Variation of Bacterial and Fungal Community Structures in $PM_{2.5}$ Collected during the 2014 APEC summit periods

Pengrui Du,[1] Rui Du,[1,*] Zedong Lu,[1] Weishan Ren[1], Pingqing Fu[2]

[1] College of Resources and Environment, University of Chinese Academy of Science, Beijing, 100049, China

[2] State Key Laboratory of Atmospheric Boundary Layer Physics and Atmospheric Chemistry (LAPC), Institute of Atmospheric Physics, Chinese Academy of Sciences, Beijing, 100029, China

ABSTRACT

The biological fraction of $PM_{2.5}$ is considered to be a major cause of various allergies and respiratory diseases. Nonetheless, differences in bacterial and fungal communities in $PM_{2.5}$ under different air quality conditions are not well known. In the present study, we collected $PM_{2.5}$ samples from October 15, 2014 to November 12, 2014 when several successive "Asia-Pacific Economic Cooperation (APEC) blue" days were recorded, following the implementation of strict emission control measures to ensure the APEC summit held during November 5–11, 2014 in Beijing. This study analyzed bacteria and fungi in $PM_{2.5}$ samples through rRNA gene high-throughput sequencing. In total, 690 genera of bacteria and 229 genera of fungi were detected. The variations of species richness and community diversity of bacteria and fungi in $PM_{2.5}$ were not affected significantly by the emission control measures adopted during the summit and different air quality levels. The bacterial and fungal community structures in $PM_{2.5}$ collected during the summit exhibited over 83.7% and 79.6% similarities respectively, with $PM_{2.5}$ collected from air graded as "good" quality (AQI ≤ 100) before the APEC summit. Bacteria and fungi in $PM_{2.5}$ samples collected at AQI levels between 101–200 and 201–300 before the APEC summit had more than 73.4% and 76.3% community structure similarity, respectively, with $PM_{2.5}$ samples collected at AQI ≤100. The difference between day and night $PM_{2.5}$ samples was very small for bacterial and fungal community structures. Furthermore, most of the inhalable bacteria and fungi were nonpathogenic and no a clear relationship between air quality levels and pathogens was observed. Our results showed that bacteria and fungi in $PM_{2.5}$ were less affected by emission control measures and different air quality levels. However, due to the limited number of samples, the relationship between air pollution levels and airborne bacteria and fungi still needs further study.

Keywords: APEC; emission control; bioaerosols; $PM_{2.5}$; AQI

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39 *Corresponding author.
40 Tel: +86-10-69672840
41 E-mail address: ruidu@ucas.ac.cn
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43 INTRODUCTION

44 With the rapid development of industry and economy in China, exhaust gas and
45 particulate matter (PM) discharged from factories and automobiles are increasing.
46 Beijing, an international megacity, has been suffering from serious $PM_{2.5}$ (aerosol
47 particles with an aerodynamic diameter of ≤ 2.5 μm) pollution (Lang et al., 2017;
48 Xu et al., 2005; Zhou et al., 2015). Although previous studies have shown that the
49 annual average concentration of $PM_{2.5}$ in some cities in China was more than 90 μg
50 m^{-3} (Feng et al., 2009; He et al., 2001; Meng et al., 2007; Zheng et al., 2005), public
51 awareness regarding $PM_{2.5}$ was low until 2011. The relationship between exposure to
52 $PM_{2.5}$ and public health has been well established in a number of studies (Boldo et al.,
53 2006; Pope III et al., 2002; Valavanidis et al., 2008; Wang et al., 2017).
54 Epidemiological data summarizes the correlation between $PM_{2.5}$ exposure and allergy,
55 morbidity, mortality, respiratory, pulmonary, and cardiovascular diseases (Dockery et
56 al., 1993; Englert, 2004; Pope III et al., 2002). Due to their small size, $PM_{2.5}$ can be
57 deposited in the nose, trachea, bronchi, and penetrate the alveoli (Brook et al., 2004;
58 Frøsig et al., 2001).Although $PM_{2.5}$-induced oxidative stress and inflammatory
59 mediator release are considered as important molecular mechanisms by which they
60 influence human health (Becker et al., 2005; Deng et al., 2013; Risom et al., 2005),
61 hitherto, their mechanisms of causation of various diseases have not been well
62 understood.

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63 Physical and chemical properties of $PM_{2.5}$ have been extensively investigated. At
64 present, studies of $PM_{2.5}$ pollution focus on the components, emission sources, and
65 spatiotemporal variation in China. $PM_{2.5}$ components such as organic carbon,
66 elemental carbon, sulfates, nitrates, and geological material have been studied in
67 different cities and regions (Xiu et al., 2015; Zhan et al., 2016; Zhao et al., 2016).
68 However, few studies have explored the biological fraction of $PM_{2.5}$ including
69 bacteria, fungi, pollen, viruses, spores, and cell debris (Cao et al., 2014; Gao et al.,
70 2017; Li et al., 2015; Yan et al., 2016). The identification of bacteria and fungi in
71 aerosol particles through culture-based methods and clone library sequencing has
72 been documented (Fahlgren et al., 2010; Haas et al., 2013; Haas et al., 2014; Stoeck et
73 al., 2007; Tarigan et al., 2017), but these results do not realistically reflect the
74 microbial community composition and diversity due to the limitation of cultivatable
75 microorganisms and number of constructed clones in an aerosol sample (Amann et al.,
76 1995; Nocker et al., 2007). Particulate matters of biological origin may contribute 15 %
77 to 30% of the total aerosol particles in the atmosphere (Matthias-Maser et al., 1995;
78 Ruprecht, 2005). Fungal spores, pollens, endotoxins, and viruses represent a major
79 part of the biological particles and may elicit diseases such as allergies, asthma,
80 airway obstruction, and inflammatory reaction (Arteaga et al., 2015; Bush and
81 Portnoy, 2001; Degobbi et al., 2011). Due to limited understanding of the variation in
82 composition and dynamics of microorganisms in $PM_{2.5}$ at different air quality levels, a
83 critical knowledge gap on the effect of $PM_{2.5}$ on human health exists.

