Title:

Variations in airborne bacterial communities at high altitudes over the Noto Peninsula (Japan) in response to Asian dust events

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1. Introduction

Airborne microorganisms (bioaerosols) associated with desert-sand and anthropogenic particles were transported through free troposphere from the Asian continents to downwind regions of East Asia and can influence climate changes, ecosystem dynamics, and human health (Iwasaka et al., 2009). Natural dust events from the Asian desert regions carry airborne microorganisms, supporting atmospheric microbial dispersals (Griffin et al., 2007; Maki et al., 2010; Pointing and Belnap, 2014). Haze days caused by anthropogenic particles from Asian continents also affect airborne microbial abundance and endotoxin levels (Wei et al., 2016). Some studies demonstrated that Asian dust events, including natural and anthropogenic particles, cause vertical mixture of bioaerosols in downwind areas, such as in Japan (Huang et al., 2015b; Sugimoto et al., 2012; Maki et al., 2015).

Bioaerosols, which include bacteria, fungi, and viruses, are transported from ground environments to the free troposphere and account for a substantial proportion of organic aerosols (Jaenicke, 2005). Bioaerosols are thought to influence atmospheric processes by participating in atmospheric chemical reactions and in the formation of cloud-nucleating particles (Pratt et al., 2009; Morris et al., 2011; Hara et al., 2016b). Indeed, airborne microorganisms act as ice nuclei that are related to ice-cloud formation processes (Möhler et al., 2007; Delort et al., 2010; Creamean et al., 2013; Joly et al., 2013). In particular, ice-nucleation activating proteins of some microorganims, such as *Pseudomonas syringae*, *Xanthomonas campestris* and *Erwinia herbicola*, exhibit high nucleation activities, initiating ice formation at relatively warm temperatures (greater than -5 °C) (Morris et al. 2004) in comparison to the inorganic ice-nucleating particles,

such as potassium feldspar (approximately -8 °C) (Atkinson et al. 2013). Ice-nucleating particles that originate from bioaerosols are believed to activate ice formation more efficiently than inorganic substances (Hoose and Möhler, 2012; Murray et al. 2012), and are primary contributors of rapid ice-cloud formation even at low concentrations in the clouds at temperatures between -8 °C and -3 °C (Hallett and Mossop, 1974). Bioaerosols are key factors for elucidating the detailed mechanisms of ice-cloud formation and precipitation over East Asia (Hara et al., 2016ab), but the microbial characteristics of bioaerosols transported over long distances by Asian-dust events are still unclear. Furthermore, the microorganisms transported by Asian dust events increase the allergenic burden, consequently inducing asthma incidences (Ichinose et al., 2005) and contributing to the dispersal of diseases such as Kawasaki disease (Rodó et al., 2011) and rust diseases (Brown and Hovmøller, 2002).

In downwind areas of East Asia, the atmospheric bacterial dynamics at high altitudes should be investigated in order to understand the ecological and meteorological influences of airborne bacteria as well as their long-range dispersion. Meteorological shifts and dust events can dramatically alter airborne bacterial communities at high altitudes in Japan (Maki et al., 2013 and 2015) because of air masses that originate from heterogeneous environments, including marine, mountainous, urban, and desert areas. The airborne microorganisms around North American mountains (2,700 m above sea level) were also found to increase their species diversities in response to Asian dust events (Smith et al., 2013). High-throughput sequencing technology can generate large numbers of nucleotide sequences and the sequencing database has played an important role for investigation of airborne bacterial compositions (Brodie et al., 2007; Woo et al.,

2013). Indeed, the analyses using high-throughput sequencing has demonstrated that airborne bacterial populations at ground levels change in response to pollutants from Beijing (Cao et al., 2014) and African dust events (Mazar et al., 2016). To investigate their long-range transported bacteria while avoiding the ground-surface contaminations, the bioaerosol samples collected at high altitudes by aircrafts were analyzed using high-throughput sequencing, showing the airborne microbial diversities at high altitudes, ranging from 1,000 m to 3,000 m (DeLeon-Rodriguez et al., 2013; Maki et al., 2015). There are also a few studies on the vertical bacterial distribution from the ground level to the troposphere (DeLeon-Rodriguez et al., 2013; Maki et al., 2015). Nonetheless, while some variations were observed, the specific changes in tropospheric bioaerosols over East Asia, and, in particular, differences between Asian dust and non-dust events remain poorly understood.

Organic aerosol particles, such as bioaerosols, account for high rates of tropospheric aerosols, ranging from 30 % to 80 % (Jaenicke, 2005), and fluctuate at high concentrations, ranging from 10^3 to 10^5 particles m^{-3}, under the boundary layer at 4,000 m above the ground (Twohy et al., 2016). Epifluorescence microscopy using fluorescent-dye staining is a useful tool for observation and determination of microbial particles in the atmosphere, demonstrating that the biomass of airborne microorganisms increased 10– to 100–fold during Asian-dust events (Hara et al., 2012, Maki et al., 2014). Under a fluorescence microscope, DNA in microbial particles fluoresce blue when stained with 4, 6-diamidino-2-phenylindole (DAPI) (Russell et al., 1974), and organic materials aggregated with proteins and microbial cell components were confirmed as yellow fluorescence particles (Mostajir et al., 1995). Mineral particles

(white particles) and black carbon (black particles) can also be observed as background fluorescence in microscopic observation fields (Maki et al., 2014). Accordingly, several DAPI-stained particles could be detected in air samples collected from all over Japan during dust events (Maki et al., 2013) and can be used as indicators for evaluating the amounts of some aerosol species during dust events.

In this study, the bacterial communities from different altitudes around the Japanese islands were compared to identify the potential influences of long-range transported air masses on tropospheric bacteria. We used a helicopter for collecting air samples at altitudes ranging from 1,200 m to 3,000 m over the Noto Peninsula, Japan. Helicopter sampling was used to collect chemical components at high altitudes, which has previously been used to avoid contamination from the downwash created by spinning rotors (Watanabe et al., 2016). This air sampling method can directly collect aerosols moving from Asian continents or marine areas to Japan. We estimated the air mass conditions using the meteorological data obtained during the sampling periods, and determined aerosol amounts by using meteorological monitoring and epifluorescence microscopic observation. Bacterial community structures were analyzed by using high-throughput sequencing targeting bacterial 16S rRNA genes (16S rDNA).

