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

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Abstract. Aerosol particles, including airborne microorganisms, are transported through the free troposphere from the Asian continental area to the downwind area in East Asia and can influence climate changes, ecosystem dynamics, and human health. However, the variations present in airborne bacterial communities in the free troposphere over downwind areas are poorly understood, and there are few studies that provide an in-depth examination of the effects of long-range transport of aerosols (natural and anthropogenic particles) on bacterial variations. In this study, the vertical distributions of airborne bacterial communities at high altitudes were investigated and the bacterial variations were compared between dust events and non-dust events.

Aerosols were collected at three altitudes from ground level to the free troposphere (upper level: 3,000 m or 2,500 m; middle level: 1,200 m or 500 m; and low level: 10 m) during Asian dust events and non-dust events over the Noto Peninsula, Japan, where westerly winds carry aerosols from the Asian continental areas. During Asian dust events, air masses at high altitudes were transported from the Asian continental area by westerly winds, and Laser Imaging Detection and Ranging (LiDAR) data indicated high concentrations of non-spherical particles, suggesting that dust-sand particles were transported from the central desert regions of Asia. The air samples collected during the dust events contained 10–100 times higher concentrations of microscopic fluorescent particles and Optical Particle Counter (OPC) measured particles than in non-dust events. The air masses of non-dust events contained lower amounts of dust-sand particles. Additionally, some air samples showed relatively high levels of black carbon, which were likely transported from the Asian continental coasts. Moreover, during the dust events, microbial particles at altitudes of >1,200 m increased to the concentrations ranging from 1.2×10^6 particles m^{-3} to 6.6×10^6 particles m^{-3}. In contrast, when dust events disappeared, the microbial particles at >1,200 m decreased slightly to microbial-particle concentrations ranging from 6.4×10^4 particles m^{-3} to 8.9×10^5 particles m^{-3}.

High-throughput sequencing technology targeting 16S rRNA genes (16S rDNA) revealed that the bacterial communities collected at high altitudes (from 500 m to 3,000 m) during dust events exhibited higher diversities and were predominantly composed of natural-sand/terrestrial bacteria, such as *Bacillus* members. During non-dust periods, airborne bacteria at high altitudes were mainly composed of anthropogenic/terrestrial bacteria, (Actinobacteria), marine bacteria (Cyanobacteria and

5   Alphaproteobacteria), and plant-associated bacteria (Gammaproteobacteria), which shifted in composition in correspondence with the origins of the air masses and the meteorological conditions. Heterogeneous bacterial populations, originating from terrestrial, marine, and plant-associated categories, were used as an indicator for the mixture levels of air masses originating from the Asian continental area, the Sea of Japan, and the Japanese islands, respectively. The airborne bacterial structures at high altitudes suggested remarkable changes in response to air mass sources, which contributed to the increases in

10   community richness and to the domination of a few bacterial taxa.

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

15   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 (Sugimoto et al., 2012; Maki et al., 2015).

20   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; Hara et al., 2016a). Indeed, airborne microorganisms were demonstrated to act as ice nuclei (Joly et al., 2013; Hara et al., 2016b) and cloud condensation nuclei that affect ice cloud processes (Möhler et al., 2007; Creamean

25   et al., 2013). Viable bacteria identified in supercooled cloud droplets were found to metabolize organic matter from the environment (Sattler et al., 2001; Vaïtilingom et al., 2012). Some airborne microorganisms synthesize organic molecules, such as mannitol, glucose, and fructose, and affect the organic-aerosol contents (Delort et al., 2010). 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

30   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 on Asian islands or peninsulas, because the air masses originate from heterogeneous environments, including marine, mountainous, urban, and desert areas (Hara and Zhang, 2012; Maki et al., 2014). 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-
5 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
10 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., 2013). 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.

15 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
20 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 microscopic observation. Bacterial community structures were analyzed by using high-throughput sequencing targeting bacterial 16S rRNA genes (16S rDNA).

2 Experiments

25 ### 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 Noto Peninsula, Japan. Both cities are located on the western coast of the Noto Peninsula where aerosols directly arrive from continental areas (Fig. 1). The helicopter traveled 20 km northwest from Kanazawa to Uchinada; air sampling was continuously conducted from Uchinada to the northern coastal areas. To
30 compare the vertical distributions of airborne bacteria during dust and non-dust events, air samples were collected using a helicopter at the 1 to 3 altitudes ranging from 500 m to 3,000 m above ground level (Table 1). Air samples from low altitude regions (10 m above ground level) were collected from the roof of a building located at Taki bay in Hakui (36º92 N, 136º76

E). To compare the vertical bacterial distribution, aerosol samples were collected during the daytime (from 9:00 Japanese standard time [JST; UTC + 9 h] to 16:30 JST) on March 19, 2013; April 28, 2013; March 28, 2014; and March 20, 2015. These samples were collected at the following altitude sets; (1) 2,500 m, 1,200 m, and 10 m; (2) 3,000 m, 1,200 m, and 10 m; (3) 3,000 m, 1,200 m, and 10 m; and (4) 2,500 m and 500 m, respectively, and sampels were labeled as shown in Table 1.

5   To investigate the bacterial changes at altitudes in response to time, temporal transect at the altitude of 1,200 m was prepared for seven days – the 23rd, 24th, 25th, and 29th of March 2014 and the 16th, 17th, and 21st of March 2015 – and the sample names are showed in Table 1.

Air samples were collected through sterilized polycarbonate filters (0.22-µm pore size; Whatman, Tokyo, Japan) with a sterilized filter holder connected to an air pump. Air sampling was performed with a flow rates of 5 L min^{-1} over sampling
10   periods from 0.2 h to 1.0 h. Triplicate sampling filters were obtained for each altitude. During helicopter sampling, outside air was transferred from a window to the bioaerosols-sampling inlet, which was sterilized by autoclaving and UV irradiation. The sterilized filter holders were inserted into the sampling inlet to avoid contamination. To collect air particles at an altitude of 10 m, we used filter holders fixed on a 3 m stick, which was placed on the roof of a building (Maki et al., 2014).

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

2.2 Characteristics and trajectories of air masses

Information regarding weather conditions (temperature, relative humidity, and pressure) was gathered. During the helicopter flight, outside air was transferred from a window into the meteorological-measurement inlet, into which the
20   adaptor of the measurement device (TR-73U; T&D Corporation, Matsumoto, Japan) was inserted, and the temperature, relative humidity, and pressures were sequentially measured. The temperature and relative humidity at an altitude of 10 m were also measured on the roof of a building in Hakui.

The depolarization rates determined by Laser Imaging Detection and Ranging (LiDAR) measurements at Toyama were used for estimating the abundances of dust mineral particles.
25   To track the transport pathways of air masses, 72 h back trajectories were calculated using the National Oceanic and Atmospheric Administration (NOAA) HYbrid Single Particle Lagrangian Integrated Trajectory (HYSPLIT) model (http://www.arl.noaa.gov/HYSPLIT.php). The coordinator of Hakui was used as the back trajectory starting point at several altitudes from 10 m to 3,000 m above ground level to estimate the trajectories of the air masses.

2.3 Determination of particle abundance

30   The air particles at each altitude were measured using an optical particle counter (OPC: Rion, Tokyo, Japan). The OPC device was connected to the meteorological-measurement inlet. The air particles at an altitude of 10 m were also counted using the OPC device placed on the roof of a building.

Fluorescent particles stained with 4, 6-diamidino-2-phenylindole (DAPI) were also counted via epifluorescence microscopy. Within 2 h of sampling, 1 mL of 1% paraformaldehyde was added to one of the filters to fix the aerosols. After a 1 h incubation, the filter was stained with DAPI at a final concentration of 0.5 μg mL^{-1} for 15 min (Russell et al., 1974). Next, the filter was placed on a slide in a drop of low-fluorescence immersion oil. A second drop of oil was added, and a coverslip was placed on top. Particles on the filter were observed using a fluorescence microscope (Olympus, Tokyo, Japan) with a UV excitation system. A filter transect was scanned, and the four categorized particles, including white fluorescent particles, blue fluorescent particles (microbial particles), yellow fluorescent particles, and black particles, on the filter transect were counted using a previously reported observational technique (Maki et al., 2014). The detection limit of aerosols was 1.1×10^4 particles m^{-3} of air.

