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► **To cite this version:**

Caihong Xu, Min Wei, Jianmin Chen, Chao Zhu, Jiarong Li, et al.. Profile of Inhalable Bacteria in PM 2.5 at Mt. Tai, China: Abundance, Community, and Influence of Air Mass Trajectories. *Ecotoxicology and Environmental Safety*, 2019, 168, pp.110-119. 10.1016/j.ecoenv.2018.10.071 . hal-02460329

HAL Id: hal-02460329

<https://hal.science/hal-02460329>

Submitted on 30 Jan 2020

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1 **Profile of Inhalable Bacteria in PM_{2.5} at Mt. Tai,**
2 **China: Abundance, Community, and Influence of Air**
3 **Mass Trajectories**

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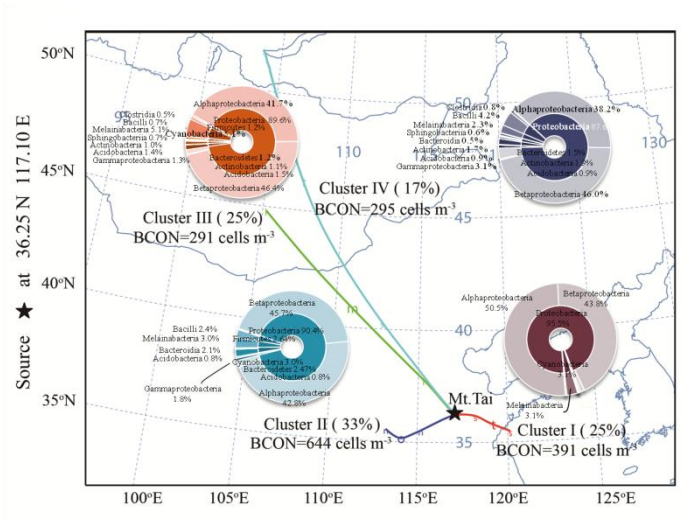
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21 **Highlights**

- 22 1. PM_{2.5} 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_{2.5} 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_{2.5} (61 μg 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 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 pneumonia¹⁻³. 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 concentration⁴. 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^{5, 6}. 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 livestock⁷⁻¹⁰. 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 balance¹¹⁻¹³.

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 ocean¹⁴. 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 century¹⁵.
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^{7, 16-18}. Franzetti et al. (2011)¹⁹ described the significant seasonal variation of
79 bacterial community associated with PM_{10} and $\text{PM}_{2.5}$ based on the Roche 454 sequencing.
80 Based on the high-throughput sequencing, Bower et al. (2013)²⁰ 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)²¹ developed a comprehensive framework of the microbial community in
83 $\text{PM}_{2.5}$ and 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^{16,22}. 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^{23,24}. 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 before²⁴⁻²⁶.
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 health²⁴⁻²⁵. 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_{2.5} at Mt. Tai, China, (2) to elucidate the environmental variables
110 (temperature, humidity, PM_{2.5}, PM_{2.5} 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_{2.5}) 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⁻¹. 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^{25,26}. 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 (NO_3^- , SO_4^{2-} , NH_4^+ , Ca^{2+} , Na^+ , Mg^{2+} ,
129 and Cl^-), meteorological data, the mass concentration of $\text{PM}_{2.5}$, DNA extraction procedure and
130 PCR amplification have been described before²⁶. 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'-TGCCAGCMGCCGCGTAA-3') and 926R (5'-CGTCAATTCMTTGGAGTTT-3'). The
140 real-time quantitative PCR was performed in a total volume of 25 μL reaction mixture
141 including 12.5 μL TransStart Green qPCR SuperMix (Transgene, AQ101-03), 1 μL forward
142 primer, 1 μL reverse primer, 5 μL DNA, and 5.5 μL double distilled H_2O . 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 (cells m^{-3}) was calculated using the method described before²⁷⁻²⁹.

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) 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) 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 database³⁰⁻³³. 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_{2.5}.