84 The Chinese government set the Technical Regulation on Ambient Air Quality

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85 Index (AQI) in 2012, which included $PM_{2.5}$ concentration for the first time. Air
86 quality were divided six grades: excellent, good, slight pollution, moderate pollution,
87 heavy pollution, and severe pollution according to AQI ≤ 50, 51–100, 101–150,
88 151–200, 201–300, and > 300, respectively. The corresponding $PM_{2.5}$ concentrations
89 of these grades were ≤ 35μg m^{-3}, 36–75 μg m^{-3}, 76–115 μg m^{-3}, 116–150 μg m^{-3},
90 151–250 μg m^{-3}, and > 250 μg m^{-3}, respectively. Cao et al.(2014) first investigated
91 different species of the airborne microbes in $PM_{2.5}$ using metagenome analysis during
92 a severe smog event in Beijing city and suggested that majority of them were
93 nonpathogenic to humans. Subsequently, correlation between various factors and
94 cultivable bacteria or fungi during haze and non-haze days in Beijing have been
95 studied (Gao et al., 2015b; Gao et al., 2016) with increasing focus on the species
96 composition and dynamics of bioaerosol particles (Wei et al., 2016). Recently,
97 high-throughput sequencing technology based on the rRNA gene of microorganisms
98 and metagenomes are being used to survey the composition and diversity of microbial
99 communities in atmospheric particles (Bowers et al., 2011a; Cao et al., 2014; Gao et
100 al., 2017; Nonnenmann et al., 2010). However, there is little information regarding the
101 transformation in the biological components of the $PM_{2.5}$ in relation to different
102 pollution levels.

103 In order to ensure good air quality during the 2014 APEC summit hosted in Beijing
104 during November 5–12, 2014, the government implemented a series of strict measures
105 to control air polluting emissions, such as reducing vehicles on roads by executing
106 odd-and-even license plate traffic bans on alternate days, suspension of activities at

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107 construction sites, and shutting down polluting factories. The term "APEC blue" was
108 coined to describe the "good" air quality during the period. This intervention provided
109 an interesting opportunity to research the impact of emission control measures on
110 atmospheric aerosol particles. Latest researches have demonstrated that inorganic and
111 organic aerosols significantly decreased during the APEC summit (Chen et al., 2015;
112 Sun et al., 2016; Tang et al., 2015). Reduction in pollutants during the summit was
113 observed and the relationship between pollutant concentrations and AQI was noted
114 (Sun et al., 2016; Tang et al., 2015). Nevertheless, the variation in the composition
115 patterns of chemical or biological fractions of $PM_{2.5}$ was not explored deeply. To the
116 best of our knowledge, our study firstly demonstrated the impact of emission control
117 on bioaerosols in $PM_{2.5}$ during the APEC summit.

118 This study investigates the abundance and similarities of bacteria and fungi in
119 $PM_{2.5}$ using high volume sampling and high-throughput genome sequencing
120 technology. Additionally, the impact of air pollution emission control and different air
121 quality levels on microorganisms in $PM_{2.5}$ were also analyzed.

122

123 # METHODS

124 ## Sampling site and $PM_{2.5}$ Collection

125 $PM_{2.5}$ samples were collected on the roof of a two-story building (~8m high)
126 between October 15 and November 12, 2014 at the Institute of Atmospheric Physics
127 (IAP), Chinese Academy of Sciences (39° 58′ 28″N, 116° 22′ 16″ E). The site is located
128 between the North 3rd and 4th Ring road and air pollution was monitored at this site

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129 for several years (Sun et al., 2012). $PM_{2.5}$ was collected using 203mm×254mm quartz
130 microfiber filters (WhatmanTM, GE, USA) with a high volume air particulate matter
131 sampler (TE-6070VFC, Tisch, USA) at a flow rate of 1.13 $m^3 min^{-1}$. The quartz filters
132 were individually wrapped in aluminum foil and sterilized by calcinating in a muffle
133 furnace at 550°C for 5 h. $PM_{2.5}$ samples were collected both during the daytime
134 (7:00AM to 18:00 PM) and nighttime (18:00 PM to 7:00 AM). Each filter capturing
135 $PM_{2.5}$ was immediately wrapped in the original aluminum foil packing after the
136 completion of each sampling and stored in a sealed bag. A blank control sample was
137 set by placing a new sterile quartz filter in the air particulate matter sampler for five
138 minutes under the shutdown mode. All $PM_{2.5}$ samples were stored at -20°C until
139 subsequent analyses were performed.
140
141 **Sample Groups**
142 A total of 58 $PM_{2.5}$ sample filters were collected during the study period. We
143 excluded two sample filters collected on October 25th as the samples had AQI values
144 above 300 and failed in DNA extraction, presumably due to severe pollution. We
145 divided the 40 sample filters collected before the APEC summit into six sample
146 groups according to the AQI and day or night sample timing. The letters D and N
147 were used to represent the day and night sampling periods, respectively, and letters of
148 G, M, and H represented excellent and good air quality (AQI ≤ 100), slight and
149 moderate pollution (100 < AQI ≤ 200) and heavy pollution (200 < AQI ≤ 300),
150 respectively. Therefore, the six sample groups were GD, GN, MD, MN, HD, and HN.

