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1 **Investigation of Diverse Bacteria in Cloud Water at Mt. Tai,**  
2 **China**

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15 **ABSTRACT**

16 Bacteria are abundant in atmospheric water phase with the potential to influence  
17 atmospheric processes and human health, yet relatively little information is known  
18 about the bacterial characteristics at high altitudes. Here we investigated the bacterial  
19 community by high throughput sequencing in 24 cloud water samples collected from  
20 September 26 to October 31, at the summit of Mt. Tai (36°15' N, 117°06' E, 1534 m  
21 a.s.l) in China. Diverse bacterial population were identified and the gram-negative  
22 bacteria contributed the majority of total bacteria including Proteobacteria (81.6%) and  
23 Bacteroidetes (3.9%), followed by gram-positive bacteria Firmicutes (7.1%) and  
24 Actinobacteria (2.3%). These gram-negative taxa mainly inhabited in leaf-surface and  
25 cold environments. Meanwhile bacteria involved in the cloud condensation nuclei and  
26 ice nuclei formation were observed such as *Sphingomonas* (6.7%), *Pseudomonas*  
27 (4.1%), and *Bacillus* (1.1%). In addition, *Sphingomonas* was more active than that in  
28 daytime and participated in the cloud chemistry process. Meanwhile O<sub>3</sub> and SO<sub>2</sub>  
29 critically contributed to the variation of bacterial community. It is the first report on the  
30 bacterial community structure of cloud water over Asian area. Our results can serve as  
31 an important reference for environmental scientists, and biologists.

32 **Keywords:** Cloud water; Bioaerosol; Bacterial community; 16S rRNA sequencing

## 33 1. Introduction

34 Airborne bacteria have gained growing attentions from scholars, mainly focusing on  
35 their potential roles in atmospheric chemistry and nucleation process such as  
36 biotransformation of organic matter, carbon cycling, photochemical reactions, and  
37 cloud formation (Amato et al., 2007a; Bowers et al., 2009; Delort et al., 2010; Despres  
38 et al., 2012; Šantl-Temkiv et al., 2015; Smets et al., 2016). These microbes also provide  
39 a medium for the spread of diseases through potential pathogens and allergens (Bowers  
40 et al., 2012; Cao et al., 2014). The previous findings have reported that these bacteria  
41 at high altitudes are metabolic active and reproductive even under a hostile super-  
42 cooled environment (Smets et al., 2016). Increasing evidence stated that the active  
43 bacteria isolated from different cloud type (continental, marine, and anthropogenic  
44 cloud) can link to the photochemistry and biological reactions (Delort et al., 2010;  
45 Vařtilingom et al., 2012). While the bacterial characteristics obtained from orographic  
46 clouds have not been fully studied.

47 Orographic clouds are generated by the forced lift of air mass under a special  
48 topographic feature, in particular mountain regions. The air mass can quickly cool down  
49 adiabatically with the increase of altitudes, and then raise the relative humidity up to  
50 100%, a fact that is likely to influence the bacterial community (Kourtev et al., 2011).  
51 So far the research at the high altitudes were restricted, mainly focused on the bacterial  
52 ice nucleation activity, concentration and functions on the nucleation process  
53 (Matthias-Maser et al., 2000; Birgit et al., 2001; Marinoni et al., 2004; Vařtilingom et  
54 al., 2012; Joly et al., 2013; Amato et al., 2015). Researches on the total bacterial  
55 community were rare due to the limited measurement technique. Vařtilingom et al.  
56 (2012) investigated the microbial population in cloud water via cultured method at the

57 puy de Dôme Mountain (about 1465m), France. Yet, this method restricted on the  
58 cultured bacteria which contributed to less than 1% of the total bacteria (Amato et al.,  
59 2007a; Hill et al., 2007). With the advances in high-throughput sequencing, many  
60 researchers employed this technology to characterize the taxonomic identification of  
61 airborne bacteria at phylum, class, order, family, genus, and species levels. Bowers et  
62 al. (2009) presented the dominant Proteobacteria and Bacteroidetes which occupied  
63 about 93% of total bacteria by tagged pyrosequencing , and evaluated their potentials  
64 to act as ice nuclei at Mt. Werner (about 3200m), USA. Afterward at the same sampling  
65 site, they found the temperature affected the seasonal variation of airborne community  
66 dramatically (Bowers et al., 2012). DeLeon-Rodriguez et al. (2013) revealed an  
67 important impact of hurricanes on the bacterial community in the middle to upper  
68 troposphere across the United States. Maki et al. (2015) reported the metrological  
69 influence on the bioaerosol collected at three different altitudes (10m, 1000m, and  
70 3000m) in downtown atmosphere over the Noto Peninsula, Japan. However, the  
71 relevant researches were mostly conducted in America and Asia based on the aerosol  
72 samples, limited eyes concentrated on the bacterial population in cloud water across  
73 Asian region. Hence, it is essential to advance the knowledge, especially in the fast  
74 developing countries. During 2013, 2014 and 2015, Northern China experienced  
75 serious air pollution associated with the inadequate use of energy in the transport,  
76 domestic, and industrial sectors. North China Plain is the region with the worst air  
77 pollution in China, including Beijing, Tianjin, Shijiazhuang, Jinan, and Qingdao. Mt.  
78 Tai (36°15' N, 117°06' E, 1534 m a.s.l), locates on the summit of North China Plain,  
79 covers a total area of approximately 426 square kilometers, faces the east sea and back  
80 on the yellow river to the west. It is frequently attacked by cloud events, especially in  
81 March, April, June, July, October, and November (Guo et al., 2012; Liu et al., 2012;