2. Experiments

2.1. Sampling

Aerosol sampling using a helicopter (R44; Robinson, CA, USA) was performed over coastal areas from Uchinada (36º67N, 136º64E) to Hakui (36º92N, 136º76E) in the

120 Noto Peninsula, Japan. Both cities are located on the western coast of the Noto
121 Peninsula where aerosols arrive from continental areas across the Sea of Japan and are
122 mixed with local aerosols (Fig. 1). The helicopter traveled 20 km northwest from
123 Kanazawa to Uchinada; air sampling was continuously conducted from Uchinada to the
124 northern coastal areas. To compare the vertical distributions of airborne bacteria during
125 dust and non-dust events, air samples were collected using a helicopter at the 1 to 3
126 altitudes ranging from 500 m to 3,000 m above ground level (Table 1). Air samples from
127 low altitude regions (10 m above ground level) were collected from the roof of a
128 building located at Taki bay in Hakui (36º92 N, 136º76 E). To compare the vertical
129 bacterial distribution, aerosol samples were collected during the daytime (from 9:00
130 Japanese standard time [JST; UTC + 9 h] to 16:30 JST) on March 19, 2013; April 28,
131 2013; March 28, 2014; and March 20, 2015. These samples were collected at the
132 following altitude sets; (1) 2,500 m, 1,200 m, and 10 m; (2) 3,000 m, 1,200 m, and 10
133 m; (3) 3,000 m, 1,200 m, and 10 m; and (4) 2,500 m and 500 m, respectively, and
134 samples were labeled as shown in Table 1. To investigate the bacterial changes at
135 altitudes in response to time, temporal transect at the altitude of 1,200 m was prepared
136 for seven days – the 23rd, 24th, 25th, and 29th of March 2014 and the 16th, 17th, and
137 21st of March 2015 – and the sample names are showed in Table 1.
138 Air samples were collected through sterilized polycarbonate filters (0.22-μm pore
139 size; Whatman, Tokyo, Japan) with sterilized filter holders (Swinnex Filter holder;
140 Merck, Darmstadt, German) connected to an air pump. At the sterilization processes, the
141 filters and the filter-holder parts were irradiated separately under UV light for 1.0 h and
142 the filter holders attached with the filters were autoclaved at 121 ºC for 20 min. Air

143 sampling was performed with a flow rates of 5 L min^{-1} over sampling periods from 0.2 h
144 to 1.0 h. Triplicate sampling filters were obtained for each altitude. During helicopter
145 sampling, outside air was transferred from a window to the bioaerosols-sampling inlet,
146 which was sterilized by autoclaving and UV irradiation. The sterilized filter holders
147 were inserted into the sampling inlet to avoid contamination. To collect air particles at
148 an altitude of 10 m, we used filter holders fixed on a 3 m stick, which was placed on the
149 roof of a building (Maki et al., 2014).

150 In total, 18 air samples were obtained during the sampling periods (Table 1). Of
151 the two filters used to collect each sample, one filter was used to determine the
152 particulate abundances under fluorescence microscopy, and the other was stored at
153 -80°C before the extraction of genomic DNA for analysis of bacterial compositions.

154

155 *2.2. Characteristics and trajectories of air masses*

156 Information regarding weather conditions (temperature, relative humidity, and
157 pressure) was gathered. During the helicopter flight, outside air was transferred from a
158 window into the meteorological-measurement inlet, into which the adaptor of the
159 measurement device (TR-73U; T&D Corporation, Matsumoto, Japan) was inserted, and
160 the temperature, relative humidity, and pressures were sequentially measured. The
161 temperature and relative humidity at an altitude of 10 m were also measured on the roof
162 of a building in Hakui. The depolarization ratio, which was measured by Laser Imaging
163 Detection and Ranging (LIDAR) measurements at Toyama, has been used for the
164 detection of non-spherical aerosols, such as mineral dust particles and/or sea salts.

165 To track the transport pathways of air masses, 72 h back trajectories were

166 calculated using the National Oceanic and Atmospheric Administration (NOAA)
167 HYbrid Single Particle Lagrangian Integrated Trajectory (HYSPLIT) model
168 (http://www.arl.noaa.gov/HYSPLIT.php). The coordinator of Hakui was used as the
169 back trajectory starting point at several altitudes from 10 m to 3,000 m above ground
170 level to estimate the trajectories of the air masses.
171
172 *2.3. Determination of particle abundance*
173 The air particles at each altitude were measured using an optical particle counter
174 (OPC: Rion, Tokyo, Japan). The OPC device was connected to the
175 meteorological-measurement inlet. The air particles at an altitude of 10 m were also
176 counted using the OPC device placed on the roof of a building.
177 Fluorescent particles stained with DAPI were also counted via epifluorescence
178 microscopy. Within 2 h of sampling, 1 mL of 1 % paraformaldehyde was added to one
179 of the filters to fix the aerosols. After a 1 h incubation, the filter was stained with DAPI
180 at a final concentration of 0.5 μg mL^{-1} for 15 min (Russell et al., 1974). Next, the filter
181 was placed on a slide in a drop of low-fluorescence immersion oil (Type-F
182 IMMOIL-F30CC, Olympus, Tokyo, Japan). A second drop of oil was added, and a
183 coverslip was placed on top. Particles on the filter were observed using a fluorescence
184 microscope (BX-51, Olympus, Tokyo, Japan) with a UV excitation system. A filter
185 transect was scanned, and the four categorized particles, including white fluorescent
186 particles, blue fluorescent particles (microbial particles), yellow fluorescent particles,
187 and black particles, on the filter transect were counted using a previously reported
188 observational technique (Maki et al., 2014). The TA connections in DNA sequences of

189 microbial particles are bound with DAPI, emitting clear blue fluorescence. However, the
190 aggregation of organic matter might also accumulate DAPI at high amounts emitting
191 yellow fluorescence, which is due to formation of a compound with DAPI. Mineral
192 particles often have white autofluorescence or emit weak-blue (mostly white)
193 fluorescence originating from residues of DAPI on the particle surfaces and can be
194 identified on the weak blight background of microscopic observation fields. The black
195 color of black carbon can be identified in the background. The detection limit of aerosol
196 particle concentration was 1.1×10^4 particles m^{-3} of air.
197
198 *2.4. Analysis of bacterial community structures using MiSeq sequencing analysis*
199 *targeting 16S rDNA sequences*
200 After the aerosol particles on the other two filters were suspended in 3 mL of
201 sterile 0.6 % NaCl solution, the particles were pelleted by centrifugation at 20,000 × *g*
202 for 10 min. The genomic DNA (gDNA) was then extracted from the particle pellets
203 using sodium dodecyl sulfate, proteinase K, and lysozyme and purified by
204 phenol-chloroform extraction as previously described (Maki et al., 2008). The bacterial
205 community structure was determined using MiSeq DNA sequencing, which facilitates
206 multiplexed partial sequencing of 16S rDNA. Fragments of 16S rDNA (approximately
207 500 bp) were amplified from the extracted gDNA by PCR using the universal 16S
208 rDNA bacterial primers 515F (5′- Seq A -TGTGCCAGCMGCCGCGGTAA-3′) and
209 806R (5′- Seq B -GGACTACHVGGGTWTCTAAT-3′) (Caporaso et al., 2011), where
210 Seq A and Seq B represent the nucleotide sequences bounded by the second set of PCR
211 primers described below. The PCR amplicon sequences covered the variable region V4

