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## **Profile of Inhalable Bacteria in PM 2.5 at Mt. Tai, China: Abundance, Community, and Influence of Air Mass Trajectories**

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1           **Profile of Inhalable Bacteria in PM<sub>2.5</sub> at Mt. Tai,**  
2           **China: Abundance, Community, and Influence of Air**  
3           **Mass Trajectories**

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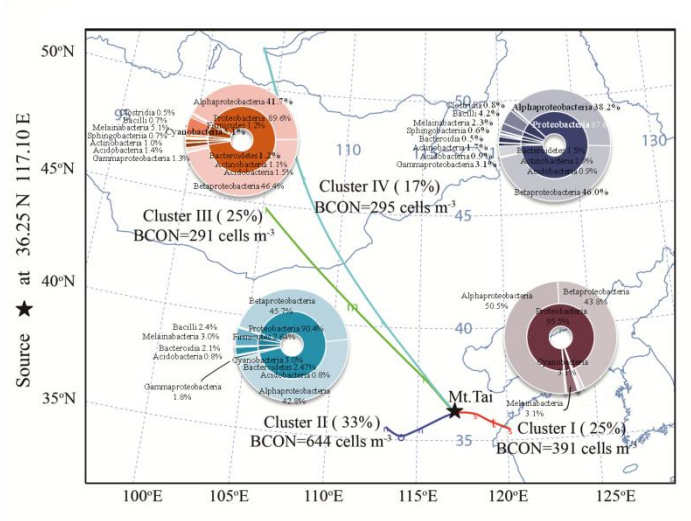
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21          **Highlights**

- 22          1. PM<sub>2.5</sub> samples exhibited a high microbial biodiversity and complex chemical composition  
23          2. Relationships between bacterial populations and PM composition were investigated.  
24          3. The highest bacterial concentration and more potential pathogens were recorded under the  
25          influence of local air pollutants.  
26          4. The long-range transported air mass exhibited highest richness and diversity with an  
27          increased abundance of dust-borne bacteria.

## 28 Graphical abstract



29

## 30 Keywords

31 Bioaerosol, PM, Air mass origin, Diversity

## 32 Abstract

33 Bacteria are ubiquitous in the near-surface atmosphere where they constitute an important  
34 component of aerosols with the potential to affect climate change, ecosystems, atmospheric  
35 process and human health. Limitation in tracking bacterial diversity accurately has thus far  
36 prevented the knowledge of airborne bacteria and their pathogenic properties. We performed a  
37 comprehensive assessment of bacterial abundance and diverse community in PM<sub>2.5</sub> collected  
38 at Mt. Tai, via high-throughput sequencing and real-time PCR. The samples exhibited a high  
39 microbial biodiversity and complex chemical composition. The dominating populations were  
40 gram-negative bacteria including *Burkholderia*, *Delftia*, *Bradyrhizobium*, and  
41 *Methylobacterium*. The PM mass concentration, chemical composition, bacterial  
42 concentration and community structure varied under the influence of different air-mass  
43 trajectories. The highest mass concentration of PM<sub>2.5</sub> (61  $\mu\text{g m}^{-3}$ ) and major chemical  
44 components were recorded during periods when marine southeasterly air masses were  
45 dominant. The local terrestrial air masses from Shandong peninsula and its adjacent areas  
46 harbored highest bacterial concentration loading (602 cells  $\text{m}^{-3}$ ) and more potential pathogens  
47 at the site. In contrast, samples influenced by the long-distance air flow from Siberia and  
48 Outer Mongolia were found to have a highest richness and diversity as an average, which was  
49 also marked by the increase of dust-associated bacteria (*Brevibacillus* and *Staphylococcus*).  
50 The primary research may serve as an important reference for the environmental  
51 microbiologist, health workers, and city planners.

## 52 1. Introduction

53 The particulate matter with aerodynamic diameters of  $\leq 2.5 \mu\text{m}$  ( $\text{PM}_{2.5}$ ) is known as a major  
54 health risk for the spread of asthma, stroke burden, and chronic diseases such as  
55 nasopharyngitis, bronchitis, and pneumonia<sup>1-3</sup>. Assessment of air pollution exposure for the  
56 global burden of disease revealed that about 29.2% of stroke burden was associated with  
57 inhaled particulate matters at a high concentration<sup>4</sup>. The environmental health scholars  
58 considered that the toxic effect of  $\text{PM}_{2.5}$  on the respiratory tract and lungs mainly induced by  
59 the suspension and reaction of gases, heavy metals, mineral element, and toxic organic  
60 matters (cancerogenic substance) in ambient air<sup>5, 6</sup>. In addition to these components, there is  
61 increasing evidence that pathogenic and allergenic microorganisms (bacteria, eukaryotes,  
62 archaea, and viruses) attached to the particles, may deposit in the epithelial cells  
63 through respiration, and thus causing various ailments in humans and livestock<sup>7-10</sup>. The  
64 pathogenic species (e.g. adenovirus, influenza, SARS, and rhinovirus) and their components  
65 (e.g. endotoxins, glucans, and mycotoxins) widely distributed in near-surface atmosphere  
66 bring a great threat to the ecosystem balance<sup>11-13</sup>.

67 Bacteria, approximately  $10^4 \text{ cells m}^{-3}$  and  $0.1 \mu\text{g m}^{-3}$  in PM, mainly originated from the  
68 emission of soil, vegetation, surface of rock, human and animals (excrements and debris from  
69 skin and hair), precipitation (surface snow and ice core), and ocean<sup>14</sup>. Investigations on the  
70 ambient bacteria experienced a recent growth in interest with the aerosol community. To our  
71 knowledge, the first study on airborne microorganisms was reported in the mid-19th century<sup>15</sup>.  
72 The occurrence of high throughput sequencing offers a unique possibility to examine the  
73 bacterial, viral, and eukaryotic diversity in the aerosol. While the understanding of airborne  
74 microbe is still being developed due to current challenges, e.g., low density of airborne  
75 microbes, variability in airborne microbial community structure, and the lack of  
76 unified regulations for the research. However, in spite of this, this method has been applied to  
77 study the airborne bacteria and the associated pathogens in Japan, USA, Korea, and China in  
78 the last few years<sup>7, 16-18</sup>. Franzetti et al. (2011)<sup>19</sup> described the significant seasonal variation of  
79 bacterial community associated with  $\text{PM}_{10}$  and  $\text{PM}_{2.5}$  based on the Roche 454 sequencing.  
80 Based on the high-throughput sequencing, Bower et al. (2013)<sup>20</sup> found the significant seasonal  
81 variability of Actinobacteridae and Pseudomonadales across  $\text{PM}_{2.5}$  and  $\text{PM}_{2.5-10}$  in Greeley,  
82 CO. Cao et al. (2014)<sup>21</sup> developed a comprehensive framework of the microbial community in  
83  $\text{PM}_{2.5}$  and  $\text{PM}_{10}$  under sever smog events by metagenomics. These findings mainly dressed  
84 the scanty composition community of bacteria in the urban air, the identification for the  
85 inhaled microbes and their pathogenic members at high altitudes were incomplete. Airborne  
86 bacteria with active metabolic have been reported in the mountains and the upper  
87 troposphere<sup>16,22</sup>. These species dissolving into cloud droplets proposed an important impact on

88 the regional precipitation distribution, biogeochemical cycling of elements in ecosystem  
89 processes, and cloud chemistry by acting as cloud condensation nucleation or ice  
90 nucleation<sup>23,24</sup>. Thus it is necessary to further the understanding for the profile of airborne  
91 bacterial community structure at high elevations.