82 Wang et al., 2011). The special terrain is conducive to the formation of orographic  
83 clouds. Across this area, researchers have described the physicochemical properties of  
84 cloud water, e.g. pH, conductivity, water soluble inorganic ions, sulfur (IV), total  
85 organic carbon, PAHs, heavy metal elements and organic acids (Li et al., 2011a; Li et  
86 al., 2011b; Li et al., 2013; Ervens et al., 2015; Li et al., 2016), and did not pay attentions  
87 on the airborne bacterial characteristics.

88 Herein, we used high-throughput sequencing to characterize the bacterial  
89 communities in cloud water collected at Mt. Tai, China, with environmental factors  
90 (PM<sub>2.5</sub>, SO<sub>2</sub>, O<sub>3</sub>, CO, and NO<sub>2</sub>) monitored in real time. The unique dataset fulfill the  
91 gap of the comprehensive analysis of bacterial communities at a high altitude site across  
92 the Asian area and allow us to address the following hypothesis: what characteristics  
93 the bacterial community in cloud water at high altitude exhibited? Does the bacterial  
94 diversity and community structure varied between daytime and nighttime? Does the  
95 bacterial community structure changed across different environmental factors?

## 96 **2. Material and methods**

### 97 *2.1 Cloud Water Collection and Environmental Factors*

98 Cloud water is formed by water condensation of atmospheric particles under  
99 supersaturation environment (Martinsson et al., 1999). We determined the cloud events  
100 based on the duration. The cloud process lasted more than one hour in a day was  
101 considered as one cloud event. For each event, continuous samples were collected once  
102 volumes got enough of 100 mL. As shown in Table 1, Mt. Tai experienced 10 cloud  
103 events, and 24 samples were collected from September 26 to October 31, 2014. The  
104 cloud water were sampled on a bank of Teflon strands at 24.3 m<sup>3</sup>/min by a fan situated

105 at the rear by by Caltech Active Strand Cloud water Collector Version 2 (CASCC2)  
106 (Collett et al., 2001; Wang et al., 2014; Modini et al., 2015). Collected samples flowed  
107 from the Teflon strands, through Teflon tube into a polyethylene bottle finally. The  
108 theoretical 50% cut-off size was equivalently drop diameter of 3.5  $\mu\text{m}$ . To avoid the  
109 artificial contamination, the Teflon strands and tube were kept in sterile criteria and the  
110 polyethylene bottles were pretreated with 75% ethanol before sampling. The pH and  
111 conductivity of the samples were measured immediately using a pH/conductivity  
112 analyzer (pH/COND/TEMP METER, 6350). An automatic meteorological station  
113 (JZYG, PC-4) and Synchronized Hybrid Ambient Real-Time Particulate monitor  
114 (SHARP, Model 5030, Thermo Fisher Scientific, USA) were used to measure the  
115 environmental temperature and PM<sub>2.5</sub> mass concentration in real time. Trace gases SO<sub>2</sub>,  
116 NO<sub>2</sub>, and O<sub>3</sub> in atmosphere were monitored by Model 42C, Model 43C, and Model 49C  
117 (Thermo Scientific, USA). CO was monitored by an API 300E analyzer (Teledyne,  
118 USA). The Hybrid Single Particle Lagrangian Integrated Trajectory (HYSPLIT) model  
119 was employed to create the twenty-four hours backward trajectories for air mass  
120 transport pathway arriving at 1534 m above ground level. The detail information about  
121 the sampling time and environmental factors were shown in Table 1. In order to identify  
122 the day/night variation of bacterial community, we specified daytime sampling from 7  
123 a.m. to 7 p.m. Beijing time and nighttime sampling from 7 p.m. to the next day 7 a.m.  
124 Beijing time. There were twelve samples sampled from daytime and nighttime.

## 125 *2.2 DNA Extraction and PCR Amplification*

126 Firstly, each sample was centrifuged for 3 min at 3000 rpm, then discarded the  
127 supernatants and extracted the pellets. Genomic DNA was extracted by a MP  
128 Biomedicals FastDNA Spin Kit for soil according to the manufacturer's directions.

129 Afterwards DNA concentration was analyzed by NanoDrop Spectrophotometer  
130 (NanoDrop Technologies, Rockland, DE, USA). The ultrapure water and a MP  
131 Biomedicals FastDNA Spin Kit blank served as negative controls to indicate any  
132 microbial contamination in the sampling and extraction kit. The V3-V4 hypervariable  
133 regions were amplified with the PCR primer pairs 341f (5'-  
134 CCTACGGGNGGCWGCAG) with adaptor B and 805r (5'-  
135 GACTACHVGGGTATCTAATCC) with adaptor A (Ye et al., 2013; Herlemann et al.,  
136 2011), and the barcode nucleotide sequences was equipped between PCR primer and  
137 adaptor. The PCR amplification was performed on an ABI GeneAmp<sup>®</sup> PCR  
138 system 9700 (Applied Biosystems, 101 Foster City, CA) using a 20 µL-mixture  
139 containing 4 µL 5×FastPfu buffer, 2 µL 2.5 mM dNTPs, 0.8 µL 5 µM forward primer,  
140 0.8 µL 5 µM reverse primer, 0.4 µL Fastfu polymerase, 10 ng template DNA, and 11  
141 µL double distilled H<sub>2</sub>O. PCR was performed according to the procedures outlined in  
142 the instruction manual of the detection kit: 95°C (3 min, hold), 95°C (30 s), 55°C (30  
143 s), 72°C (42 s), and 10°C (hold). The PCR products were separated by 1% agarose gel  
144 electrophoresis and purified with an axygen nucleic acid purification Kit (Axygen,  
145 USA). Finally, the purified products were prepared for sequencing on the Illumina  
146 Miseq<sup>™</sup> platform (Illumina Inc., San Diego, CA, USA).

