

## **Bacterial characterization in ambient submicron particles during severe haze episodes at Ji'nan, China**

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1           **Bacterial Characterization in Ambient Submicron Particles during**  
2                                   **Severe Haze Episodes at Ji'nan, China**

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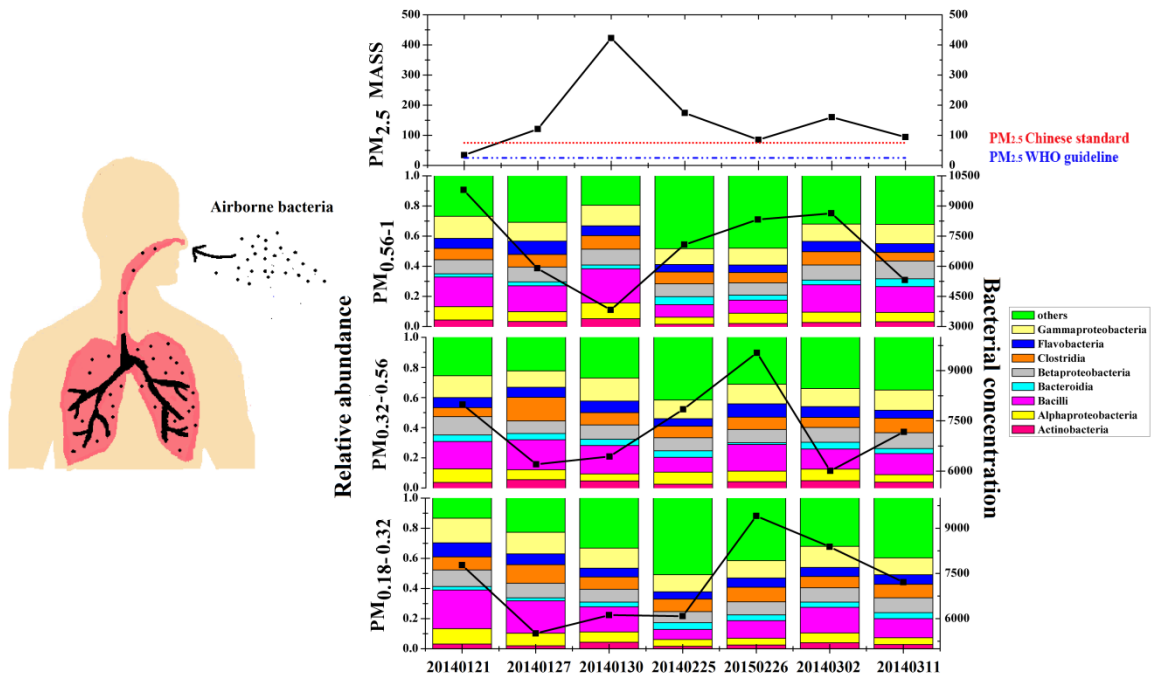
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15 **Graphic Abstract**



16

17 **HIGHLIGHTS**

- 18 1. High bacterial concentration and diverse bacterial community in submicron particles  
 19 (PM<sub>0.18-0.32</sub>, PM<sub>0.32-0.56</sub>, and PM<sub>0.56-1</sub>) during haze episodes were observed.  
 20 2. The bacterial community varied significantly via different size fractions.  
 21 3. Source track analysis showed that the ambient bacteria mainly originated from soils, leaf  
 22 surfaces, and feces.

23 **ABSTRACT**

24 In January 2014, severe haze episodes which sweep across Chinese cities have attracted  
25 public concern and interest at home and abroad. In addition to the physicochemical properties  
26 of air pollutants, bacteria are thought to be responsible for the spread of respiratory diseases  
27 and various allergies. We attempted the bacterial characterization of submicron particles  
28 (PM<sub>0.18-0.32</sub>, PM<sub>0.32-0.56</sub>, and PM<sub>0.56-1</sub>) under severe haze episodes using high-throughput  
29 sequencing and real-time quantitative PCR detecting system based on 21 samples collected  
30 from January to March 2014 at Ji'nan, China. The high bacterial concentration in PM<sub>0.32-0.56</sub>  
31 (7314 cells·m<sup>-3</sup>), PM<sub>0.18-0.3</sub> (7212 cells·m<sup>-3</sup>), and PM<sub>0.56-1</sub> (6982 cells·m<sup>-3</sup>) showed significant  
32 negative correlations with SO<sub>2</sub>, NO<sub>2</sub>, and O<sub>3</sub>. Under sufficient sequencing depth, 37 phyla,  
33 71 classes, 137 orders, 236 families, and 378 genera were classified, and the bacterial  
34 community structure varied significantly in different size fractions. For example,  
35 Holophagaceae (Acidobacteria) in PM<sub>0.32-0.56</sub> showed 6-fold higher abundance than that in  
36 PM<sub>0.18-0.32</sub>. Moreover, functional categories and bacterial species (*Lactococcus piscium*,  
37 *Pseudomonas fragi*, *Streptococcus agalactiae*, and *Pseudomonas cichorii*) that may  
38 potentially be responsible for infections and allergies were also discovered. Source track  
39 analysis showed that the ambient bacteria mainly originated from soils, leaf surfaces, and  
40 feces. Our results highlighted the importance of airborne microbial communities by  
41 understanding the concentration, structure, ecological and health effects, especially those in  
42 submicron particles during haze episodes.

43 **Keywords:** Bioaerosol; Haze; Bacterial community; Submicron particles

## 44 1. Introduction

45 In the last few decades, the rapid economic growth and energy consumption, along with the  
46 lack of measures for protecting atmospheric environments, has resulted in continuous haze  
47 episodes in China (Yang et al., 2015; Li et al., 2014). In severe haze episodes, the daily  
48 average PM<sub>2.5</sub> mass concentration of Jing-Jin-Ji regions (Wang et al., 2013; Han et al., 2016)  
49 largely exceeded 25  $\mu\text{g}\cdot\text{m}^{-3}$ , which is specified as the limit of the World Health  
50 Organization(WHO) PM<sub>2.5</sub> health guideline, by about 20-fold. Exposure to such high  
51 concentrations of airborne particles leads to high morbidity and mortality due to infectious  
52 diseases such as cardiovascular diseases, respiratory infections, and lung cancer (Bower et  
53 al., 2013; Esposito et al., 2012). Based on the definition of haze by the State Standard of the  
54 People's Republic of China (QX/T 113-2010), haze is defined as a complex air pollution  
55 process include the following conditions: (1) visibility less than 10 km and relative humidity  
56 lower than 80%, and (2) the PM<sub>2.5</sub> mass concentration higher than 75  $\mu\text{g}\cdot\text{m}^{-3}$  (Leng et al.,  
57 2013; Kong et al., 2014; Jansen et al., 2014; China Meteorological Administration, 2010). In  
58 January of 2013 and 2014, severe haze episodes were reported in Beijing (Wei et al., 2016),  
59 Hebei (Wang et al., 2013), Nanjing (Kong et al., 2015), and Ji'nan (Wang et al., 2015a) which  
60 caused great economic losses and public panic across China. Ji'nan is the capital of Shandong  
61 Province and covers an area of 8177 km<sup>2</sup>. It is surrounded by hills on three sides which may  
62 exacerbate the accumulation of airborne pollutants including atmospheric particles, sulfur  
63 dioxide, nitrogen oxide, trace gases, and volatile organic compounds (Liu et al., 2015; Zhang  
64 et al., 2014; Li et al., 2011). Majority of existing studies focussed on the bacteria which  
65 occupied about 80.8% and 86.1% of the total microbes (Cao et al., 2014) in PM<sub>2.5</sub> and PM<sub>10</sub>.  
66 Limited studies investigated bacteria in submicron particles (Gou et al. 2016), which can  
67 easily penetrate lungs or even the blood stream (Janssen et al., 2011; Visser et al., 2015; Gao  
68 et al., 2015b). Hence it is essential to study the bacterial characteristics of such

69    aforementioned submicron particles in atmosphere.