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151 The 16 sample filters collected during the APEC summit were divided into two
152 sample groups based on the sampling period conducted during the day or night, and
153 were named as AD and AN, respectively. Thus, a total of eight sample groups were
154 established and each group consisted of four or more individual filter membranes
155 (Table 1). The AQI data used in our study was acquired from the Olympic Sports
156 Centre Air Monitoring Station, which was the nearest monitoring station, situated 2
157 km to the northeast of our sampling site. Figure 1 presents the AQI values and the
158 dominant pollutants on various days during the sampling period.
159

160 **DNA extraction and Sequencing**

161 We merged the filters in the same sample group to extract DNA of the biological
162 components in $PM_{2.5}$. Based on the different number of $PM_{2.5}$ sample filters in each
163 sample group, pieces of different areas were cut from each filter as follows: GD (4
164 cm^2), GN (4 cm^2), MD (10 cm^2), MN (10 cm^2), HD (6.6 cm^2), HN (6.6 cm^2), AD (5
165 cm^2), and AN (5 cm^2). The total area of the quartz filters in each sample group was
166 about 50 cm^2. The filter pieces from the same sample group were ground together to a
167 powder using an agate mortar, which was sterilized using an autoclave sterilizer. The
168 quartz filter powders were loaded into a bead tube provided in a PowerSoil DNA
169 isolation kit (MoBio Laboratories, Carlsbad, CA, USA). The remaining steps of the
170 DNA extraction were performed according to the manufacturer's instructions except
171 for the vortex adapter oscillation step, which was replaced with FastPrep instrument
172 (MP Biomedicals, Solon, USA) for enhancing mechanical cell breaking to improve

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173 DNA yield. We detected the DNA concentration using NanoDrop 2000 (Thermo
174 Fisher Scientific, Wilmington, DE, USA). The range of DNA concentration was 10-80
175 ng μl^{-1}, and it was generally sufficient to ensure the completion of subsequent
176 experiments.

177 The 16S rRNA gene of bacteria and internal transcribed spacer 1 (ITS1) rRNA gene
178 of fungi were sequenced through high-throughput sequencing to determine the
179 bacterial and fungal community composition in $PM_{2.5}$. The V3-V4 hyper-variable
180 region of the bacterial 16S rRNA gene was amplified using the primers 338F
181 (5'-ACTCCTACGGGAGGCAGCA-3') and 806R (5'-GGACTACHVGGGTWTCTA
182 AT-3'). The ITS1 region of the fungal rRNA gene was amplified using the primers
183 1737F (5'-GGAAGTAAAAGTCGTAACAAGG-3') and 2043R (GCTGCGTTCTG
184 CATCGATGC). Each primer included a unique barcode sequence used for
185 distinguishing different samples in the subsequent analyses. Polymerase chain
186 reactions (PCRs) were performed in a 25 μl reaction mixture containing 1× PCR
187 buffer, 0.2 mM dNTPs, 0.2 μM primer, 0.6 Units taq DNA polymerase, and nearly
188 10ng template DNA under the following cycling conditions: pre-denaturation at 94°C
189 for 5 min; 30 cycles of denaturation at 94°C for 30 s, annealing at 50°C for 30 s (for
190 bacteria) or 56°C for 30s (for fungi), extension at 72°C for 45 s, with a final extension
191 at 72°C for 5 min. Each $PM_{2.5}$ sample group had three repeated PCRs and the
192 products were pooled together on the completion of the PCRs. Blank control samples
193 were treated as above for DNA extraction and PCR amplification and the results
194 showed that nothing was produced after the PCR processing. Subsequent purification

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195 and sequencing of the PCR products were performed using the Ilumina Miseq PE300
196 platform (Illumina, San Diego, CA, USA) by the Majorbio company (Shanghai,
197 China). Sequences obtained from the Miseq sequencing platform were deposited in
198 the NCBI Sequence Read Archive under accession number SRR3822027.
199

200 **Sequence analysis**

201 Sequences were processed and analyzed using the open-source software package
202 Mothur v.1.34.4 (http://www.mothur.org/wiki). Sequences were removed if they were
203 < 400 bp or > 470 bp (for 16s rRNA gene), < 200 bp or > 300 bp (for ITS rRNA gene),
204 contained ambiguous bases, or had homopolymer stretches of more than 8 bp. PCR
205 chimeras were filtered out using the Chimer Uchime command in the Mothur
206 (http://www.mothur.org/wiki/Chimera.uchime). The subsequent processing and
207 analysis steps of the 16s rRNA gene were performed as described by Kozich et al
208 (2013). Sequences of the ITS rRNA gene were aligned using MAFFT version 7
209 (http://mafft.cbrc.jp/alignment/server), which was a target-independent alignment tool
210 as alignment database was unavailable. Sequences were assigned into operational
211 taxonomic units (OTUs) at 97% similarity. In order to compare the alpha and beta
212 diversities in different $PM_{2.5}$ sample groups fairly, the minimum number of sequences
213 in the eight sample groups was selected as the standard of normalization. Abundance
214 based coverage estimator (ACE), Chao1 species richness estimator, Shannon diversity
215 index, and Good's coverage were calculated based on the abundance of each OTU
216 using Mothur. The bacterial sequences were assigned to phylotypes using a Bayesian

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217 approach against the Ribosomal Database Project (RDP) 16s rRNA gene training
218 database with a confidence threshold of 70%. The method and database employed in
219 the taxonomic classification process for fungal sequences were the K-Nearest
220 Neighbor algorithm and UNITE ITS database, respectively (Kessy et al., 2010; Wang
221 et al., 2007). The alpha and beta diversity of the $PM_{2.5}$ samples were calculated using
222 the operating procedure described at http://www.mothur.org/wiki/MiSeq_SOP
223 (Kozich et al., 2013). Beta-diversity indexes (Bray-Curtis similarity index and Yue &
224 Clayton similarity index) were determined to highlight the difference in each pair of
225 $PM_{2.5}$ sample groups and were calculated using the formulas:

$$S_{bray\text{-}curtis} = 2\frac{\sum_{i=1}^{n} min(S_{A,i}, S_{B,i})}{\sum_{i=1}^{n} S_{A,i} + \sum_{i=1}^{n} S_{B,i}}$$

$$S_{yue\&clayton} = \frac{\sum_{i=1}^{n} a_i b_i}{\sum_{i=1}^{n} (a_i - b_i)^2 + \sum_{i=1}^{n} a_i b_i}$$

226

227 *where,*

228 S_{Ai} is the number of sequences of the OTU i in community A;

229 S_{Bi} is the number of sequences of the OTU i in community B;

230 "n" is the total number of OTUs in communities A and B;

231 a_i is the relative abundance of OTU i in community A;

232 b_i is the relative abundance of OTU i in community B.