### 147 *2.3 Sequencing Data*

148 Raw sequences were processed with the Mothur version 1.25.0  
149 (<http://www.mothur.org/wiki>) and FastX Toolkit software  
150 ([http://hannonlab.cshl.edu/fastx\\_toolkit](http://hannonlab.cshl.edu/fastx_toolkit)) based on the reference in Ribosomal Database  
151 Project (RDP) database for bacteria. All sequences were trimmed by removing the  
152 barcodes and low-quality sequences. Total 577523 effective sequences were obtained



153 for 24 samples and the sequence number of each sample was normalized to 9100 reads  
154 (the lowest in the 24 samples) in order to compare the different samples effectively after  
155 quality control. The high-quality sequences were clustered into operational taxonomic  
156 units (OTUs) at a 97% sequence similarity cutoff by uCLUST software  
157 (<http://www.drive5.com/usearch/index.html>). The alpha-diversity estimators including  
158 Chao1, Coverage, Simpson, and Shannon index were calculated based on the 97%  
159 species similarity. At this similarity level, the variation tendencies across the different  
160 samples were shown clearly and in details (Di Bella et al., 2013). The similarity trees  
161 were constructed according to the bray-curtis distance coefficient. The Ribosomal  
162 Database Project (RDP) was employed to determine the abundance of bacterial taxa at  
163 phylum, class, order, family and genus level using similarity cutoff values at 80, 85, 91,  
164 92 and 95% similarity, respectively (Quadros et al., 2016). MetaPhlAn ((Metagenomic  
165 Phylogenetic Analysis) was used to create phylogenetic tree of the bacteria identified  
166 from cloud water samples. The nucleotide sequences obtained in this study have been  
167 deposited in the Sequence Read Archive (SRA) under accession numbers SRA267745.  
168 In order to determine the specific taxa between daytime and nighttime, linear  
169 discriminant analysis effect size (LEfSe) was employed in this study  
170 (<http://huttenhower.sph.harvard.edu/galaxy/>). It is able to analyze two or more sets  
171 groups at any taxonomic level (Mahnert et al., 2015), and then establishes statistical  
172 significance, biological consistency, and effect-size estimation of predicted biomarkers  
173 through bacterial taxa explanations. We use the default logarithmic (LDA) value higher  
174 than 4 for clarity.

### 175 **3. Result and Discussion**

#### 176 *3.1 Bacterial Diversity*

177 Total 128 to 1910 OTUs were generated from high quality 16S rRNA sequences with  
178 an average of 664 OTUs per sample. The saturation rarefaction curves for OTUs  
179 approached an asymptote (Figure 1A), indicating the entire diversity was acquired.  
180 Notably, the OTUs were higher than that in aerosol collected in the upper troposphere  
181 (314; Deleon-Rodriguez et al., 2013), implying that the cloud water at Mt. Tai had a  
182 higher bacterial richness. It was also confirmed by Chao1 richness index. The value  
183 was higher than that in aerosols obtained in 1000 m over the Noto Peninsula, Japan  
184 (410, Maki et al., 2015) and other hurricane-related aerosols at high altitudes across the  
185 United States (241, DeLeon-Rodriguez et al., 2013). The Shannon index for estimation  
186 of bacterial diversity showed a daily variation tendency. The daytime samples had a  
187 higher Shannon index (3.8) than the nighttime samples (3.0), even higher than the Asian  
188 dust aerosols (3.51, Jeon et al., 2011). It means that higher richness and diversity of  
189 bacteria were observed in cloud water than that in aerosol obtained at high altitude or  
190 aerosol collected obtained at the earth ground affected by the Asian-dust events.

191 Based on the hierarchical clustering analysis, samples containing different OTUs  
192 were divergent into two groups (Figure 1B). Eight samples obtained in Sep. 26, Sep.  
193 29 and Oct. 12, 2014 clustered together forming Group I (OTUs: 199, Chao 1: 517.5,  
194 and Shannon: 2.0). The remaining samples making up to the largest group II which had  
195 a more neutral pH (5.8) and conducted to a greater bacterial diversity (OTUs: 866,  
196 Chao1: 1694.2; Shannon: 4.1).