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_{2.5}

189 During the sampling period, the PM_{2.5} mass concentration, ranging from 3 to 111 $\mu\text{g m}^{-3}$
190 m^{-3} with a mean value of 40 $\mu\text{g m}^{-3}$ at Mt. Tai, were much lower than that in summer 2006
191 (123 $\mu\text{g m}^{-3}$)³² and comparable with that in summer 2007 (59 $\mu\text{g m}^{-3}$)³⁰ that conducted at the
192 same site. Table 1 summarizes the corresponding bacterial concentrations in a different size.
193 The bacterial concentration of PM_{2.5} varied from 40 to 1728 cells m^{-3} with an average of 490
194 cells m^{-3} at Mt. Tai. It was much lower than those TSP from the high-altitude air, urban Japan,
195 Korea, and Italy, PM_{2.5} and PM₁ from China. The lower bacterial concentration were found to
196 be correlated with PM_{2.5} mass concentration ($r=0.526, p < 0.05$), SO_4^{2-} ($r=0.549, p < 0.01$),
197 NO_3^- ($r=0.458, p < 0.05$), NH_4^+ ($r=0.570, p < 0.01$), K^+ ($r=0.508, p < 0.01$), Na^+
198 ($r=0.585, p < 0.01$), Mg^{2+} ($r=0.608, p < 0.01$), and Ca^{2+} ($r=0.432, p < 0.05$) 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 10^7 cells m^{-3} , varied in a degree with
202 many aspects^{11,23,29,34}, like sampling time, site (altitude, temperature, humidity, etc.), sample
203 type (TSP, PM₁₀, PM_{2.5}, PM₁, cloud/fog water, etc.), and analysis methods (culture methods,
204 bio-molecular technology, and quick estimation model)^{14,35,36}. And these indexes effectively
205 represented the situation of bacterial community on particles under different conditions.
206 Borodulin et al. (2005)³⁷ 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 PM³. 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 size³⁸. 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_{2.5}

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 Despr es
219 et al. (2007)⁵⁴, 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_{2.5} and PM₁, 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 $>7.1 \mu\text{m}$)⁵⁵.
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_{2.5}. 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^{7,56}, Italy³⁴,
242 and East Asian Regions^{15,21,48,49,51,56}, 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%)¹⁸, and haze (16.5%)²⁷ 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)⁵⁷ 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_{2.5} were different with Fang
254 et al. (2007)⁵⁸ 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 aerosolization⁵⁹. 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 attention⁶⁰⁻⁶³. An outbreak of soil-borne bacteria *Pseudomonas putida* was
268 involved in clinic infections in patients with medical devices and immunosuppressed hosts⁶⁴⁻
269 ⁶⁵. 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 rosy spoilage of bread and rots in
272 potatoes⁶⁶⁻⁶⁷. *R. equi*, mainly thrive on water and soils, were connected with pneumonia in
273 immunocompromised patients and animal hosts⁶⁸⁻⁶⁹. *S. anginosus* were able to cause diseases
274 including bloodstream infections, pyogenic liver abscess, brain and liver abscesses⁷⁰⁻⁷¹. 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)²⁴, 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_{2.5} to the preponderance of
285 *Pseudomonas* in cloud water. The distant discrepancy of dominating bacterial distribution
286 also found between PM_{2.5} of Mt. Tai and PM₁ 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)⁷² considered the relative humidity, wind
289 scale, CO, and SO₂ 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^{3,10,73}. The chemical and biological data were jointly processed and
294 verified that the varied NH_4^+ , NO_3^- , Mg^{2+} , Ca^{2+} , Cl^- and Na^+ explained about 53.7%~63.1% of
295 the variance of species-environment correlations⁷⁴. 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 Ca^{2+} in ambient
298 $\text{PM}_{2.5}$. Local emission and air pollutants were more favorable for formation of secondary
299 pollutants (SO_4^{2-} , NH_4^+ , and NO_3^-) and reactions between particulate species. We also
300 detected the obvious correlation between airborne bacterial community and Ca^{2+} , 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, Cl^- (4.35
318 $\mu\text{g m}^{-3}$) and Na^+ ($3.73 \mu\text{g m}^{-3}$) can be assumed mostly to originate from sea salt aerosols. The
319 Cl^-/Na^+ molar ratios obtained (0.75) was lower than that of bulk seawater (1.18)⁷⁵, 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^{24,45}.