233 In order to explore the potential effect of bioaerosol in $PM_{2.5}$ on human health,
234 pathogenic bacteria and fungi were identified at the genus level according to the

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235 directory of pathogenic microorganisms infecting humans promulgated by the
236 Ministry of Health of the People's Republic of China. Meanwhile, metabolic function
237 pathway of bacterial communities, involving the human diseases caused by bacteria,
238 was predicted through the PICRUSt (Phylogenetic Investigation of Communities by
239 Reconstruction of Unobserved States). Community metabolic function was annotated
240 against with the Kyoto Encyclopedia of Gene and Genomes (KEGG) database. The
241 variation in species richness and community diversity in different experimental
242 treatments was assessed using analysis of variance (ANOVA) test conducted using
243 SPSS 19.0 software.

244

245 **RESULTS AND DISCUSSION**

246

247 **Impact of emission control and air quality levels on species richness and**
248 **community diversity**

249 Nearly 110331 high-quality sequences of bacteria and 103810 of fungi were
250 gathered and the optimized sequences were assigned to different OTUs at 97%
251 similarity. Following segmentation of OTUs, each $PM_{2.5}$ sample was normalized by
252 singling out 11245 and 9181 sequences, which were the least sequence numbers for
253 bacteria and fungi among the eight sample groups, respectively, to ensure fairness in
254 downstream analyses. A total of 7043 OTUs for bacterial sequences and 1008 OTUs
255 for fungal sequences were obtained and used to calculate the alpha diversity indexes
256 and the coverage index reflecting the sequencing depth. The Illumina Miseq

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257 sequencing results for bacteria shown in Table 2 indicate that the depth of sequencing
258 for bacteria was more than 90.3% in most samples, except for AN, which was 88.5%.
259 Table 3 lists the Illumina Miseq sequencing results for fungi and shows that all
260 sequencing depths were greater than 98.0%.

261 Emission control implemented during the APEC summit had effectively reduced all
262 forms of pollutants and rendered the air to a "good" quality (Chen et al., 2015; Sun et
263 al., 2016). Bacterial and fungal communities in the $PM_{2.5}$ samples collected during
264 that period were compared with the $PM_{2.5}$ samples collected prior to the summit. The
265 results for bacterial communities indicated that community species richness indexes
266 ACE and Chao1 for AN were 8145 and 4915, respectively, and greater than GN (5905
267 for ACE, 3768 for Chao1). Additionally, the Shannon index for AN was 5.878 and
268 greater than the GN sample (5.786). However, AD collected in daytime during the
269 APEC summit showed lower ACE and Chao1 indexes compared with GD. The result
270 of statistical analysis showed no significant difference between samples collected
271 during APEC summit and samples collected before the summit (ANOVA, P=0.43,
272 F=0.95 for ACE; P=0.39, F=1.22 for Chao1; P=0.69, F=0.22 for Shannon). Similarly,
273 the fungal communities harbored in $PM_{2.5}$ samples collected during APEC summit
274 and before the summit showed no significantly different for indexes ACE (ANOVA,
275 P=0.78, F=0.10), Chao1 (ANOVA, P=0.80, F=0.08), and Shannon (ANOVA, P=0.79,
276 F=0.09). Therefore, emission control measures probably did not affect the species
277 richness and community diversity of microbial community harbored in $PM_{2.5}$.

278 $PM_{2.5}$ samples collected before the summit, in the absence of emission control

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279 measures, were compared based on different pollution levels and sampling times. The
280 results of bacterial communities showed that no significant variation occurred
281 between sample groups collected under different pollution for indexes ACE (ANOVA,
282 P=0.29, F=1.89), Chao1 (ANOVA, P=0.26, F=2.16), and Shannon (ANOVA, P=0.29,
283 F=1.96). Likewise, the fungal communities showed no significant difference between
284 them for fungal species richness and community diversity indexes (ANOVA, P>0.05
285 for every index). Furthermore, the results of ANOVA analysis showed that bacterial
286 and fungal communities had no significant difference between daytime and nighttime
287 (ANOVA, P>0.05 for every index). On the whole, at a short period of time, the
288 species richness and community diversity of microbial communities in $PM_{2.5}$ would
289 not vary significantly because of the impacts of emission control measures, air quality
290 levels, and sampling time.

291 Previous studies have briefly discussed the relationship between bioaerosol and air
292 pollution. Gao et al.(2015a; 2015b) showed that the concentration of viable
293 bioaerosols decreased with increasing haze severity in Beijing city. However, Huang
294 et al.(2017) showed that airborne bacterial and fungal concentrations were positive
295 correlated with particle mass and number concentrations. Li et al.(2015) suggested
296 that bioaerosols exhibited higher concentration on hazy days than on non-hazy days in
297 Xi'an city. Thus, previous studies have presented inconsistent results on the
298 relationship between the concentration of airborne bioaerosols and pollution levels
299 (Gao et al., 2015a; Gao et al., 2015b; Li et al., 2015). Our results revealed that the
300 community richness and diversity were not affected by the air quality levels and

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301 emission control measures. This might be because community richness was
302 independent of the concentration of airborne bioaerosols. Several factors may have
303 influenced the bioaerosol concentration. For example, chemical pollutants,
304 meteorological conditions, and biological sources affected the characteristics of
305 airborne microbes (Mouli et al., 2005). Some air pollutants might have potential to
306 impair some sensitive bioaerosols (Adhikari et al., 2006; Gingell et al., 1976).
307 Additionally, physiological function of plants, which were major sources of
308 bioaerosols (Bertolini et al., 2013; Bowers et al., 2013), might be influenced by
309 atmospheric particle pollution, and this might cause change of biological activity
310 bacteria and fungi inhabiting the surface of the plant. These impacts would result in
311 changes on the concentration of culturable microorganisms and their biological
312 activity. However, community richness calculated through gene sequencing
313 technology was independent of concentration and activity of microorganisms, and it
314 estimated that how many different kinds of microorganisms in the community.
315 Therefore, even though the concentration of micororganisms in $PM_{2.5}$ varied
316 according with the air pollution level, the community richness would still remain
317 stable in a short period of time.