92 Here, we expound on our initial efforts to advance the knowledge of airborne bacteria over  
93 Mt. Tai, the highest site at the North China Plain. It is located on the transport path of the  
94 Asian continent outflow and be affected by air mass from the Asian dust area. Mt. Tai is a  
95 tilted fault block mountain with height increasing from the north to the south, facing to the  
96 East China Sea and back on the yellow river to the west. In this region, the airborne bacterial  
97 populations in cloud system and fungal populations in particles have been reported before<sup>24-26</sup>.  
98 The preponderant gram-negative bacteria inhabited in the cold and leaf-surface environments  
99 which were involved in the cloud condensation/ice nuclei formation and pathogenicity,  
100 demonstrating their importance in ecology, epidemiology and human health<sup>24-25</sup>. The  
101 pioneering studies were lack of a comprehensive and compound research on the bacteria in  
102 particles. The chemical composition in particles have been proved to be related to the variable  
103 airborne microbe. Further, they also highlighted the crucial influence of environmental factors  
104 humidity, and temperature on the bacterial community in cloud water and fungal community  
105 in particles. Generally the chemical composition of particles may be influenced by different  
106 air mass origin with the input of local aerosol or long-range transported aerosol. Whether the  
107 different air trajectories do play a role in shaping bacterial community of particles still  
108 remains a question. In this study, we aim to: (1) profile the bacterial abundance and  
109 community of PM<sub>2.5</sub> at Mt. Tai, China, (2) to elucidate the environmental variables  
110 (temperature, humidity, PM<sub>2.5</sub>, PM<sub>2.5</sub> chemistry), and the air mass trajectories during sampling  
111 time, (3) to estimate whether the different air mass trajectories effect on the bacterial  
112 characteristics.

## 113 **2. Materials and method**

### 114 *2.1 Aerosol collection and DNA Extraction*

115 Fine particulate matter (PM<sub>2.5</sub>) were sampled on quartz fiber filters (PALL, NY, U.S.  
116 88mm) with a middle-volume sampler (TH-100A, Wuhan Tianhong Instruments Co., Ltd.,  
117 China) and a flux speed of 100 L min<sup>-1</sup>. The blank filters were obtained by placing a sterilized  
118 quartz microfiber filter inside of the sampler without operation. The sampling campaigns  
119 were carried out in 8~13 day periods in summer, autumn, and winter during July 2014 to  
120 August 2015. The sampler was located at the summit of Mt. Tai (36.25°N and 117.10°E, 1534  
121 m above sea level), a typical tourist area in the North China Plain (Figure 1). The  
122 geographical characteristics of the sampling site have been previously described<sup>25,26</sup>. Each

123 collection period lasted 23 h. Before sampling, all the filters were baked at a Muffle furnace at  
124 500°C for 5 h and then placed into sterilized aluminum foil before deposited into a sealed bag.  
125 To avoid the contamination, the sampling filter holder and materials used for changing filters  
126 were treated with 75% ethanol before sampling every day. After sampling, the samples were  
127 stored at -80 °C until the next analysis. The environmental factors including temperature,  
128 relative humidity, visibility, water-soluble inorganic ions ( $\text{NO}_3^-$ ,  $\text{SO}_4^{2-}$ ,  $\text{NH}_4^+$ ,  $\text{Ca}^{2+}$ ,  $\text{Na}^+$ ,  $\text{Mg}^{2+}$ ,  
129 and  $\text{Cl}^-$ ), meteorological data, the mass concentration of  $\text{PM}_{2.5}$ , DNA extraction procedure and  
130 PCR amplification have been described before<sup>26</sup>. The final product was ready for resolving  
131 for the abundance and community of airborne bacteria by real-time quantitative PCR and  
132 Illumina Miseq sequencing.

### 133 2.2 Quantification of 16S rRNA gene copy number

134 The bacterial abundance was determined by the quantification of the copy numbers of the  
135 gene 16S rRNA. This value is not directly related to bacterial number due to the presence of  
136 multiple ribosomal operons in the bacterial genomes; however, it can be used to look at  
137 relative shifts in microbial biomass as the bias in copy number is likely constant across  
138 samples. The V4-V5 hypervariable regions were PCR-amplified with universal primers 515F  
139 (5'-TGCCAGCMGCCGCGGTAA-3') and 926R (5'-CGTCAATTCMTTGTGAGTTT-3'). The  
140 real-time quantitative PCR was performed in a total volume of 25  $\mu\text{L}$  reaction mixture  
141 including 12.5  $\mu\text{L}$  TransStart Green qPCR SuperMix (Transgene, AQ101-03), 1  $\mu\text{L}$  forward  
142 primer, 1  $\mu\text{L}$  reverse primer, 5  $\mu\text{L}$  DNA, and 5.5  $\mu\text{L}$  double distilled  $\text{H}_2\text{O}$ . The amplification  
143 was run on a BioRad CFX96 Real-Time PCR system under the following conditions: 95 °C  
144 for 5 min, followed by 40 cycles of 95 °C for 30 s, 56 °C for 30 s and 72 °C for 30 s, with  
145 acquisition of the fluorescence at the end of each 72 °C elongation step. The deionized water  
146 was employed as the negative controls and was pipetted into the wells in a 96-well microplate  
147 with the DNA extracted from the samples. All the samples and controls were tested in  
148 triplicate in each run. Based on the average rRNA gene copy number (3.98), the bacterial cell  
149 concentration ( $\text{cells m}^{-3}$ ) was calculated using the method described before<sup>27-29</sup>.

### 150 2.3 Sequence Analyses

151 A DNA sequencing library was constructed on the basis of the TruSeq DNA PCR-Free  
152 Sample Prep Kit (Illumina) and was sequenced by the MiSeq reagent kit v2 (Illumina) on an  
153 Illumina MiSeq instrument (Illumina, San Diego, CA, USA). The raw sequences were  
154 analyzed using standard Mothur software. Variable sequence numbers (7044 to 32,293) were  
155 obtained after the quality control of raw reads by removing the chimeric sequences, primers,  
156 barcodes, and low-quality reads (average quality score < 20) using FASTX-ToolKit. We

157 normalized the sequences number to 7044 for the subsequent analysis. The Operational  
158 Taxonomic Units (OTUs) were defined using a distance-based cutoff method by Usearch  
159 (version 7.1; <http://drive5.com/uparse/>). The alpha-diversity estimators Chao1 and Shannon  
160 indexes, which indicated the richness and diversity of the bacterial community, were  
161 calculated by QIIME (version 1.17; [http://qiime.org/scripts/assign\\_taxonomy.html](http://qiime.org/scripts/assign_taxonomy.html)) after  
162 removing the low-abundance OTUs (<0.01%). The taxonomic classification was aligned to  
163 the Silva database (<http://www.arb-silva.de/>) using uCLUST  
164 ([http://www.drive5.com/uclust/downloads1\\_2\\_22q.html](http://www.drive5.com/uclust/downloads1_2_22q.html)) at different similarity levels to  
165 determine the diverse bacteria at phylum, class, order, family, genus, and species levels. The  
166 sequences obtained in this study have been deposited in the Sequence Read Archive (SRA)  
167 under accession numbers SRR51461. The potential pathogenic sequences corresponding to  
168 human health were screened referenced to the previous database<sup>30-33</sup>. The human pathogenic  
169 bacteria list, including species names and the diseases caused, was used to find the potential  
170 pathogens sequences from total sequences. We selected the operational taxonomic units  
171 (OTUs) belonged to these genera mentioned in the reference list. The nearest phylogenetic  
172 species were obtained by NCBI BLAST. Finally, the bacterial species belonging to the  
173 reference list were determined as the potential pathogens in PM<sub>2.5</sub>.