197 3.2 *Bacterial Community Taxonomy*

198 About 99.6% of OTUs were classified into 29 phyla and the most abundant one was  
199 Proteobacteria (81.6%). Similar result was observed in other samples including cloud  
200 water (50%, Vaithilingom et al., 2012) and aerosol collected at high altitudes (83%,  
201 Bowers et al., 2009; 49.4%, Bowers et al., 2013; over 50%, DeLeon-Rodriguez et al.,  
202 2013). The following phyla were Firmicutes (7.1%), Bacteroidetes (3.9%),  
203 Actinobacteria (2.3%), and Cyanobacteria (1.1%). The abundant of Proteobacteria and  
204 Bacteroidetes implied a large proportion of gram-negative fractions in total bacteria.  
205 While in cultivated bacteria, gram-positive bacterial population were preponderant due  
206 to those gram-negative strains lose cultivability after aerosolization (Amato et al.,  
207 2007b; Heidelberg et al., 1997). Hence the bacteria in cloud water were mainly  
208 occupied by the gram-negative bacteria. At the class level, 89.8% of sequences were  
209 classified into 45 taxa, and the Gammaproteobacteria, Betaproteobacteria,  
210 Alphaproteobacteria were dominant with relative abundance of 44.5%, 23.6%, and  
211 12.9%. Since there were no comparable surveys about cloud microbe using high-  
212 throughput sequencing currently, we compared the results with airborne bacterial  
213 community at high altitude sites analyzed by high-throughput sequencing. The  
214 community was slightly different with aerosols collected at different altitudes. The  
215 marine bacterial members Alphaproteobacteria and Cyanobacteria were dominant in  
216 aerosols obtained at 1000m, while terrestrial bacteria Bacilli and Actinobacteria  
217 contributed more sequences to aerosols collected at 3000m (Maki et al., 2015). In  
218 present study, Cyanobacteria was not predominant, but it showed a higher abundance  
219 (2.1%) in samples B and H which was related to a back trajectory of air mass come  
220 from Yellow Sea than other samples from terrestrial air mass (1.0%). Bacilli were more

221 abundant in the Asian dust mineral particles caused by Chinese desert region (49.9%;  
222 Maki et al., 2014) than that obtained in this study (3%). According to the 24 h back  
223 trajectory prior to sampling, the air mass of Sep 26 (sample G) moved from the Chinese  
224 mineral region Shanxi Province (Figure 3). Hence, the abundance of Bacilli in sample  
225 G was 2.2 times higher than that in non-mineral-influenced samples. It is coincident  
226 with the result reported by Maki (2015) that members of Bacilli at high altitudes have  
227 potential to form resistant endospores and take part in long distance transmission.

228 At the order level, Pseudomonadales (Gammaproteobacteria), which is commonly  
229 found in the leaf-surface environment (Bowers et al., 2012), was most abundant (over  
230 30%) in cloud water, may be resulting from approximately 80% vegetation coverage at  
231 Mt. Tai (Figure 2). Further, 192 families were identified in cloud samples and the taxa  
232 of dominant families were similar to those groups identified in other airborne studies  
233 (Bowers et al., 2012 and Maki et al., 2014). However the abundance was significantly  
234 different. Moraxellaceae family affiliating to Pseudomonadales was considered to be  
235 the most abundant family at Mt. Tai (35.4%). This value was much higher than that  
236 obtained at the summit of Mt. Werner (40°27' N, 106°43' W, 3220 m a.s.l), USA (less  
237 than 10%; Bowers et al., 2012). This bacterium is also an important part of bacterial  
238 community in other cold environments such as Antarctic soil (Bowman et al., 1996),  
239 Antarctic sea ice (Bozal et al., 2003), and snow (Amato et al., 2007b). At the genus  
240 level, members of Gamma-Proteobacteria have been proved to participate in the  
241 nucleation process by act as cloud condensation nuclei (CCN) and ice nuclei (IN).  
242 Through the high throughput sequencing, we observed sequences with homology to the  
243 following known cloud condensation nucleating and ice nucleating bacteria:  
244 *Sphingomonas* (6.7%), *Pseudomonas* (4.1%), *Bacillus* (1.1%), *Rhodococcus* (0.5%),

245 and *Psychrobacter* (0.4%). These bacteria cycling in near-surface atmosphere  
246 experience aerosolization processes by emission from surface cover (soil, ocean, and  
247 vegetation) and deposition processes by rain and cloud. First, bacteria take part in the  
248 cloud formation via offering a surface for the water vapor condensation at the  
249 supersaturation over the particle surface. The presence of lipopolysaccharides or  
250 exopolymeric substances on bacterial surface can enhance its activity ability to cloud  
251 droplets (Delort et al., 2010). For instance, several bacteria (such as *Pseudomonas*  
252 strains) can produce biosurfactants which may decrease the surface tension of cloud  
253 droplets and result in the supersaturation (Ahern et al., 2007). The pioneering works  
254 have found strains belonging to gram-negative bacterium *Pseudomonas* and gram-  
255 positive bacteria (*Rhodococcus* and *Bacillus*) are biosurfactant producers and play a  
256 role in cloud condensation nuclei formation (Maki et al., 1974; Hirano et al., 2000;  
257 Bauer et al., 2003; Santl-Temkiv et al., 2015). Secondly, the ice-nuclei-active bacteria  
258 in cloud water have strong ice nucleation efficiency between -7 and -9°C, especially  
259 epiphytic bacteria (Ponder et al., 2005, Delort et al., 2010).