318

319 **Impact of emission controls and air quality levels on the bacterial community**
320 **structures**

321 The Bray-Curtis similarity index and Yue&Clayton similarity index were calculated
322 based on the OTUs to quantify the impact of emission control and air quality on the

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323 bacterial community structure in the $PM_{2.5}$ samples. There were 7 dominant OTUs
324 (abundance > 1% in all $PM_{2.5}$ sample groups), 13 major OTUs (abundance > 0.5% in
325 all $PM_{2.5}$ sample groups), and 60 common OTUs (abundance > 0.1% in all $PM_{2.5}$
326 sample groups) in the bacterial communities of the $PM_{2.5}$ samples (SI Table S1).
327 Based on the dominant OTUs, the Bray-Curtis similarity and Yue & Clayton
328 similarity between AD and GD was 91.5% and 95.6%, respectively, and the
329 Bray-Curtis similarity and Yue & Clayton similarity between AN and GN was 94.8%
330 and 98.3%, respectively (Table 4). Additionally, based on the major or common OTUs,
331 the similarity of AD and GD was more than 86.8%, and the similarity of AN and GN
332 was more than 83.7%. The high similarity between AD-GD and AN-GN demonstrated
333 that the emission control measures implemented during the summit had little effect on
334 the bacterial community structure. Chen et al. (2015) suggested the aerosol chemical
335 composition pattern was relatively similar before and during APEC summit although
336 various aerosol pollutants decreased significantly by 40% – 80% following the
337 emission control measures. Furthermore, the similarities of GD-MD, GD-HD, and
338 MD-HD were more than 84.2%, 82.3%, and 78.9%, respectively. At the same time,
339 the similarities of GN-MN, GN-HN, and MN-HN were more than 83.1%, 73.4 %, and
340 65.2%, respectively. Thus, high similarity between bacterial community structures at
341 different pollution levels was demonstrated. Hence, the impact of air pollution levels
342 on bacterial structures was very little. Coincidentally, Wei et al. (2016) indicated that
343 no significant difference was discovered for the dominant bacterial genera between
344 haze and sunny days and this might be due to transformation of bacteria depending on

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345 their source (Bertolini et al., 2013). Additionally, bacterial communities in $PM_{2.5}$
346 collected during day and night times were compared for four pairs of sample groups
347 (AD-AN, GD-GN, MD-MN, HD-HN), and the average value of similarities
348 calculated based on three levels of OTUs using two methods was 86.9%. These results
349 demonstrated that the bacterial community structures in $PM_{2.5}$ altered slightly between
350 day and night times. The concentration of bioaerosol is likely to vary (Dong et al.,
351 2015; Gao et al., 2015a; Li et al., 2015) along with the haze pollution levels, but the
352 main biological composition pattern in $PM_{2.5}$ was probably relatively stable within
353 different pollution levels. This phenomenon indicates that emission control measures
354 and pollution levels slightly affected the community structures of bacteria, and this
355 would implied that the sources of airborne bacteria were relatively stable in a short
356 period of time.

357 A total of 24 of bacterial phyla were identified in the eight sample groups (SI
358 Figure S1). The phyla constituting more than 1% in all sequences included
359 *Proteobacteria* (39.55%), *Actinobacteria* (28.56%), *Firmicutes* (15.85%),
360 *Bacteroidetes* (9.19%), and *Cyanobacteria_Chloroplast* (3.60%), with the rest of
361 phyla constituting 3.26% (SI Figure S2). *Actinobacteria* was the most abundant
362 phylum (36.81%) in the MN sample, whereas, *Proteobacteria* was the most dominant
363 in the other seven sample groups. These dominant bacterial compositions were similar
364 to the taxa identified in other studies (Bowers et al., 2013; Cao et al., 2014; Liao et al.,
365 2013). Furthermore, there were as many as 690 different genera of bacteria identified
366 in the $PM_{2.5}$ samples, although 18224 sequences were sorted as unclassified. The

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top-ten dominant genera were *Sphingomonas*(6.84%), *Kocuria*(5.77%), *Paracoccus*(5.50%), *Rubellimicrobium*(3.17%), *Curtobacterium*(2.75%), *Streptophyta* (2.45%), *Methylobacterium* (2.26%), *Bacillus* (2.04%), *Clostridium_sensu_stricto* (2.00%), and *Hymenobacter* (2.00%). Nevertheless, 124 genera were identified only once in any sample and 384 genera accounted for less than 0.01% in the total sequences. At the genus level, four genera *Clostridium_XI, Deinococcus, Ralstonia,* and *Thermoactinomyces* were more abundant in the $PM_{2.5}$ samples with AQI >100, than the samples with AQI ≤100 (SI Figure S3). Simultaneously, the bacterial communities in the $PM_{2.5}$ samples collected at AQI ≤ 100 exhibited higher proportions of four genera, which were *Adhaeribacter, Flavisolibacter, Segetibacter,* and*Acidobacteria_Gp4*, compared to samples collected at AQI > 100. The results seemed to show that some bacteria could be responsive to the AQI, but their contribution to the variability of community structures was insufficient. Other studies also indicated that some bacteria showed considerable variation with the pollution levels (Cao et al., 2014; Li et al., 2015), although most airborne bacteria remained stable in the atmospheric environment during the sampling period (Bowers et al., 2011a; Bowers et al., 2011b; Wei et al., 2016). So far, characteristics of most airborne bacteria, including cellular activity, pathogenicity, resistance to unfavorable meteorological factors, and biotransformation mechanism are not very clear. Therefore, research focusing on the relationship between pollution levels and bacterial community structure in $PM_{2.5}$ are restricted by several knowledge and technology gaps which might result in contradictory results from different researches.