#### 174 *2.4 Statistical Analyses*

175 The difference in bacterial concentration across different air mass trajectories were  
176 assessed by an ANOVA test, followed by post-hoc tests. The ANOVAs test was also  
177 conducted to determine whether season had a significant impact on the relative abundances of  
178 specific microbial groups. The weighted UniFrac analysis was employed to estimate the  
179 significant differences of the microbial community among three seasons. The relationship  
180 between the airborne microbial concentrations and environmental factors including PM  
181 concentrations and chemical compositions was assessed with a Pearson correlation by SPSS  
182 16.0. The results that Bonferroni adjusted P-value for each pairwise comparison higher than  
183 0.05 were considered to be significant. The correlation coefficient between NMDS plot and  
184 environmental elements was calculated via metadata analysis with Mothur software.  
185 Statistical values, including Pearson and Spearman correlation coefficients and independent t-  
186 tests, were calculated using R software with the R package.

### 187 3. Results and discussion

#### 188 3.1 Bacterial abundance in PM<sub>2.5</sub>

189 During the sampling period, the PM<sub>2.5</sub> mass concentration, ranging from 3 to 111 µg  
190 m<sup>-3</sup> with a mean value of 40 µg m<sup>-3</sup> at Mt. Tai, were much lower than that in summer 2006  
191 (123 µg m<sup>-3</sup>)<sup>32</sup> and comparable with that in summer 2007 (59 µg m<sup>-3</sup>)<sup>30</sup> that conducted at the  
192 same site. Table 1 summarizes the corresponding bacterial concentrations in a different size.  
193 The bacterial concentration of PM<sub>2.5</sub> varied from 40 to 1728 cells m<sup>-3</sup> with an average of 490  
194 cells m<sup>-3</sup> at Mt. Tai. It was much lower than those TSP from the high-altitude air, urban Japan,  
195 Korea, and Italy, PM<sub>2.5</sub> and PM<sub>1</sub> from China. The lower bacterial concentration were found to  
196 be correlated with PM<sub>2.5</sub> mass concentration ( $r=0.526, p < 0.05$ ), SO<sub>4</sub><sup>2-</sup> ( $r=0.549, p < 0.01$ ),  
197 NO<sub>3</sub><sup>-</sup> ( $r=0.458, p < 0.05$ ), NH<sub>4</sub><sup>+</sup> ( $r=0.570, p < 0.01$ ), K<sup>+</sup> ( $r=0.508, p < 0.01$ ), Na<sup>+</sup>  
198 ( $r=0.585, p < 0.01$ ), Mg<sup>2+</sup> ( $r=0.608, p < 0.01$ ), and Ca<sup>2+</sup> ( $r=0.432, p < 0.05$ ) significantly  
199 based on the Spearman rank correlations. The ambient pollutants attached to the particles may  
200 provide nutrients (sulfur, nitrogen, and ammonia) and thus affect the bacterial characteristics.  
201 In general, the airborne bacteria, ranging between 10 and 10<sup>7</sup> cells m<sup>-3</sup>, varied in a degree with  
202 many aspects<sup>11,23,29,34</sup>, like sampling time, site (altitude, temperature, humidity, etc.), sample  
203 type (TSP, PM<sub>10</sub>, PM<sub>2.5</sub>, PM<sub>1</sub>, cloud/fog water, etc.), and analysis methods (culture methods,  
204 bio-molecular technology, and quick estimation model)<sup>14,35,36</sup>. And these indexes effectively  
205 represented the situation of bacterial community on particles under different conditions.  
206 Borodulin et al. (2005)<sup>37</sup> observed the varied concentration of culturable microorganisms for  
207 about more than two orders of magnitude over an altitude range of 0.5~7 km in southwestern  
208 Siberia. They found the overall bacterial concentration in the atmosphere where not only  
209 dependent on altitude but also related to the cell size of bacteria and particulate matters.  
210 Different bacteria have respective sizes ranging from 0.1~5 µm, and each bacterium has a  
211 particular shape feature that plays an important role in the adhesion on PM<sup>3</sup>. Since the study  
212 environments were breeding settings in which bacteria had better conditions to grow and  
213 propagate, the bacteria concentration may decrease with decreasing particulate size<sup>38</sup>. Hence  
214 more high-throughput sequencing researches for the bacterial community distribution were  
215 vital to understanding their diversity fully.

#### 216 3.2 Inhalable bacterial community in PM<sub>2.5</sub>

217 After quality control and normalization, a number of OTUs ranged from 237 to 1579 were  
218 detected. The alpha diversity estimators were summarized in Table 2. Similar with Després  
219 et al. (2007)<sup>54</sup>, the airborne bacteria at Mt. Tai commonly exhibited lower richness and



220 diversity compared with those from urban, marine, sand, and dust sites. The diversity  
221 estimators can reflect the state of the bacteria and their relationship to the environment, and  
222 microbial resources developments, ecosystem functioning, global change and human health  
223 are all closely related to microbial diversity. In addition to the changing sampling sites, this  
224 discrepancy may be caused by the different analysis platform (Miseq sequencing,  
225 pyrosequencing, and clone library). Using the same method (Miseq Sequencing), TSP seems  
226 to have higher OTUs, chao1, and Shannon indexes than PM<sub>2.5</sub> and PM<sub>1</sub>, indicating more and  
227 various bacterial species in the larger particulate matter. We attribute this increasement to the  
228 larger surface area of TSP for bacterial adhesion. As mentioned above, the size of a single  
229 bacterial cell is smaller than particles, there is a mass of nutrients and bacterial slime coat on  
230 the bacteria that make the bacterial size bigger but still smaller than particles, so there were  
231 more bacteria presenting in particles with a larger size. Actually different bacteria had  
232 respective variation tendency for particle sizes, although there were some differences on size  
233 distribution, bacteria mainly attached to particles with two peaks (1.1~2.1 μm and >7.1 μm)<sup>55</sup>.  
234 Except for the bacterial size, the structure of bacterial community can also exert a certain  
235 extent influence on the diversity characteristics.