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_{2.5} mass was 37 μg m⁻³.
327 The secondary inorganic ions, i.e., SO₄²⁻ (9.92 μg m⁻³), NO₃⁻ (4.23 μg m⁻³), and NH₄⁺ (2.29 μg
328 m⁻³), which mostly originated from the transformation of their precursors (SO₂, NO₂, and NH₃)
329 were found as the dominant species, followed by Cl⁻ (0.75 μg m⁻³), K⁺ (0.63 μg m⁻³), Ca²⁺
330 (0.51 μg m⁻³), Na⁺ (0.31 μg m⁻³), and Mg²⁺ (0.07 μg m⁻³). In order to investigate the
331 continental influence, non-sea-salt sulfate (nss-SO₄²⁻) concentrations are estimated as:
332 $nss - SO_4^{2-} (\mu g m^{-3}) = [SO_4^{2-}] - 0.245 \times [Na^+]$. The mass fraction of nss-SO₄²⁻ ranged
333 from 1.17 μg m⁻³ to 18.89 μg m⁻³ (mean 9.85 μg m⁻³), accounted for 99.02% of total sulfate.
334 SO₄²⁻ mainly originates from anthropogenic and biogenic sources, while nss-SO₄²⁻ originates
335 mainly from stationary sources. We often use the mass ratio of [NO₃⁻]/[nss-SO₄²⁻] as an
336 indicator of the relative importance of stationary versus mobile sources of sulfur and nitrogen
337 in the atmosphere. High [NO₃⁻]/[nss-SO₄²⁻] 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 [NO₃⁻]/[nss-SO₄²⁻] mass ratios in China were ascribed lower value⁷⁶. The
340 measured [NO₃⁻]/[nss-SO₄²⁻] mass ratios at Mt. Tai (0.25) was higher or comparable with
341 those reported in other cities, e.g., 0.17 in Hongkong⁷⁷, 0.20 in Guangzhou⁷⁸ and 0.35 in
342 Qingdao⁷⁹. However, the ratio was lower than those cities where the use of coal was limited,
343 for example, Los Angeles (2.0)⁸⁰ and Seoul (0.73)⁸¹. 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 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 studies⁸²⁻⁸⁵. 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_{2.5}. The
368 long-range transported air parcels seem to be able to harbor diverse and dynamic microbial
369 populations⁵³. 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 event⁸⁶. Maki et al., (2017) also observed the
372 variable microbial community in response to the Asian dust events²². 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, *Pandora*, and *Phyllobacterium* associated with plant bodies or
379 surface. The prevalence of *Pandora* had a significant positive relationship with the highest
380 content of SO₄²⁻ (***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⁻ in PM_{2.5} (***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_{2.5} 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_{2.5},

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_{2.5} pollutants from July 2014 to August 2015. Results demonstrated the lower
399 bacterial concentration and diverse bacterial community in PM_{2.5} 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_{2.5} 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_{2.5}, 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.

418 **Acknowledgements**

419 This work was supported by the Ministry of Science and Technology of China (No.
420 2016YFC0202700), the National Natural Science Foundation of China (No. 91743202,
421 21527814). This project has received funding from the European Union's Horizon 2020
422 research and innovation programme under grant agreement No 690958.