212 of the 16S rRNA gene. Thermal cycling was performed using a thermocycler (Program
213 Temp Control System PC-700; ASTEC, Fukuoka, Japan) under the following
214 conditions: denaturation at 94°C for 1 min, annealing at 52°C for 2 min, and extension
215 at 72°C for 2 min for 20 cycles. Fragments of 16S rDNA in PCR products were
216 amplified again using the second PCR forward primer (5′- Adaptor C - xxxxxxxx - Seq
217 A -3′) and reverse primer (5′- Adaptor D - Seq B -3′), where Adaptors C and D were
218 used for the Miseq sequencing reaction. The sequences "xxxxxxxx" comprise an 8
219 nucleotide sequence tag designed for sample identification barcoding. Thermal cycling
220 was performed under the following conditions: denaturation at 94°C for 1 min,
221 annealing at 59°C for 2 min, and extension at 72°C for 2 min for 15 cycles. PCR
222 amplicons were purified using the MonoFas DNA purification kit (GL Sciences, Tokyo,
223 Japan). PCR amplicons from each sample were pooled at approximately equal amounts
224 into a single sequencing tube on a MiSeq Genome Sequencer (Illumina, CA, USA)
225 machine. The sequences obtained for each sample were demultiplexed based on the tag,
226 including the 8 nucleotide sequence. After removal of the tags, an average read length of
227 450 bp was obtained. Negative controls (no template and extraction products from
228 unused filters) were prepared in the DNA extraction process to check for contamination.
229 The amount of gDNA extracted from air samples ranged from the detection limit (<0.5
230 ng/samples) to approximately 50 ng/samples and cannot be determined directly by light
231 absorbance measurements. Accordingly, quantities of gDNA were estimated using the
232 PCR products after the first amplification step, and compared with the
233 microbial-particle concentrations that were determined by fluorescence microscopic
234 observation. The efficiency of the gDNA extraction from air samples was more than

80 %.

Before the analysis of bacterial community structures, USEARCH v.8.01623 (Edgar, 2013) was used to process the raw Illumina sequencing reads. Anomalous sequences were removed with the following workflow. First, the forward and reverse paired-end reads were merged, and the merged reads with lengths outside of the 200-500 bp range or those exceeding 6 homopolymers were discarded using Mothur v1.36.1 (Schloss et al., 2009). Next, the sequences were subjected to Q-score filtering to remove reads with more than one expected error. Reads occurring only once in the entire dataset (singleton) were then removed. Theses sequences were clustered *de novo* (with a minimum identity of 97 %) into 204 operational taxonomic units (OTUs) among the 18 samples. The taxonomy of the representative OTU sequences was assigned using the RDP classifier (Wang et al., 2007) implemented in QIME v1.9.1 (Caporaso et al., 2010). Non-metric multidimensional scaling (NMDS) plot of the pairwise Bray-Curtis distance matrix were used for the classification of all air samples. Greengenes release 13_8 (McDonald et al., 2012) was used as the reference taxonomic database.

2.5. Accession numbers

All data obtained from MiSeq sequencing data have been deposited in the DDBJ/EMBL/GenBank database (accession number of the submission is PRJEB17915).

3. Results

3.1. Air mass analyses using LIDAR measurements, back trajectories, and metrological data

The vertical distributions of the depolarization ratio determined by LIDAR measurements were assessed for the four sampling events (March 19, 2013; March 20, 2015; April 28, 2013; and March 28, 2014). The depolarization ratio increased at the altitude of 3,000 m on March 19, 2013 (Fig. 2a), while it decreased at the middle altitude of 1,000 m. The air mass on March 20, 2015 showed high values of depolarization ratio at altitudes of 2,500 m and 500 m, consistent with the vertical distribution of non-spherical (mineral dust) particles over the Noto Peninsula (Fig. 2d). A 3-day back trajectory analysis indicated that the air mass at 3,000 m on both sampling dates came from the Asian desert region to the Noto Peninsula (Hakui) immediately across the Sea of Japan (Fig. 3). These results indicated the dust event occurrence on March 19, 2013 was specific to the upper altitude of 3,000 m, while the dust event on March 20, 2015 occurred between the altitudes of 2,500 m and 500 m. Moreover, samples collected on April 28, 2013 and March 28, 2014 exhibited low depolarization ratio (Fig. 2b-c), and the air masses on these two sampling dates came from areas of North Asia, including eastern Siberia (Fig. 3).

The air-sampling periods from the March 2014 time series (from the 23rd to the 29th of March 2014) and the March 2015 time series (from the 16th to the 21st of March 2015) showed different patterns of depolarization ratio and air mass trajectory roots between the two series (Figs. 4 and 5). Depolarization ratio from March 2014 maintained lower values (Fig. 4a) and the trajectory lines changed the roots from eastern Siberia to the Korean Peninsula before surrounding the Japanese islands (Fig. 4c). In

281 contrast, the sampling period during March 2015 had substantially higher depolarization
282 ratio, indicating a strong presence of mineral dust particles (Fig. 5a), and air masses at
283 3,000 m consistently originated from the Asian desert regions (Fig. 5c).
284 Temperatures from March 19, 2013; April 28, 2013; March 28, 2014; and March 20,
285 2015 increased from approximately 290 K to approximately 300 K at middle altitudes
286 (500 m and 1,200 m) (Fig. 2). The temperature profile clearly indicated the presence of
287 a thin boundary under the upper altitudes (2,500 m and 3,000 m), which suggested that
288 there is a difference in air qualities between the middle and upper altitudes (Table 1).
289 During the March 2014 time series, temperatures dynamically changed at altitudes of
290 approximately 1,200 m, while those from the March 2015 time series (the 16th, 17th,
291 and 21st of March 2015) were stable at 1,200 m (Figs. S1 and S2). These results
292 indicate that the boundary layers were located at 1,200 m during the March 2014 time
293 series, whereas the tropospheric air transported by westerly winds was suspended at the
294 sampling altitudes (500 m and 1,200 m) used during the March 2015 time series.
295
296 *3.2. Vertical distributions and sequential variations of aerosol particles*
297 Aerosol particle concentrations from the ground level to the troposphere were
298 measured using OPC to compare the vertical distributions of aerosols from the four
299 sampling events. The OPC-measured particles on March 19, 2013 and March 20, 2015
300 maintained similar concentrations below the troposphere (Fig. 2ad), while the
301 concentrations on April 28, 2013 and March 28, 2014 decreased one or two orders of
302 magnitude between the troposphere and ground level (Fig. 2bc). At high altitudes (2,000
303 m to 2,500 m), the course particles (greater 1.0 μm) observed on March 19, 2013 and

March 20, 2015 were one or two orders of magnitude higher (10^5 to 10^6 particles m^{-3}) than those on April 28, 2013 and March 28, 2014 (no more than 1.2×10^4 particles m^{-3}). The fine particles (0.3 μm to 1.0 μm) showed similar concentrations between the four sampling events, fluctuating between 1.2×10^6 to 3.5×10^7 particles m^{-3}. At lower altitudes (130 m to 510), the aerosol particles had similar concentrations and size distributions between the four sampling periods; the course particle concentration ranged from 8.4×10^5 particles m^{-3} to 1.2×10^6 particles m^{-3}, and the fine particles ranged from 1.3×10^7 particles m^{-3} to 1.2×10^8 particles m^{-3}.