2.4 Analysis of bacterial community structures using MiSeq sequencing analysis targeting 16S rDNA sequences

After the aerosol particles on the other two filters were suspended in 3 mL of sterile 0.6 % NaCl solution, the particles were pelleted by centrifugation at $20,000 \times g$ for 10 min. The genomic DNA (gDNA) was then extracted from the particle pellets using sodium dodecyl sulfate, proteinase K, and lysozyme and purified by phenol-chloroform extraction as previously described (Maki et al., 2008). The bacterial community structure was determined using MiSeq DNA sequencing, which facilitates multiplexed partial sequencing of 16S rDNA. Fragments of 16S rDNA (approximately 500 bp) were amplified from the extracted gDNA by PCR using the universal 16S rDNA bacterial primers 515F (5′- Seq A - TGTGCCAGCMGCCGCGGTAA-3′) and 806R (5′- Seq B -GGACTACHVGGGTWTCTAAT-3′) (Caporaso et al., 2011), where Seq A and Seq B represent the nucleotide sequences bounded by the second set of PCR primers described below. The PCR amplicon sequences covered the variable region V4 of the 16S rRNA gene. Thermal cycling was performed using a thermocycler (Program Temp Control System PC-700; ASTEC, Fukuoka, Japan) under the following conditions: denaturation at 94°C for 1 min, annealing at 52°C for 2 min, and extension at 72°C for 2 min for 20 cycles. Fragments of 16S rDNA in PCR products were amplified again using the second PCR forward primer (5′- Adaptor C - xxxxxxxx - Seq A - 3′) and reverse primer (5′- Adaptor D - Seq B -3′), where Adaptors C and D were used for the Miseq sequencing reaction. The sequences "xxxxxxxx" comprise an 8 nucleotide sequence tag designed for sample identification barcoding. Thermal cycling was performed under the following conditions: denaturation at 94°C for 1 min, annealing at 59°C for 2 min, and extension at 72°C for 2 min for 15 cycles. PCR amplicons were purified using the MonoFas DNA purification kit (GL Sciences, Tokyo, Japan). PCR amplicons from each sample were pooled at approximately equal amounts into a single sequencing tube on a MiSeq Genome Sequencer (Illumina, CA, USA) machine. The sequences obtained for each sample were demultiplexed based on the tag, including the 8 nucleotide sequence. After removal of the tags, an average read length of 450 bp was obtained. Negative controls (no template and extraction products from unused filters) were prepared in the DNA extraction process to check for contamination.

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

5   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). 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

10   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 rates 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 rate increased

15   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 rates at altitudes of 2,500 m and 500 m, consistent with the vertical distribution of non-spherical (dust mineral) 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

20   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 rates (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 rates and air mass

25   trajectory roots between the two series (Figs. 4 and 5). Depolarization rates 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 contrast, the sampling period during March 2015 had substantially higher depolarization rates, indicating a strong presence of dust mineral particles (Fig. 5a), and air masses at 3,000 m consistently originated from the Asian desert regions (Fig. 5c).

30   Temperatures from March 19, 2013; April 28, 2013; March 28, 2014; and March 20, 2015 increased from approximately 290 K to approximately 300 K at the middle altitudes (500 m and 1,200 m) (Fig. 2), indicating the free

troposphere formed at the upper altitudes (2,500 m and 3,000 m) over boundary layers (Table 1). During the March 2014 time series, temperatures dynamically changed at altitudes of approximately 1,200 m, while those from the March 2015 time series (the 16th, 17th, and 21st of March 2015) were stable at 1,200 m (Figs. S1 and S2). These results indicate that the boundary layers were located at 1,200 m during the March 2014 time series, whereas the tropospheric air transported by

5 westerly winds was suspended at the sampling altitudes (500 m and 1,200 m) used during the March 2015 time series.

3.2 Vertical distributions and sequential variations of aerosol particles

Aerosol particle concentrations from the ground level to the troposphere were measured using OPC to compare the vertical distributions of aerosols from the four sampling events. The OPC-measured particles on March 19, 2013 and March 20, 2015 maintained similar concentrations below the troposphere (Fig. 2ad), while the concentrations on April 28, 2013 and

10 March 28, 2014 decreased one or two orders of magnitude between the troposphere and ground level (Fig. 2bc). At high altitudes (2,000 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

15 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})

20 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}.

25 Based on the above observations, the sampled air masses of 13H319-u, 15H316-m, 15H317-m, 15H320-u, 15H320-m, and 15H321-m were influenced by dust events and categorized as "dust samples" The sampled air masses of 13H319-m, 13H319-l, 13H428-u, 13H428-m, 13H428-l, 14H328-u, 14H328-m, 14H328-l, 14H323-m, 14H324-m, 14H325-m, and 14H329-m were not influenced by dust events and categorized as "non-dust samples" in relation to the presence or absence of dust events as the source of the aerosol samples (Table 1).

30 3.3 Fluorescent microscopic observation of aerosol particles

Uning epifluorescence microscopy with DAPI staining, the aerosol particles in the 18 air samples emitted several types of fluorescence, categorized as white, blue, yellow, or black (Fig. S3). White fluorescence particles, (white particles) were

indicative of mineral particles originating from the sand or soil. Microbial (prokaryotic) particles stained with DAPI emitted blue fluorescence, forming coccoid- or bacilli-like particles with a diameter <3 μm. Yellow fluorescence particles (yellow particles) stained with DAPI were organic matter and ranged from 1.0 μm to 10 μm in diameter. Most of the yellow particles disappeared in the aerosol-particle suspending solutions after protease treatment, suggesting that the yellow particles

5   consisted mainly of proteins. Black particles were indicative of an anthropogenic black carbon originating from East Asian regions, produced by biomass burning, industrial activities, and vehicle exhaust.

    The dust samples from upper altitudes (2,500 m and 3,000 m) contained 5 to 100 times higher concentrations of microbial, organic, and white particles than the concentrations detected in the non-dust samples (Fig. 2). In the upper altitude dust samples, the concentration of mineral particles ranged from 7.77×10^5 particles m^{-3} to 1.08×10^6 particles m^{-3} (Fig. 2ad),

10   whereas the concentrations of the non-dust samples ranged from 3.14×10^4 particles m^{-3} to 1.48×10^5 particles m^{-3} (Fig. 2bc). The microbial particles in the high altitude dust samples exhibited concentrations of approximately 1.5×10^6 particles m^{-3} that were two orders of magnitude higher than in the non-dust samples (approximately 6.0×10^4 particles m^{-3}). The organic particles in the high altitude dust samples were also found at higher concentrations of 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 $5.30 \times$

15   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-

20   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

25   $\times 10^5$ 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

30   particles m^{-3}). The ratio of organic particles to the total number of organic and microbial particles detected during March 2015 (71.5 % to 95.6 %) were higher than those during March 2014 series (8.0 % to 36.2 %) (Fig. S4). The black particles were randomly observed in all samples from March 2015 and March 2014.

3.4 Analysis of bacterial communities using MiSeq sequencing analysis

For the analysis of the prokaryotic composition in the 18 samples, 645,075 quality-filtrated merged paired-end sequences were assessed with lengths ranging from 244 bp to 298 bp, and the sequence library size for each sample was normalized at 1,500 reads. The 16S rDNA sequences were divided into 204 phylotypes (sequences with >97 % similarity).

5 Phylogenetic assignment of sequences resulted in an overall diversity of 16 phyla and candidate divisions, 32 classes (and class-level candidate taxa), and 72 families (and family-level candidate taxa). The majority (>90 %) of the sequences were represented by 9 bacterial classes and 33 families (Figs. 6 and 7). The bacterial compositions varied during the sampling periods and included the phylotypes belonging to the classes Cyanobacteria, Actinobacteria, Bacilli, Bacteroidetes, SBRH58, and Proteobacteria (Alpha, Beta, Gamma, and Deltaproteobacteria), which are typically generated from atmospheric,

10 terrestrial and marine environments. On the box plots, the numbers of bacterial species estimated by Chao I were similar at average levels between the dust samples and non-dust samples, while the Chao I and Shannon values of the non-dust samples showed a wider range than that of Dust samples (Fig. 8a). A non-metric multidimensional scaling plot with weighted UniFrac distances, demonstrated the distinct clustering of prokaryotic communities separating the Dust samples and the non-dust samples (Fig. 8b). For the PCR-analysis steps, negative controls (no template and template from unused

15 filters) did not contain 16S rDNA amplicons demonstrating the absence of artificial contamination during experimental processes.

3.5 Vertical distributions of bacterial communities in dust and non-dust samples

The vertical distributions of bacterial compositions showed different patterns between dust event days and non-dust days (Fig. 6). In the dust samples collected at upper altitudes, phylotypes belonging to the phylum Bacilli accounted for more than

20 60.5 % of the total, and mainly consisted of members of the families Bacillaceae and Paenibacilliaceae family members (Fig. 6). Bacterial numbers from the phylum Bacilli decreased at lower altitudes during dust events, and the phylotypes of Cyanobacteria, Actinobacteria, and Protobacteria increased in relative abundance in the samples collected at middle and low altitudes (13H319-m, 13H319-l, and 15H320-m).