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

690 * Copies m⁻³; ** CFU m⁻³; *** 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 _{2.5}	2457	4915	5.89	Miseq Sequencing	⁴⁶
Urban/Terrestrial	PM _{2.5}	552	571	3.80	Miseq Sequencing	⁴⁴
Urban/Terrestrial	PM _{0.56-1}	222	272	2.44	Miseq Sequencing	²⁷
Dust/Terrestrial	PM _{2.5}	62	200	3.51	Clone Library	⁴⁷
Dust/Terrestrial	TSP		270		Pyrosequencing	⁴⁸
Dust/Terrestrial	Dust	869	1485		Pyrosequencing	⁴⁹
Dust/Terrestrial	PM ₁₀	733	1265		Pyrosequencing	¹⁸
Sand/Terrestrial	Sand	1445	1858	5.99	Pyrosequencing	⁵⁰
Marine/Coastal	TSP	753			Pyrosequencing	⁵¹
Marine/Coastal	TSP	5015		7.66~9.28	Miseq Sequencing	⁵²
High altitude	TSP	410			Pyrosequencing	²²
High altitude	TSP	314	241		Miseq Sequencing g	⁵³
High altitude	CW*	664	1302	3.80	Miseq Sequencing	²⁴
High altitude	PM _{2.5}	580	1282	3.01		
Cluster I	PM _{2.5}	491	1151	2.67		
Cluster II	PM _{2.5}	567	1322	2.93	Miseq Sequencing	This Study
Cluster III	PM _{2.5}	677	1397	3.09		
Cluster IV	PM _{2.5}	625	1234	3.63		

694

*

Cloud

Water

695 **List of Figures**

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

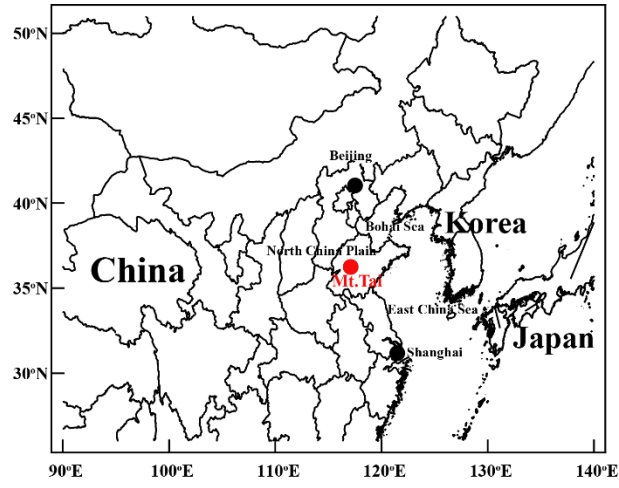
697 **Figure 2** The co-occurrence network of top 100 OTUs of PM_{2.5} at Mt. Tai, China. The bigger
698 the circle, the greater the number of OTUs.

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_{2.5}

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

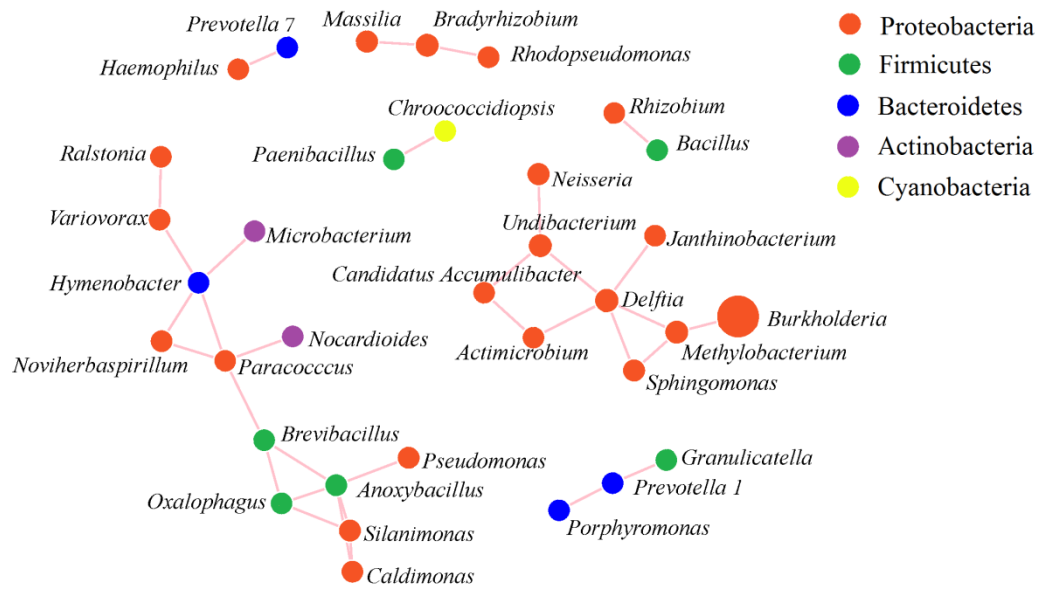
705 **Figure 6** Variance analysis of genera species based on the Kruskal–Wallis rank sum test (A)
706 and the Spearman's rank correlations with environmental factors (B; * $p < 0.05$; ** $p < 0.01$).