OPC measurements indicated that air samples collected at 1,200 m during the March 2015 time series consistently contained course particles at one or two orders of magnitude higher in concentration (1.4×10^6 to 3.4×10^6 particles m^{-3}) than detected in the March 2014 time series, which had concentrations of no more than 1.8×10^5 particles m^{-3} (Fig. 4b). The concentration of relatively large particles (>5.0 μm) in March 2015 maintained relatively higher concentrations (from 1.4×10^4 to 8.2×10^5 particles m^{-3}) than those observed in March 2014 (no more than 3.74×10^3 particles m^{-3}). In contrast, the fine particles measured in March 2014 and March 2015 fluctuated around similar concentrations ranging from 10^7 to 10^8 particles m^{-3}.

Based on the above observations, the sampled air masses that were influenced by Asian dust events and included dust particles were categorized as “dust samples”. The sampled air masses that were not influenced by dust events or contained less dust particles were categorized as “non-dust samples”, in relation to the presence or absence of dust events as the source of the aerosol samples (Table 1).

327 *3.3. Fluorescent microscopic observation of aerosol particles*

328 Using epifluorescence microscopy with DAPI staining, the aerosol particles in the
329 18 air samples emitted several types of fluorescence, categorized as white, blue, yellow,
330 or black (Fig. S3). White fluorescence particles, (white particles) were indicative of
331 mineral particles originating from the sand or soil. Microbial (prokaryotic) particles
332 stained with DAPI emitted blue fluorescence, forming coccoid- or bacilli-like particles
333 with a diameter <3 μm. Yellow fluorescence particles (yellow particles) stained with
334 DAPI were organic matter and ranged from 1.0 μm to 10 μm in diameter. Most of the
335 yellow particles disappeared in the aerosol-particle suspending solutions after protease
336 treatment, suggesting that the yellow particles consisted mainly of proteins. Black
337 particles were indicative of an anthropogenic black carbon originating from East Asian
338 regions, produced by biomass burning, industrial activities, and vehicle exhaust.

339 The dust samples from upper altitudes (2,500 m and 3,000 m) contained 5 to 100
340 times higher concentrations of microbial, organic, and white particles than the
341 concentrations detected in the non-dust samples (Fig. 2). In the upper altitude dust
342 samples, the concentration of mineral particles ranged from 7.77×10^5 particles m^{-3} to
343 1.08×10^6 particles m^{-3} (Fig. 2ad), whereas the concentrations of the non-dust samples
344 ranged from 3.14×10^4 particles m^{-3} to 1.48×10^5 particles m^{-3} (Fig. 2bc). The
345 microbial particles in the high altitude dust samples exhibited concentrations of
346 approximately 1.5×10^6 particles m^{-3} that were two orders of magnitude higher than in
347 the non-dust samples (approximately 6.0×10^4 particles m^{-3}). The organic particles in
348 the high altitude dust samples were also found at higher concentrations of
349 approximately 4.2×10^6 particles m^{-3} than those from the non-dust samples 13H428-u

and 14H328-u, which were 2.12×10^4 particles m^{-3} and 5.30×10^4 particles m^{-3}, respectively. In contrast, the air samples collected at the low altitude of 10 m exhibited a random or stochastic pattern between 10^5 and 10^6 particles m^{-3}, regardless of the sampling dates (Fig. 2). Black particles were observed in the four air samples from 10 m and fluctuated around concentrations of less than 8.48×10^4 particles m^{-3}. Finally, the percentage of organic particles out of the total number of particles (organic and microbial particles) in the dust samples 13H319-u, 15H320-u, and 15H320-m ranged between approximately 71.5 % and 73.6 %, which was higher than in the non-dust samples, which ranged from 4.6 % to 46.3 % (Fig. S4).

All types of fluorescence particles were also observed in the sequentially collected air samples at 1,200 m in the March 2015 time series (except for 2,500 m on March 20th) and the March 2014 series. The dust samples examined from the March 2015 series had higher concentrations of total particles than the non-dust samples of the March 2014 series (Figs. 4 and 5). The mineral particles detected in the March 2014 series fluctuated at low concentrations from 3.39×10^4 particles m^{-3} to 2.62×10^5 particles m^{-3} (Fig. 4), while in the March 2015 series the mineral particles showed higher values from 1.80×10^5 particles m^{-3} to 1.77×10^7 particles m^{-3} (Fig. 5). High levels of organic particles were detected in the March 2015 series samples, ranging from 3.13×10^5 to 3.75×10^7 particles m^{-3}, which decreased to below 2.28×10^5 particles m^{-3} in the March 2014 series samples. The microbial particle concentrations in the March 2015 series samples (ranging from 4.75×10^5 to 2.06×10^6 particles m^{-3}) were higher than those of in the March 2014 series samples (ranging from 3.31×10^5 to 1.25×10^6 particles m^{-3}). The ratio of organic particles to the total number of organic and microbial

373 particles detected during March 2015 (71.5 % to 95.6 %) were higher than those during
374 March 2014 series (8.0 % to 36.2 %) (Fig. S4). The black particles were randomly
375 observed in all samples from March 2015 and March 2014.
376
377 *3.4. Analysis of bacterial communities using MiSeq sequencing analysis*
378 For the analysis of the prokaryotic composition in the 18 samples, we obtained
379 645,075 merged paired-end sequences with the lengths ranging from 244 bp to 298 bp
380 after quality filtering, and the sequence library size for each sample was normalized at
381 1,500 reads. The 16S rDNA sequences were divided into 204 phylotypes (sequences
382 with >97 % similarity). Phylogenetic assignment of sequences resulted in an overall
383 diversity of 16 phyla and candidate divisions, 32 classes (and class-level candidate taxa),
384 and 72 families (and family-level candidate taxa). The majority (>90 %) of the
385 sequences were represented by 9 bacterial classes and 33 families (Figs. 6 and 7). The
386 bacterial compositions varied during the sampling periods and included the phylotypes
387 belonging to the classes Cyanobacteria, Actinobacteria, Bacilli, Bacteroidetes, SBRH58,
388 and Proteobacteria (Alpha, Beta, Gamma, and Deltaproteobacteria), which are typically
389 generated from atmospheric, terrestrial and marine environments. On the box plots, the
390 numbers of bacterial species estimated by Chao I were similar at average levels between
391 the dust samples and non-dust samples, while the Chao I and Shannon values of the
392 non-dust samples showed a wider range than that of dust samples (Fig. 8a). A
393 non-metric multidimensional scaling (NMDS) plot demonstrated the distinct clustering
394 of prokaryotic communities separating the dust samples and the non-dust samples (Fig.
395 8b). For the PCR-analysis steps, negative controls (no template and template from