Cyanobacteria, Actinobacteria, and Proteobacteria sequences also dominated in the air samples collected during non-

25 dust events (13H428-m, 14H328-u, 14H328-m, and 14H328-l). Specifically, Actinobacteria phylotypes increased in their relative abundance, ranging from 14.1 % to 24.7 % in the non-dust samples collected on March 28, 2014. Proteobacteria phylotypes containing several bacterial families occupied a high relative abundance, ranging from 60.5 % to 85.3 % in the non-dust samples 13H428-u, 13H428-m, 14H328-u, 14H328-m, and 14H328-l. In particular, the non-dust samples collected on March 28, 2014 included the Alphaproteobacteria phylotypes, which have composed of members of the families

30 Phyllobacteriaceae and Sphingomonadaceae. Most Betaproteobacteria, phylotypes including the families Oxalobacteraceae and 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 between 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 Actinobacteria phylotypes, Alpha and Gammaproteobacteria phylotypes showed an increasing trend from 30.6 % to 96.8 % between the 23rd and the 29th of March 2014 (Fig. 7a). Alphaproteobacteria phylotypes belonging to the families Sphingomonadaceae, and Phyllobacteriaceae, consistently appeared throughout the sampling periods of the March 2014 series and occupied a maximum relative abundance of 72.9 % and 22.3 % respectively. For Gammaproteobacteria, the Xanthomonadaceae sequences dominated at a relative abundance of 18.3 % and 5.4 % in the non-dust samples 14H325-m and 14H329-m, respectively, during the air mass was suspended the Japanese islands for a few days.

4 Discussion

4.1 Air mass conditions during Asian dust and non-dust events

Westerly winds blowing over Asian continents disperse airborne microorganisms associated with dust mineral particles (Maki et al., 2008) and anthropogenic particles (Cao et al., 2014; Wei et al., 2016), influencing the airborne microbial variations at high altitudes over East Asia (Maki et al., 2013). The microorganisms transported at high altitudes by westerly winds include ice-nucleation bacteria, potentially related to climate change (Hara et al., 2016b). Asian dust events have been reported to increase the airborne bacterial abundance in ground-surface air (Hara and Zhang, 2012) and at high altitudes (Maki et al., 2013; Maki et al., 2015). In this investigation, the increases in aerosol particles (dust particles) and associated microbial particles were observed over the Noto Peninsula during the dust events of March 19, 2013 and March 20, 2015 (Figs. 2 and 4). At the two sampling dates, the air mass including microbial particles had traveled from the Asian desert

region throughout the anthropogenic polluted areas (Fig. 2), and the dust 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

5   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

10   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. 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). Organic (biological) aerosol particles are reported to account for high rates of tropospheric aerosols, ranging from 30 % to 80 % (Jaenicke, 2005), and to

15   fluctuate at high concentrations from 10^3 to 10^5 particles m^{-3} under the boundary layer at 4,000 m above the ground (Twohy et al., 2016). When fungi (*Bjerkandera adusta*) and bacteria (*Bacillus* spp.) isolated from aerosol samples were incubated, the dead-phase microbial cells mainly irradiated yellow fluorescence instead of blue fluorescence (Liu et al., 2014; Fig. S3). The relative numbers of organic particles to the total number of microbial and organic particles in the dust samples showed significantly higher values (82.9 ± 32.3 %) than in the non-dust samples (23.3 ± 13.7 %) (Figs. S4). Hara and Zhang reported

20   that dust events in Kyushu, Japan, resulted in an increased ratio of damaged microbial cells in the ground-surface air (Hara and Zhang, 2012). Microbial cells coming from Asian continents to Japan would be exposed to environmental stressors during their long-range transport, increasing the ratios of damaged and dead cells.

The appearance of black carbon most likely originated from anthropogenic activities, such as biomass burning, industrial activities, and vehicle exhaust (Chung and Kim, 2008). In the anthropogenic regions of eastern China,

25   anthropogenic particles originating from human activities are expected to comprise more than 90 % of dust particles (Huang et al., 2015). When the westerly winds are strongly blowing over the Noto Peninsula, the black carbon particles at upper altitudes (3,000 m) are thought to mainly derive from continental anthropogenic regions.

4.3 Comparing the community structures of atmospheric bacteria between Asian dust and non-dust events

Asian dust events and pollution occurrences changed the airborne bacterial communities over Beijing (Jeon et al., 2011;

30   Cao et al., 2014). The westerly winds transported airborne bacteria to the high-altitude atmosphere over the Noto Peninsula (Maki et al., 2015) and over North American mountains (Smith et al., 2013). African dust events also contribute to changes in the airborne microbial composition in European continents (Mazar et al., 2016). Our box plots analysis suggested that

changes in the bacterial diversity in the dust samples would be more stable than in the non-dust samples (Fig. 8a). Furthermore, using a non-metric multidimensional scaling plot, the bacterial compositions in the dust samples could be distinguished from non-dust samples (Fig. 8b). Thus, the aerosol particles transported by Asian dust events changed the atmospheric bacterial composition at higher altitudes over downwind areas.

5   The phylotypes in the dust samples were predominately clustered into the classes Bacilli and Cyanobacteria (Fig. 4a), while the non-dust samples mainly included the phylotypes of the classes Alpha, Beta, and Gammaproteobacteria and Actinobacteria. Our previous investigations indicated that the bacterial communities at an altitude of 3,000 m over the Noto Peninsula included more than 300 phylotypes, which were predominantly composed of Bacilli phylotypes (Maki et al., 2015). Bacterial groups belonging to Bacilli, Proteobacteria, and Actinobacteria have been reported as airborne bacteria around

10   European mountains (Vaïtilingom et al., 2012) as well as over Asian rural regions (Woo et al., 2013). Some Bacilli isolates were found to act as ice-nucleation agents and to be involved in ice cloud (Matulova et al., 2014; Mortazavi et al., 2015). Isolates of Gammaproteobacteria isolates were obtained from dust mineral particles (Hara et al., 2016b), glaciated high-altitude clouds (Sheridan et al., 2003), and plant bodies (Morris et al., 2008), and some isolate species, such as *Pseudomonas*, were confirmed to have the ice-nucleation activity. Accordingly, Bacilli and Proteobacteria members associated with dust

15   events could potentially contribute to climate change resulting from dust events.

4.4 Dominant bacterial populations in the air masses transported from Asian continents

     In some dust-event samples collected at high altitudes (13H319-u, 15H320-u, and 15H320-m), Bacilli sequences accounted for more than 52.7 % of the total number of sequences (Fig. 6). Back trajectories on March 19, 2013 and March 20, 2015 came from the Asian desert region to the Noto Peninsula. Some *Bacillus* species were predominantly detected at

20   high altitudes above the Taklimakan Desert (Maki et al., 2008) and above downwind areas during Asian dust events (Maki et al., 2010; Smith et al., 2012; Maki et al., 2013; Jeon et al., 2011; Tanaka et al., 2011). *Bacillus* species are the most prevalent isolates obtained from dust mineral particles collected over downwind areas (Hua et al., 2007; Gorbushina et al., 2007).

     Bacilli members can form resistant endospores that support their survival in the atmosphere (Nicholson et al., 2000). The *Bacillus* isolates obtained from atmospheric samples showed higher-level resistance to UV irradiation than normal

25   isolates (Kobayashi et al., 2015). In the Gobi Desert, dust events increase the diversity of airborne microbial communities; after dust events, spore-forming bacteria, such as *Bacillus*, increase in their relative abundances (Maki et al., 2016). Accordingly, in the atmosphere, selected Bacilli members associated with dust particles would be transported over long distances.

     The Bacilli sequences showed different vertical variations between the two dust events on March 19, 2013 and March

30   20, 2015. On March 19, 2013 (13H319-m), the relative abundances of Bacilli sequences decreased dynamically from 3,000 m to 1,200 m, while unstable atmospheric layers on March 20, 2015 most likely mixed the transported bacteria with the regional bacteria over the Noto Peninsula. A previous investigation also demonstrated the vertical mixture of airborne bacteria over Suzu in the Noto Peninsula (Maki et al., 2010).