236 At Mt. Tai, the diverse bacterial community including 40 phyla, 78 classes, 154 orders, 286  
237 families, 724 genera was classified in PM<sub>2.5</sub>. About 97.82% of OTUs belongs to the phylum  
238 Proteobacteria, Cyanobacteria, Firmicutes, and Bacteroidetes. The co-occurrence network  
239 were used to reveal the complex bacterial community structure based on the top 100 OTUs  
240 (Figure 2). The species of dominant phyla (Proteobacteria, Actinobacteria, Cyanobacteria,  
241 Firmicutes, Acidovacteria, Fusobacteria, and Bacterioidetes) were similar in USA<sup>7,56</sup>, Italy<sup>34</sup>,  
242 and East Asian Regions<sup>15,21,48,49,51,56</sup>, but the relative abundance were different. Furthermore,  
243 the microbiome is a dynamic aspect of the near-surface layer among extreme  
244 weather incidents such as dust, haze, rain, and tropical storms. Proteobacteria members were  
245 the dominant species in the atmosphere over mountains and from the troposphere. The  
246 relative abundance of this bacterium (91.42%) were richer than that in the dust (69.0%), non-  
247 dust (53.5%)<sup>18</sup>, and haze (16.5%)<sup>27</sup> days. It was dominated by a few taxa throughout the  
248 whole sampling period, with a marked prevalence of Alphaproteobacteria, Betaproteobacteria,  
249 and Gammaproteobacteria. At higher taxonomic levels, a bacterium in the nitrogen fixing,  
250 gram-negative pathogenic bacteria genus *Burkholderia* appeared to be the most abundant.  
251 Bowers et al. (2009)<sup>57</sup> also showed the dominant Burkholderiales in particles collected at the  
252 top of Mt. Werner near Steamboat Springs CO, USA at 3220 m above sea level via tagged  
253 pyrosequencing. The preponderant gram-negative bacteria in PM<sub>2.5</sub> were different with Fang  
254 et al. (2007)<sup>58</sup> who reported the 80%~86% culturable bacteria in outdoors environment were  
255 gram-positive. They considered the gram-negative bacteria cannot adapt to the higher air

256 pollutants concentration, stronger solar radiation, and complicated meteorological  
257 environment outdoors. We speculate this gap attributed to the different analysis platform. The  
258 Illumina Miseq sequencing method gained the comprehensive airborne bacteria no matter  
259 culturable or unculturable. Actually, the proportion of culturable bacteria is very small.  
260 Another reason is that those gram-negative bacteria in the atmosphere lose cultivability after  
261 aerosolization<sup>59</sup>. Hence the majority of airborne bacteria outdoors were gram-negative.

262 Out of all the gram-negative species, eight opportunistic pathogens were determined based  
263 on the human pathogenic bacteria list as described before. *Acinetobacter baumannii*,  
264 primarily associated with hospital-acquired infections, commonly found in water and soil.  
265 Over the past decades, reports on community-acquired *A. baumannii* infections (wound  
266 infection, ventilator-associated pneumonia, and bloodstream infection) have increased and  
267 attracted public attention<sup>60-63</sup>. An outbreak of soil-borne bacteria *Pseudomonas putida* was  
268 involved in clinic infections in patients with medical devices and immunosuppressed hosts<sup>64-</sup>  
269 <sup>65</sup>. Besides, five gram-positive potential pathogens were detected including *Bacillus subtilis*,  
270 *Rhodococcus equi*, *Streptococcus anginosus*, *Bacillus pumilus*, and *Rhodococcus fascians*.  
271 Some strains of *B. subtilis* cause food poisoning through the ropy spoilage of bread and rots in  
272 potatoes<sup>66-67</sup>. *R. equi*, mainly thrive on water and soils, were connected with pneumonia in  
273 immunocompromised patients and animal hosts<sup>68-69</sup>. *S. anginosus* were able to cause diseases  
274 including bloodstream infections, pyogenic liver abscess, brain and liver abscesses<sup>70-71</sup>. These  
275 bacterial species suspending in the atmosphere diffuse downwind, yielding a threat to the  
276 health of plants and animals in local and downstream area.

277 Other than the pathogenicity, the diverse bacteria demonstrates a diverse metabolism in the  
278 atmospheric process and ecosystem interactions. The bacteria with ice nucleation activity  
279 suspending in the atmosphere can take part in the cloud chemistry via act as cloud  
280 condensation or ice nuclei. During the same project, we captured some typical cloud events  
281 (Event F and G)<sup>24</sup>, incidentally. The special terrain of Mt. Tai is conducive to the formation of  
282 orographic clouds. The tiny droplets could dissolve or adsorb the particles suspending in the  
283 atmosphere and then accumulated into the cloud water. Though the same sampling site, the  
284 dominant bacterial species varied from *Burkholderia* in PM<sub>2.5</sub> to the preponderance of  
285 *Pseudomonas* in cloud water. The distant discrepancy of dominating bacterial distribution  
286 also found between PM<sub>2.5</sub> of Mt. Tai and PM<sub>1</sub> of Ji'nan (Figure 3A). As to this phenomenon,  
287 different bacterial suitable inhabits and growing media (water, soil, atmosphere, etc.) may  
288 be important factors. Additionally, Liu et al. (2018)<sup>72</sup> considered the relative humidity, wind  
289 scale, CO, and SO<sub>2</sub> were the main factors, which affected the diversity of total bacteria and  
290 the proportion of pathogenic bacteria. The PM's chemical components serve as the

291 nutritional ingredient, coupled with surrounding environments such as temperature, humidity,  
292 wind direction, and atmospheric pressure seems to shift on the bacterial growth and  
293 metabolism in atmosphere<sup>3,10,73</sup>. The chemical and biological data were jointly processed and  
294 verified that the varied  $\text{NH}_4^+$ ,  $\text{NO}_3^-$ ,  $\text{Mg}^{2+}$ ,  $\text{Ca}^{2+}$ ,  $\text{Cl}^-$  and  $\text{Na}^+$  explained about 53.7%~63.1% of  
295 the variance of species-environment correlations<sup>74</sup>. The chemical composition of  $\text{PM}_{2.5}$   
296 correlated with the air mass back trajectories. Air mass originating from Mongolia region had  
297 strong correlations with dust pollution, along with high concentration of  $\text{Ca}^{2+}$  in ambient  
298  $\text{PM}_{2.5}$ . Local emission and air pollutants were more favorable for formation of secondary  
299 pollutants ( $\text{SO}_4^{2-}$ ,  $\text{NH}_4^+$ , and  $\text{NO}_3^-$ ) and reactions between particulate species. We also  
300 detected the obvious correlation between airborne bacterial community and  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ , and  
301 temperature at Mt. Tai, while the influence of atmospheric circulation using the back-  
302 trajectory analysis was not evaluated fully. Therefore how does the air mass trajectories vary  
303 and whether the air mass trajectories evoked the change of concentration and structure of  
304 bacterial community is worth discussing.

### 305 3.3 Cluster analysis of backward trajectory and $\text{PM}_{2.5}$ chemistry

306 At Mt. Tai, the air mass back trajectories have been categorized into four types, typified by  
307 the examples in Figure 4, based on trajectories terminating at the site on the day of  
308 measurements. One trajectory per day was, in our experience, adequate to represent the flow  
309 patterns which tended to change over timescales of a few days. Inevitably, there were a few  
310 days on which assignment of trajectory type was difficult, but the predominant type from the  
311 four synoptic hours was adopted in these circumstances. The four types of back trajectory  
312 were as follows:

313 *Cluster I (25%)*: The air mass had passed across the marginal seas in the western Pacific  
314 Ocean and had been in contact with the land immediately prior to arrival in the study area.  
315 Broadly speaking, the highest mass concentration of  $\text{PM}_{2.5}$  ( $61 \mu\text{g m}^{-3}$ ) and major water  
316 inorganic ions were recorded followed the decreasing order:  $\text{SO}_4^{2-} > \text{NO}_3^- >$   
317  $\text{Cl}^- > \text{Na}^+ > \text{Ca}^{2+} > \text{K}^+ > \text{NH}_4^+ > \text{Mg}^{2+}$ . In addition to the secondary inorganic ions,  $\text{Cl}^-$  ( $4.35$   
318  $\mu\text{g m}^{-3}$ ) and  $\text{Na}^+$  ( $3.73 \mu\text{g m}^{-3}$ ) can be assumed mostly to originate from sea salt aerosols. The  
319  $\text{Cl}^-/\text{Na}^+$  molar ratios obtained (0.75) was lower than that of bulk seawater (1.18)<sup>75</sup>, suggesting  
320 that the chlorine loss of marine aerosols during transport to some extent. These ions are  
321 proved to play essential roles in atmospheric processes. e.g., cloud formation, visibility  
322 degradation, solar radiation, acidification of cloud, rain, fog and haze formation and served as  
323 the nutrients for the growth and development of airborne bacteria<sup>24,45</sup>.