### 260 3.3 Differences between Daytime and Nighttime.

261 Across different sampling time (daytime and nighttime), bacterial community  
262 structure exhibited obvious disparities as shown in Figure 4. Total 31 taxa were clearly  
263 distinguishable using the default logarithmic (LDA) value higher than 2. Total 14 taxa  
264 enriched in daytime, while no fine lineages had an LDA value higher than 4. At  
265 daytime, the active bacteria not only participate in the atmospheric biological process  
266 (biotransformation and biochemical degradation pathways), but also have the ability to  
267 absorb or reflect the sunlight, which may induce photochemistry reaction (Delort et al.,  
268 2010; Despres et al., 2012). Photochemistry reaction was limited during nighttime. And

269 low temperature may cause the shift of bacterial community. During nighttime five  
270 major indicators affiliated to the phylum of Proteobacteria enriched including  
271 Alphaproteobacteria (class) and the cluster of Sphingomonadales (from class to  
272 species). Vaitilingom et al. (2010) found that biodegradation by *Sphingomonas* strains  
273 at nighttime contribute more to cloud chemistry than that during the daytime due to the  
274 competition with photochemistry during the daytime. Amato et al. (2005) also proved  
275 that *Sphingomonas* was psychrophilic and able to grow better at low temperature (5°C)  
276 due to that they synthesis yellow or off-white color pigment colonies likely belonging  
277 to the carotenoid family which has been described to protect bacteria from low  
278 temperatures (Vincent et al., 2000; Asker et al., 2007). Thus, the *Sphingomonas* under  
279 the conditions of no light and low temperature may enriched during nighttime

#### 280 *3.4 Interrelationship between Bacterial Community and Environmental factors*

281 The categorization classified by the hierarchical clustering analysis in the bacterial  
282 community structures could be identified at the order level. Pseudomonadales  
283 contributed almost 39.2% of the bacteria in group II. While in group I, this order  
284 reduced to less than 17.8% and replaced by members of the Burkholderiales (35.7%)  
285 and Enterobacteriales (28.7%) apparently which as an effect of marine especially in  
286 sample B. Previous researchers have presented the discrepancies of bacterial  
287 community across different samples. Bowers et al., (2011) reported that the shifts of  
288 the airborne bacterial community in three land-use types, in part, driven by the  
289 differences in the relative contributions of bacteria from specific source habitats. Jeon  
290 et al., (2011) observed that the Asian dust directly affect the airborne bacterial  
291 communities by air mass transport pathways. Maki et al., (2015) proved the effect of  
292 air mass and metrological conditions on the bacterial community at different altitudes

293 in downwind atmosphere. Amato et al. (2007c) reported that bacteria emitted by ocean  
294 could transmit into cloud droplets in the free troposphere. Here the abundance of marine  
295 bacterium *Pseudomonas* (Lee et al., 1977) in marine-influenced samples was found to  
296 be 9-times higher than the non-marine influenced cloud samples. Owing to the lack of  
297 standardization in the sample collection and analysis process, it is hard to explain these  
298 differences with previous publications. The discrepancies may be due to the different  
299 analysis methods (Denaturing Gradient Gel Electrophoresis, cultured method, and  
300 BLASTN application, and high throughput sequencing), sampling methods,  
301 metrological conditions (temperature, UV radiation, wind speed, and humidity),  
302 sampling location (geographic location, land use, traffic nearby, and altitude), and  
303 environmental factor (pH, conductivity, PM<sub>2.5</sub>, SO<sub>2</sub>, O<sub>3</sub>, CO, NO<sub>2</sub>, and temperature).

304 Herein the redundancy discriminant analysis showed that the environmental factors  
305 distinctly related to the variations of species-environment relation in different samples.  
306 The first axis explained 66.7% of the variation of species-environment relationship, and  
307 the second axes explained 14.0% (Figure 5). The RDA with original data shows that O<sub>3</sub>  
308 and SO<sub>2</sub>, which made 0.001 Pr and 0.019 Pr with 999 permutations number based on  
309 1% level in a permutation test, was the critical factors for the species-environment  
310 relationship. Corresponding values were higher than 0.05 for other environmental  
311 factors. Therefore, O<sub>3</sub> and SO<sub>2</sub> in atmosphere contributed statistically significant to  
312 variation of bacterial community. The O<sub>3</sub> and SO<sub>2</sub> gases participated in the disinfection  
313 of bacteria and formation of SO<sub>4</sub><sup>2-</sup> in cloud water. Zuma et al. (2009 and 2010) showed  
314 that ozone have bigger germicidal effectiveness to gram-negative and gram-positive  
315 strains than the free radicals. Kim et al., (2000) observed a lower survivors fraction of  
316 *Pseudomonas* strains with increasing ozone treatment. H<sub>2</sub>SO<sub>4</sub> generated by SO<sub>2</sub> in

317 atmosphere were known to be toxic for micrograms such as Rhodobacteraceae and  
318 Desulfobacterales (Gao et al., 2016; Won et al., 1969). In the present study, these two  
319 factors mainly have a positive influence on the relative abundance of *Noviherbasirillum*,  
320 *Serratia*, and *Massilia*, and then act on the distribution of samples dominated by these  
321 genera, such as sample A, B, and C in group I. In addition, PM<sub>2.5</sub>, conductivity, and  
322 temperature were also important to positive correlation with the relative abundance of  
323 *Sphingomonas*, which may be explained by their relatively tolerance of high PM  
324 pollutants and capability to degrade the organic matter in atmosphere (Gingell et al.,  
325 1976; Ye et al., 1995). It was consistent with Sun et al. (2014) that temperature was a  
326 key factor in the aquatic bacterial community's composition. While in group II, pH and  
327 NO<sub>2</sub> have also great impact on the *Acinetobacter* and *Acidovorax*. Further  
328 investigations combination culture techniques and bacterial concentration analysis are  
329 required to accurately evaluate the detail influencing mechanisms on the growth and  
330 development of bacteria.

