderiales, with the highest relative abundance at both study sites, contains many potential pathogenic bacteria, and its variation over time in the short-term period were noteworthy. The Sphingomonadales and Deinococcales at the SHAA site increased, while Sphingobacteriales and Rhodobacterales were stable over time. The relative abundance of dominant bacteria at order levels at the IHAA site, including Rhodobacterales, Micrococcales and Bacillales, increased constantly during the study period. Bacillus could form a dormant stage, enabling it to survive in harsh environmental conditions[64]. Increased relative abundance of Bacillales, containing many potential pathogenic bacteria, also increased the potential risks to human health from the RDS. The relative abundance of Pseudomonadales decreased initially and then increased over time. Therefore, the relative abundance of various bacteria could vary considerably even within a short-term period. However, there was less literature on the characteristics of microbial communities on RDS in short term.

3.5 Chemical Components and Interspecific Relationships Affected the Bacterial Abundance

The correlations between the relative abundance of dominant bacteria (top 20) at order level and the considered environmental factors were obtained by Canonical Correlation Analysis (CCA), and are shown in Figure 7. The interpretation rates of the first and second sorting axes were 36% and 39.2% at urban (Figure 7a) and suburban (Figure 7b) study sites, respectively. The season factor presented the highest

417 explanation (18.2 %) to the bacterial relative abundance in urban areas (In the CCA,
418 the values of 1, 2, 3, 4 were assigned to spring, summer, autumn and winter in sequence).
419 This showed positive correlations between winter and the relative abundance of
420 Pseudomonadales, Rhodobacterales, Propionibacteriales and Corynebacteriales, while
421 negative correlations were between winter and the relative abundance of
422 Sphingomonadales, Xanthomonadales and so on. The land use (11.9%) also showed
423 strong correlations with the relative abundance of Sphingomonadales, Clostridiales,
424 Bacteroidales and Rhizobiales. Among the chemical components, the explanation of Fe,
425 Mn, and Cu (35.8%) was higher to the bacterial relative abundance, followed by NO_3-
426 N, NH_3-N and Pb (22.7%). In particular, a strong positive correlation was revealed
427 between the metals (Fe, Mn, Cu and Pb) and the relative abundance of Rhodobacterales,
428 Propionibacteriales and Corynebacteriales. The fact that the four metals were
429 interlinked may be related to the preferential association of Pb and Cu with the oxides
430 of Fe and Mn, which have been noticed by researchers in the past[65]. Compared with
431 suburbs, urban areas have more traffic and commercial activities. Therefore, the
432 cumulative concentration of metals in the RDS may be higher[66], which may have a
433 greater association on the microbial community diversity. Other chemical components
434 (TOC, Cr, TP and Zn) had little difference in explaining the bacterial relative abundance,
435 and were relatively low. In particular, TOC showed strong correlations with the relative
436 abundance of Rhizobiales, Rhodospirillales, Deinococcales, Frankiales, Micrococcales,
437 Sphingobacteriales and Burkholderiales. In addition, the bioavailability of metals is one
438 important aspect in considering the toxicity for bacteria which needs more efforts to

investigate in the future. This could help us to better understand the impact of metal pollution level on the microbial communities.