Actinobacteria sequences decreased in relative abundance between the 23rd and 29th of March 2014 corresponding with changes in the air mass trajectory roots from north Asian regions, such as eastern Siberia and Japan (Fig. 7). Furthermore, Actinobacteria sequences appeared in the samples collected from air masses that were transported throughout the Korean Peninsula on March 19, 2013; April 28, 2013; and March 20, 2015. Actinobacteria members are frequently dominant in

5 terrestrial environments but seldom survive in the atmosphere for a long time, because they cannot form spores (Puspitasari et al., 2015). However, the family Micrococcaceae in Actinobacteria was primarily detected from anthropogenic particles collected in Beijing, China (Cao et al., 2014). Over anthropogenic source regions for Asian continents, anthropogenic particles occupy more than 90 % of dust particles and originate from soils disturbed by human activities in cropland, pastureland, and urbanized regions (Huang et al., 2015; Guan et al., 2016). Air masses transported from the continental

10 coasts are expected to include a relatively high abundance of Actinobacteria members associated with anthropogenic particles.

Natural dust particles from Asian desert areas (Taklimkan and Gobi Deserts) are transported in the free troposphere (Iwasaka et al., 1988) and vertically mixed with anthropogenic particles during the transportation processes (Huang et al., 2015). In some cases, short-range transport of air masses would carry only anthropogenic particles to Japan, because the

15 anthropogenic particles are often dominant in Asian continental coasts (Huang et al., 2015). Actinobacteria members may have been transported with anthropogenic particles from continental coasts.

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

Proteobacteria sequences increased in their relative abundances at high altitudes during non-dust sampling dates (13H428-u, 13H428-m, 14H328-u, 14H328-m, and March 2014 series), when air mass origins at 1,200 m changed from the

20 Korean Peninsula to Japan (Fig. 7). Proteobacteria members were the dominate species in the atmosphere over mountains (Bowers et al., 2012; Vaïtilingom et al., 2012; Temkiv et al., 2012), in the air samples collected on a tower (Fahlgren et al., 2010), and from the troposphere (DeLeon-Rodriguez et al., 2013; Kourtev et al., 2011). In the phylum proteobacteria, the families Phyllobacteriaceae, Methylobacteriaceae, and Xanthomonadaceae were predominately detected from the non-dust samples and are associated with plant bodies or surfaces (Mantelin et al., 2006; Fürnkranz et al., 2008; Khan and Doty, 2009;

25 Fierer and Lennon, 2011). The Betaproteobacteria sequences in the non-dust samples mainly contained the Oxalobacteraceae and Comamonadaceae families, which are commonly dominate in freshwater environments (Nold and Zwart, 1998) as well as on plant leaves (Redford et al., 2010). In addition, the class Alphaproteobacteria in the non-dust samples also included marine bacterial sequences belonging to the family Sphingomonadaceae (Desriac et al., 2013; Cavicchioli et al., 2003). Bacterial populations originating from marine areas are prevalent in cloud droplets (Amato et al., 2007), in air samples

30 collected from the seashores of Europe (Polymenakou et al., 2008), in storming troposphere (DeLeon-Rodriguez et al., 2013), and at high altitudes in Japanese regions (Maki et al., 2014), suggesting that the marine environments represent a major source of bacteria in clouds. The air masses suspended over the Sea of Japan or Japanese islands during non-dust events (the

March 2014 series) could include a high relative abundance of airborne bacteria, which were transported from the surface-level air over the marine environments and the regional phyllosphere.

4.6 Bacterial populations commonly detected during dust events and the non-dust events

Sequences originating from Synechococcaceae (in the class Cyanobacteria) randomly appeared in the MiSeq sequencing databases results obtained from air samples, regardless of dust event occurrences. *Synechococcus* species in the family Synechococcaceae can eliminate excess peroxide from photosynthesis to resist UV radiation and oxygenic stress (Latifi et al., 2009), suggesting that these bacteria resist environmental stressors in the atmosphere. In a previous study, the air samples transported from marine environments to Japan predominately contained *Synechococcus* species (Maki et al., 2014), which were dominant marine bacteria in the Sea of Japan and the East China Sea (Choi and Noh, 2009). The cloud water at approximately 3,000 m above ground level was also dominated by Cyanobacteria populations, indicating their atmospheric transport (Kourtev et al., 2011). In addition to Alphaproteobacteria, marine cyanobacterial cells can be transported from seawater to the atmosphere, thereby contributing to the airborne bacterial variations over the Noto Peninsula.

Bacteroidetes sequences were detected in some air samples collected during Asian dust and non-dust events. Members of the phylum Bacteroidetes, which were composed of the families Cytophagaceae, associate with organic particles in terrestrial and aquatic environments (Turnbaugh et al., 2011; Newton et al., 2011). Furthermore, these bacterial populations dominate the atmosphere and sand of desert areas, where plant bodies and animal feces are sparsely present (Maki et al., 2016). These bacterial groups possibly originated from organic-rich microenvironments in several areas, such as desert and marine areas.

4.7 Air mass tracking using the composition of bioaerosols

Bacterial communities at high altitudes over the Noto Peninsula change depending on the origins of the bacterial populations, such as from the Asian continental area, marine environments, and Japanese regional areas. Some scientists attempt to apply the airborne bacterial composition to the tracking tracers of air mass sources at ground level (Bowers et al., 2011; Mazar et al., 2016). This study suggested that the bacterial compositions in the atmosphere can be used as air mass tracers, which could identify the mixture levels of air masses transported for long-distances. Bacilli and Actinobacteria members were dominant bacteria in the air samples transported from the Asian continental area (Fig. 9a), and were reported to originate from terrestrial environments (Bowers et al., 2011; Bottos et al., 2014). Some members of the classes Alphaproteobacteria (Phyllobacteriaceae and Methylobacteriaceae), Gammaproteobacteria, and Betaproteobacteria increased their relative abundances in the non-dust samples collected when the air mass was suspended around the Japanese islands. They are commonly associated with plant leaves (Redford et al., 2010; Fierer and Lennon, 2011) or the dominant species of bacteria in freshwater environments (Nold and Zwart, 1998; Nemergut et al., 2011). In addition, atmospheric aerosols arriving via marine areas include a high relative abundances of marine bacteria belonging to the classes Cyanobacteria (Choi and Noh, 2009) and Alphaproteobacteria (Sphingomonadaceae) (Desriac et al., 2013; Cavicchioli et al., 2003).

The relative abundance of Cyanobacteria, Bacilli, Actinobacteria, and Alpha, Beta, and Gammaproteobacteria in each sample provides a prediction regarding the mixture levels of air masses originating from several regions (Fig. 9b). The abundance of Bacilli and Actinobacteria would be used as a tracer, indicating the long-range transport of air masses from the Asian continental area. The abundance of Cyanobacteria and Alphaproteobacteria (Sphingomonadaceae) would be used as a

5   tracer for air masses influenced by marine environments in the Sea of Japan. Finally, the abundance of Gammaproteobacteria and Alphaproteobacteria (Phyllobacteriaceae and Methylobacteriaceae) would be used as a tracer for air masses including regional aerosols originating from regions surrounding Japan islands.

5 Conclusion

Air samples including airborne bacteria were sequentially collected at high altitudes over the Noto Peninsula during dust

10   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. 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,

15   marine areas, and Japan regional areas, showing different variations in bacterial compositions between the sampling dates. However, one limitation of our investigation is that the number of samples analyzed was not sufficient to cover entire changes in airborne bacteria at high altitudes over the Noto Peninsula. Although the airborne bacterial composition during non-dust periods was found to change dynamically, only a few types of variation were followed in this investigation. In the future, greater numbers of samples, which are sequentially collected at high altitudes using this sampling method, will need

20   to be originated to more accurately evaluate bioaerosol tracers. Since helicopter sampling procedures require sophisticated techniques and are expensive, the sample numbers at high altitudes are difficult to increase. Air sampling at high altitudes should be combined with sequential ground-air sampling to advance the understanding of the influence of westerly winds on airborne bacterial dynamics in downwind areas. Metagenomic analyses and microbial culture experiments would also provide valuable information about airborne microbial functions relating to ice-nucleation activities, chemical metabolism,

25   and pathogenic abilities.