324 *Cluster II* (33%): The air mass had come in from a generally western direction bringing it  
325 across Shandong peninsula and its adjacent areas, a typical densely polluted areas of northern  
326 China. Influenced by the local terrestrial air flow, the average of PM<sub>2.5</sub> mass was 37  $\mu\text{g m}^{-3}$ .  
327 The secondary inorganic ions, i.e., SO<sub>4</sub><sup>2-</sup> (9.92  $\mu\text{g m}^{-3}$ ), NO<sub>3</sub><sup>-</sup> (4.23  $\mu\text{g m}^{-3}$ ), and NH<sub>4</sub><sup>+</sup> (2.29  $\mu\text{g}$   
328  $\text{m}^{-3}$ ), which mostly originated from the transformation of their precursors (SO<sub>2</sub>, NO<sub>2</sub>, and NH<sub>3</sub>)  
329 were found as the dominant species, followed by Cl<sup>-</sup> (0.75  $\mu\text{g m}^{-3}$ ), K<sup>+</sup> (0.63  $\mu\text{g m}^{-3}$ ), Ca<sup>2+</sup>  
330 (0.51  $\mu\text{g m}^{-3}$ ), Na<sup>+</sup> (0.31  $\mu\text{g m}^{-3}$ ), and Mg<sup>2+</sup> (0.07  $\mu\text{g m}^{-3}$ ). In order to investigate the  
331 continental influence, non-sea-salt sulfate (nss-SO<sub>4</sub><sup>2-</sup>) concentrations are estimated as:  
332  $\text{nss-SO}_4^{2-} (\mu\text{g m}^{-3}) = [\text{SO}_4^{2-}] - 0.245 \times [\text{Na}^+]$ . The mass fraction of nss-SO<sub>4</sub><sup>2-</sup> ranged  
333 from 1.17  $\mu\text{g m}^{-3}$  to 18.89  $\mu\text{g m}^{-3}$  (mean 9.85  $\mu\text{g m}^{-3}$ ), accounted for 99.02% of total sulfate.  
334 SO<sub>4</sub><sup>2-</sup> mainly originates from anthropogenic and biogenic sources, while nss-SO<sub>4</sub><sup>2-</sup> originates  
335 mainly from stationary sources. We often use the mass ratio of [NO<sub>3</sub><sup>-</sup>]/[nss-SO<sub>4</sub><sup>2-</sup>] as an  
336 indicator of the relative importance of stationary versus mobile sources of sulfur and nitrogen  
337 in the atmosphere. High [NO<sub>3</sub><sup>-</sup>]/[nss-SO<sub>4</sub><sup>2-</sup>] mass ratios represent the predominance of mobile  
338 sources over stationary sources. Due to the extensive use of sulfur-containing coal, the  
339 average ratio [NO<sub>3</sub><sup>-</sup>]/[nss-SO<sub>4</sub><sup>2-</sup>] mass ratios in China were ascribed lower value<sup>76</sup>. The  
340 measured [NO<sub>3</sub><sup>-</sup>]/[nss-SO<sub>4</sub><sup>2-</sup>] mass ratios at Mt. Tai (0.25) was higher or comparable with  
341 those reported in other cities, e.g., 0.17 in Hongkong<sup>77</sup>, 0.20 in Guangzhou<sup>78</sup> and 0.35 in  
342 Qingdao<sup>79</sup>. However, the ratio was lower than those cities where the use of coal was limited,  
343 for example, Los Angeles (2.0)<sup>80</sup> and Seoul (0.73)<sup>81</sup>. The result implied that stationary source  
344 emissions were likely more important than mobile sources at Mt. Tai under the influence of  
345 local terrestrial air flow.

346 *Cluster III* (25%) and *Cluster IV* (17%): The air mass arrived from a northwesterly sector  
347 outer Mongolia and having crossed dust area (Inner Mongolia and Shanxi Province) and high  
348 anthropogenic emission area (Henan and Shandong Province). This type air mass  
349 moves quickly and the corresponding mass concentration of coarse particulate matter was  
350 usually high. Typically, these westerly and northwesterly trajectories had not been in contact  
351 with any land for several days prior to arriving at the site.

### 352 3.4 Influence of air mass trajectories on bacterial populations and community

353 Obviously, no obvious variation for the bacterial concentration was observed under the  
354 influence of air mass trajectories. The terrestrial air masses from the Shandong peninsula and  
355 its adjacent areas exhibited a little higher bacterial concentration loading (644 cells  $\text{m}^{-3}$ ) and  
356 more potential pathogens. While the bacterial concentration of PM influenced by the marine  
357 air mass was lower, though the highest chemical composition (Figure 4). In contrast, samples

358 influenced by the long-distance air flow from Siberia and Outer Mongolia were found to have  
359 a highest richness and diversity as an average (Table 2). The effects of long-transported air  
360 parcels on the aerosol particles and its composition and sources have been discussed in  
361 previous studies<sup>82-85</sup>. The Asian dust particles blow from these areas in the westerly wind and  
362 play an important role in the leeward ecosystem. The bacterial community at phyla, class, and  
363 order levels under the influence of four air mass trajectories was shown in Figure 5. However,  
364 the bacterial abundance made no significant difference among the top 20 OTUs (Figure 3B),  
365 suggesting the stable community structure of airborne bacteria. Yet, some non-dominant  
366 bacterial groups that exhibited pronounced changes in relative abundance across the different  
367 air mass trajectories (Figure 6) can be linked to specific source environments of PM<sub>2.5</sub>. The  
368 long-range transported air parcels seem to be able to harbor diverse and dynamic microbial  
369 populations<sup>53</sup>. Kakikawa et al., (2008) verified the injection of dustborne bacteria  
370 (*Brevibacillus*, *Staphylococcus*, *Rhodococcus*, *Delftia*, *Pseudomonas*, and *Agrobacterium*  
371 *tumefaciens*) into the atmosphere during the dust event<sup>86</sup>. Maki et al., (2017) also observed the  
372 variable microbial community in response to the Asian dust events<sup>22</sup>. The number of microbial  
373 particles at high altitudes (>1200m) increased about 5.5-folds. The corresponding bacterial  
374 community was predominantly composed of natural-sand/terrestrial bacteria during dust  
375 events instead of anthropogenic/terrestrial bacteria, marine bacteria, and plant-associated  
376 bacteria during non-dust events. The prevalent existence of marine bacteria in cloud droplets,  
377 the air masses suspended from the Yellow Sea over the marine environment harbored more  
378 proteobacterial populations, *Pandoraea*, and *Phyllobacterium* associated with plant bodies or  
379 surface. The prevalence of *Pandoraea* had a significant positive relationship with the highest  
380 content of SO<sub>4</sub><sup>2-</sup> (\*\**p*<0.01). Consistent with these findings, the long-range transported  
381 westerly air mass increase the diversity and richness of airborne bacterial community, with the  
382 increase of some dustborne species such as genus *Brevibacillus*, *Staphylococcus*, and  
383 *Anoxybacillus* (Figure 6A). The Bacilli members obtained from mineral dust particles over  
384 downwind areas were positively correlated to temperature and Cl<sup>-</sup> in PM<sub>2.5</sub> (\*\**p*<0.01),  
385 excepting the obvious effect of humidity and temperature. They showed a stronger ability to  
386 resist ultraviolet light and be able to survive in the atmosphere by the formation of resistant  
387 endospores. The natural dust particles from dust areas can be transported to the free  
388 troposphere and vertically mixed with the anthropogenic particles during the transportation  
389 processes. Together, the airborne bacteria community at Mt. Tai contains a mixture of dust-  
390 borne bacteria, terrestrial bacteria, marine bacteria, and others. The community of dominant  
391 bacteria in PM<sub>2.5</sub> was relatively stable, and little affected by the sampling environment. In  
392 other words, ambient bacterial community structure were stable in atmosphere. Four air mass  
393 trajectories from various origin environments may lead to the discrepant composition in PM<sub>2.5</sub>,