70       The near-surface and upper troposphere contain thousands to millions of bacterial cells per  
71    cubic meter (Bower et al. 2012). Active bacteria can serve as a medium for the spread of  
72    allergens and pathogens in a crowd (Creamean et al., 2013; Husman et al., 1996). There are  
73    also increasing evidences indicating that bacteria can act as cloud condensation nuclei,  
74    absorbing or reflecting sunlight, or even participating in N-cycling and C-cycling in the  
75    ecosystem (Bauer et al., 2003). So far, many investigations on the active bacterial  
76    concentration and bacterial community in airborne particles have been conducted (Bertolini  
77    et al., 2013; Hospodsky et al., 2015; Prussin et al., 2015). The airborne bacterial concentration  
78    in the near-surface ranged from  $10^4$  to  $10^6$  cells·m<sup>-3</sup> (Bowers et al., 2012; Haas et al., 2013;  
79    Murata et al., 2014; Goudarzi et al. 2014; Murata et al., 2016). Bower et al. (2013) reported  
80    detailed information on the airborne microbial community and sources in PM<sub>2.5</sub> and PM<sub>2.5-10</sub>  
81    and found that the bacterial richness and communities structures showed a significant  
82    distinction across these two size fractions. In China, Cao et al. (2014) described the microbial  
83    communities of PM<sub>2.5</sub> and PM<sub>10</sub> using metagenomics during a serious smog event and found  
84    that bacteria were the dominant one which was mostly terrestrial-related. Wei et al. (2016)  
85    investigated the concentration and size distribution of bioaerosols during haze and sunny  
86    days in Beijing. Compared to the sunny day, the fluorescent particle concentrations increased  
87    during the haze episodes and decreased with the dissipation of haze occurrences in 3-5 days.  
88    Furthermore no obvious difference in the airborne bacterial abundance and community  
89    structure were observed between haze and sunny days. Although these studies have  
90    illustrated the concentration and community compositions of cultured or uncultured bacteria  
91    in atmospheric fine particles, studies on bacterial characterizations in submicron particles are  
92    rare, especially during severe haze episodes.

93       Herein, we first characterized severe haze episodes to reveal the nutrients in submicron

94 particles from January to March 2014 in Ji'nan. The bacterial concentration and community  
95 structure of different particle size fractions was analyzed subsequently. Third, we performed  
96 functional analysis of the bacteria in the submicron particles to assess their potential to cause  
97 risk to human health. Our study draws a framework of bacterial community in Jinan's  
98 submicron particles during haze episodes and emphasizes the health risks of long-term  
99 exposure to high concentrations bacteria.

## 100 **2. Materials and methods**

### 101 *2.1. Aerosol Collection.*

102 Aerosol samples were collected from the rooftop of the Lizong building in the central  
103 campus of Shandong University located in Ji'nan (36°40'N, 117°3'E). The Lizong building  
104 is a six-floored teaching building where classes are conducted from 08:00 to 17:00 from  
105 Monday to Friday. To avoid the interference from local anthropogenic emissions on the  
106 ground, a Micro-Orifice Uniform Deposit Impactor (MOUDI) and on-line monitoring  
107 instruments such as SO<sub>2</sub> analyzer (Model 43C, Thermo, USA), NO<sub>x</sub> analyzer (Model 42C,  
108 Thermo, USA), O<sub>3</sub> analyzer (Model 49C, Thermo, USA) were placed in the rooftop of  
109 Lizong building about 20 m from the ground. We sterilized the quartz membrane by baking  
110 in a Muffle furnace at 500 °C for 6 h before sampling. After the filter cooled, it was packaged  
111 into sterilized aluminum foil and stored in a sealed bag. Before sampling, the inside surfaces  
112 of the MOUDI were kept sterile and 75% ethanol was used to sterilize the impactor. Seven  
113 sets of aerosol samples were obtained on the 47-mm quartz membrane of the MOUDI for  
114 23h (9:00 am to 8:00 am next day) at a flow rate of 30 lpm during Jan. 20, 2014 to Mar. 31,  
115 2014; these samples were stored at -80 °C until analysis. Each set contained nine samples in  
116 nine size-resolved ranges as follows: stage1,  $\geq 18 \mu\text{m}$ ; stage2, 10–18  $\mu\text{m}$ ; stage3, 5.6–10  
117  $\mu\text{m}$ ; stage4, 3.2–5.6  $\mu\text{m}$ ; stage5, 1.8–3.2  $\mu\text{m}$ ; stage6, 1.0–1.8  $\mu\text{m}$ ; stage7, 0.56–1.0  $\mu\text{m}$ ;

118 stage8, 0.32–0.56  $\mu\text{m}$ ; and stage9, 0.18–0.32  $\mu\text{m}$ . The  $\text{PM}_{1.0}$  can easily penetrate thoracic  
119 and pulmonary airways and plays an important role in haze formation and visibility  
120 degradation (Shi et al., 2014). Meanwhile the fact that specific surface area of  $\text{PM}_{1.0}$  is greater  
121 than  $\text{PM}_{2.5}$  provides evidences that  $\text{PM}_{1.0}$  containing more health risks. Therefore we used  
122 the stage7, stage8, and stage9 samples for the following experiments. An automatic  
123 meteorological station (JZYG, PC-4) was employed to measure meteorological factors (wind  
124 direction, wind speed, humidity, and temperature) in real time. Meanwhile, a Synchronized  
125 Hybrid Ambient Real-Time Particulate monitor (SHARP, Model 5030, Thermo Fisher  
126 Scientific, USA) and a Monitor for AeRosols and GAses analyzer (MARGA, ADI20801,  
127 Applikon-ECN, Netherlands) were used to analyze the hourly average mass concentration of  
128  $\text{PM}_{2.5}$ , water-soluble ions, and trace gases as described previously. Based on the definition  
129 of haze, seven days including six haze days (January 27, 2014; January 30, 2014; February  
130 25, 2014; February 26, 2014; March 2, 2014; and March 11, 2014) and one clear day (January  
131 21, 2014) were selected. Details about sampling time and the chemical characteristics  
132 including  $\text{PM}_{2.5}$ , trace gases ( $\text{SO}_2$ ,  $\text{NO}_2$ , and  $\text{NH}_3$ ), water soluble inorganic ions ( $\text{NH}_4^+$ ,  $\text{SO}_4^{2-}$ ,  
133  $\text{NH}_4^+$ ,  $\text{K}^+$ ,  $\text{Cl}^-$ , and  $\text{Na}^+$ ), and meteorological factors (wind direction and wind speed) of  
134 sampling time are summarized in Figure 1.