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Table 1 Sampling information during the sampling periods.							
Sample name	Sampling date	Collection time (JST)	Total time (min)	Air volume	Sampling method	Sampling location[*1]	Free troposphere[*2]
13H319-u	19 March 2013	14:04 − 15:04	60	700 L	helicopter	2500m	FT
13H319-m		15:19 − 16:19	60	700 L	helicopter	1200m	ABL
13H319-l		14:25 − 15:25	60	700 L	building	10m	GL
13H428-u	28 April 2013	12:10 − 13:04	56	653 L	helicopter	2500m	FT
13H428-m		13:13 − 14:03	50	583 L	helicopter	1200m	ABL
13H428-l		12:03 − 13:03	60	700 L	building	10m	GL
14H328-u	28 March 2014	12:50 − 13:50	60	700 L	helicopter	3000m	FT
14H328-m		14:04 − 15:04	60	700 L	helicopter	1200m	ABL
14H328-l		13:00 − 14:00	60	700 L	building	10m	GL
15H320-u	20 March 2015	12:26 − 13:23	47	548 L	helicopter	2500m	FT
15H320-m		13:39 − 14:40	60	711 L	helicopter	500m	ABL
14H323-m	23 March 2014	10:45 − 11:02	17	11.1 L	helicopter	1200m	ABL
14H324-m	24 March 2014	9:09 − 9:30	21	13.7 L	helicopter	1200m	ABL
14H325-m	25 March 2014	9:31 − 9:50	29	18.9 L	helicopter	1200m	ABL
14H328-m	28 March 2014	14:04 − 15:04	60	700 L	helicopter	1200m	ABL
14H329-m	29 March 2014	9:06 − 9:24	15	9.75 L	helicopter	1200m	PT
15H316-m	16 March 2015	11:21 − 11:43	22	14.3 L	helicopter	1200m	FT
15H317-m	17 March 2015	11:04 − 11:31	27	17.6 L	helicopter	1200m	FT
15H320-u	20 March 2015	12:26 − 13:23	47	548 L	helicopter	2500m	FT
15H321-m	21 March 2015	15:35 − 15:55	20	13.0 L	helicopter	1200m	FT
*1 Height above the ground.							
*2 Free troposhere: FT, Atmospheric boundary layer: ABL, Phase transiens: PT, GL: Ground level							
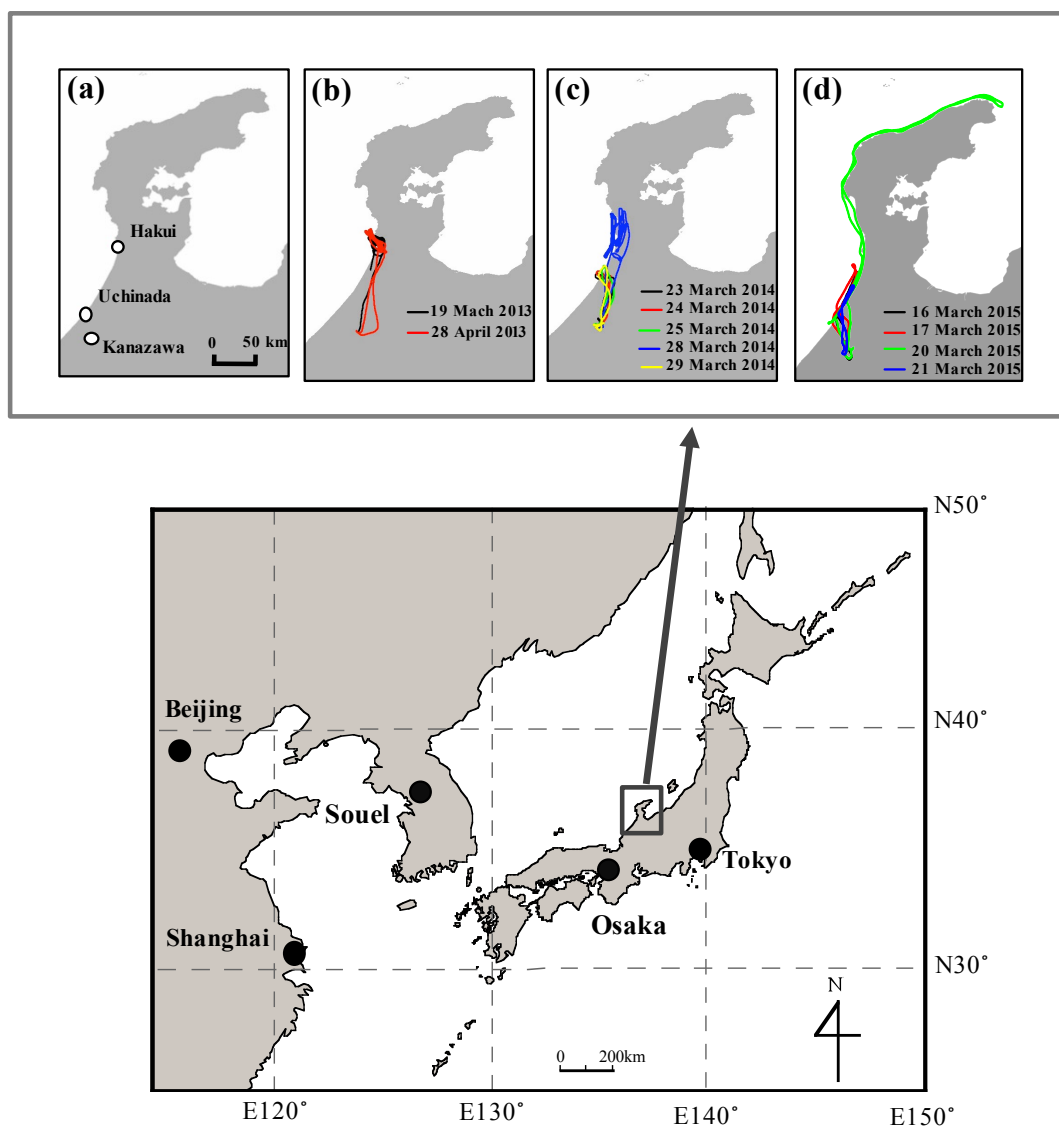
Figure 1: Sampling location (a) and helicopter flight routes during the sampling periods on March 19, 2013, and April 28, 2013 (b); the 23rd, 24th, 25th, and 29th of March 2014 (c); and the 16th, 17th, 20th, and 21st of March 2015 (d).
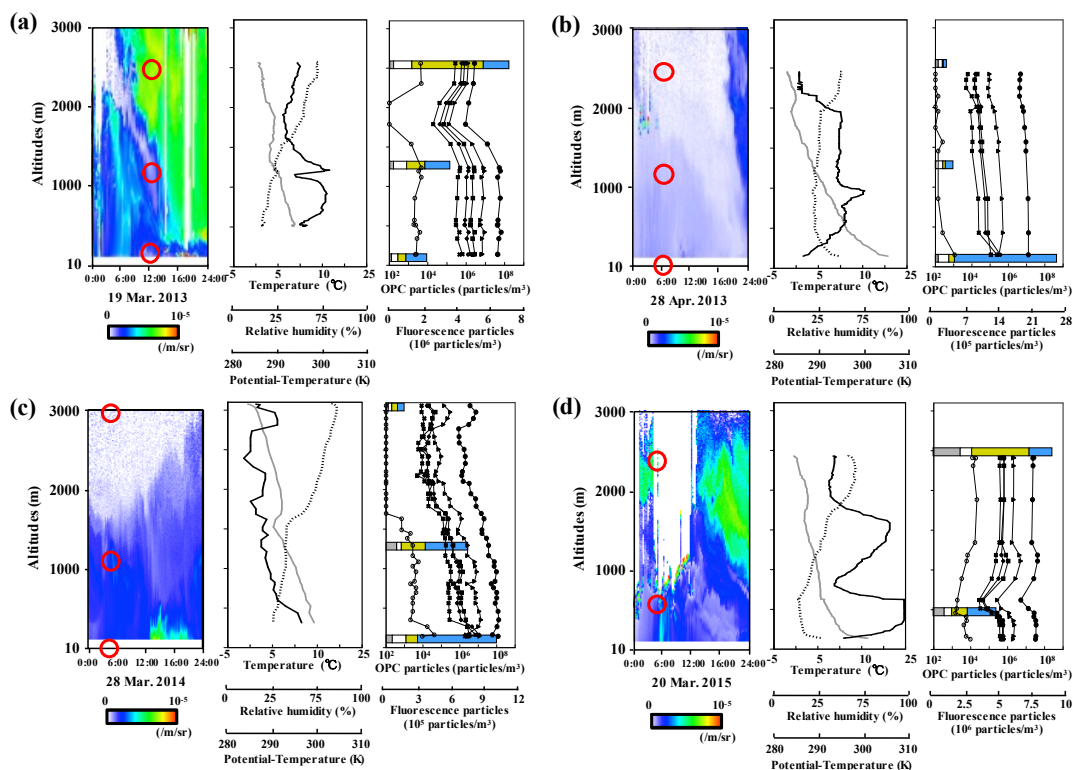