396 unused filters) did not contain 16S rDNA amplicons demonstrating the absence of
397 artificial contamination during experimental processes.
398
399 *3.5. Vertical distributions of bacterial communities in dust and non-dust samples*
400 The vertical distributions of bacterial compositions showed different patterns
401 between dust event days and non-dust days (Fig. 6). In the dust samples collected at
402 upper altitudes, phylotypes belonging to the phylum Bacilli accounted for more than
403 60.5 % of the total and were mainly composed of members of the families Bacillaceae
404 and Paenibacilliaceae (Fig. 6). Bacterial numbers from the phylum Bacilli decreased at
405 lower altitudes during dust events, and the phylotypes of Cyanobacteria, Actinobacteria,
406 and Protobacteria increased in relative abundance in the samples collected at middle and
407 low altitudes (13H319-m, 13H319-l, and 15H320-m).
408 Cyanobacteria, Actinobacteria, and Proteobacteria sequences also dominated in the
409 air samples collected during non-dust events (13H428-m, 14H328-u, 14H328-m, and
410 14H328-l). Specifically, Actinobacteria phylotypes increased in their relative abundance,
411 ranging from 14.1 % to 24.7 % in the non-dust samples collected on March 28, 2014.
412 Proteobacteria phylotypes containing several bacterial families occupied a high relative
413 abundance, ranging from 60.5 % to 85.3 % in the non-dust samples 13H428-u,
414 13H428-m, 14H328-u, 14H328-m, and 14H328-l. In particular, the non-dust samples
415 collected on March 28, 2014 included the Alphaproteobacteria phylotypes, which have
416 composed of members of the families Phyllobacteriaceae and Sphingomonadaceae.
417 Most Betaproteobacteria, phylotypes including the families Oxalobacteraceae and
418 Comamonadaceae, were specific to the non-dust samples collected at 1,200 m and 2,500

m on April 28, 2013.

Cyanobacteria phylotypes, which were randomly detected from both dust samples and non-dust samples, particularly increased in both the non-dust sample collected at 10 m on April 28, 2013 and the dust sample collected at 3,000 m on March 20, 2015, with a relative abundance of 15.3 % and 74.6 %, respectively. Bacteroidia phylotypes also randomly appeared in several air samples, regardless of the dust event influences and were present at maximal levels in the non-dust sample 13H319-m, with a relative abundance of 35.6 %.

3.6. Variations in bacterial communities during dust events and non-dust events

Sequential variations in the bacterial composition of air samples at altitudes of 1,200 m or 2,500 m were compared between dust event periods (March 2015 series) and non-dust periods (March 2014 series). During the March 2015 dust event, phylotypes of the family Bacillaceae in the class Bacilli occupied more than 53.0 % of the relative abundance in the four dust samples collected (Fig. 7). Cyanobacteria phylotypes related to the marine cyanobacterium Synechococcaceae uniquely appeared in the dust samples of the March 2015 series; their abundance fluctuated the values ranging from 12.5 % to 14.8 % between the 16th and the 20th of March 2015 before decreasing to 1.5 % on March 20.

During the non-dust periods of the March 2014 series at the middle altitude, the relative abundance of Actinobacteria phylotypes belonging to the family Micrococcaceae was occupied 59.9 % on March 23, decreased to 19.5 % on March 24, and disappeared from samples collected on March 29. Corresponding to the decrease in

442 Actinobacteria phylotypes, Alpha and Gammaproteobacteria phylotypes showed an
443 increasing trend from 30.6 % to 96.8 % between the 23rd and the 29th of March 2014
444 (Fig. 7a). Alphaproteobacteria phylotypes belonging to the families Sphingomonadaceae,
445 and Phyllobacteriaceae, consistently appeared throughout the sampling periods of the
446 March 2014 series and occupied a maximum relative abundance of 72.9 % and 22.3 %
447 respectively. For Gammaproteobacteria, the Xanthomonadaceae sequences dominated at
448 a relative abundance of 18.3 % and 5.4 % in the non-dust samples 14H325-m and
449 14H329-m, respectively, during the air mass was suspended the Japanese islands for a
450 few days.
451
452 **4. Discussion**
453
454 *4.1 Air mass conditions during Asian dust and non-dust events*
455 Westerly winds blowing over East Asia disperse airborne microorganisms
456 associated with dust mineral particles (Maki et al., 2008) and anthropogenic particles
457 (Cao et al., 2014; Wei et al., 2016), influencing the abundances and taxon compositions
458 of airborne bacteria at high altitudes over downwind areas, such as Noto Peninsula
459 (Maki et al., 2013). In this investigation, the increases in aerosol particles (dust
460 particles) and associated microbial particles were observed over the Noto Peninsula
461 during the dust events of March 19, 2013 and March 20, 2015 (Figs. 2 and 4). At the
462 two sampling dates, the air mass including microbial particles had traveled from the
463 Asian desert region throughout the anthropogenic polluted areas (Fig. 2), and the dust
464 particles entered the Japanese troposphere and were maintained at high altitudes (March

19, 2013) or mixed with the ground-surface air (March 20, 2015). During non-dust days, the air masses at high altitudes came from several areas, including the eastern region of Siberia, Asian continental coasts (Korean Peninsula), the Sea of Japan, or surrounding Japanese islands, and mixed with ground-surface air over the Noto Peninsula. The air samples collected during dust and non-dust events were valuable for understanding the westerly wind influences on vertical distributions and sequential dynamics of airborne bacteria at high altitudes over the downwind regions.

4.2 Aerosol dynamics during Asian dust and non-dust event

The microscopic fluorescence particles of all samples could be separated into four categories: mineral (white), microbial (blue), organic (yellow), and black-carbon (black) particles (Fig. S3), which were observed in the previous air samples collected during dust events (Maki et al., 2015). The amount of microbial particles increased at high altitudes during dust events, suggesting that the dust events directly carried bacterial particles to the troposphere over downwind areas. At low altitudes, similar concentrations of fluorescent particles were observed in air samples collected between dust events (13H319-l) and non-dust events (13H428-l) (Fig. 2) because the dust particles did not reach the ground surface on the dust-event days. In the absence of the influences of dust-events, the aerosols mainly originated from local environments in Japanese areas.