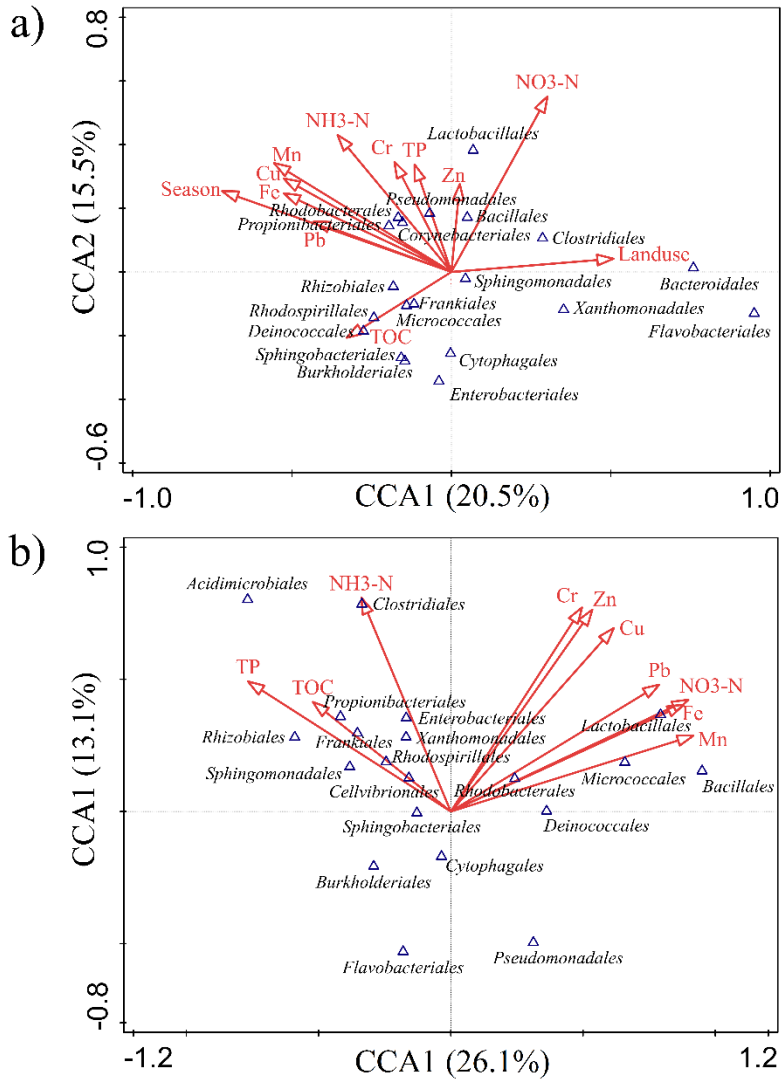


Fig. 7. The CCA analysis between relative abundance of bacteria at order level and chemical composition in the RDS in urban areas a) and suburban areas b).

There was a strong correlation between Zn, Cr, Cu as group 1, and Pb, Fe, Mn and NO_3-N as group 2 in the suburbs, which had positive correlations with the relative abundance of Rhodobacterales, Micrococcales, Bacillales, Lactobacillales and Deinococcales, and negative correlations with Burkholderiales, Cytophagales, and Sphingobacteriales (Figure 7b). However, the higher explanation for the bacterial

449 relative abundance was NO_3-N (27.7%), TP (14%), and NH_3-N (4.5%). The TOC, TP
450 and NH_3-N on the RDS showed positive correlations with the relative abundance of
451 Sphingobacteriales, Cellvibrionales, Sphingomonadales, Enterobacteriales, Frankiales,
452 Clostridiales, Rhodospirillales, Xanthomonadales, Propionibacteriales, and
453 Rhizobiales. This might be associated with the surrounding soil and plants being the
454 sources of these bacteria. The green areas around the sampling sites in the suburbs were
455 comparatively large, so that nitrogen and phosphorus were needed for maintenance.
456 The relative abundance of Rhizobiales was directly related to the plants[67]. Given this,
457 the chemical components may have important associations with the microbial
458 communities on RDS.