Figure 2: LiDAR observation of the depolarization ratio in Toyama city as well as vertical changes in temperature, relative humidity, and potential temperature, and vertical distributions of concentrations of OPC-counted particles and DAPI-stained particles from the four sampling events on March 19, 2013 (a); April 28, 2013 (b); March 28, 2014 (c); and March 20, 2015 (d). OPC-counted particles were categorized according to diameter sizes of 0.3–0.5 µm (closed squares), 0.5–0.7 µm (closed triangles),
10   0.7–1.0 µm (closed circles), 1.0–2.0 µm (closed diamonds), 2.0–5.0 µm (crosses), and >5.0 µm (open circles). DAPI-stained particles were classified into microbial particles (blue bars), white particles (white bars), yellow fluorescent particles (yellow bars), and black carbon (black bars).
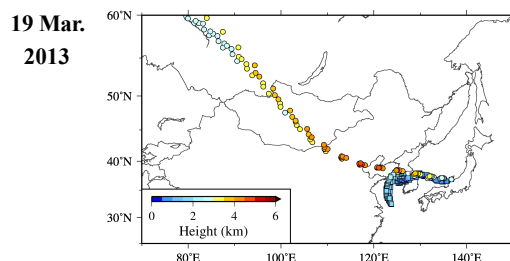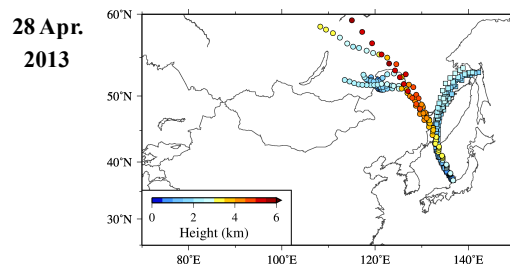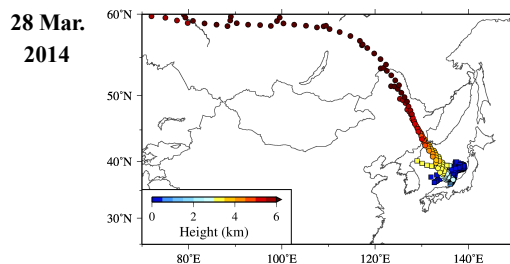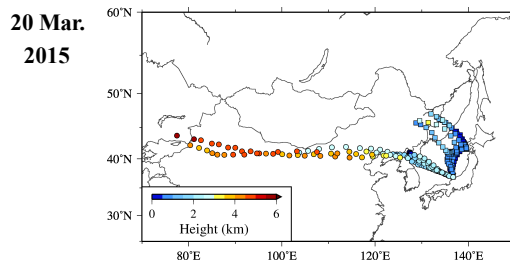
Figure 3: Three-day back trajectories of the aerosols that arrived at 2,500 m (blue-type lines) and 1,200 m (red-type lines) in Hakui, Japan, during the sampling periods at the four sampling events on March 19, 2013; April 28, 2013; March 28, 2014; and March 20, 2015.
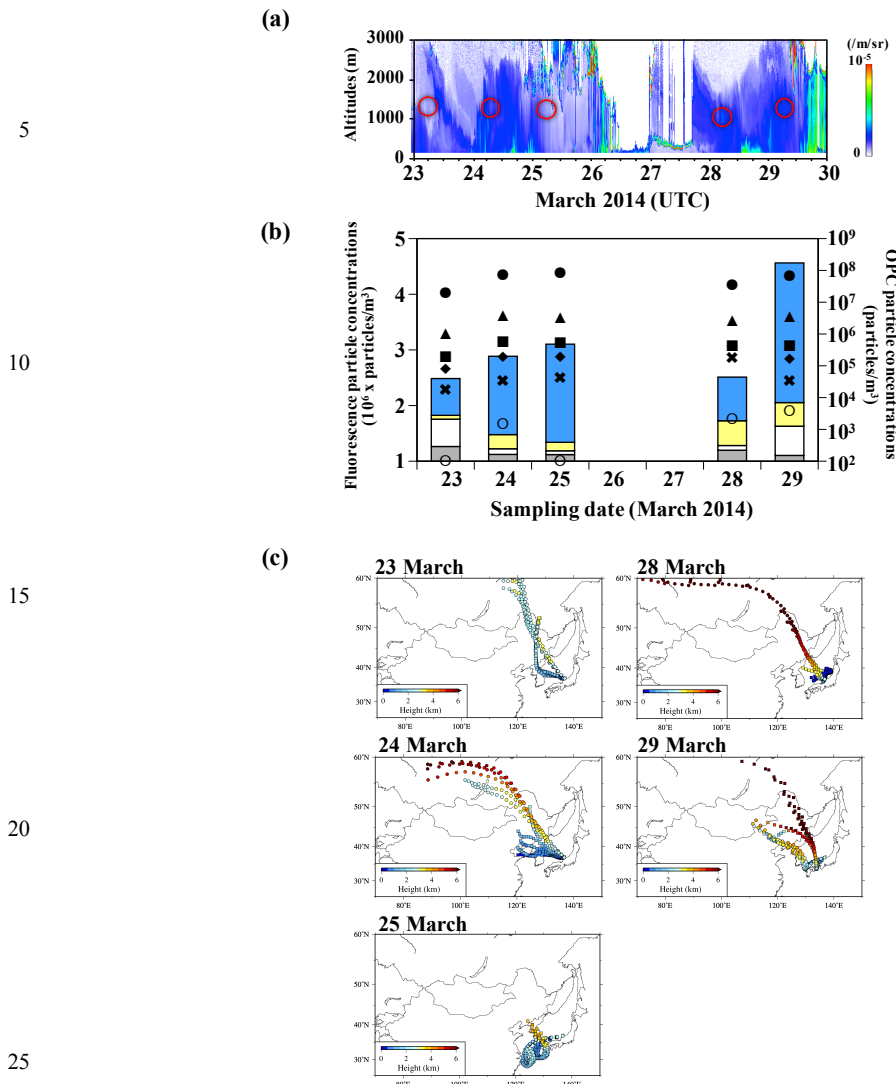
Figure 4: (a) LiDAR observation of the depolarization ratio in Toyama city and concentrations of OPC-counted particles and DAPI-stained particles from 0:00 UTC on March 23rd to 0:00 UTC on March 30th 2014. **(b)** OPC-counted particles were categorized according to diameter sizes of 0.3–0.5 μm (closed squares), 0.5–0.7 μm (closed triangles), 0.7–1.0 μm (closed circles), 1.0–2.0 μm (closed diamonds), 2.0–5.0 μm (crosses), and >5.0 μm (open circles). DAPI-stained particles were classified into microbial particles (blue bars), white particles (white bars), yellow particles (yellow bars), and black particles (gray bars). **(c)** Three-day back trajectories of the aerosols that arrived at 2,500 m (blue-type lines) and 1,200 m (red-type lines) in Hakui, Japan, during the sampling periods on the 23rd, 24th, 25th, 28th, and 29th of March 2014.
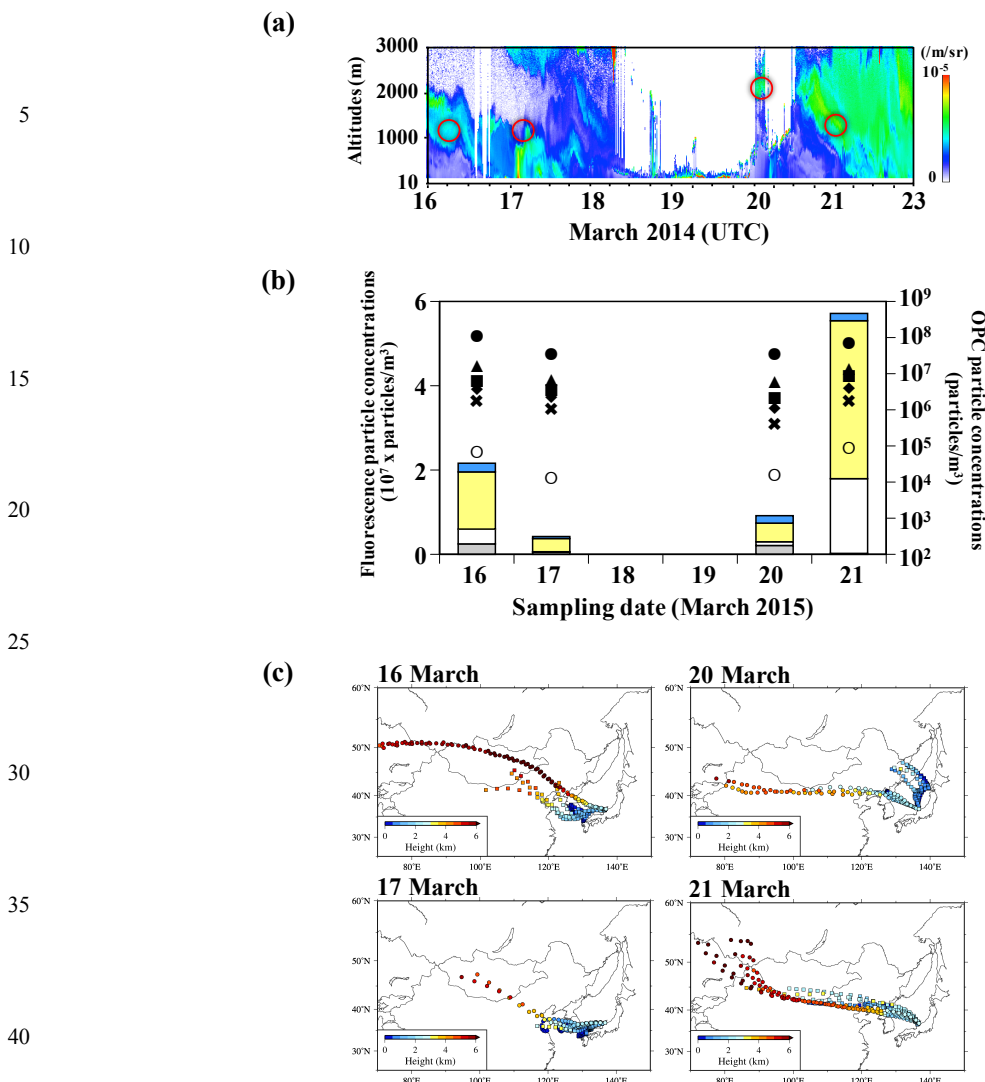