#### 331 **4. Conclusion**

332 In order to describe the bacterial community in atmospheric cloud system and its  
333 variation, cloud water were collected at the summit of Mt. Tai and analyzed by high  
334 throughput sequencing. Diverse bacterial population at high altitude was dominated by  
335 gram-negative fractions, e.g. *Acinetobacter*, *Serratia*, *Massilia*, *Sphingomonas*,  
336 *Acidovorax*, and *Pseudomonas*. Some member of these taxa are able to participate in  
337 the nucleation process by act as cloud condensation nuclei (CCN) and ice nuclei (IN)  
338 in cloud system. The bacterial community showed a significant daily tendency between  
339 daytime and nighttime. Meanwhile environmental factors including O<sub>3</sub>, and SO<sub>2</sub>  
340 critically contributed to the variation of bacterial community structure. In the future the



341 real time quantitative PCR and cultured experiments should be combined to target the  
342 detail impact mechanisms of environmental factors on the variation of bacteria  
343 community.

#### 344 **Conflict of Interest**

345 The authors declare no conflict of interest.

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537 **Table 1** Sampling time and environmental parameters over the sampling period.

Sample ID	Sampling Time	pH	PM <sub>2.5</sub> ( $\mu\text{g m}^{-3}$ )	Conductivity	CO(ppm)	SO <sub>2</sub> (ppb)	O <sub>3</sub> (ppb)	NO <sub>2</sub> (ppb)
A1	09/26/2014 0:00-2:00	5.8	32.9	813.5	1.0	0.9	72.1	
A2	09/26/2014 2:00-3:00	6.3	12.9	365.7	0.7	0.6	66.4	
B1	09/29/2014 0:00-3:00	6.9	0.9	50.5	0.4	0.1	55.9	
B2	09/29/2014 3:00-5:00	6.6	0.7	66.0	0.5	0.2	44.7	
B3	09/29/2014 5:00-7:00	6.7	2.0	44.9	0.4	0.1	46.6	
C1	10/12/2014 8:30-10:00	3.8	28.0	330.8	1.2	4.2	51.1	8.7
C2	10/12/2014 10:00-11:00	4.2	16.3	236.8	1.0	1.6	43.0	6.8
C3	10/12/2014 11:00-12:00	5.4	3.9	71.7	0.7	0.8	26.6	7.5
D1	10/19/2014 10:43-13:23	6.6	3.8	141.7	0.6	0.5	8.9	4.9
D2	10/19/2014 13:23-15:02	6.1	4.4	109.7	0.6	0.4	6.5	5.3
E1	10/20/2014 16:55-18:15	5.1	10.6	236.8	1.6	1.1	11.9	9.0
E2	10/20/2014 18:15-19:35	4.9	23.4	295.2	1.5	0.6	11.9	6.0
F1	10/21/2014 0:10-2:20	4.3	2.6	154.7	0.8	0.4	10.3	3.1

F2	10/21/2014 2:00-4:55	5.1	1.5	42.2	0.7	0.3	12.5	3.5
G1	10/23/2014 20:15-23:30	5.8	12.5	370.7		0.3	7.7	13.7
G2	10/23/2014 23:30-3:30	5.7	13.2	354.9		0.2	3.5	13.1
H1	10/29/2014 19:40-1:30	6.4	26.0	352.6	1.2	0.4	8.3	6.0
J1	10/30/2014 1:30-4:00	6.2	4.0	60.4	0.7	0.2	8.4	6.4
J2	10/30/2014 12:00-14:00	6.6	2.3	20.4	0.4	0.1	5.5	6.6
J3	10/30/2014 14:00-16:00	6.2	1.4	13.4	0.4	0.1	9.5	5.6
J4	10/30/2014 16:00-17:30	6.2	2.4	22.0	0.4	0.1	12.1	5.9
J5	10/30/2014 17:30-19:00	6.3	4.9	22.3	0.6	0.1	12.5	6.7
J6	10/30/2014 19:00-20:30	6.9	2.3	184.7	0.6	0.1	12.4	6.0
K1	10/31/2014 15:55-17:00	4.4	4.8	128.1	0.8	0.3	3.2	10.5

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## List of Figures

**Figure 1.** The saturation rarefaction curves (A) and hierarchical clustering (B) of the abundant communities at OTU level of 24 cloud water samples..

**Figure 2.** Bacterial community structure of cloud water identified at the order level. Only bacteria with relative abundance  $\geq 1\%$  were labeled.

**Figure 3.** Geographical location of Mt. Tai and HYSPLIT back trajectories of air masses during sampling period. The 24 h backward trajectories were obtained from the NOAA ARL (<http://ready.arl.noaa.gov/>).

**Figure 4.** Indicator microbial groups within the two types of cloud water with LDA values higher than 2 (red indicating daytime and green indicating nighttime).