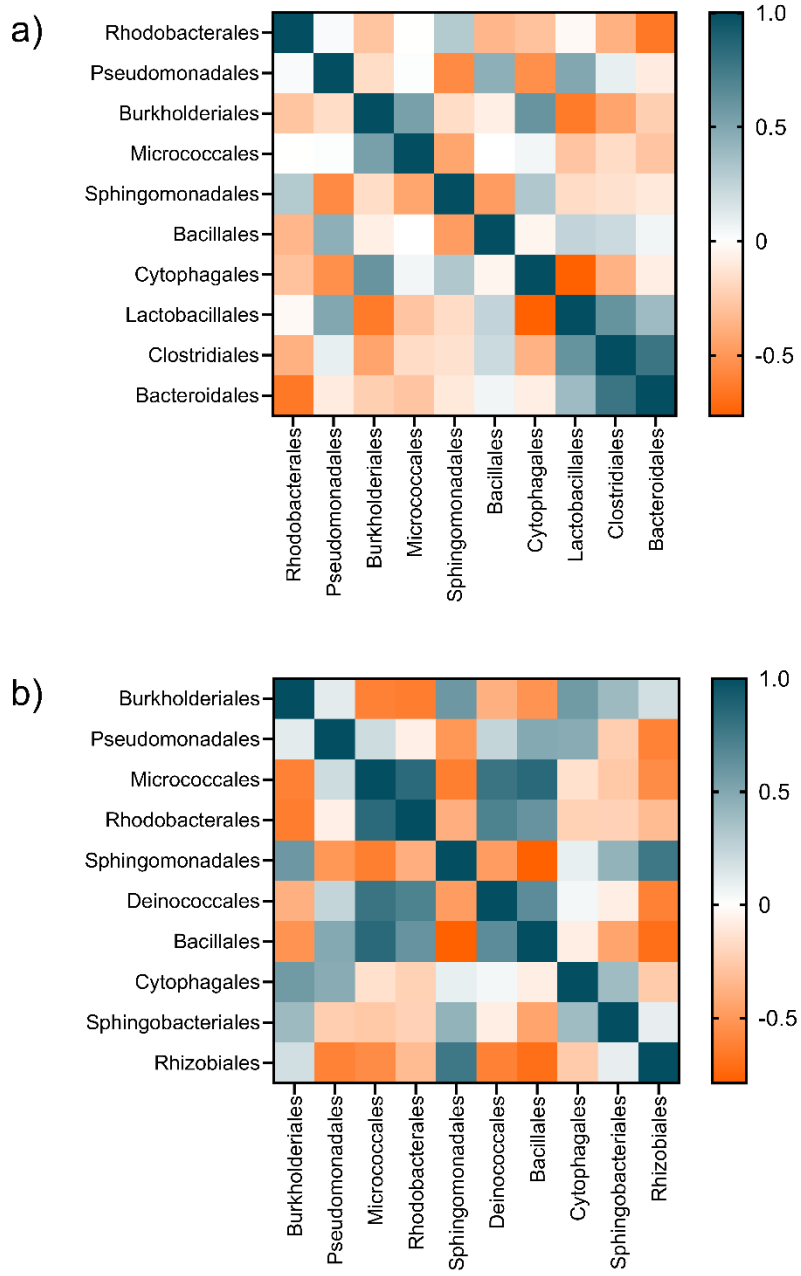


Fig. 8. Spearman correlation matrices between dominant bacterial orders (top 10) on RDS from urban a) and suburban b) areas.

Except for chemical components, the correlations between the dominant bacteria at the order level (top 10) was assessed using the Spearman's rank correlation coefficient method (Figure 8). This could identify these dominant bacteria with the same or opposite abundance variations. The result analysis will help future research on

466 the assembly process of bacterial communities[48]. Compared with the suburbs, the
467 dominant bacteria in urban areas showed more negative correlations with other species.
468 There were significant negative correlations between each of the pairs: Rhodobacterales
469 and Bacteroidales, Cytophagales and Lactobacillales, Lactobacillales and
470 Burkholderiales in the urban areas. In the suburban sites, significant negative
471 correlations were displayed between Burkholderiales and Micrococcales,
472 Rhodobacterales, Bacillales; Rhizobiales and Bacillales. Sphingomonadales had
473 negative correlations with Bacillales, Pseudomonadales and Micrococcales in both
474 urban and suburbs. The negative correlations between the pairs of bacterial species
475 might attribute to the adaptive ability to the environmental conditions, or the
476 competition between them. For example, Micrococcales were concentrated in autumn
477 (Figure S5), and the relative abundance in autumn increased significantly (Figure S10).
478 Sphingomonadales were mainly concentrated in winter, and the relative abundance in
479 autumn were stable. Therefore, the different adaptability to seasons made them show a
480 negative correlation with one another. However, Pseudomonadales were also mainly
481 concentrated in winter, and there might be a competitive relationship between
482 Sphingomonadales and Pseudomonadales.