## 135 2.2. DNA Extraction and PCR Amplification.

136 DNA was extracted from the quartz membrane fragments (cut into 1.1  $\text{cm}^2$  filter area) using  
137 the E.Z.N.A.® Soil DNA Kit (Omega Bio-tek, Norcross, GA, U.S.) according to the  
138 manufacturer's instructions. DNA concentration was determined using NanoDrop 2000  
139 (Thermo, Wilmington, Delaware, USA). Extracted DNA samples were stored at  $-80^\circ\text{C}$  until  
140 further analysis. The V3-V4 region of 16S rRNA was amplified using a bacterial universal  
141 PCR primer set 515F (5'-GTGYCAGCMGCCGCGGTAA-3') and 907R (5'-



142 CCYCAATTCMTTTRAGTTT-3') (Gallagher et al., 2013). PCR amplification was  
143 performed on an ABI GeneAmp® PCR system 9700 (Applied Biosystems, 101 Foster City,  
144 CA) using a 20 µL reaction mixture contained 4 µL 5×FastPfu buffer, 2 µL 2.5 mM dNTPs,  
145 0.8 µL 5 µM forward primer, 0.8 µL 5 µM reverse primer, 0.4 µL Fastfu polymerase, 10 ng  
146 template DNA, and 11 µL double distilled H<sub>2</sub>O. PCR was performed at 95 °C for 3 min; 27  
147 cycles of 95 °C for 10 s, 55 °C for 30 s and 72 °C for 45 s; 72 °C for 10 min; and hold at  
148 10 °C. The final products were separated by 1% agarose gel electrophoresis and purified  
149 using an Axygen nucleic acid purification Kit (Axygen, Biosciences, CA, USA). The purified  
150 PCR products were prepared for sequencing on the Miseq™ platform (Illumina, San Diego,  
151 CA, USA). The nucleotide sequences were deposited in the Sequence Read Archive (SRA)  
152 under the accession number SRA385099.

### 153 2.3. Real-time Quantitative PCR.

154 To determine the absolute content of 16S genes in the particles of three sizes, Real-time  
155 Quantitative PCR Detecting System (qPCR) was applied in this study. For the qPCR reaction  
156 mixtures contained 12.5 µL of the ABI Power SybrGreen qPCR Master Mix (Promega, USA),  
157 0.5 µL of each primer, 2 µL of sample DNA, and 9.5 µL of double distilled H<sub>2</sub>O. The PCR  
158 program was performed in the ABI 7500 Real-Time PCR system (Applied Biosystems, 101  
159 Foster City, CA) as follows: 50 °C for 2 min; 95 °C for 15 min; 40 cycles of 95 °C for 15 s;  
160 and 60 °C for 1 min. The deionized water was employed as the negative controls in this work  
161 and was pipetted into the wells in a 96-well microplate with the DNA extracted from the  
162 samples. All the samples were tested in triplicate and cycle thresholds worked by ABI 7500  
163 software (Applied Biosystems). Based on the average rRNA gene copy number (3.98), the  
164 bacterial cell concentration (cells·m<sup>-3</sup>) was calculated using the method described by Doorn  
165 et al. (2007).

166 *2.4. High-throughput Sequence Analysis.*

167 After sequencing, the primers and barcodes were trimmed from the end of the raw sequences,  
168 and low-quality reads (length less than 200 bp and average quality score less than 20) were  
169 removed using FASTX-ToolKit. The sequences that passed quality control were processed  
170 using QIIME (version 1.17). Alpha-diversity using the Chao1, Coverage, Simpson, and  
171 Shannon indexes was also calculated at 97% similarity level. Operational taxonomic units  
172 (OTU) were clustered at 97% similarity level using Uclust Soft. The ribosomal database  
173 project was employed for taxonomic classification (phylum, class, order, family, and genus)  
174 (Amato et al., 2013). To predict the potential functions of the bacterial communities,  
175 PICRUST (Phylogenetic Investigation of Communities by Reconstruction of Unobserved  
176 States, <http://picrust.github.com>) was performed using the 16S rRNA gene data. These  
177 predictions were rarefied and analyzed in the Clusters of Orthologous Groups of proteins  
178 (COGs) database (<http://www.ncbi.nlm.nih.gov/COG/>).

179 *2.5. Data Analysis*

180 Spearman correlation coefficients were used to visualize the relationships between bacterial  
181 concentration and environmental factors (temperature, humidity, visibility, NO, NO<sub>2</sub>, SO<sub>2</sub>,  
182 CO and O<sub>3</sub>). The variation in bacterial abundances across three size fractions at phyla, family,  
183 class, and genus level was assessed using analysis of variance (ANOVA) test. Spearman  
184 correlation analysis and ANOVA test were conducted by SPSS 16.0 software (SPSS Inc.,  
185 Chicago, IL). Results were considered statistically significant at P value < 0.05 and P value  
186 <0.01. To describe the origin bacteria habitats, we aligned the Hiseq sequences of top 21  
187 OTUs (relative abundance more than 94.5%) in NCBI database. The habitants of top five  
188 highest similarities bacteria were linked to the source of airborne bacteria.

### 189 3. Results

#### 190 3.1. General Characteristics of the Haze Episodes in Ji'nan.

191 The environmental factors including PM<sub>2.5</sub>, water soluble inorganic ions, trace gases, wind  
192 speed and wind direction were determined during sampling period. The PM<sub>2.5</sub> daily average  
193 mass concentration ranged from 34.3 to 422.4  $\mu\text{g}\cdot\text{m}^{-3}$  and the highest value was about 17-  
194 fold higher than the daily average specified by the WHO guideline for PM<sub>2.5</sub>. The mass  
195 concentration of water-soluble inorganic ions and trace gases in PM<sub>2.5</sub> were in the order of  
196  $\text{SO}_4^{2-} > \text{NO}_3^- > \text{NH}_4^+ > \text{Cl}^- > \text{Ca}^{2+} > \text{K}^+ > \text{Mg}^{2+} > \text{Na}^+$ , and  $\text{SO}_2 > \text{NO}_2 > \text{NH}_3$ , respectively  
197 (Figure 1). The total water-soluble inorganic ions accounted for 64% of the PM<sub>2.5</sub> mass  
198 concentration and sulfate, nitrate, and ammonia were the most abundant compositions, which  
199 were consistent with the previous report by Wang et al. (2014) who showed that  $\text{SO}_4^{2-}$ ,  $\text{NH}_4^+$   
200 and  $\text{NO}_3^-$  accounted for majority of the total water-soluble ions in PM<sub>2.5</sub> during the winter  
201 haze in Ji'nan.  $\text{Cl}^-$  ( $8.9 \mu\text{g}\cdot\text{m}^{-3}$ ) and  $\text{K}^+$  ( $1.5 \mu\text{g}\cdot\text{m}^{-3}$ ) derived from the biomass burning cannot  
202 be ignored in the heating season. Du et al. (2011) determined that it was the higher  
203 concentration of  $\text{K}^+$  from the biomass burning induced pollution events. They also implied  
204 that  $\text{K}^+$  was the main existing form of KCl format in Shanghai. Meanwhile the use of  
205 firecrackers and fireworks on the eve of Chinese New Year was also identified as the cause  
206 for the same phenomenon (Zhang et al., 2010). The mass concentration of  $\text{K}^+$  in the eve of  
207 2014 Chinese New Year in Jinan was 18-folds higher than that in February 26, 2014. In  
208 addition to these three major ions,  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  from continental crust sources were also  
209 important during haze episodes in Ji'nan.