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389

390 **Impact of emission control and air quality levels on the fungal community**
391 **structure**

392 There were 3 dominant OTUs (abundance > 1% in all $PM_{2.5}$ sample groups), 4
393 major OTUs (abundance > 0.5% in all $PM_{2.5}$ sample groups), and 11 common OTUs
394 (abundance > 0.1% in all $PM_{2.5}$ sample groups) in the fungal communities of $PM_{2.5}$
395 samples (SI Table S2). Though the similarity of AD-GD was less than 37.5%, AD was
396 similar to the other two $PM_{2.5}$ samples collected during day time, with more than 80%
397 for AD-MD and more than 85% for AD-HD (Table 5, SI Table S5). The similarity
398 between AN and GN was also more than 78.9%. The results showed that emission
399 control measures had little impact on the fungal community structures when ingoring
400 the GD sample. Comparison between paired $PM_{2.5}$samples collected in daytime and
401 nighttime revealed a high similarity of over 84.1%, except for GD-GN. The average
402 similarity of the three pairs of samples AD-AN, MD-MN, and HD-HN, was 91.5%.
403 Thus, the impact of sampling time was not obvious on the fungal community structure.
404 Oddly, we found that similarities of any random paired comparison were less than 43%
405 when GD sample was included. However, other paired comparisons, excluding GD,
406 showed more than 76% similarity regardless of the pollution level. These results
407 showed that GD was different from the other $PM_{2.5}$ samples because of some
408 unknown factors. When GD was ignored, these high similarities of paired
409 comparisons showed that the impacts of emission control measures during the APEC
410 summit, air quality levels, and timing of sampling were very little on the fungal

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411 community structures in $PM_{2.5}$. Gao et al. (2015a) also indicated that there was no
412 obvious relationship between cultivable fungi and haze levels over the four seasons.
413 However, previous studies showed fungal communities in the atmosphere were
414 influenced by many factors such as wind, sunlight, pollutants, sources and
415 geographical location (Gao et al., 2016; Woo et al., 2013; Yan et al., 2016). Our
416 results implied that the stability of sources of airborne fungi were the most important
417 factor for variation of community structure in a short period of time.

418 At the phylum level, *Ascomycota* was predominant, comprising 94.728% of the
419 103810 fungal sequences in $PM_{2.5}$. The other three detected fungal phyla were
420 *Basidiomycota*, *Chytridiomycota,* and *Zygomycota*, which accounted for 1.122%,
421 0.009%, and 0.013% of the total fungal sequences, respectively. The predominance of
422 *Ascomycota* fungi in airborne particles was identified in many studies (Bowers et al.,
423 2013; Li et al., 2015; Yan et al., 2016), although *Basidiomycota* was more abundant
424 than *Ascomycota* at some times (Fröhlich-Nowoisky et al., 2009). Table S4 lists the 19
425 detected classes of fungi and indicates that *Dothideomycetes* was the most abundant
426 with more than 49% in all $PM_{2.5}$ sample groups. Several classes of fungi in the GD,
427 including *Sordariomycetes*, *Tremellomycetes*, *Microbotryomycetes*,
428 *Crystobasidiomycetes*, and *Exobasidiomycetes* had higher abundance compared to
429 other sample groups. At the genus level, the fungal sequences could be assigned to
430 229 genera. *Epicoccum* was the largest fungal genus (11.85%) and other major genera
431 (>1% of sequence reads) included *Selenophoma* (4.40%), *Cladosporium* (1.38%), and
432 *Mycosphaerella* (1.33%). The proportion of *Selenophoma* in the GD (18.72%) was far

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433 more than other sample groups (0.71% - 5.73%). The above differences in fungal
434 taxonomy between the GD and other sample groups resulted in different fungal
435 community structure. Several earlier studies (Dannemiller et al., 2014;
436 Fröhlich-Nowoisky et al., 2009; Haas et al., 2013; Li et al., 2015; Oh et al., 2014; Yan
437 et al., 2016) have usually reported less than five fungal genera that were more than 1%
438 abundant, but their categories varied across different studies. These could be related to
439 different emission sources of fungal spores at different research sites.
440

441 **Variation of Microbial pathogens in $PM_{2.5}$ samples**

442 According to the directory of pathogenic microorganisms infecting humans
443 promulgated by the Ministry of Health of the People's Republic of China (MOHC),
444 we detected six pathogenic bacterial genera and nine pathogenic fungal genera in the
445 $PM_{2.5}$ samples (SI Figure S4). The most abundant pathogenic bacteria were
446 *Streptococcus* (0.440%), followed by *Mycobacterium* (0.10%), *Prevotella* (0.082%),
447 *Escherichia* (0.037%), *Rickettsia* (0.015%), and *Erysipelothrix* (0.009%). At the genus
448 level, the directory of human infection by pathogenic microorganisms indicates that
449 *Erysipelothrix*, *Prevotella*, and *Rickettsia* are capable of causing certain diseases.
450 However, the directory also lists only a few species, belonging to the genera
451 *Escherichia*, *Mycobacterium,* and *Streptococcus*, which are known to cause diseases.
452 Therefore, we submitted several representative sequences of the three genera to the
453 National Center for Biotechnology Information (NCBI), and the results indicated that
454 some *Streptococcus* sequences were exactly similar to *Streptococcus gallolyticus,*