483 In addition, Clostridiales and Bacteroidales, Burkholderiales and Micrococcales
484 showed significant positive correlations in urban areas. Micrococcales and
485 Rhodobacterales, Deinococcales and Bacillales, Sphingomonadales and Rhizobiales
486 showed clear positive correlations in the suburbs. Moreover, Pseudomonadales and
487 Bacillales, Burkholderiales and Cytophagales had significant positive correlations in

both urban and suburban sites. The positive correlations between the bacterial species indicated that their susceptibility to environmental factors may be consistent, or that there were symbiotic relationships between them. Many strains of Pseudomonadales and Bacillales were hosted in plants as beneficial bacteria. Studies have demonstrated that the regulatory mechanism of coexistence of Pseudomonadales and Bacillales, and also hinted at their possible coexistence in the natural environment[68]. However, the symbiotic and competitive relationships between microbial communities on RDS requires further study to verify it. This study proposed the hypotheses for the variations in the microbial communities, and provided a strong basis for future research.

4. Conclusions

This study verified that the diversity and abundance of bacteria (including potential pathogenic bacteria) on RDS varied with the season, land use, urbanization, particle size and chemical components. The bacterial abundance at seasonal scale displayed the most significant variations than land use and particle size. The bacterial community diversity raised substantially at daily scale and the variation of dominant bacteria could be fitted by quadratic function. Urbanization notably increased the bacterial community diversity, while the potential pathogenic bacteria were more abundant in the suburban areas. The bacterial community diversity on coarse particles was greater than that on fine particles. In addition, the chemical components on RDS showed special correlations with the relative abundance of dominant bacteria. This study provided a deep and comprehensive understanding on the bacterial communities on RDS, and provided a solid basis on which to fill the knowledge gap. This helps to

further study the influence of the bacteria on RDS on that in nearby waters and air. It is of great significance for studying the source and sink process. In addition, it was worth noting that the abundance results in this study were based on relative values, and further research are needed in the future by using qPCR to quantify the absolute abundance.

Data Availability

The authors declare that all the data supporting the findings of this study are available within the article and its SI Appendix.

Acknowledgments

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References

1. Cao, C.; Jiang, W.; Wang, B.; Fang, J.; Lang, J.; Tian, G.; Jiang, J.; Zhu, T. F., Inhalable Microorganisms in Beijing's PM2.5 and PM10 Pollutants during a Severe Smog Event. Envrion. Sci. Technol. 2014, 48, (3), 1499-1507.
2. Polymenakou, P. N., Atmosphere: A Source of Pathogenic or Beneficial Microbes? Atmosphere 2012, 3, (1), 87-102.
3. Wijesiri, B.; Egodawatta, P.; McGree, J.; Goonetilleke, A., Understanding the uncertainty associated with particle-bound pollutant build-up and wash-off: A critical review. Water Res. 2016, 101, 582-596.
4. Zhang, X.; Zhi, X.; Chen, L.; Shen, Z., Spatiotemporal variability and key influencing factors of river fecal coliform within a typical complex watershed. Water Res. 2020, 178, 115835.
5. Zhou, Z.; Dionisio, K. L.; Verissimo, T. G.; Kerr, A. S.; Coull, B.; Howie, S.; Arku, R. E.; Koutrakis, P.; Spengler, J. D.; Fornace, K.; Hughes, A. F.; Vallarino, J.; Agyei-Mensah, S.; Ezzati, M., Chemical Characterization and Source Apportionment of Household Fine Particulate Matter in Rural, Peri-urban, and Urban West Africa. Envrion. Sci. Technol. 2014, 48, (2), 1343-1351.
6. Amato, F.; Schaap, M.; van der Gon, H.; Pandolfi, M.; Alastuey, A.; Keuken, M.; Querol, X., Short-term variability of mineral dust, metals and carbon emission from road dust resuspension. Atmospheric Environment 2013, 74, 134-140.
7. Sterk, A.; Schijven, J.; de Roda Husman, A. M.; de Nijs, T., Effect of climate change on runoff of Campylobacter and Cryptosporidium from land to surface

water. Water Res. 2016, 95, 90-102.