#### 210 3.2. Bacterial concentration and community structure.

211 The bacterial concentration in PM<sub>0.18-1</sub> was in the range of 17624-25573  $\text{cells}\cdot\text{m}^{-3}$ , with an

212 average of 21509 cells·m<sup>-3</sup>. The maximum of bacterial concentration occurred in PM<sub>0.32-0.56</sub>,  
213 with a value of 7314 cells·m<sup>-3</sup>, followed by PM<sub>0.18-0.32</sub> (7212 cells·m<sup>-3</sup>) and PM<sub>0.56-1</sub> (6982  
214 cells·m<sup>-3</sup>) (Figure 2). After sequencing and homogenization, 27094 reads were gained for  
215 each sample and a total of 236, 188, and 222 OTUs were obtained in PM<sub>0.18-0.32</sub>, PM<sub>0.32-0.56</sub>,  
216 and PM<sub>0.56-1</sub>, respectively. The PM<sub>0.32-0.56</sub> had the the minimum bacterial species which also  
217 confirmed by the lowest Chao1 index (Table 1). The coverage of more than 99% indicates  
218 the reliability of the experimental data. Meanwhile the  $S_{obs}/S_{Chao1}$  reaches 78% saturation,  
219 indicating a sufficient sampling (Toti et al., 2000). In addition, PM<sub>0.32-0.56</sub> and PM<sub>0.56-1</sub> had  
220 the same Shannon index, suggesting the similar bacterial diversity for these two particle sizes.  
221 This finding was different from a previous report, where bacterial richness in fine particulate  
222 matter (PM<sub>2.5</sub>) was lower than that in coarse particulate matter (PM<sub>2.5-10</sub>) (Bower et al., 2013).

223 In addition to bacterial concentration and alpha diversity estimators, the species comprising  
224 the bacterial community also play an essential role in the evaluation of health risks. In this  
225 study, 37 phyla, 71 classes, 137 orders, 236 families, and 378 genera were observed during  
226 the haze episodes. The predominant bacterial phyla (relative abundance more than 1%) were  
227 Firmicutes (78.9%), Proteobacteria (16.5%), Bacteroidetes (2.4%), and Actinobacteria  
228 (1.7%), which corresponded to the following bacterial classes: Bacilli (78.6%),  
229 Gammaproteobacteria (15.1%), Flavobacteria (2.0%), and Actinobacteria (1.7%) (Figure 3).  
230 With regard to the bacteria identified at the order level, Lactobacillales, Bacillales, and  
231 Pseudomonadales were found to be dominant with the relative abundances of 46.1%, 32.5%,  
232 and 10.5%, respectively. At a higher taxonomic level, the most abundant genera were found  
233 to be *Lactococcus*, *Bacillus*, *Pseudomonas*, and *Psychrobacter*, with relative abundances of  
234 43.4%, 28.4%, 7.6%, and 2.3%, respectively (Figure 4). The dominant taxa in the bacterial  
235 community in PM<sub>0.18-0.32</sub>, PM<sub>0.32-0.56</sub>, and PM<sub>0.56-1</sub> present a similar distribution at the phyla,  
236 order, and genus levels. It was consistent with those of previous studies and indicated that

237 the bacterial community structure was similar within the same seasons (Bertolini et al., 2013).  
238 However, the structure of non-dominant bacteria community at different levels disclose a  
239 remarkable variation (Figure 5, ANOVA,  $P=0.05$ ,  $F=2.3$ ). Holophagaceae (Acidobacteria)  
240 were abundant in  $PM_{0.32-0.56}$ , displaying 6-fold times higher abundance than in  $PM_{0.18-0.32}$ .  
241 *Acetobacter* (Proteobacteria) showed a higher relative abundance in  $PM_{0.32-0.56}$ , which was  
242 5-fold higher than that in  $PM_{0.18-0.32}$ . TA06, an uncultivated candidate phylum, was found  
243 only in  $PM_{0.18-0.32}$ . And some taxa could be found only in  $PM_{0.18-0.32}$  and  $PM_{0.56-1}$  such as  
244 Candidate division WS3, *Rheinheimera*, and *Fastidiosipila*.

### 245 3.3. Implications on Human health Risks and COG Analysis.

246 Given the high diversity of inhalable bacteria, the potential influence of these bacteria on  
247 humans and nature is worth noticing. 43.7% of the identified bacteria, including *Lactococcus*  
248 *piscium*, *Pseudomonas fragi*, *Streptococcus agalactiae*, and *Pseudomonas cichorii*,  
249 identified at the species level appeared to have interactive effects on human, animal, and  
250 plant health with an average coverage of 35.4%, 7.1%, 0.7%, and 0.5%, respectively. In  
251 addition to these pathogens, we also determined functional gene distribution in the airborne  
252 bacterial community in this study. The COGs were considered to be related to four functional  
253 groups and twenty-five function descriptions (Figure 6). Almost 80% of multi-copied genes  
254 with known functions were assigned with COG codes; for example, 9.3% of the genes were  
255 assigned a COG code of 'E', which represents amino acid transport and metabolism, and 7.1%  
256 of the genes were found to be functionally involved in carbohydrate transport and metabolism.  
257 Other codes such as K (transcription), J (translation, ribosomal structure and biogenesis), P  
258 (inorganic ion transport and metabolism), M (cell wall/membrane/envelope biogenesis), and  
259 C (energy production and conversion) also occupied a large proportion of the multi-copied  
260 genes (more than 5%). In addition, some of the genes detected in the airborne particulate

261 matter were poorly characterized with unknown function.

### 262 **3. Discussion**

263 Air pollution has been studied extensively by aerosol chemistry and physics. However the  
264 correlation between haze episodes and bacterial community has not been fully understood.  
265 The high concentration of airborne pollutants in haze episodes may provide nutrients (sulfur,  
266 nitrogen, and ammonia) and thus affect the bacterial community structure in submicron  
267 particles. For example,  $\text{SO}_4^{2-}$  has a distinct ability to influence the existence and growth of  
268 microbes and thus affect on their relative abundance (Scherer et al., 1981).  $\text{Ca}^{2+}$  is recognized  
269 to be associated with cellular processes such as the cell cycle and cell division in bacteria and  
270 can affect protein stability, enzymatic activity, and signal transduction, thus controlling  
271 protein functions (Michiels et al., 2002).  $\text{Cl}^-$ , employed in chemical agents, is related to water  
272 disinfection processes and thus causes bacterial stress injury. The high concentration of  $\text{K}^+$   
273 and  $\text{Na}^+$  may disturb the structure and function of bacteria and then cause the death of bacteria.  
274 Previous studies have also showed that certain bacteria were related to atmospheric dynamics.  
275 For example, *Pseudomonas* species were likely to be involved in atmospheric processes such  
276 as desulfuration and denitration (Robinson et al., 2012). *Bacillus* species could participate in  
277 the nitrogen and carbon cycling in ecosystems (Ulrich et al., 2008). Therefore, under  
278 conditions of severe air pollution, the increased concentrations of ambient pollutants in  
279 submicron particles could be associated with the variation in bacterial concentration and  
280 community structure.