**Figure 5.** Redundancy discriminant analysis (RDA) biplot of the distribution of bacterial communities with environmental variables in cloud water at Mt. Tai. The upper left legend shows color-coding indicating ten cloud events.

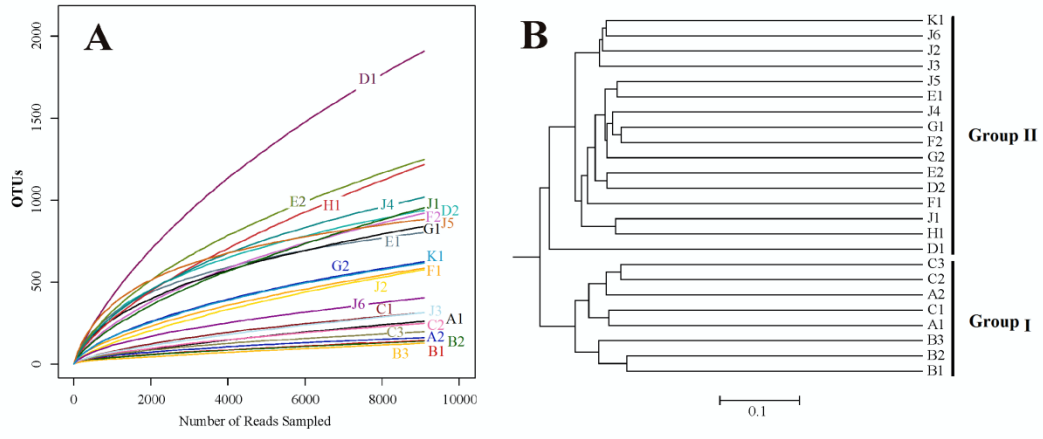
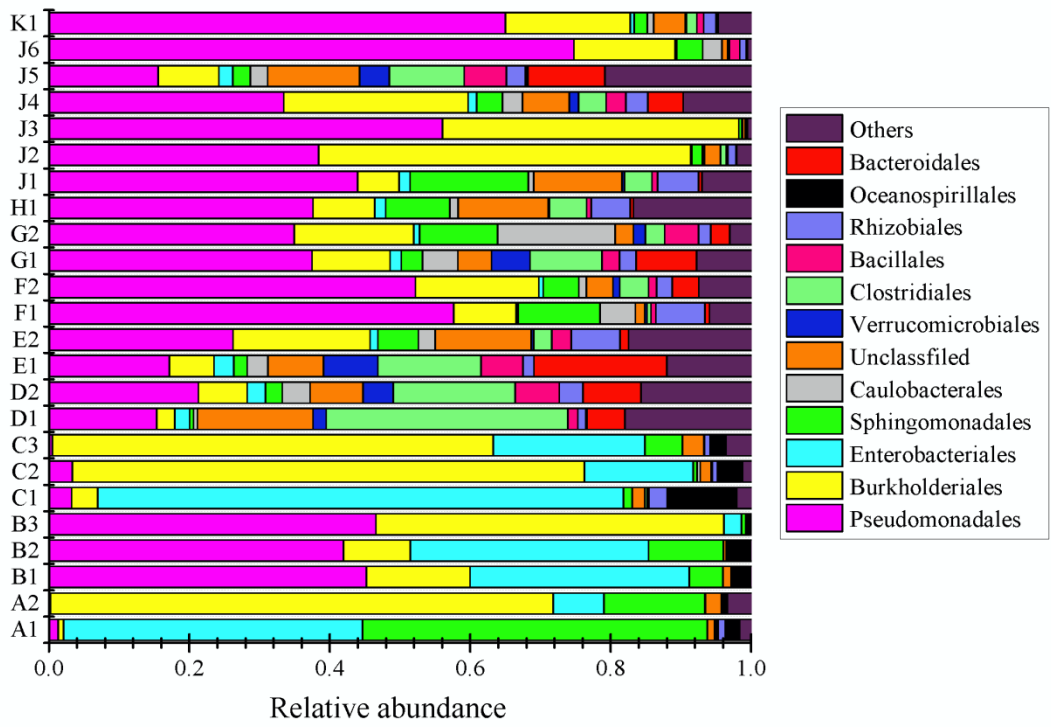