8. Womack, A. M.; Bohannan, B. J. M.; Green, J. L., Biodiversity and biogeography of the atmosphere. Philosophical Transactions of the Royal Society B: Biological Sciences 2010, 365, (1558), 3645-3653.

9. Dueker, M. E.; French, S.; O'Mullan, G. D., Comparison of Bacterial Diversity in Air and Water of a Major Urban Center. Frontiers in Microbiology 2018, 9, (2868).

10. Zeng, X.; Kong, S.; Zheng, S.; Cheng, Y.; Wu, F.; Niu, Z.; Yan, Q.; Wu, J.; Zheng, H.; Zheng, M.; Zeng, X.-c.; Chen, N.; Xu, K.; Zhu, B.; Yan, Y.; Qi, S., Variation of airborne DNA mass ratio and fungal diversity in fine particles with day-night difference during an entire winter haze evolution process of Central China. Sci. Total Environ. 2019, 694, 133802.

11. Brodie, E. L.; DeSantis, T. Z.; Parker, J. P. M.; Zubietta, I. X.; Piceno, Y. M.; Andersen, G. L., Urban aerosols harbor diverse and dynamic bacterial populations. Proceedings of the National Academy of Sciences 2007, 104, (1), 299.

12. Fierer, N.; Liu, Z.; Rodríguez-Hernández, M.; Knight, R.; Henn, M.; Hernandez, M. T., Short-Term Temporal Variability in Airborne Bacterial and Fungal Populations. Applied and Environmental Microbiology 2008, 74, (1), 200.

13. Jang, G. I.; Hwang, C. Y.; Cho, B. C., Effects of heavy rainfall on the composition of airborne bacterial communities. Frontiers of Environmental Science & Engineering 2017, 12, (2), 12.

14. Ancion, P.-Y.; Lear, G.; Lewis, G. D., Three common metal contaminants of urban runoff (Zn, Cu & Pb) accumulate in freshwater biofilm and modify embedded

bacterial communities. Environ. Pollut. 2010, 158, (8), 2738-2745.

15. Qi, Y.; Li, Y.; Xie, W.; Lu, R.; Mu, F.; Bai, W.; Du, S., Temporal-spatial variations of fungal composition in PM2.5 and source tracking of airborne fungi in mountainous and urban regions. Sci. Total Environ. 2019, 135027.
16. Xie, J.; Jin, L.; He, T.; Chen, B.; Luo, X.; Feng, B.; Huang, W.; Li, J.; Fu, P.; Li, X., Bacteria and Antibiotic Resistance Genes (ARGs) in PM2.5 from China: Implications for Human Exposure. Envrion. Sci. Technol. 2019, 53, (2), 963-972.
17. Zhai, Y.; Li, X.; Wang, T.; Wang, B.; Li, C.; Zeng, G., A review on airborne microorganisms in particulate matters: Composition, characteristics and influence factors. Environment International 2018, 113, 74-90.
18. Adams, R. I.; Miletto, M.; Lindow, S. E.; Taylor, J. W.; Bruns, T. D., Airborne bacterial communities in residences: similarities and differences with fungi. PloS one 2014, 9, (3), e91283-e91283.
19. Besemer, K.; Moeseneder, M. M.; Arrieta, J. M.; Herndl, G. J.; Peduzzi, P., Complexity of Bacterial Communities in a River-Floodplain System (Danube, Austria). Applied and Environmental Microbiology 2005, 71, (2), 609.
20. Kim, K.-H.; Kabir, E.; Kabir, S., A review on the human health impact of airborne particulate matter. Environment International 2015, 74, 136-143.
21. Wang, J.; Huang, J. J.; Li, J., A study of the road sediment build-up process over a long dry period in a megacity of China. Sci. Total Environ. 2019, 696, 133788.
22. Kawanaka, Y.; Tsuchiya, Y.; Yun, S.-J.; Sakamoto, K., Size Distributions of Polycyclic Aromatic Hydrocarbons in the Atmosphere and Estimation of the

Contribution of Ultrafine Particles to Their Lung Deposition. Envrion. Sci. Technol. 2009, 43, (17), 6851-6856.