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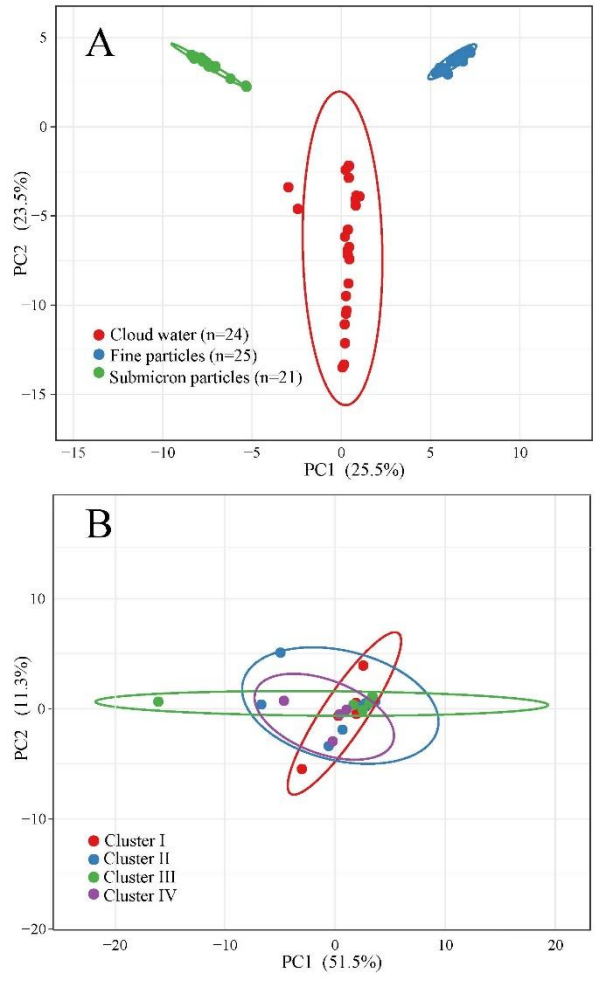
Figure 1