Organic particles also increased during dust events and in the ratios between all particles related to the dust events. The organic particles originate from proteins and other biological components (Mostajir et al., 1995). The tropospheric aerosols would be

488 composed of organic particles at high rates ranging from 30 % to 80 % (Jaenicke, 2005),
489 and organic particle concentrations fluctuated from 10^3 to 10^5 particles m^{-3} at high
490 altitudes of 4,000 m above the ground (Twohy et al., 2016). The dead-phase cells of
491 microbial isolates obtained from aerosol samples mainly irradiated yellow fluorescence
492 instead of blue fluorescence (Liu et al., 2014). When fungi (*Bjerkandera adusta*) and
493 bacteria (*Bacillus* spp.) isolated from aerosol samples were incubated, the dead-phase
494 microbial cells mainly irradiated yellow fluorescence instead of blue fluorescence (Liu
495 et al., 2014; Fig. S3). The relative numbers of organic particles to the total number of
496 microbial and organic particles in the dust samples showed significantly higher values
497 (82.9 ± 32.3 %) than in the non-dust samples (23.3 ± 13.7 %) (Fig. S4). Hara and Zhang
498 reported that dust events in Kyushu, Japan, resulted in an increased ratio of damaged
499 microbial cells in the air at the ground-surface and that the ratio increased to
500 approximately 80 % (Hara and Zhang, 2012). Furthermore, organic molecules
501 associated with dust aerosols are reported to be composed of mannitol, glucose, and
502 fructose, which are part of cell components of airborne microorganisms and contribute
503 to the formation of secondary organic aerosols (SOA) (Fu et al., 2016). Microbial cells
504 or their components coming from Asian continents to Japan would be exposed to air at
505 high-altitudes during their long-range transport, increasing the ratios of damaged and
506 dead cells or SOA.

507 The appearance of black carbon most likely originated from anthropogenic
508 activities, such as biomass burning, industrial activities, and vehicle exhaust (Chung and
509 Kim, 2008). In the anthropogenic regions of eastern China, anthropogenic particles
510 originating from human activities are expected to comprise more than 90 % of dust

511 particles (Huang et al., 2015a). When the westerly winds are strongly blowing over the
512 Noto Peninsula, the black carbon particles at upper altitudes (3,000 m) are thought to
513 mainly derive from continental anthropogenic regions.
514
515 *4.3 Comparing the community structures of atmospheric bacteria between Asian dust*
516 *and non-dust events*
517 Dust events and air-pollutant occurrences changed the airborne bacterial
518 communities over the downwind areas, such as Beijing (Jeon et al., 2011; Cao et al.,
519 2014) and east Mediterranean areas (Mazar et al., 2016). The westerly winds blowing
520 over East Asia would transport airborne bacteria to the high-altitude atmosphere over
521 the Noto Peninsula (Maki et al., 2015) and North American mountains (Smith et al.,
522 2013). Our box plots analysis suggested that changes in the bacterial diversity in the
523 dust samples would be more stable than in the non-dust samples (Fig. 8a). Furthermore,
524 using a NMDS plot, the bacterial compositions in the dust samples could be
525 distinguished from non-dust samples (Fig. 8b). Thus, the aerosol particles transported
526 by Asian dust events changed the atmospheric bacterial composition at higher altitudes
527 over downwind areas.
528 The phylotypes in the dust samples were predominately clustered into the class
529 Bacilli (Fig. 4a), while the non-dust samples mainly included the phylotypes of the
530 classes Alpha, Beta, and Gammaproteobacteria and Actinobacteria. Our previous
531 investigations indicated that the bacterial communities at an altitude of 3,000 m over the
532 Noto Peninsula included more than 300 phylotypes, which were predominantly
533 composed of Bacilli phylotypes (Maki et al., 2015). Bacterial groups belonging to

534 Bacilli, Proteobacteria, and Actinobacteria have been reported as airborne bacteria
535 around European mountains (Vaïtilingom et al., 2012) as well as over Asian rural
536 regions (Woo et al., 2013). Some Bacilli isolates were found to act as ice-nucleating
537 agents and to be involved in ice cloud (Matulova et al., 2014; Mortazavi et al., 2015).
538 Isolates of Gammaproteobacteria isolates were obtained from mineral dust particles
539 (Hara et al., 2016a), glaciated high-altitude clouds (Sheridan et al., 2003), and plant
540 bodies (Morris et al., 2008), and some isolate species, such as *Pseudomonas*, were
541 confirmed to have the ice-nucleation activity. Accordingly, Bacilli and Proteobacteria
542 members associated with dust events could potentially contribute to climate change
543 resulting from dust events.
544
545 *4.4 Dominant bacterial populations in the air masses transported from Asian continents*
546 In some dust-event samples collected at high altitudes (13H319-u, 15H320-u, and
547 15H320-m), Bacilli sequences accounted for more than 52.7 % of the total number of
548 sequences (Fig. 6). Back trajectories on March 19, 2013 and March 20, 2015 came from
549 the Asian desert region to the Noto Peninsula. Some *Bacillus* species were
550 predominantly detected at high altitudes above the Taklimakan Desert (Maki et al.,
551 2008) and above downwind areas during Asian dust events (Maki et al., 2010 and 2013;
552 Smith et al., 2013; Jeon et al., 2011; Tanaka et al., 2011). *Bacillus* species are the most
553 prevalent isolates obtained from mineral dust particles collected over downwind areas
554 (Hua et al., 2007; Gorbushina et al., 2007).
555 Bacilli members can form resistant endospores that support their survival in the
556 atmosphere (Nicholson et al., 2000). The *Bacillus* isolates obtained from atmospheric

557 samples showed higher-level resistance to UV irradiation than normal isolates
558 (Kobayashi et al., 2015). In the Gobi Desert, dust events increase the diversity of
559 airborne microbial communities; after dust events, spore-forming bacteria, such as
560 *Bacillus*, increase in their relative abundances (Maki et al., 2016). Accordingly, in the
561 atmosphere, selected Bacilli members associated with dust particles would be
562 transported over long distances.

563 The Bacilli sequences showed different vertical variations between the two dust
564 events on March 19, 2013 and March 20, 2015. On March 19, 2013 (13H319-m), the
565 relative abundances of Bacilli sequences decreased dynamically from 3,000 m to 1,200
566 m, while unstable atmospheric layers on March 20, 2015 most likely mixed the
567 long-range transported bacteria with the regional bacteria over the Noto Peninsula. A
568 previous investigation also demonstrated the vertical mixture of airborne bacteria over
569 Suzu in the Noto Peninsula (Maki et al., 2010).