23. Rajasekar, A.; Balasubramanian, R., Assessment of airborne bacteria and fungi in food courts. Building and Environment 2011, 46, (10), 2081-2087.

24. Gao, M.; Jia, R.; Qiu, T.; Han, M.; Wang, X., Size-related bacterial diversity and tetracycline resistance gene abundance in the air of concentrated poultry feeding operations. Environ. Pollut. 2017, 220, 1342-1348.

25. Wei, M.; Xu, C.; Xu, X.; Zhu, C.; Li, J.; Lv, G., Size distribution of bioaerosols from biomass burning emissions: Characteristics of bacterial and fungal communities in submicron (PM1.0) and fine (PM2.5) particles. Ecotoxicology and Environmental Safety 2019, 171, 37-46.

26. Raisi, L.; Aleksandropoulou, V.; Lazaridis, M.; Katsivela, E., Size distribution of viable, cultivable, airborne microbes and their relationship to particulate matter concentrations and meteorological conditions in a Mediterranean site. Aerobiologia 2013, 29, (2), 233-248.

27. Bovallius, A.; Bucht, B.; Roffey, R.; Anäs, P., Three-year investigation of the natural airborne bacterial flora at four localities in sweden. Applied and Environmental Microbiology 1978, 35, (5), 847.

28. Lighthart, B.; Shaffer, B. T., Viable bacterial aerosol particle size distributions in the midsummer atmosphere at an isolated location in the high desert chaparral. Aerobiologia 1995, 11, (1), 19-25.

29. 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. Sci. Total Environ. 2011, 409, (19), 3812-3819.

30. Li, H.; Zhou, X.-Y.; Yang, X.-R.; Zhu, Y.-G.; Hong, Y.-W.; Su, J.-Q., Spatial and seasonal variation of the airborne microbiome in a rapidly developing city of China. Sci. Total Environ. 2019, 665, 61-68.
31. Laforest-Lapointe, I.; Messier, C.; Kembel, S. W., Tree Leaf Bacterial Community Structure and Diversity Differ along a Gradient of Urban Intensity. mSystems 2017, 2, (6), e00087-17.
32. Yan, L.; Herrmann, M.; Kampe, B.; Lehmann, R.; Totsche, K. U.; Küsel, K., Environmental selection shapes the formation of near-surface groundwater microbiomes. Water Res. 2020, 170, 115341.
33. Guo, J.; Zheng, Y.; Teng, J.; Song, J.; Wang, X.; Zhao, Q., The seasonal variation of microbial communities in drinking water sources in Shanghai. Journal of Cleaner Production 2020, 265, 121604.
34. Huang, L.; Bai, J.; Wen, X.; Zhang, G.; Zhang, C.; Cui, B.; Liu, X., Microbial resistance and resilience in response to environmental changes under the higher intensity of human activities than global average level. Global Change Biology 2020, n/a, (n/a).
35. Wang, J.; Huang, J. J.; Li, J., Characterization of the pollutant build-up processes and concentration/mass load in road deposited sediments over a long dry period. Sci. Total Environ. 2020, 718, 137282.
36. Jayarathne, A.; Mummullage, S.; Gunawardana, C.; Egodawatta, P.; Ayoko, G. A.;

Goonetilleke, A., Influence of physicochemical properties of road dust on the build-up of hydrocarbons. Sci. Total Environ. 2019, 694, 133812.

37. Miguntanna, N. P.; Goonetilleke, A.; Egodowatta, P.; Kokot, S., Understanding nutrient build-up on urban road surfaces. J. Environ. Sci. 2010, 22, (6), 806-812.

38. Liu, A.; Gunawardana, C.; Gunawardena, J.; Egodawatta, P.; Ayoko, G. A.; Goonetilleke, A., Taxonomy of factors which influence heavy metal build-up on urban road surfaces. J. Hazard. Mater. 2016, 310, 20-29.

39. Mahbub, P.; Goonetilleke, A.; Ayoko, G. A., Prediction Model of the Buildup of Volatile Organic Compounds on Urban Roads. Envrion. Sci. Technol. 2011, 45, (10), 4453-4459.