281 In the present study, the bacterial concentration in submicron particles were much higher  
282 than the China Scientific Ecology Center guideline ( $1000 \text{ cells}\cdot\text{m}^{-3}$ ) and those reported in  
283 other haze studies in China (Beijing:  $224 \pm 186 \text{ CFU}\cdot\text{m}^{-3}$ , and Xi'an: 1102–1736  $\text{CFU}\cdot\text{m}^{-3}$ )  
284 (Gao et al., 2015a; Li et al., 2015), but slightly lower or similar to those reported in some

285 previous studies in other countries (Italy:  $4.6 \times 10^5$  ribosomal operons per cubic meter, about  
286  $1.19 \times 10^5$  cells·m<sup>-3</sup>, and USA:  $2.72 \times 10^4$  cells·m<sup>-3</sup>) (Bertolini et al., 2013; Bowers et al.,  
287 2012). The difference was possibly because most of the previous studies on airborne bacterial  
288 concentrations were performed by the culture method, while the qPCR method was used in  
289 this study. The culture method aims at the cultured bacteria which occupied only 1% of the  
290 total bacteria, e.g. Gao et al. (2016) calculated the bacterial concentration  
291 ( $705 \pm 474$  CFU m<sup>-3</sup>) during summer based on the count method. It is therefore not surprising  
292 to find that the bacterial concentration reported here was much higher than that reported in  
293 the other studies. This high concentration indicated that the residents in Ji'nan face higher  
294 health risks. The size distribution of these airborne bacteria in submicron particles were  
295 similar to the trend observed in coarse particles that Dong et al. reported, with two peaks at  
296 1.1–2.1  $\mu\text{m}$  and 4.7–7.0  $\mu\text{m}$ , which occupied 18.6% and 19.2% of the total airborne microbes  
297 collected from October 2013 to August 2014 in Qingdao (Dong et al.,2015). Dong et al.  
298 (2015) believed that 71.5% of the microbes existed in the coarse particles ( $>2.1 \mu\text{m}$ ) and  
299 confirmed a distinct unimodal distribution with one peak at 2.1–3.2  $\mu\text{m}$  during the heating  
300 period in winter, which may be caused by a combined effect of coal combustion, higher PM,  
301 and dust from the ground. Undoubtedly, in this study, all the samples were collected during  
302 the heating seasons in Ji'nan; therefore, we hypothesized that the single peak observed at  
303 0.32–0.56  $\mu\text{m}$  may have been due to the same reason and the additional emission caused by  
304 the fireworks. However, it is not clear whether airborne bacterial concentration varied with  
305 changes in environmental factors. Previous study showed that PM<sub>2.5</sub> and visibility showed a  
306 positive and negative correlation with airborne bacterial concentration during haze episodes  
307 (Li et al., 2015; Alghamdi et al.,2014; Gao et al.,2016). Goffau et al. (2009) reported that  
308 gram-positive bacteria grow faster under lower relative humidity in the atmosphere. Cao et  
309 al. (2014) found that the relative abundance of microbial pathogens in PM<sub>2.5</sub> increased with

310 increase of air pollution level. While in this study, no obvious correlation with visibility,  
311 relative humidity and PM<sub>2.5</sub> were observed. The bacterial concentration exhibited a  
312 significant negative correlation with NO<sub>2</sub> and SO<sub>2</sub>, and O<sub>3</sub> (Table 2). It is likely that SO<sub>2</sub>,  
313 NO<sub>2</sub>, and O<sub>3</sub> have a toxic effect on microorganisms (Abdel Hameed et al., 2012). High  
314 concentration of SO<sub>2</sub>, NO<sub>2</sub>, and O<sub>3</sub> will inhibit the growth and breeding of the bacteria.

315 Due to the limit reports on the bacterial composition in PM<sub>1.0</sub>, we compared the results with  
316 several other studies emphasized on the bacterial composition in PM<sub>2.5</sub>, PM<sub>10</sub>, and TSP. At  
317 the phylum level, Cao et al. (2014) showed that Actinobacteria, Proteobacteria, Chloroflexi,  
318 Firmicutes, Bacteroidetes, and Euryarchaeota were the most abundant phyla in PM<sub>2.5</sub> and  
319 PM<sub>10</sub> during severe haze episodes. At the order level, lower abundance of Bacillales (2.0%  
320 and 3.0%) and higher Actinomycetales (80.0% and 60.0%) were reported in PM<sub>2.5</sub> and PM<sub>10</sub>  
321 samples obtained in Milan, Italy, during winter (Franzetti et al., 2011). However, the  
322 abundance of Actinomycetales in the submicron particles in Ji'nan was very low in this study  
323 (less than 1%, about 0.001%). This result was also markedly different from that reported by  
324 Bertolini et al. (2013) who showed that Actinobacteridae, Clostridiales, and  
325 Sphingobacteriales were the major taxa in the airborne bacterial community in an urban area  
326 of Northern Italy. The results may be caused by the different analysis method and sequencing  
327 platform. Bertolini et al. (2013) target on the V5-V6 region based on the primer 783F-1046R  
328 using the Illumina GA-IIx sequencing, while our target area were V3-V4 based on the  
329 universal primer (515F-907R) by Illumina Miseq platform. The different sampling and  
330 analysis method produce a difference in these two studies. At the genus level, distinct  
331 different bacterial community were found compared to Wang et al. (2015b) that *Arthrobacter*  
332 and *Frankia* from the phylum Actinobacteria were the dominant genus in PM<sub>2.5</sub> during haze  
333 episodes in Beijing. From another perspective, the abundant genera (*Lactococcus*, *Bacillus*,  
334 *Arthrobacter*, *Streptococcus*, *Leuconostoc*, and *Lactobacillus*) implying that about 76.4% of



335 the bacteria were recognized to be gram-positive, which is consistent with the report of Fang  
336 et al. (2007) who illustrated that 80–86% of the total airborne bacteria were gram-positive in  
337 outdoor environments in Beijing. The authors stated that the reason for this was that gram-  
338 positive bacteria had stronger resistance and survival ability than gram-negative bacteria  
339 under adverse conditions (aerosolized chemical pollutants, strong sunlight, and lower  
340 humidity). While in this study, no obvious difference was observed in the abundances of  
341 most abundant genus, no matter whether gram-positive or gram-negative bacteria (Figure 4).  
342 The results were consistent with previous investigation by Wei et al. (2016), in that the  
343 dominant bacterial community showed no significant difference between haze and clear days  
344 during Jan. 2014 in Beijing. The possible explanation was the short-time sampling (less than  
345 one year). Bower et al. (2012) found that the local terrestrial source environments influence  
346 is more in shifting the bacterial communities, than the atmospheric condition. In future  
347 studies, the quarterly and annual sampling were essential to analyze the variation between  
348 haze and non-haze days.

349 Among these identified taxa, some specific bacteria were identified to be linked to human  
350 health risks. *Lactococcus piscium*, a well-known pathogen to affect salmonid fish (Williams  
351 et al., 1990), *Pseudomonas fragi* is responsible for bacteriological spoilage in dairy products  
352 and causes great economic losses in the dairy industry (Pereira et al., 1957). *Streptococcus*  
353 *agalactiae* can result in invasive infections such as skin and skin structure infections, urinary  
354 tract infections, osteomyelitis, endocarditis, and meningitis in adults (Farley et al., 2001).  
355 *Pseudomonas cichorii*, which is usually isolated from the soil, shows pathogenicity in plants  
356 including eggplant, lettuce, celery, and chrysanthemum crops and has important economic  
357 effects (Hojo et al., 2008; Pauwelyn et al., 2010). Our results show that long-term exposure  
358 to high concentrations of these ambient bacteria would pose a risk to people living in such  
359 hostile environments. On the other hand, the well-known beneficial bacterium *Streptococcus*

360 *thermophiles*, which has the ability to reduce the risks of antibiotic-associated diarrhea  
361 and lung cancer in mice, was also detected and showed an average abundance of 0.7%  
362 (Beniwal et al., 2003). In addition, bacteria with the aforementioned functions during the  
363 haze episodes may have an important role in the degradation of high concentrations of  
364 pollutants. For example, some species belonging to the *Streptococcus* genus have been shown  
365 to be able to degrade organic acids (Amato et al., 2007); certain strains of *Sphingomonas* can  
366 degrade organic matter such as polynuclear aromatic hydrocarbons (Ye et al., 1995). *Bacillus*  
367 *badius* (0.5%), a well-known alkaliphilic bacterium, can degrade organic matter such as  
368 aniline and anthracene (Ahmed et al., 2012).