Figure 1



**Figure 2**

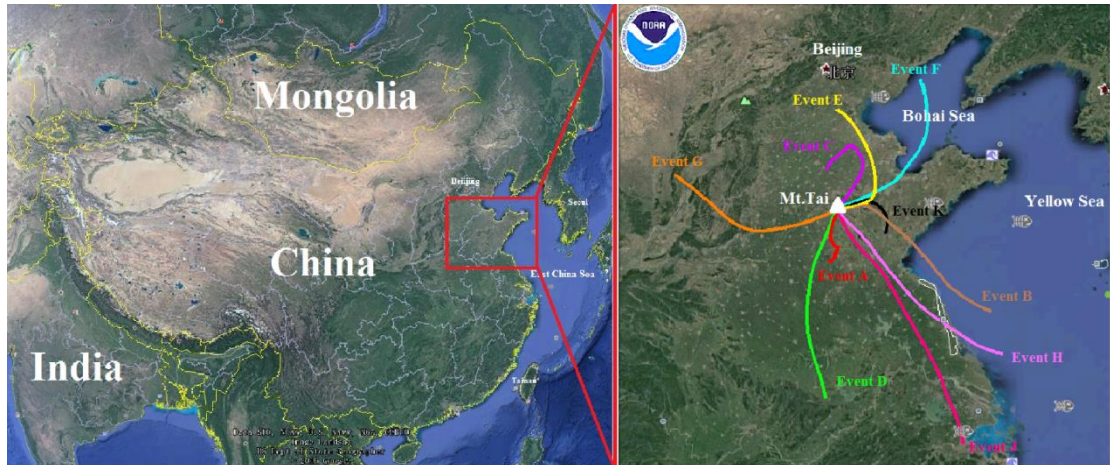


Figure 3



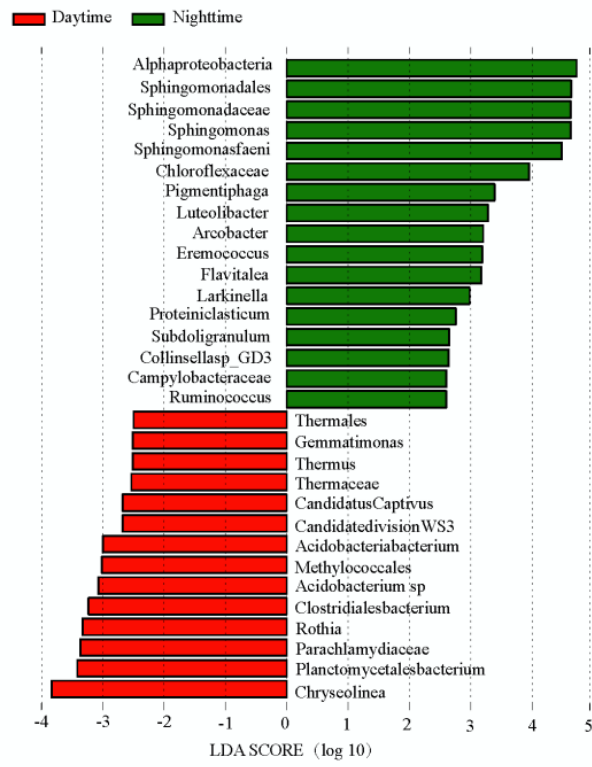


Figure 4

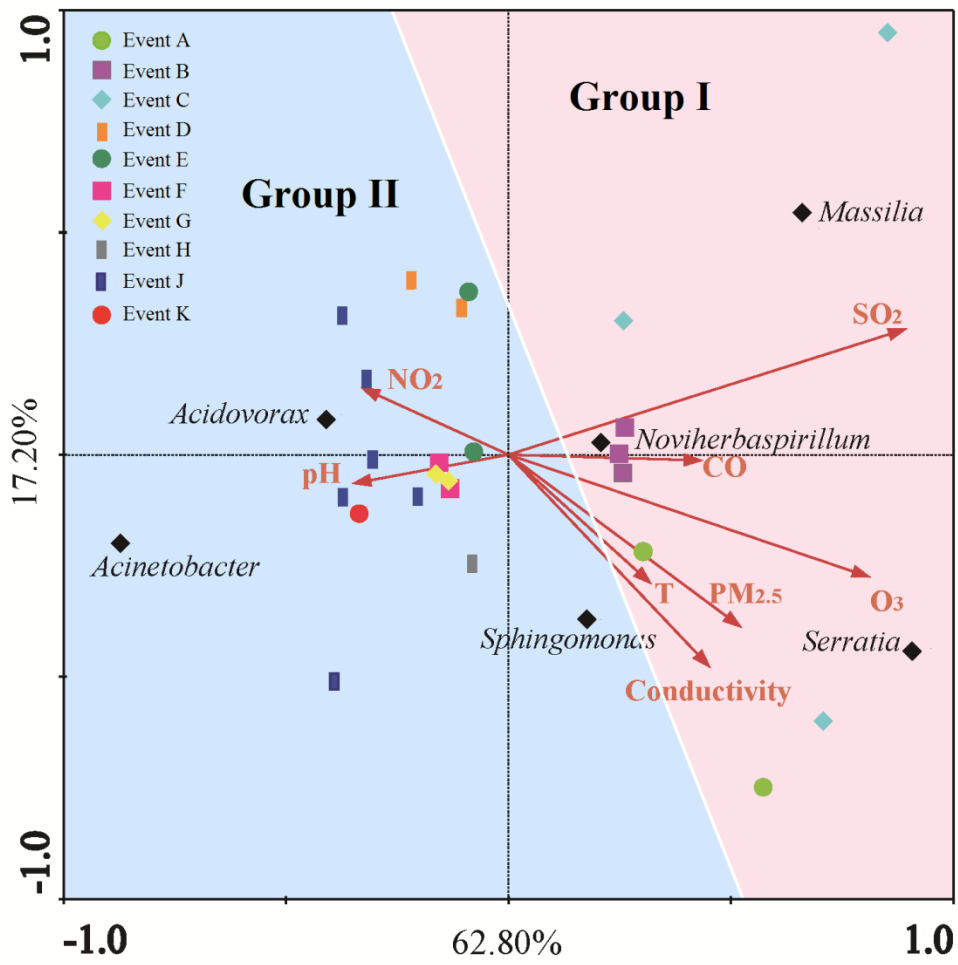


Figure 5