40. Bowers, R. M.; Clements, N.; Emerson, J. B.; Wiedinmyer, C.; Hannigan, M. P.; Fierer, N., Seasonal Variability in Bacterial and Fungal Diversity of the Near-Surface Atmosphere. Envrion. Sci. Technol. 2013, 47, (21), 12097-12106.

41. Tang, Q.; Huang, K.; Liu, J.; Shen, D.; Dai, P.; Li, Y.; Li, C., Seasonal variations of microbial assemblage in fine particulate matter from a nursery pig house. Sci. Total Environ. 2019, 134921.

42. Fan, X.-Y.; Gao, J.-F.; Pan, K.-L.; Li, D.-C.; Dai, H.-H.; Li, X., More obvious air pollution impacts on variations in bacteria than fungi and their co-occurrences with ammonia-oxidizing microorganisms in PM2.5. Environ. Pollut. 2019, 251, 668-680.

43. Jayarathne, A.; Wijesiri, B.; Egodawatta, P.; Ayoko, G. A.; Goonetilleke, A., Role of adsorption behavior on metal build-up in urban road dust. J. Environ. Sci. 2019,

83, 85-95.

44. Després, V.; Huffman, J. A.; Burrows, S. M.; Hoose, C.; Safatov, A.; Buryak, G.; Fröhlich-Nowoisky, J.; Elbert, W.; Andreae, M.; Pöschl, U.; Jaenicke, R., Primary biological aerosol particles in the atmosphere: a review. Tellus B: Chemical and Physical Meteorology 2012, 64, (1), 15598.
45. Ortega-Retuerta, E.; Joux, F.; Jeffrey, W. H.; Ghiglione, J.-F., Spatial variability of particle-attached and free-living bacterial diversity in surface waters from the Mackenzie River to the Beaufort Sea (Canadian Arctic). Biogeosciences 2013, 10, (4), 2747-2759.
46. Stegen, J. C.; Lin, X.; Konopka, A. E.; Fredrickson, J. K., Stochastic and deterministic assembly processes in subsurface microbial communities. The ISME Journal 2012, 6, (9), 1653-1664.
47. Friedman, J.; Higgins, L. M.; Gore, J., Community structure follows simple assembly rules in microbial microcosms. bioRxiv 2016, 067926.
48. Goldford, J. E.; Lu, N.; Bajić, D.; Estrela, S.; Tikhonov, M.; Sanchez-Gorostiaga, A.; Segrè, D.; Mehta, P.; Sanchez, A., Emergent simplicity in microbial community assembly. Science 2018, 361, (6401), 469.
49. Liddicoat, C.; Weinstein, P.; Bissett, A.; Gellie, N. J. C.; Mills, J. G.; Waycott, M.; Breed, M. F., Can bacterial indicators of a grassy woodland restoration inform ecosystem assessment and microbiota-mediated human health? Environment International 2019, 129, 105-117.
50. Abinandan, S.; Subashchandrabose, S. R.; Venkateswarlu, K.; Megharaj, M., Soil

microalgae and cyanobacteria: the biotechnological potential in the maintenance of soil fertility and health. Critical Reviews in Biotechnology 2019, 39, (8), 981-998.

51. Stewart, W. D. P.; Rowell, P.; Rai, A. N., Cyanobacteria-eukaryotic plant symbioses. Annales de l'Institut Pasteur / Microbiologie 1983, 134, (1, Supplement B), 205-228.

52. Dila, D. K.; Corsi, S. R.; Lenaker, P. L.; Baldwin, A. K.; Bootsma, M. J.; McLellan, S. L., Patterns of Host-Associated Fecal Indicators Driven by Hydrology, Precipitation, and Land Use Attributes in Great Lakes Watersheds. Envrion. Sci. Technol. 2018, 52, (20), 11500-11509.

53. Xiao, S.; Yin, P.; Zhang, Y.; Zhao, X.; Sun, L.; Yuan, H.; Lu, J.; Hu, S., Occurrence, genotyping, and health risk of Cryptosporidium and Giardia in recreational lakes in Tianjin, China. Water Res. 2018, 141, 46-56.