369 The impact of airborne bacterial communities on bio-ecosystems and human health needs to  
370 be investigated in future studies using bacterial cultures and metagenomics analysis.

371 Apart from the bacterial community structure in submicron particles, the source of the  
372 bacteria should be identified in order to understand the inhalable microbes further. The  
373 natural biosphere provides various natural environment sources for primary biological  
374 aerosol particles. Generally, the primary biological aerosol reside mainly in soil, plant, rock  
375 surface, leaf surface, animal secreta, skin or hair, human activity, and ocean (Bower et al.  
376 2012). The microorganisms aerosolized into atmosphere and rapidly deposited rather than  
377 suspended due to the high settling velocities (Despres et al. 2011). Since unique taxa may  
378 exist in specific environments, the potential source of the bacteria can be identified by  
379 identification of the unique taxa. In other words, the bacteria, which are derived from a  
380 specific habitat can be linked to the source in the environments. During the cold season, soil  
381 was an important source for the atmospheric bacteria, which was indicated by the high  
382 abundance of soil-inhabiting bacteria such as *Lactococcus*, *Bacillus*, and *Arthrobacter* during  
383 this season (Bowers et al., 2011). Bacteria originating from the surfaces of leaves  
384 (*Pseudomonas*) have been found to be abundant in warm temperate regions (Yashiro et al.,

385 2011). Furthermore, bacteria linked to feces have been observed such as members of  
386 *Escherichia* and *Streptococcus* (DeLeon-Rodriguez et al., 2013). Nevertheless, the high  
387 similarities between the bacterial genera detected in the diverse seasons and locations suggest  
388 that part of the airborne bacterial community may change by the spread of bacteria by long-  
389 term transport (air flow from ocean, dust events, or precipitation). Jeon et al. (2011) showed  
390 that airborne bacterial concentration increased significantly and the ambient bacterial  
391 community structure changed markedly during dust events in Asia. However, this did not  
392 seem to be the case in this study. Our results indicated that the sources of the airborne  
393 bacterial community in particulate matter may be environments such as soils, leaf surfaces,  
394 and feces.

#### 395 **4. Conclusion**

396 Bacteria, including their concentration, community characteristics, correlation with  
397 environmental factors, and role in infectious process of diseases and ecological process  
398 may have been underestimated. In the present study, high bacterial concentration and  
399 significant negative correlation with the NO<sub>2</sub>, SO<sub>2</sub>, and O<sub>3</sub> in atmosphere were detected.  
400 The diverse bacteria and pathogens in submicron particles during haze episodes was  
401 observed for the first time by the high-throughput sequencing, yet no significant difference  
402 for the dominant bacterial genus between haze and non-haze days were observed. The results  
403 also indicate that the most abundant genera show highly similarity across three size  
404 fraction, while bacteria with low abundance show a significant difference such as  
405 *Acetobacter* and *Fastidiosipila*. We also acknowledge that the ambient bacteria mainly  
406 originated from soils, leaf surfaces, and feces. This knowledge helps for the comprehensive  
407 understanding of bacterial community biodiversity in submicron particles particularly those  
408 potential pathogens during haze episodes.

409 **Conflict of Interest**

410 The authors declare no conflict of interest.

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620 **Table 1.** Alpha-diversity indexes (97%) from PM<sub>0.18-0.32</sub>, PM<sub>0.32-0.56</sub>, and PM<sub>0.56-1</sub>: Sobs  
 621 (number of OTUs), ACE, Chao1, Coverage, and Shannon.

<b>Sample</b>	<b>N<sub>sequences</sub></b>	<b>Sobs</b>	<b>ACE</b>	<b>Chao1</b>	<b>Coverage</b>	<b>Shannon</b>
PM <sub>0.18-0.32</sub>	27094	236	302	289	0.99	2.48
PM <sub>0.32-0.56</sub>	27094	188	293	249	0.99	2.40
PM <sub>0.56-1</sub>	27094	222	292	272	0.99	2.44

622

623 **Table 2.** Spearman's correlation coefficients between airborne pollutants and meteorological  
 624 parameters with bacterial concentration in PM<sub>0.18-0.32</sub>, PM<sub>0.32-0.56</sub>, PM<sub>0.18-0.32</sub> and PM<sub>0.18-1</sub> (\*P  
 625 < 0.05).

<b>Bacteria</b>	<b>Temperature</b>	<b>Humidity</b>	<b>Visibility</b>	<b>PM<sub>2.5</sub></b>	<b>NO</b>	<b>NO<sub>2</sub></b>	<b>SO<sub>2</sub></b>	<b>CO</b>	<b>O<sub>3</sub></b>
B <sub>0.56-1</sub>	0.429	-0.393	0.464	-0.571	-0.071	-0.750	-0.607	0.321	-0.643
B <sub>0.32-0.56</sub>	0.071	0.071	0.464	-0.571	0.429	-0.071	-0.571	-0.214	-0.143
B <sub>0.18-0.32</sub>	0.571	-0.036	0.393	-0.500	-0.214	-0.821*	-0.750	0.321	-0.786*
B <sub>0.18-1</sub>	0.500	-0.143	0.536	-0.679	0.000	-0.786*	-0.821*	0.250	-0.571

626

627 **List of Figures**

628 **Figure 1** Time series of the daily average ionic concentration and meteorological  
629 parameters during sampling days.

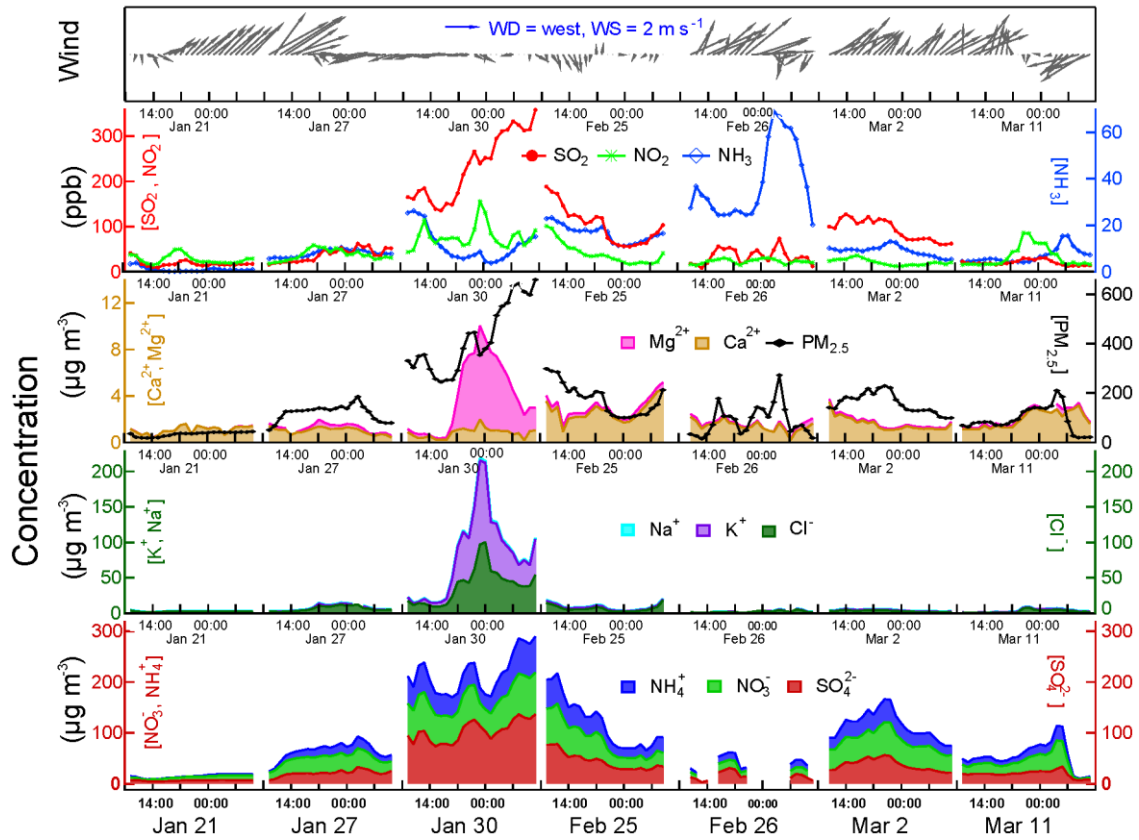
630 **Figure 2** Daily average bacterial concentration and PM mass concentration in PM<sub>0.56-1</sub>,  
631 PM<sub>0.32-0.56</sub>, and PM<sub>0.18-0.32</sub>. (a-PM<sub>0.56-1</sub>, b-PM<sub>0.32-0.56</sub>, c-PM<sub>0.18-0.32</sub>).