Figure **5**: **(a) LiDAR observation of the depolarization ratio in Toyama city and concentrations of OPC-counted particles and DAPI-stained particles from 0:00 UTC on March 16th to 0:00 UTC on March 23, 2015. (b) OPC-counted particles were categorized based on diameter sizes of 0.3–0.5 µm (closed squares), 0.5–0.7 µm (closed triangles), 0.7–1.0 µm (closed circles), 1.0–2.0 µm (closed diamonds), 2.0–5.0 µm (crosses), and >5.0 µm (open circles). DAPI-stained particles were classified into microbial particles (blue bars), white particles (white bars), yellow particles (yellow bars), and black particles (gray bars). (c) Three-day back trajectories of the aerosols that arrived at 2,500 m (blue-type lines) and 1,200 m (red-type lines) in Hakui, Japan, during the sampling periods on the 16th, 17th, 20th, and 21st of March 2015.**
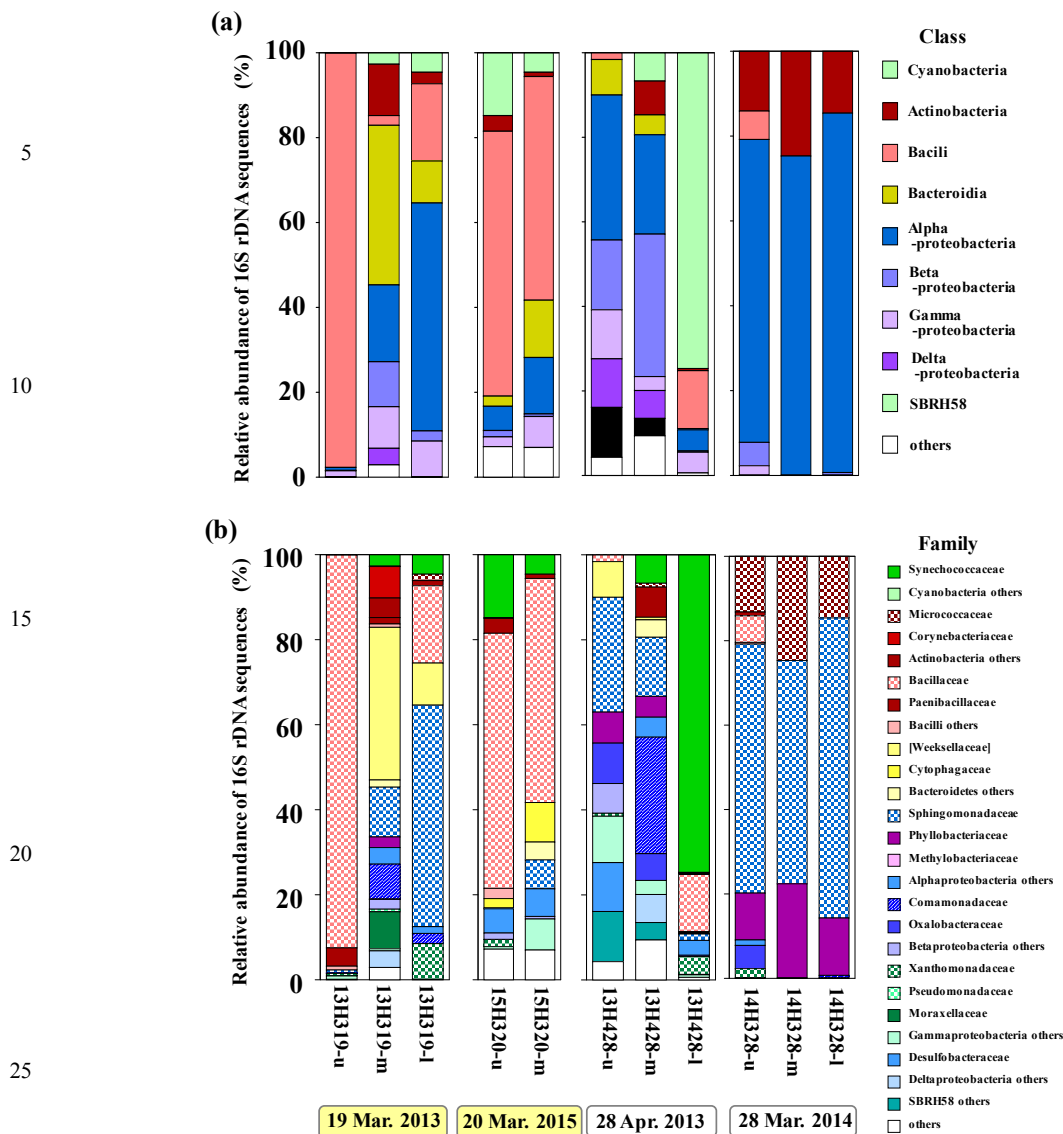
Figure 6: Vertical variations in bacterial compositions at (a) the class level and (b) the family level of the partial sequences obtained in the MiSeq sequencing database (ca. 400 bp) obtained from air samples collected at different altitudes over the Noto Peninsula on March 19, 2013; April 28, 2013; March 28, 2014; and March 20, 2015.
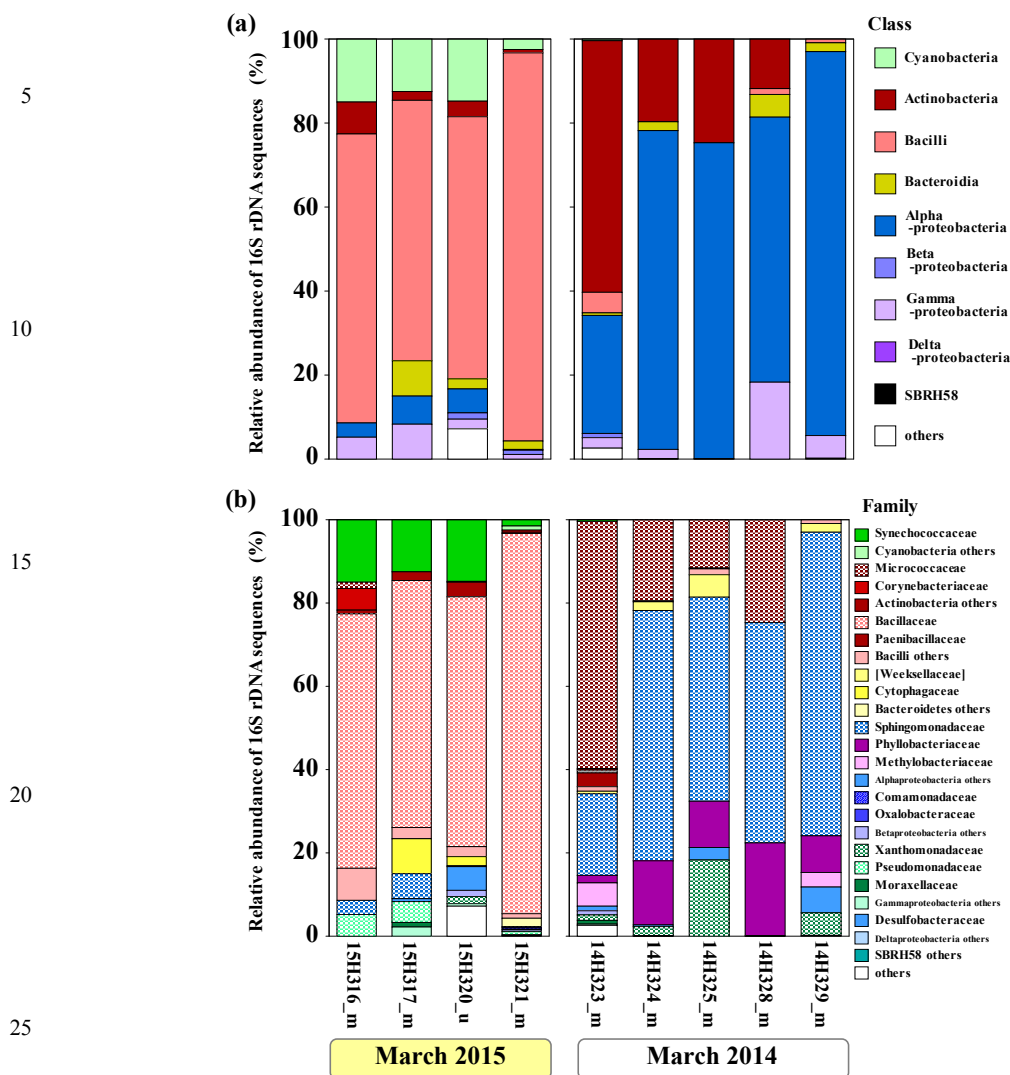
Figure 7: Changes in bacterial compositions at (a) the class level and (b) the family level of the partial sequences obtained in the MiSeq sequencing database (ca. 400 bp) from air samples collected at altitudes of 1,200 m (except for the sample collected at 500 m on March 20, 2015) over the Noto Peninsula from the 16th to the 23rd of March 2015 and from the 23rd to the 29th of March 2014.
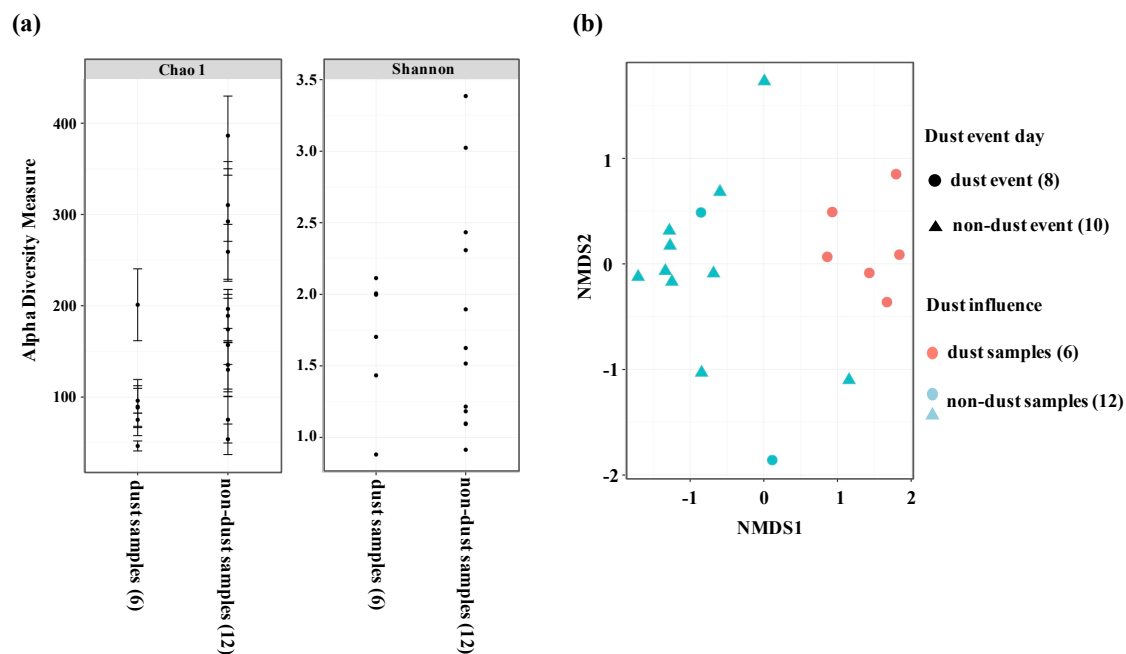
Figure 8: Comparison of the bacterial compositions among all air samples collected over the Noto Peninsula. (a) Box plots of Chao 1 and Shannon analyses indicating the bacterial diversity observed in dust samples and non-dust samples. Species were binned at the 97 % sequence similarity level. (b) Principal coordinate analysis of the pairwise weighted UniFrac distance matrix displaying phylogenetic clustering of all air samples.