570 Actinobacteria sequences decreased in relative abundance between the 23rd and
571 29th of March 2014 corresponding with changes in the air mass trajectory roots from
572 north Asian regions, such as eastern Siberia and Japan (Fig. 7). Furthermore,
573 Actinobacteria sequences appeared in the samples collected from air masses that were
574 transported throughout the Korean Peninsula on March 19, 2013; April 28, 2013; and
575 March 20, 2015. Actinobacteria members are frequently dominant in terrestrial
576 environments but seldom survive in the atmosphere for a long time, because they cannot
577 form spores (Puspitasari et al., 2015). However, the family Micrococcaceae in
578 Actinobacteria was primarily detected from anthropogenic particles collected in Beijing,
579 China (Cao et al., 2014). Over anthropogenic source regions for Asian continents,

580 anthropogenic particles occupy more than 90 % of dust particles and originate from
581 soils disturbed by human activities in cropland, pastureland, and urbanized regions
582 (Huang et al., 2015a; Guan et al., 2016). Air masses transported from the continental
583 coasts are expected to include a relatively high abundance of Actinobacteria members
584 associated with anthropogenic particles.

585 Natural dust particles from Asian desert areas (Taklimkan and Gobi Deserts) are
586 transported in the free troposphere (Iwasaka et al., 1988) and vertically mixed with
587 anthropogenic particles during the transportation processes (Huang et al., 2015a). In
588 some cases, short-range transport of air masses would carry only anthropogenic
589 particles to Japan, because the anthropogenic particles are often dominant in Asian
590 continental coasts (Huang et al., 2015a). Actinobacteria members may have been
591 transported with anthropogenic particles from continental coasts.

592

593 *4.5 Dominant bacterial populations in the air masses originated from marine*
594 *environments and Japanese islands*

595 Proteobacteria sequences increased in their relative abundances at high altitudes
596 during non-dust sampling dates (13H428-u, 13H428-m, 14H328-u, 14H328-m, and
597 March 2014 series), when air mass origins at 1,200 m changed from the Korean
598 Peninsula to Japan (Fig. 7). Proteobacteria members were the dominate species in the
599 atmosphere over mountains (Bowers et al., 2012; Vaïtilingom et al., 2012; Temkiv et al.,
600 2012), in the air samples collected on a tower (Fahlgren et al., 2010), and from the
601 troposphere (DeLeon-Rodriguez et al., 2013; Kourtev et al., 2011). In the phylum
602 proteobacteria, the families Phyllobacteriaceae, Methylobacteriaceae, and

603 Xanthomonadaceae were predominately detected from the non-dust samples and are
604 associated with plant bodies or surfaces (Mantelin et al., 2006; Fürnkranz et al., 2008;
605 Khan and Doty, 2009; Fierer and Lennon, 2011). The Betaproteobacteria sequences in
606 the non-dust samples mainly contained the Oxalobacteraceae and Comamonadaceae
607 families, which are commonly dominate in freshwater environments (Nold and Zwart,
608 1998) as well as on plant leaves (Redford et al., 2010). In addition, the class
609 Alphaproteobacteria in the non-dust samples also included marine bacterial sequences
610 belonging to the family Sphingomonadaceae (Cavicchioli et al., 2003). Bacterial
611 populations originating from marine areas are prevalent in cloud droplets (Amato et al*.,*
612 2007), in air samples collected from the seashores of Europe (Polymenakou et al., 2008),
613 in storming troposphere (DeLeon-Rodriguez et al., 2013), and at high altitudes in
614 Japanese regions (Maki et al., 2014), suggesting that the marine environments represent
615 a major source of bacteria in clouds. The air masses suspended over the Sea of Japan or
616 Japanese islands during non-dust events (the March 2014 series) could include a high
617 relative abundance of airborne bacteria, which were transported from the surface-level
618 air over the marine environments and the regional phyllosphere.
619
620 *4.6. Bacterial populations commonly detected during dust events and the non-dust*
621 *events*
622 Sequences originating from Synechococcaceae (in the class Cyanobacteria)
623 randomly appeared in the MiSeq sequencing databases results obtained from air samples,
624 regardless of dust event occurrences. *Synechococcus* species in the family
625 Synechococcaceae can eliminate excess peroxide from photosynthesis to resist UV

626 radiation and oxygenic stress (Latifi et al., 2009), suggesting that these bacteria resist
627 environmental stressors in the atmosphere. In a previous study, the air samples
628 transported from marine environments to Japan predominately contained *Synechococcus*
629 species (Maki et al., 2014), which were dominant marine bacteria in the Sea of Japan
630 and the East China Sea (Choi and Noh, 2009). The cloud water at approximately 3,000
631 m above ground level was also dominated by Cyanobacteria populations, indicating
632 their atmospheric transport (Kourtev et al., 2011). In addition to Alphaproteobacteria,
633 marine cyanobacterial cells can be transported from seawater to the atmosphere, thereby
634 contributing to the airborne bacterial variations over the Noto Peninsula. Marine
635 bioaerosols originated from cyanobacteria and gram-negative bacteria (including
636 Alphaproteobacteria) are reported to contribute the increase of endotoxin levels in
637 coastal areas influencing human health by inflammation and allergic reaction
638 (Lang-Yona et al., 2014).
639 Bacteroidetes sequences were detected in some air samples collected during Asian
640 dust and non-dust events. Members of the phylum Bacteroidetes, which were composed
641 of the families Cytophagaceae, associate with organic particles in terrestrial and aquatic
642 environments (Turnbaugh et al., 2011; Newton et al., 2011). Furthermore, these
643 bacterial populations dominate the atmosphere and sand of desert areas, where plant
644 bodies and animal feces are sparsely present (Maki et al., 2016). These bacterial groups
645 possibly originated from organic-rich microenvironments in several areas, such as desert
646 and marine areas.
647
648 **5. Conclusion**

Air samples including airborne bacteria were sequentially collected at high altitudes over the Noto Peninsula during dust events and non-dust events. The sampled air masses could be categorized based on sample types with (dust samples) and without (non-dust samples) dust event influences. Bacterial communities in the air samples displayed different compositions between dust events and non-dust events. The dust samples were dominated by terrestrial bacteria, such as Bacilli, which are thought to originate from the central desert regions of Asia, and the bacterial compositions were similar between the dust samples. In contrast, the air masses of non-dust samples came from several areas, including northern Asia, continental coasts, marine areas, and Japan regional areas, showing different variations in bacterial compositions between the sampling dates. Some scientists have attempted to apply airborne bacterial composition as tracers of air mass sources at ground level (Bowers et al., 2011; Mazar et al., 2016). In this study, the terrestrial bacteria, such as Bacilli and Actinobacteria members (Bottos et al., 2014), were dominant populations in the air samples transported from Asian continental areas. The air samples when the air mass was suspended around Japanese islands, mainly included the members of the classes Alpha (Phyllobacteriaceae and Methylobacteriaceae), Gamma, and Betaproteobacteria, which are commonly dominated in phyllosphere (Redford et al., 2010; Fierer and Lennon, 2011) or freshwater environments (Nold and Zwart, 1998). The atmospheric aerosols transported via marine areas include a high relative abundances of marine bacteria belonging to classes Cyanobacteria (Choi and Noh, 2009) and Alphaproteobacteria (Sphingomonadaceae) (Cavicchioli et al., 2003). This study suggested that bacterial compositions in the atmosphere can be used as air mass tracers, which could identify the

672 levels of mixed air masses transported from different sources.