394 and then driving the shift on the pronounced changes observed in some non-dominant  
395 bacterial groups.

#### 396 **4. Conclusions**

397 In this study, 16S rRNA gene sequencing was conducted to study the bacterial communities  
398 at Mt. Tai's PM<sub>2.5</sub> pollutants from July 2014 to August 2015. Results demonstrated the lower  
399 bacterial concentration and diverse bacterial community in PM<sub>2.5</sub> compared with some  
400 aforementioned studies. We also detected thirteen potential pathogenic species, such as *A.*  
401 *baumannii*, *P. putida*, *B.subtilis*, and *R. equi* which effect on the immunocompromised  
402 populations and livestock. In future studies, we intend to analyze the changing bacterial  
403 community structure affected by different air mass trajectories, combined with the  
404 meteorological data, PM mass concentration, water soluble ion and other physical and  
405 chemical properties. The highest mass concentration of PM<sub>2.5</sub> and major chemical components  
406 was recorded during periods when marine southeasterly air masses were dominant. The local  
407 terrestrial air masses from Shandong peninsula and its adjacent areas harbored highest  
408 bacterial concentration loading and more potential pathogens at the site. In contrast, samples  
409 influenced by the long-distance air flow from Siberia and Outer Mongolia were found to have  
410 a highest richness and diversity as an average, which was also marked by the increased  
411 abundance of dust-associated bacteria (*Brevibacillus* and *Staphylococcus*). We conclude that  
412 the air flow from different origin environments may lead to the discrepant chemical  
413 composition in PM<sub>2.5</sub>, and then driving the shift on the pronounced changes observed in some  
414 non-dominant bacterial groups. We hope our research can provide for some reference value in  
415 the global air microbial propagation and air microbial research at higher elevation site.

#### 416 **Conflict of interest**

417 The authors declare that they have no competing financial interests.

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688 Asian dust source region, Dunhuang. *Air Quality, Atmosphere and Health* **2008**, *1*: 195-202.

689 **Table 1 Bacterial abundance in PM with different size fraction.**

Type	Site	Abundance Range	Average	Method	Reference
		Cells m <sup>-3</sup>	Cells m <sup>-3</sup>		
TSP	High-altitude air, Caribbean Sea	3.0×10 <sup>3</sup> ~9.0×10 <sup>4</sup>	5.1×10 <sup>3</sup>	qPCR	39
TSP	High-altitude air, Japan	1.0×10 <sup>3</sup> ~2×10 <sup>5</sup>		qPCR	40
TSP	Toyama, Japan	1.1×10 <sup>3</sup> ~1.3×10 <sup>5</sup>	1.8×10 <sup>4</sup>	qPCR	41
TSP	Seoul, Korea	5.2×10~4.3×10 <sup>3</sup>		qPCR	29
PM <sub>10</sub>	Milan and Venice, Italy	2.4×10 <sup>2</sup> ~4.9×10 <sup>5*</sup>	3.0×10 <sup>4*</sup>	qPCR	42
PM <sub>2.5</sub>	BTH, China	8.0×10~5.8×10 <sup>3**</sup>	1.1×10 <sup>3**</sup>	Culture method	43
PM <sub>2.5</sub>	BTH, China	4.8×10 <sup>4</sup> ~2.6×10 <sup>5</sup>	1.2×10 <sup>5</sup>	qPCR	44
PM <sub>1</sub>	Ji'nan, China	1.8×10 <sup>4</sup> ~2.6×10 <sup>4</sup>	2.2×10 <sup>4</sup>	qPCR	27
CW <sup>***</sup>	Mt.Tai, China	5.8×10 <sup>4</sup> ~1.6×10 <sup>5</sup>	1.2×10 <sup>5</sup>	qPCR	45
PM <sub>2.5</sub>	Mt.Tai, China	4.0×10~1.728×10 <sup>3</sup>	4.9×10 <sup>2</sup>	qPCR	This study

690 \* Copies m<sup>-3</sup>; \*\* CFU m<sup>-3</sup>; \*\*\* Cloud Water

691

692 **Table 2 Statistical summary of alpha diversity estimators from different sample type**  
 693 **and study environments.**

Environment	Sample Type	OTUs	Chao1	Shannon	Method	Reference
Urban/Terrestrial	PM <sub>2.5</sub>	2457	4915	5.89	Miseq Sequencing	<sup>46</sup>
Urban/Terrestrial	PM <sub>2.5</sub>	552	571	3.80	Miseq Sequencing	<sup>44</sup>
Urban/Terrestrial	PM <sub>0.56-1</sub>	222	272	2.44	Miseq Sequencing	<sup>27</sup>
Dust/Terrestrial	PM <sub>2.5</sub>	62	200	3.51	Clone Library	<sup>47</sup>
Dust/Terrestrial	TSP		270		Pyrosequencing	<sup>48</sup>
Dust/Terrestrial	Dust	869	1485		Pyrosequencing	<sup>49</sup>
Dust/Terrestrial	PM <sub>10</sub>	733	1265		Pyrosequencing	<sup>18</sup>
Sand/Terrestrial	Sand	1445	1858	5.99	Pyrosequencing	<sup>50</sup>
Marine/Coastal	TSP	753			Pyrosequencing	<sup>51</sup>
Marine/Coastal	TSP	5015		7.66~9.28	Miseq Sequencing	<sup>52</sup>
High altitude	TSP	410			Pyrosequencing	<sup>22</sup>
High altitude	TSP	314	241		Miseq Sequencing g	<sup>53</sup>
High altitude	CW <sup>*</sup>	664	1302	3.80	Miseq Sequencing	<sup>24</sup>
High altitude	PM <sub>2.5</sub>	580	1282	3.01		
Cluster I	PM <sub>2.5</sub>	491	1151	2.67		
Cluster II	PM <sub>2.5</sub>	567	1322	2.93	Miseq Sequencing	This Study
Cluster III	PM <sub>2.5</sub>	677	1397	3.09		
Cluster IV	PM <sub>2.5</sub>	625	1234	3.63		

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\*

Cloud

Water



695 **List of Figures**

696 **Figure 1** The geographic situation of Mt. Tai in the North China Plain.