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Figure 1

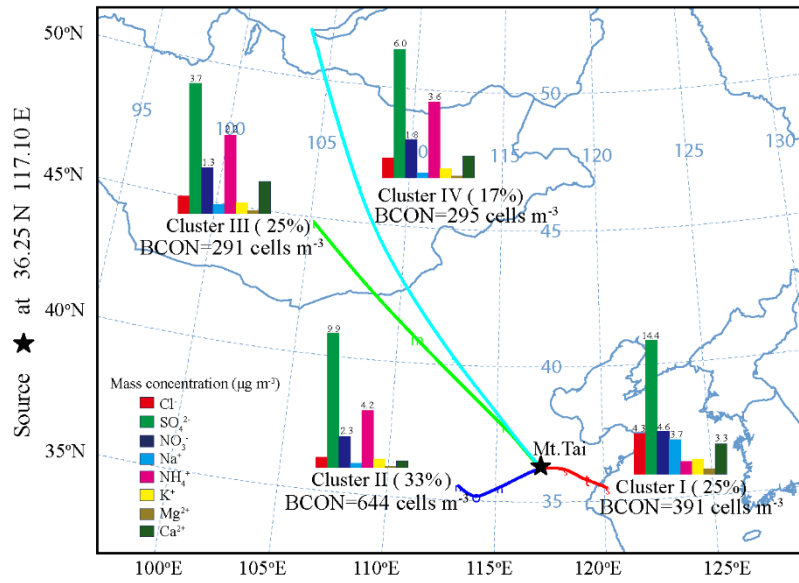


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Figure 2



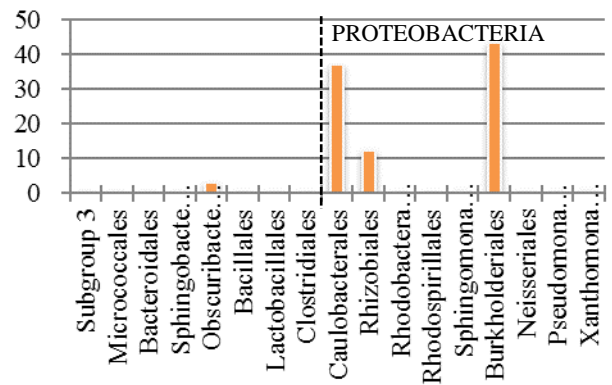
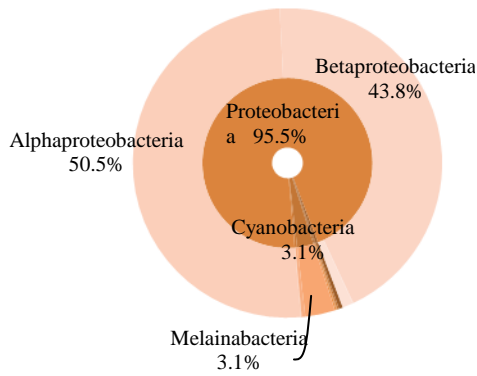
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Figure 3

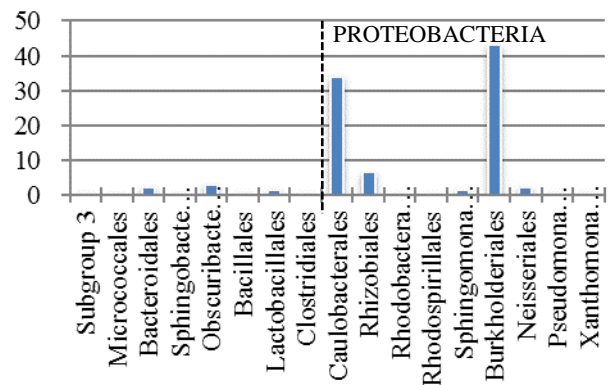
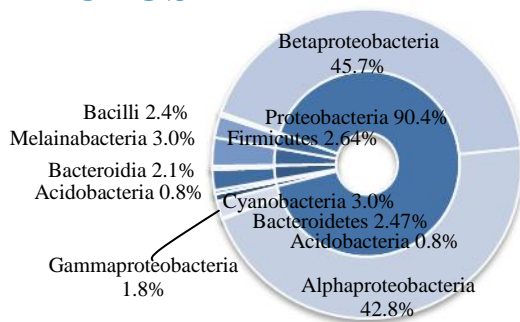
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CLUSTER I