673 However, one limitation of our investigation is that the number of samples
674 analyzed was not sufficient to cover entire changes in airborne bacteria at high altitudes
675 over the Noto Peninsula. Although the airborne bacterial composition during non-dust
676 periods was found to change dynamically, only a few types of variation were followed
677 in this investigation. In the future, greater numbers of samples, which are sequentially
678 collected at high altitudes using this sampling method, will need to be originated to
679 more accurately evaluate bioaerosol tracers. Since helicopter sampling procedures
680 require sophisticated techniques and are expensive, the sample numbers at high altitudes
681 are difficult to increase. Air sampling at high altitudes should be combined with
682 sequential ground-air sampling to advance the understanding of the influence of
683 westerly winds on airborne bacterial dynamics in downwind areas. Metagenomic
684 analyses and microbial culture experiments would also provide valuable information
685 about airborne microbial functions relating to ice-nucleation activities, chemical
686 metabolism, and pathogenic abilities.

687

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702

703 **Competing Interests**

704 The authors declare that they have no conflict of interest.
705

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984

985 **Figure Legends**

986

987 Fig. 1. Sampling location (a) and helicopter flight routes during the sampling periods on
988 March 19, 2013, and April 28, 2013 (b); the 23rd, 24th, 25th, and 29th of March 2014
989 (c); and the 16th, 17th, 20th, and 21st of March 2015 (d).

990

991 Fig. 2. LIDAR observation of the depolarization ratio in Toyama city as well as vertical
992 changes in temperature, relative humidity, and potential temperature, and vertical
993 distributions of concentrations of OPC-counted particles and DAPI-stained particles
994 from the four sampling events on March 19, 2013 (a); April 28, 2013 (b); March 28,
995 2014 (c); and March 20, 2015 (d). The red circles in the LIDAR images indicate that the
996 sampling air included dust mineral particles (solid line) or that dust-event influences are
997 absent at the altitudes on the sampling time (dotted line). OPC-counted particles were
998 categorized according to diameter sizes of 0.3–0.5 μm (closed squares), 0.5–0.7 μm
999 (closed triangles), 0.7–1.0 μm (closed circles), 1.0–2.0 μm (closed diamonds), 2.0–5.0
1000 μm (crosses), and >5.0 μm (open circles). DAPI-stained particles were classified into
1001 microbial particles (blue bars), white particles (white bars), yellow fluorescent particles
1002 (yellow bars), and black carbon (gray bars).

1003

1004 Fig. 3. Trajectories 3 days ago of aerosols that arrived at 2,500 m (blue-type lines) and
1005 1,200 m (red-type lines) in Hakui, Japan, every hour for 5 h before the completion of
1006 sampling time at the four dates; March 19, 2013; April 28, 2013; March 28, 2014; and
1007 March 20, 2015.

1008

1009 Fig. 4. (a) LIDAR observation of the depolarization ratio in Toyama city and
1010 concentrations of OPC-counted particles and DAPI-stained particles during no-dust
1011 days from 0:00 UTC on March 23 to 0:00 UTC on March 30, 2014. The red circles with
1012 dotted lines in the LIDAR images indicate dust-event influences are absent at the
1013 altitudes on the sampling time. (b) OPC-counted particles were categorized according to
1014 diameter sizes of 0.3–0.5 μm (closed squares), 0.5–0.7 μm (closed triangles), 0.7–1.0
1015 μm (closed circles), 1.0–2.0 μm (closed diamonds), 2.0–5.0 μm (crosses), and >5.0 μm
1016 (open circles). DAPI-stained particles were classified into microbial particles (blue bars),
1017 white particles (white bars), yellow particles (yellow bars), and black particles (gray
1018 bars). (c) Trajectories 3 days ago of aerosols that arrived at 2,500 m (blue-type lines)
1019 and 1,200 m (red-type lines) in Hakui, Japan, every hour for 5 h before the completion
1020 of sampling time during sampling periods on the 23rd, 24th, 25th, 28th, and 29th of
1021 March 2014.

1022

1023 Fig. 5. (a) LIDAR observation of the depolarization ratio in Toyama city and
1024 concentrations of OPC-counted particles and DAPI-stained particles during dust event
1025 days from 0:00 UTC on March 16 to 0:00 UTC on March 23, 2015. The red circles with
1026 solid lines in the LIDAR images indicate that the sampling air included dust mineral
1027 particles. (b) OPC-counted particles were categorized based on diameter sizes of
1028 0.3–0.5 μm (closed squares), 0.5–0.7 μm (closed triangles), 0.7–1.0 μm (closed circles),
1029 1.0–2.0 μm (closed diamonds), 2.0–5.0 μm (crosses), and >5.0 μm (open circles).
1030 DAPI-stained particles were classified into microbial particles (blue bars), white

1031 particles (white bars), yellow particles (yellow bars), and black particles (gray bars). (c)
1032 Trajectories 3 days ago of aerosols that arrived at 2,500 m (blue-type lines) and 1,200 m
1033 (red-type lines) in Hakui, Japan, every hour for 5 h before the completion of sampling
1034 time during sampling periods on the 16th, 17th, 20th, and 21st of March 2015.
1035
1036 Fig. 6. Vertical variations in bacterial compositions at (a) the class level and (b) the
1037 family level of the partial sequences obtained in the MiSeq sequencing database (ca.
1038 400 bp) obtained from air samples collected at different altitudes over the Noto
1039 Peninsula at dust-event days (March 19, 2013; March 20, 2015) and non-dust-event
1040 days (March 19, 2013; March 20, 2015).
1041
1042 Fig. 7. Changes in bacterial compositions at (a) the class level and (b) the family level
1043 of the partial sequences obtained in the MiSeq sequencing database (ca. 400 bp) from
1044 air samples collected at altitudes of 1,200 m (except for the sample collected at 500 m
1045 on March 20, 2015) over the Noto Peninsula during dust-event days from the 16th to the
1046 23rd of March 2015 and during non-dust-event days from the 23rd to the 29th of March
1047 2014.
1048
1049 Fig. 8. Comparison of the bacterial compositions among all air samples collected over
1050 the Noto Peninsula. (a) Box plots of Chao 1 and Shannon analyses indicating the
1051 bacterial diversity observed in dust samples and non-dust samples. Species were binned
1052 at the 97 % sequence similarity level. (b) NMDS of the pairwise Bray-Curtis distance
1053 matrix displaying clustering by all the air samples. Red indicates the samples that were

collected during dust-events and blue indicates those collected during non-dust-events as determined by meteorological data. Circle indicates that the sample contained dust particles as identified via microscopic observation, and triangle indicates that dust particles were absent from the sample. The confidence ellipses are based on a multivariate t-distribution, and represents the 95 % confidence interval of the occurrence of dust vs. non-dust events when the samples were collected.