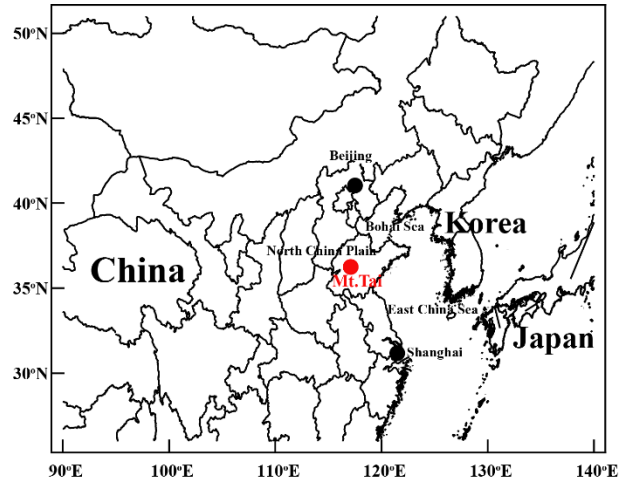
697 **Figure 2** The co-occurrence network of top 100 OTUs of PM<sub>2.5</sub> at Mt. Tai, China. The bigger  
698 the circle, the greater the number of OTUs.

699 **Figure 3** The principal component analysis showed the overall distribution pattern of A  
700 bacterial communities in different air samples and B profiles among four air mass cluster.

701 **Figure 4** Cluster analysis of air-mass trajectories and corresponding chemical composition  
702 and bacterial abundance in PM<sub>2.5</sub>

703 **Figure 5** Bacterial community at phyla, class, order levels under the influence of four air  
704 mass trajectories.

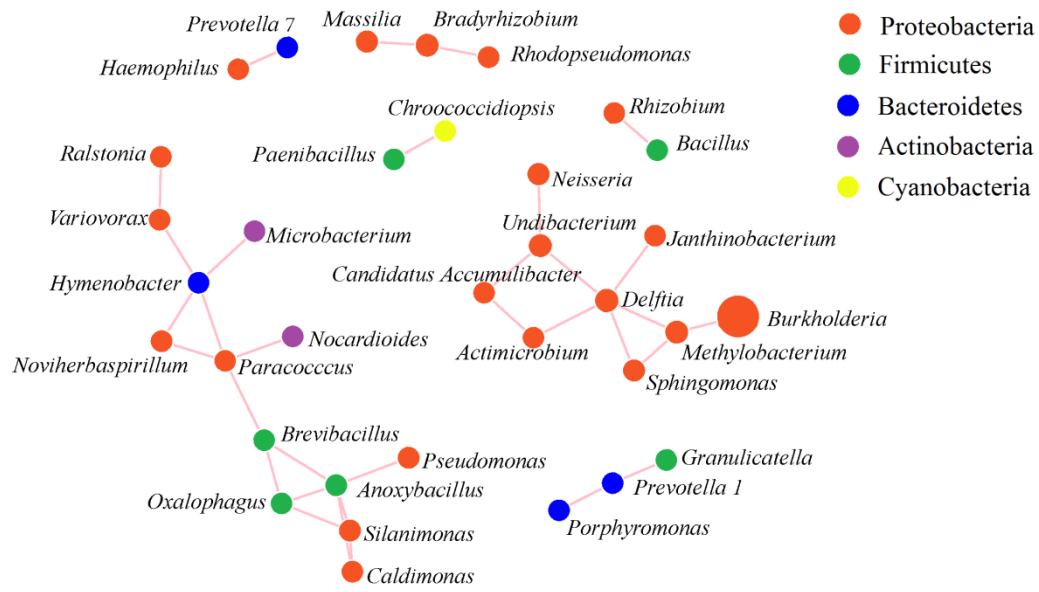
705 **Figure 6** Variance analysis of genera species based on the Kruskal–Wallis rank sum test (A)  
706 and the Spearman's rank correlations with environmental factors (B; \* $p < 0.05$ ; \*\*  $p < 0.01$ ).



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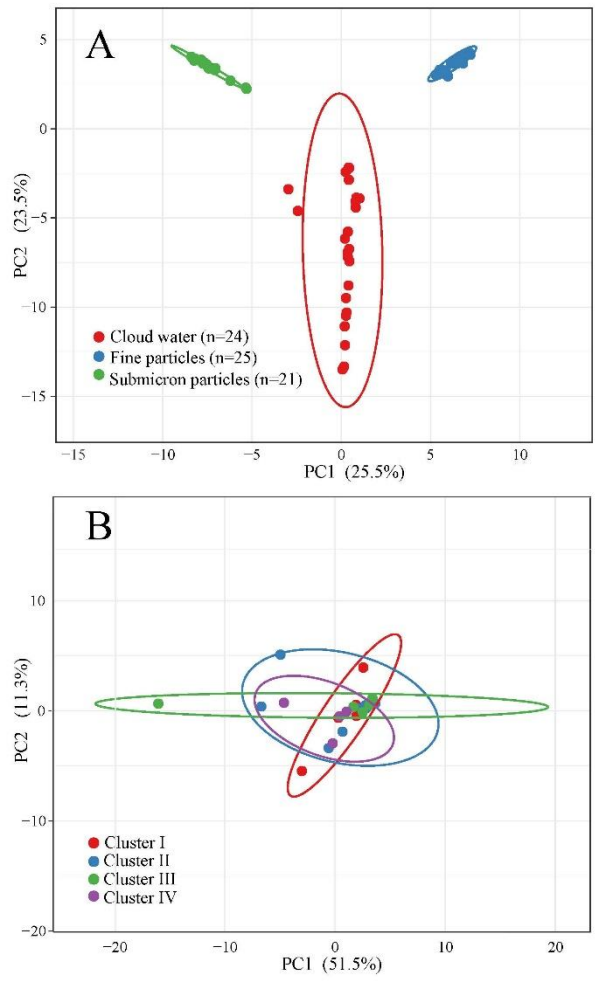
Figure 1