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455 which was associated with colorectal tumors (Abdulamir et al., 2011). Some
456 *Escherichia* and *Mycobacterium* sequences were homologous with *Escherichia coli*
457 and *Mycobacterium simiae*, respectively. *Mycobacterium simiae* causes respiratory
458 infections (Ramos et al., 2006; Samra et al., 2005) and *Escherichia coli* is a common
459 pathogen that causes gastrointestinal diseases (Paton and Paton, 1998). Compared
460 with pathogenic bacteria, potential pathogenic fungi were more abundant. Genus
461 *Aspergillus* was the most abundant, up to 0.54% in the fungal communities.
462 *Penicillium* was the second richest and some sequences in the genus had a 100%
463 similarity with *Penicillium citrinum*. Other pathogenic genera included *Alternaria*
464 (0.097%), *Arthrinium* (0.004%), *Dactylaria* (0.003%), *Fusarium* (0.186%),
465 *Stachybotrys* (0.035%), *Trichoderma* (0.063%), and *Trichothecium* (0.087%). At the
466 species level, *Fusarium oxysporum*, *Fusarium poae*, *Aspergillus flavus*, and
467 *Aspergillus ochraceus* were identified through sequence comparison in the NCBI at
468 more than 97% similarity and are responsible for some plant and human diseases
469 (Hedayati et al., 2007; Michielse and Martijn, 2009; Musa et al., 2000). The total
470 abundance of pathogenic bacteria in the AD and AN sample groups collected during
471 the summit were 0.524% and 0.616%, and pathogenic fungi were 1.406% and 1.143%,
472 respectively. Meanwhile, pathogenic bacteria accounted for 0.4% –1.183% and
473 pathogenic fungi accounted for 0.382%–2.788% in the other sample groups. Hence,
474 the proportion of pathogens in $PM_{2.5}$ was relatively rare and the impact of emission
475 control and pollution level was not apparent. Impacts of airborne particulate matter
476 pollution on human health were clearly recognized (Cheng et al., 2013; Ouyang,

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477 2013), although Cao et al. (2014) declared that most of the airborne bacteria and fungi
478 were nonpathogenic to humans. Comparing the abundance of pathogenic bacteria and
479 fungi in different samples collected during the summit and under different air
480 pollution levels, no clear relationship between the emission control measures or air
481 pollution levels and pathogenic microorganisms was demonstrated. Therefore, the
482 pathogenic microorganisms did not seem to be related to the emission control and air
483 pollution levels. Our results again indicate that there is no evident association between
484 these pathogens and air quality levels.

485 Bacterial community functions involving with human diseases caused by bacteria
486 were predicted based on 16S rRNA gene sequences using the PICRUSt algorithm. A
487 total of 9 KEGG pathways were identified. However, their proportions in total KEGG
488 pathways were all less than 0.28%. Table S6 listed the details of pathways in every
489 sample group. Wei et al. (2017) and (Xu et al. (2017)) predicted the bacterial
490 community function in cloud water and airborne particles. However, they did not
491 indicate how much community functions were associated with human diseases. Our
492 results showed that most of community functions of bacteria harbored in $PM_{2.5}$ were
493 irrelevant with human diseases.

494

495 CONCLUSION

496

497 This work demonstrated the distribution characteristics of bacteria and fungi in
498 $PM_{2.5}$ at different air pollution levels. Our results showed that APEC emission control

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499 measures had no significant influence on the bacterial and fungal community
500 structures. Additionally, high similarities in bacterial and fungal community structures
501 in the $PM_{2.5}$ samples at different pollution levels were observed, suggesting that there
502 was no significant relationship between air pollution levels and the variations of
503 community structures of $PM_{2.5}$ bioaerosols. We also found that most bacteria and
504 fungi were nonpathogenic to humans and the proportion of pathogens in the total
505 sequences was scarce. Our finding would serve as an important reference for
506 researches working on the characteristics of bioaerosols. Considering the possible
507 reactions between bioaerosols and air pollutants might affect human health, further
508 research should focus on understanding the interaction of pollutants and microbes,
509 and their combined effect on human health.
510
511
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513
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518
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Figure 1. Air quality indexes and chief pollutants during sampling period. Labels of chief pollutants: Square corresponds to PM_{10}; diamond corresponds to $PM_{2.5}$; star corresponds to NO_2. Dash line corresponds to second grade air quality (AQI = 100)

Table 1. Sampling information for the eight sample groups

Table 2. Richness and diversity indices of bacteria in the eight sample groups

Table 3. Richness and diversity indices of fungi in the eight sample groups

Table 4. Similarities of bacterial communities in $PM_{2.5}$ collected under different air pollution levels

Table 5. Similarities of fungal communities in $PM_{2.5}$ collected under different air pollution levels

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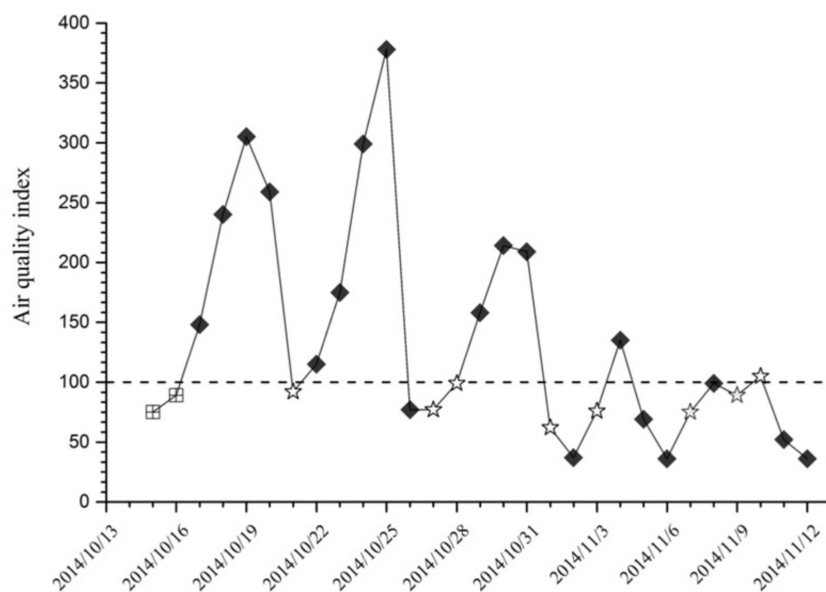

Figure 1. Air quality indexes and dominant pollutants during sampling period. Labels of dominant pollutants: Square corresponds to PM_{10}; diamond corresponds to $PM_{2.5}$; star corresponds to NO_2. Dash line corresponds to second grade air quality (AQI = 100).