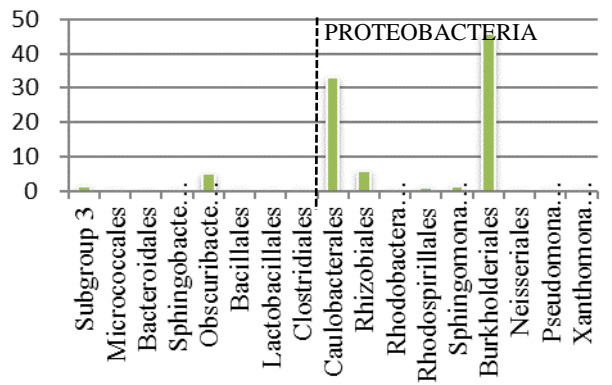
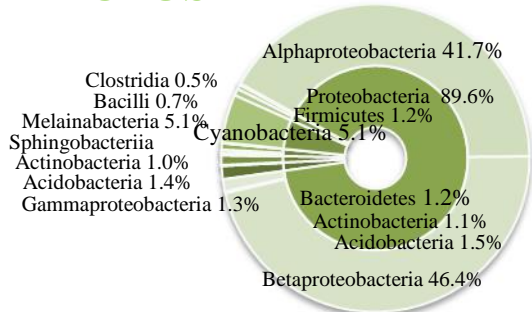
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CLUSTER II



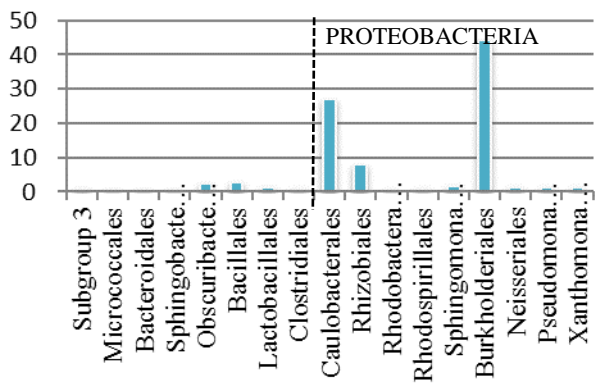
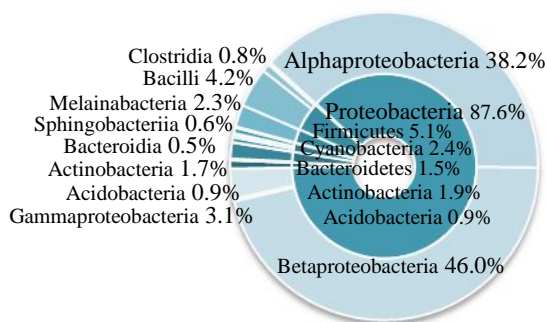
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CLUSTER III



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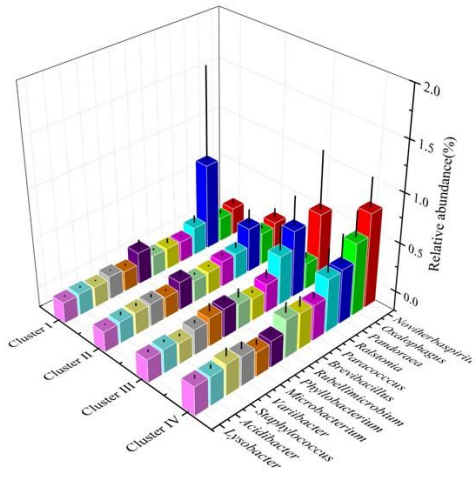
CLUSTER IV



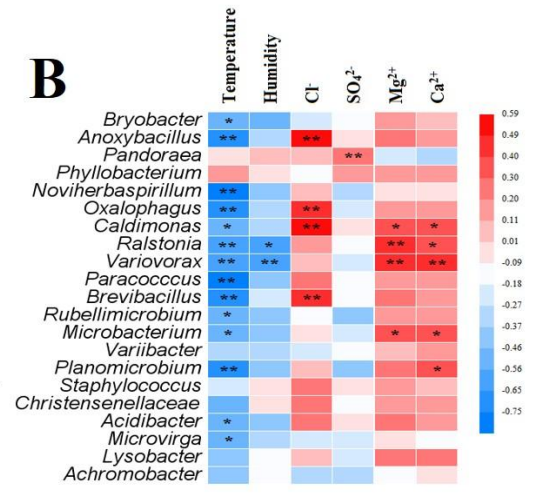
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Figure 4

A



B



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Figur 5