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Figure 1

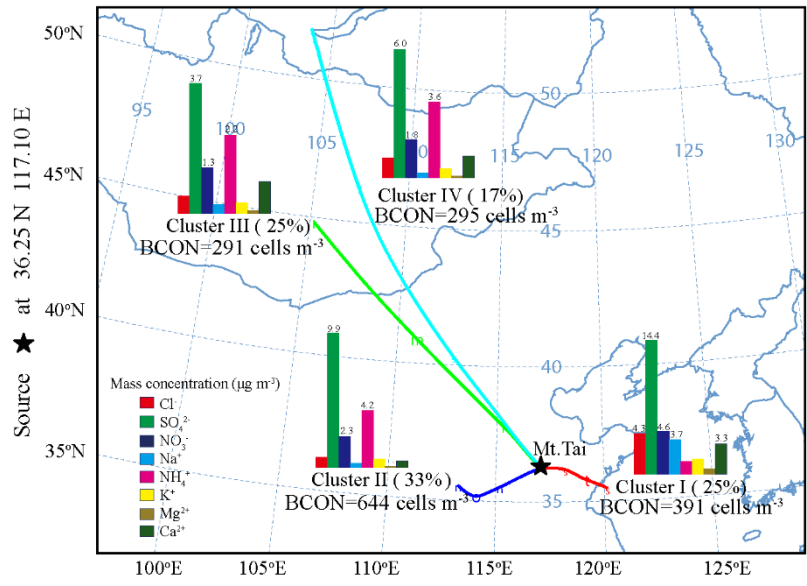


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Figure 2



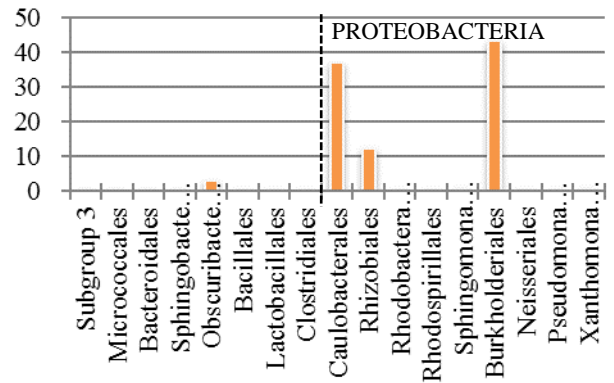
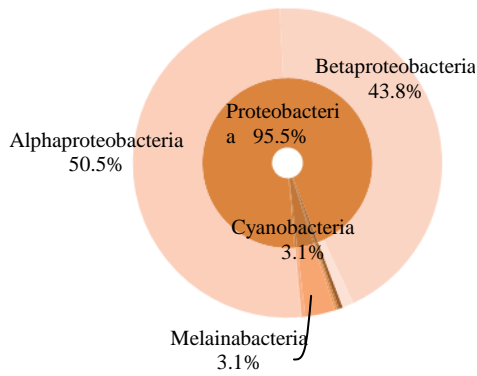
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Figure 3

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### CLUSTER I



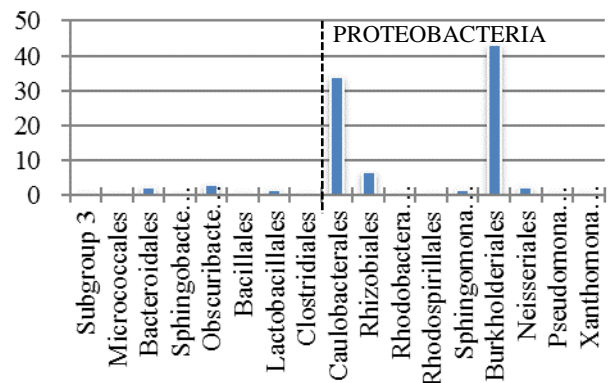
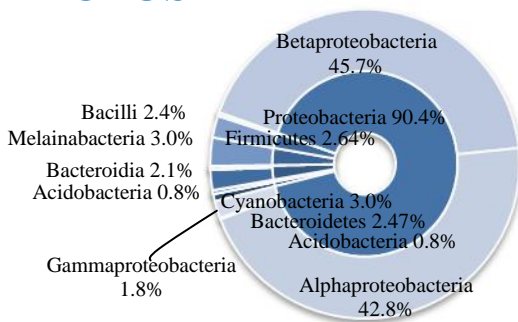
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### CLUSTER II



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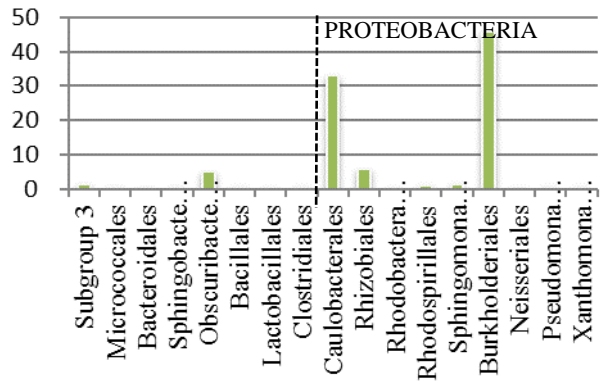
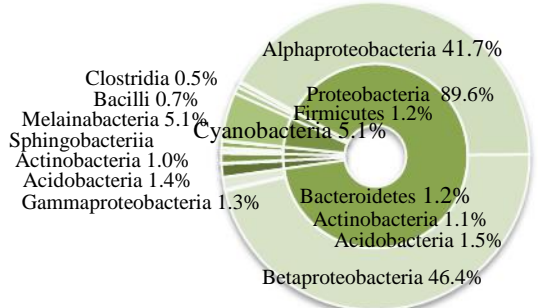
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### CLUSTER III



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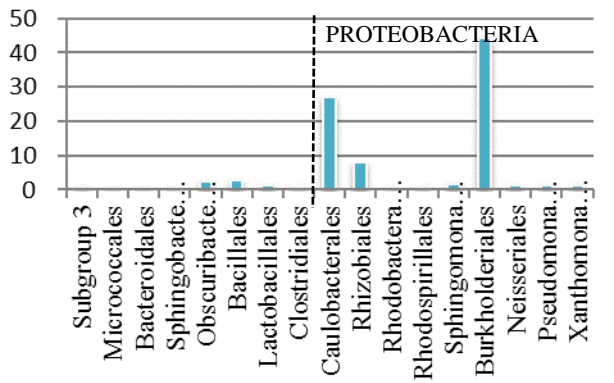
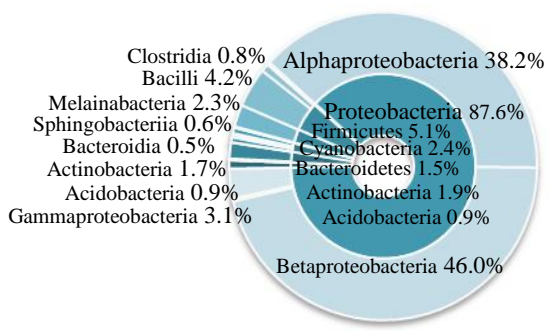
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### CLUSTER IV



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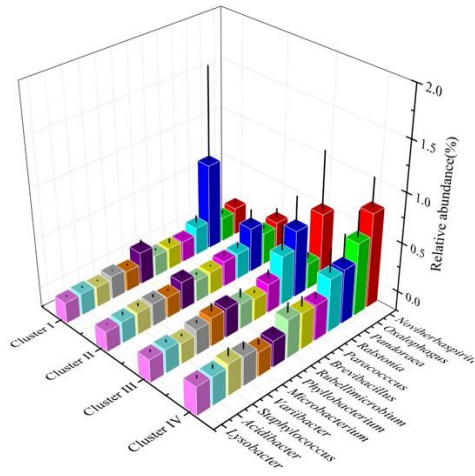
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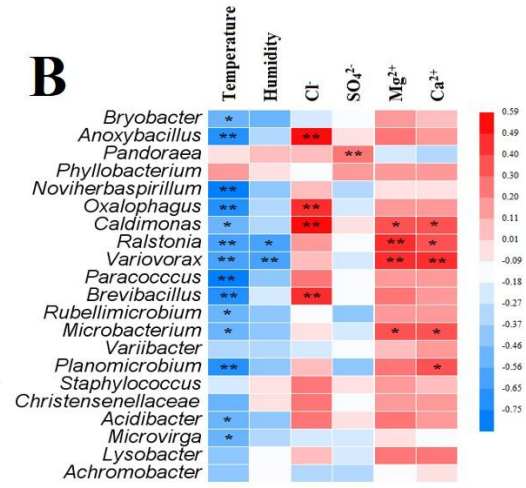
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Figure 4

**A**



**B**



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Figur 5