54. Xie, Z.; Fan, C.; Lu, R.; Liu, P.; Wang, B.; Du, S.; Jin, C.; Deng, S.; Li, Y., Characteristics of ambient bioaerosols during haze episodes in China: A review. Environ. Pollut. 2018, 243, 1930-1942.

55. Hiraoka, S.; Miyahara, M.; Fujii, K.; Machiyama, A.; Iwasaki, W., Seasonal Analysis of Microbial Communities in Precipitation in the Greater Tokyo Area, Japan. Frontiers in Microbiology 2017, 8, (1506).

56. Gong, J.; Qi, J.; E, B.; Yin, Y.; Gao, D., Concentration, viability and size distribution of bacteria in atmospheric bioaerosols under different types of pollution. Environ. Pollut. 2019, 113485.

57. Wang, J., Huang, J. J., Chu, X., Snowmelt Runoff Pollution of Different Functional Areas in Tianjin. CHINA WATER & WASTEWATER 2018, 34(11):114-118.

58. Du, P.; Du, R.; Ren, W.; Lu, Z.; Zhang, Y.; Fu, P., Variations of bacteria and fungi in PM2.5 in Beijing, China. Atmospheric Environment 2018, 172, 55-64.

59. Du, P.; Du, R.; Ren, W.; Lu, Z.; Fu, P., Seasonal variation characteristic of inhalable microbial communities in PM2.5 in Beijing city, China. Sci. Total Environ. 2018, 610-611, 308-315.

60. Liu, H.; Zhang, X.; Zhang, H.; Yao, X.; Zhou, M.; Wang, J.; He, Z.; Zhang, H.; Lou, L.; Mao, W.; Zheng, P.; Hu, B., Effect of air pollution on the total bacteria and pathogenic bacteria in different sizes of particulate matter. Environ. Pollut. 2018, 233, 483-493.

61. Yamamoto, N.; Bibby, K.; Qian, J.; Hospodsky, D.; Rismani-Yazdi, H.; Nazaroff, W. W.; Peccia, J., Particle-size distributions and seasonal diversity of allergenic and pathogenic fungi in outdoor air. The ISME Journal 2012, 6, (10), 1801-1811.

62. Wijesiri, B.; Egodawatta, P.; McGree, J.; Goonetilleke, A., Incorporating process variability into stormwater quality modelling. Sci. Total Environ. 2015, 533, 454-461.

63. Mhuireach, G.; Johnson, B. R.; Altrichter, A. E.; Ladau, J.; Meadow, J. F.; Pollard, K. S.; Green, J. L., Urban greenness influences airborne bacterial community composition. Sci. Total Environ. 2016, 571, 680-687.

64. Plomp, M.; Leighton, T. J.; Wheeler, K. E.; Malkin, A. J., The High-Resolution Architecture and Structural Dynamics of Bacillus Spores. Biophysical Journal

2005, 88, (1), 603-608.

65. Elliott, H. A.; Liberati, M. R.; Huang, C. P., Competitive Adsorption of Heavy Metals by Soils1. J. Environ. Qual. 1986, 15, 214-219.

66. Jin, Y.; O'Connor, D.; Ok, Y. S.; Tsang, D. C. W.; Liu, A.; Hou, D., Assessment of sources of heavy metals in soil and dust at children's playgrounds in Beijing using GIS and multivariate statistical analysis. Environment International 2019, 124, 320-328.

67. Dombrecht, B.; Marchal, K.; Vanderleyden, J.; Michiels, J., Prediction and overview of the RpoN-regulon in closely related species of the Rhizobiales. Genome Biology 2002, 3, (12), research0076.1.

68. Molina-Santiago, C.; Pearson, J. R.; Navarro, Y.; Berlanga-Clavero, M. V.; Caraballo-Rodriguez, A. M.; Petras, D.; García-Martín, M. L.; Lamon, G.; Haberstein, B.; Cazorla, F. M.; de Vicente, A.; Loquet, A.; Dorrestein, P. C.; Romero, D., The extracellular matrix protects Bacillus subtilis colonies from Pseudomonas invasion and modulates plant co-colonization. Nature Communications 2019, 10, (1), 1919.