632 **Figure 3** (A-B) Relative abundance of bacteria at the phylum and class level in PM<sub>0.56-1</sub>,  
633 PM<sub>0.32-0.56</sub>, and PM<sub>0.18-0.32</sub>. (a-PM<sub>0.56-1</sub>, b-PM<sub>0.32-0.56</sub>, c-PM<sub>0.18-0.32</sub>).

634 **Figure 4** Heatmap of the dominant genus (relative abundance higher than 0.05%) in PM<sub>0.56-</sub>  
635 <sub>1</sub>, PM<sub>0.32-0.56</sub>, and PM<sub>0.18-0.32</sub> at the genus level. (a-PM<sub>0.56-1</sub>, b-PM<sub>0.32-0.56</sub>, c-PM<sub>0.18-0.32</sub>).

636 **Figure 5** Relative abundance of the taxa (at the phyla, family, or genus levels) that were  
637 found to show significant difference across aerosol size fractions: \*P < 0.05, \*\*P < 0.01.

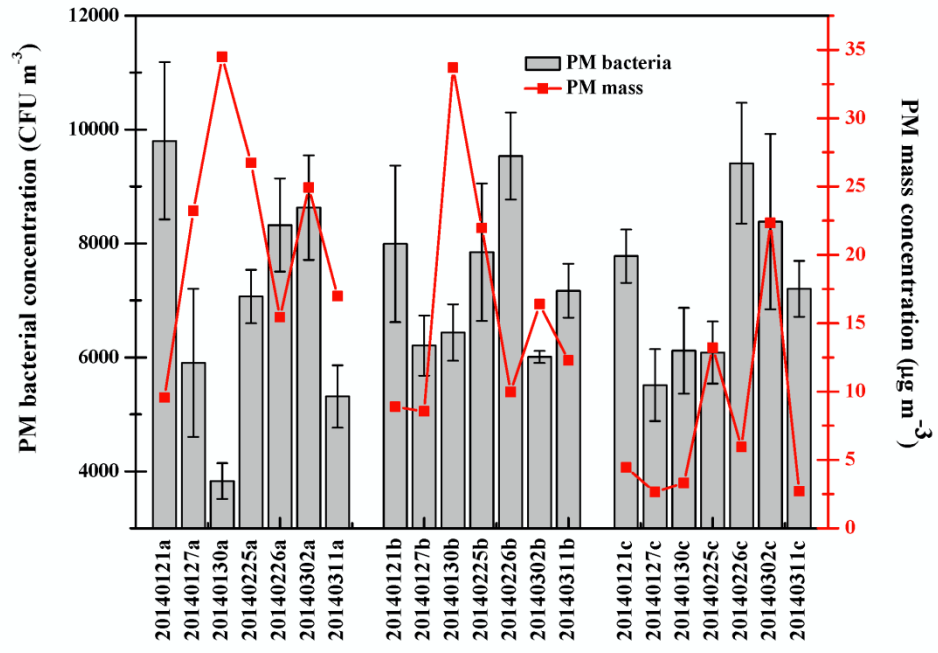
638 **Figure 6** Categorization of the microbial community genome contigs according to COGs  
639 functional categories.