(a)

(b)
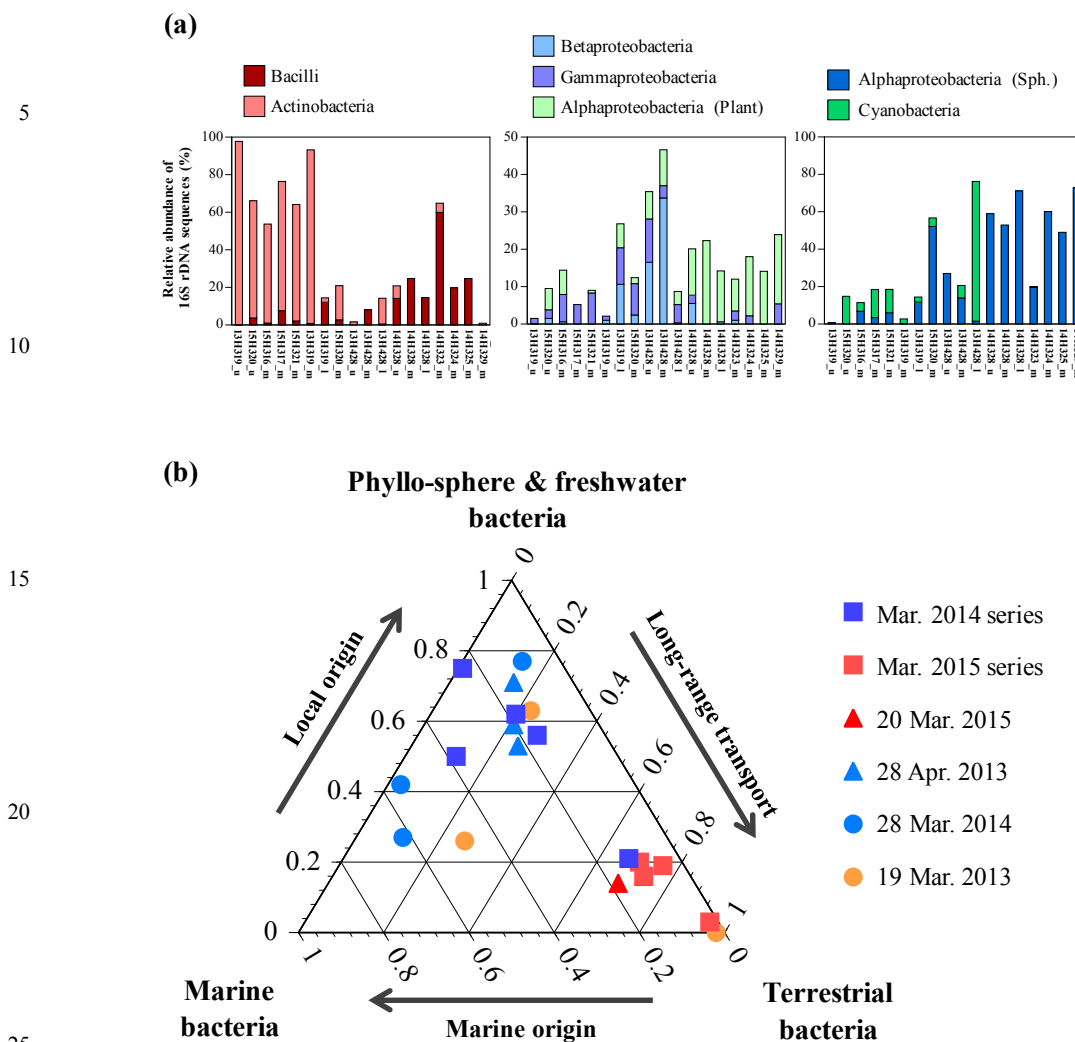
Figure 9: (a) Composition of bacterial population thought to originate from terrestrial environments (Bacilli and Actinobacteria), phyllosphere or freshwater environments (Betaproteobacteria, Gammaproteobacteria, and Alphaproteobacteria [Plant]), and marine environments (Cyanobacteria and Alphaproteobacteria [Sph.]), in the 18 air samples. **(b)** Relative abundances of bacterial sequences of the three categories that were used indicators, suggesting the long-range transport from Asian continental area, the contamination from regions surrounding Japanese islands, and the transport from marine environments in the Sea of Japan.