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Table 1
Sampling information for the eight sample groups

Sample group	Sampling duration	AQI range	Pollution Level	Sampling Date
GD	7:00-18:00	0-100	excellent and good	Oct 15, 16, 21, 22, 26, 27, 28, and Nov 1,2, 3
GN	18:00-7:00(next day)	0-100	excellent and good	Oct 15, 16, 21, 22, 26, 27, 28, and Nov 1,2, 3
MD	7:00-18:00	101-200	slight and moderate pollution	Oct 17, 23, 29, and Nov 4
MN	18:00-7:01(next day)	101-200	slight and moderate pollution	Oct 17, 23, 29, and Nov 4
HD	7:00-18:00	201-300	heavy pollution	Oct 18, 19, 20, 24, 30, 31
HN	18:00-7:00(next day)	201-300	heavy pollution	Oct 18, 19, 20, 24, 30, 31
AD	7:00-18:03	0-100	excellent and good	Nov 5, 6, 7, 8, 9, 10, 11, 12
AN	18:00-7:03(next day)	0-100	excellent and good	Nov 5, 6, 7, 8, 9, 10, 11, 12

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Table 2
Richness and diversity indices of bacteria in the eight sample groups

Sample group	Number of sequences [a]	OTUs [b]	ACE	Chao 1	Shannon	Coverage
GD	11245	1762	5934	3939	5.506	0.907
GN	13521	2034	5905	3768	5.786	0.906
MD	15300	2028	6498	3868	5.435	0.909
MN	15823	1894	3967	2863	5.525	0.926
HD	11790	945	1148	1178	5.227	0.976
HN	12620	1705	4429	3038	5.460	0.922
AD	15268	2212	5892	3913	5.599	0.903
AN	14764	2457	8145	4915	5.878	0.885

[a] sequences that passed quality controls.

[b] The operational taxonomic units (OTU) were determined at 97% similarity.

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Table 3
Richness and diversity indices of fungi in the eight sample groups

Sample group	Number of sequences [a]	OTUs [b]	ACE	Chao 1	Shannon	Coverage
GD	9181	421	868	672	3.147	0.980
GN	13704	277	714	461	1.854	0.986
MD	13876	252	552	399	1.883	0.988
MN	10269	252	571	396	2.012	0.987
HD	12777	233	319	298	1.999	0.991
HN	17783	186	551	282	1.525	0.992
AD	13444	419	878	634	2.273	0.980
AN	12776	378	765	564	2.331	0.983

[a] sequences that passed quality controls.

[b] The operational taxonomic units (OTU) were determined at 97% similarity.

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Table 4
Similarities of bacterial communities in $PM_{2.5}$ collected under different air pollution levels

Pair-wise comparison	Bray-Curtis[a]	Yue & Clayton[a]	Bray-Curtis[b]	Yue & Clayton[b]	Bray-Curtis[c]	Yue & Clayton[c]
AD-GD	0.914912	0.955613	0.916032	0.957033	0.868348	0.936608
AN-GN	0.947849	0.982889	0.919668	0.963863	0.837341	0.910845
GD-MD	0.908037	0.961903	0.850908	0.89565	0.841817	0.886187
GD-HD	0.932844	0.968033	0.920558	0.955089	0.822717	0.884695
MD-HD	0.853416	0.91819	0.838573	0.90311	0.789131	0.85567
GN-MN	0.90302	0.961346	0.851547	0.872949	0.83129	0.857736
GN-HN	0.759697	0.784182	0.753448	0.749472	0.74311	0.73434
MN-HN	0.697861	0.651964	0.717455	0.65651	0.74909	0.675205
AD-AN	0.891519	0.922154	0.861354	0.902938	0.834444	0.87979
GD-GN	0.972138	0.996219	0.941933	0.987747	0.853723	0.926934
MD-MN	0.890453	0.95094	0.88508	0.949011	0.841441	0.91216
HD-HN	0.716415	0.723817	0.75655	0.730088	0.772534	0.752735

[a] Similarity calculation based on the dominant OTUs (relevant abundance > 1% in every sample)
[b] Similarity calculation based on the major OTUs (relevant abundance > 0.5% in every sample)
[c] Similarity calculation based on the common OTUs (relevant abundance > 0.1% in every sample)

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Table 5

Similarities of fungal communities in $PM_{2.5}$ collected under different air pollution levels

Pair-wise comparison	Bray-Curtis[a]	Yue & Clayton[a]	Bray-Curtis[b]	Yue & Clayton[b]	Bray-Curtis[c]	Yue & Clayton[c]
AD-GD	0.375024	0.251044	0.34911	0.248425	0.367261	0.248399
AN-GN	0.803206	0.865689	0.796432	0.861222	0.789347	0.857304
GD-MD	0.333305	0.205676	0.256547	0.171263	0.239242	0.151586
GD-HD	0.429283	0.309638	0.347122	0.245168	0.34131	0.21122
MD-HD	0.761001	0.814871	0.770362	0.82289	0.760723	0.829711
GN-MN	0.847834	0.919818	0.842343	0.923918	0.834636	0.928067
GN-HN	0.786562	0.800175	0.785901	0.816972	0.763659	0.836315
MN-HN	0.850206	0.911182	0.855147	0.918957	0.857222	0.932885
AD-AN	0.961054	0.993477	0.958643	0.993158	0.956328	0.993285
GD-GN	0.330195	0.202926	0.272359	0.182548	0.256265	0.165806
MD-MN	0.84083	0.911587	0.846184	0.916539	0.841285	0.92218
HD-HN	0.8609	0.91667	0.85663	0.92227	0.841591	0.931041

[a] Similarity calculation based on the dominant OTUs (relevant abundance > 1% in every sample)

[b] Similarity calculation based on the major OTUs (relevant abundance > 0.5% in every sample)

[c] Similarity calculation based on the common OTUs (relevant abundance > 0.1% in every sample)

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