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Figure 1

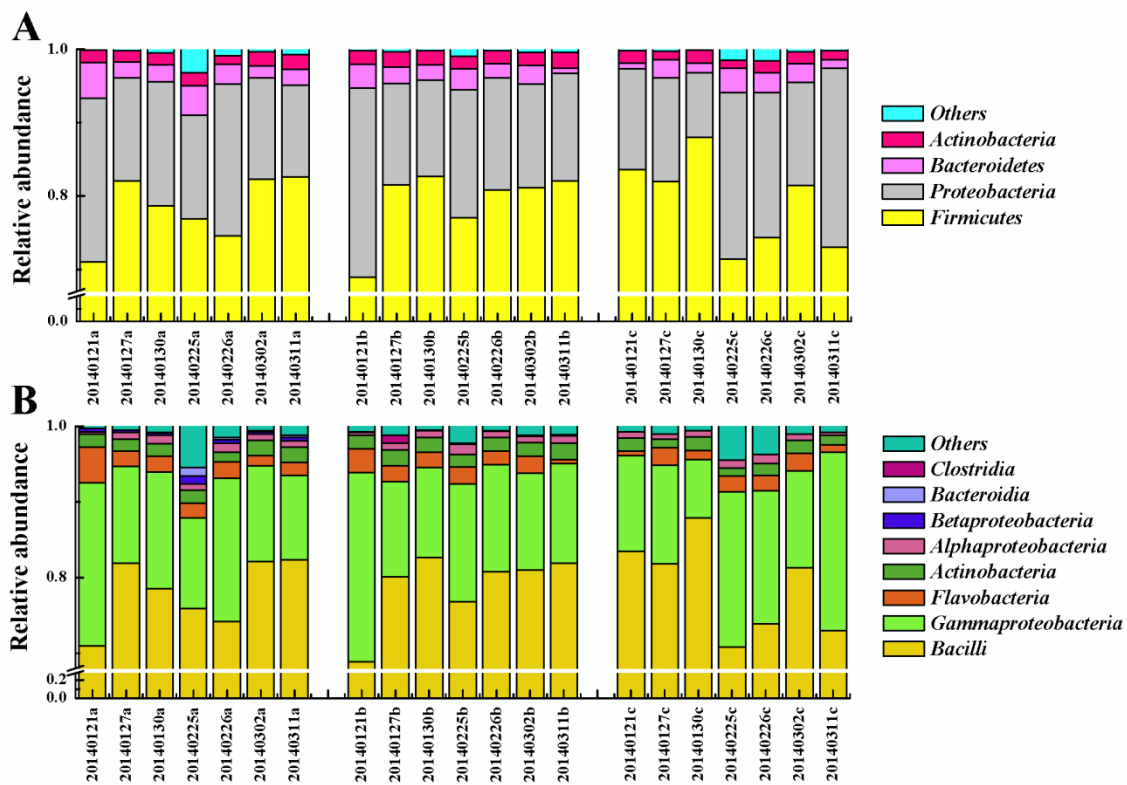


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Figure 2

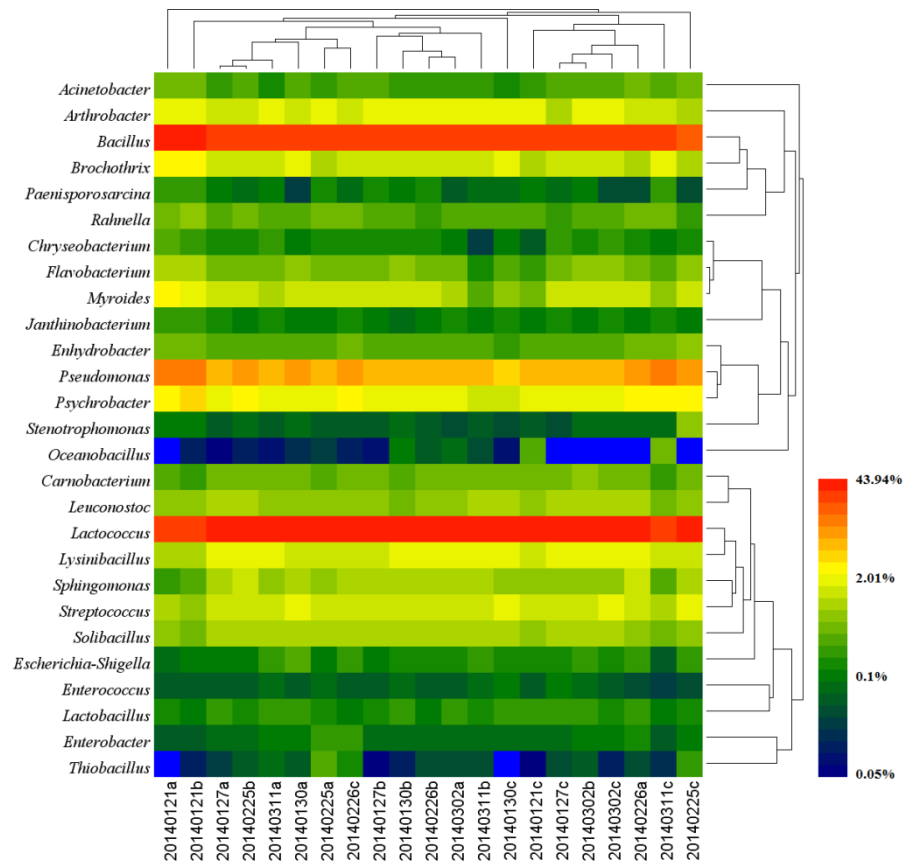




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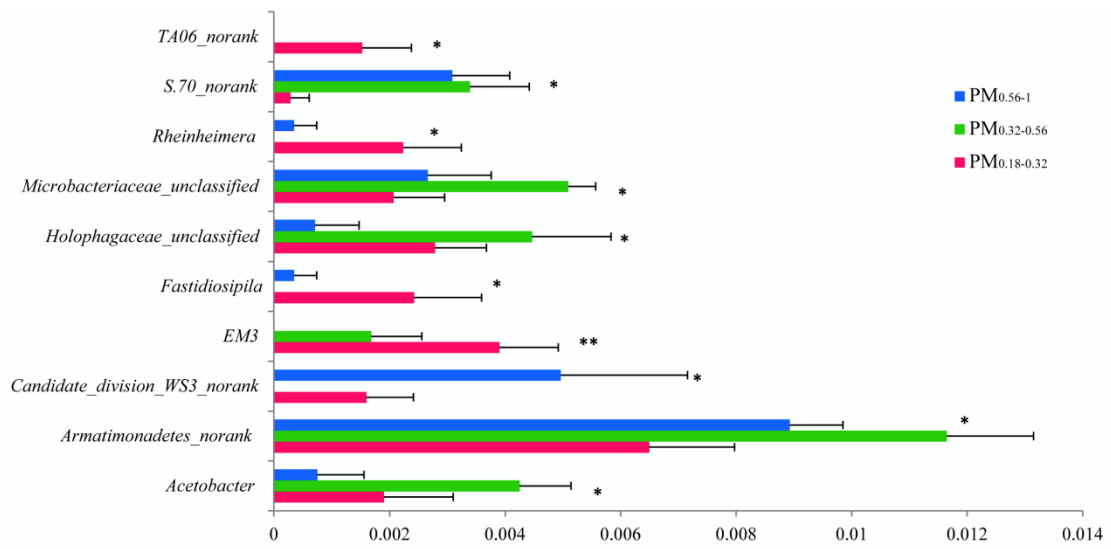
Figure 3



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Figure4

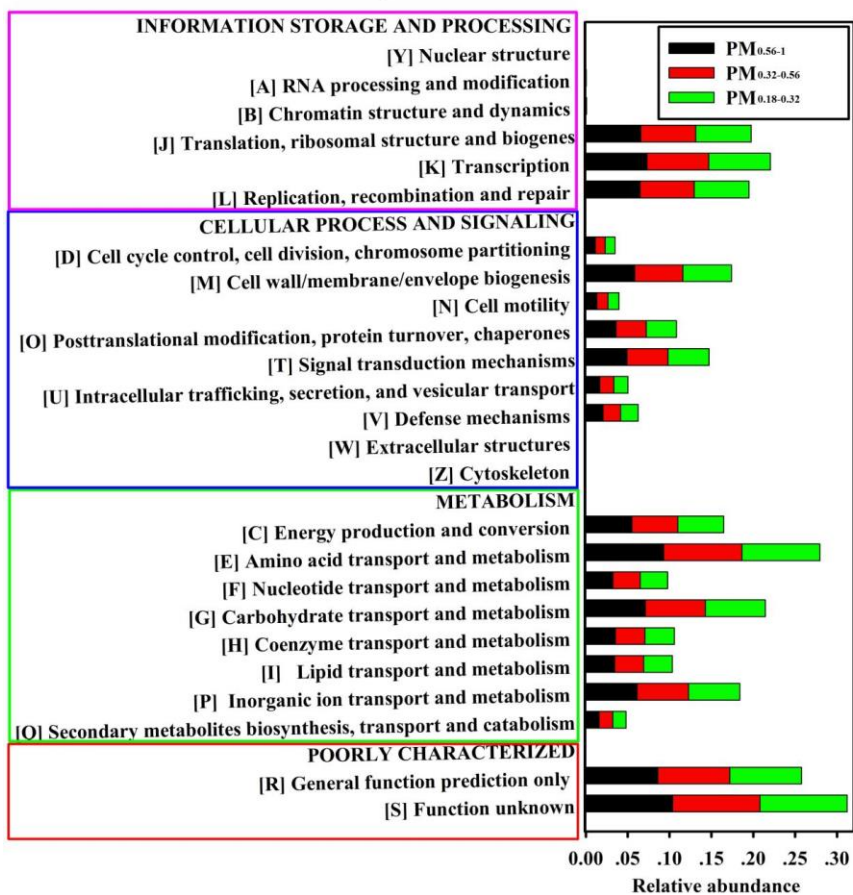


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**Figure 5**

### COG categories



650

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Figure 6