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## Long-range transport of airborne bacteria over East Asia: Asian dust events carry potentially nontuberculous *Mycobacterium* populations

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### ABSTRACT

The nontuberculous mycobacterial pulmonary disease (NTM-PD) caused by *Mycobacterium* species has increased in prevalence all over the world. The distributions of NTM-PD are possibly determined by the westerly wind traveling at high altitudes over East Asia. However, the long-range transport of *Mycobacterium* species has not been demonstrated by analyzing the bacterial communities in aerosols such as desert mineral particles and anthropogenic pollutants transported by the westerly wind. Here, airborne bacterial compositions were investigated including *Mycobacterium* species in high-elevation aerosols, which were captured in the snow cover at 2,450 m altitude on Mt. Tateyama. This was further compared to the ground-level or high-altitude aerosols collected at six sampling sites distributed from Asian-dust source region (Tsogt-Ovoo) to downwind areas in East Asia (Asian continental cities; Erenhot, Beijing, Yongin, Japanese cities; Yonago, Suzu, Noto Peninsula). The cell concentrations and taxonomic diversities of airborne bacteria decreased from the Asian continent to the Japan area. Terrestrial bacterial populations belonging to Firmicutes and Actinobacteria showed higher relative abundance at high-elevation and Japanese cities. Additionally, *Mycobacterium* species captured in the snow cover on Mt. Tateyama increased in relative abundance in correspondence to the increase of black carbon concentrations. The relative abundance of *Mycobacterium* sequences was higher in the aerosol samples of Asian continental cities and Japanese cities than in the desert area. Presumably, anthropogenic pollution over East Asia carries potential *Mycobacterium* species, which induce NTM-PD, thereby impacting upon the public health.

### 1. Introduction

Nontuberculous mycobacterial pulmonary disease (NTM-PD) has increased incidence globally and is addressed as a critical public health issue (Adjemian et al. 2012; Thomson 2010; Prevots and Marras 2015; Thomson et al. 2017). The recent epidemiologic investigation estimated the prevalence of NTM-PD as a chronic infection, which was more prevalent than tuberculosis disease (Raju et al. 2016). In particular, the

incidence rates of NTM-PD in Japan were observed to be at a high level among the industrialized countries (Namkoong et al. 2016), thereby suggesting that Asian regions are particularly susceptible to NTM-PD (Koh et al. 2006; Lai et al. 2010; Morimoto et al. 2014). Nontuberculous mycobacteria are normal bacterial inhabitants in the environment and could cause aerosol infections occasionally in the living environments of human (Nishiuchi et al. 2017). The most frequent etiology of NTM-PD is known to be *M. avium* complex (MAC), which

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predominantly consists of *M. avium* and *M. intracellulare* (Griffith et al. 2007; Kasperbauer and Daley 2008). The MAC pulmonary disease caused by *M. avium* exhibited a higher ratio of occurrence in the northern and eastern parts of Japan, whereas that of *M. intracellulare* appeared at a higher ratio in the southern and western parts of Japan (Kasperbauer and Daley 2008). Additionally, *M. intracellulare* were the predominant causative agents of NTM-PD in the Chinese continents (Griffith et al. 2007).

In East Asia, natural desert dust and anthropogenic pollution carry mineral particles and black carbons as well as microorganisms from the Asian continent to the downwind region (Iwasaka et al. 1983; Huang et al. 2015a,b; Šanti-Temkiv et al. 2021). In Japan, Asian dust events come to the southern and western parts of Japan more frequently than the northern and eastern parts of Japan (Yamaguchi and Takemura 2011; Huang et al. 2015a). The NTM-PD caused by *Mycobacterium* populations is known to spread via exposure to environmental terrestrials (Fujita et al. 2013; Hamada et al. 2016). Accordingly, there is a hypothesis that *M. intracellulare* was dispersed to the southern and western parts of Japan by Asian dust events (Morimoto et al. 2014). Indeed, in the Japanese southern and western parts, airborne microbial abundances increase in response to atmospheric depressions, which travel from the Chinese continent (Murata and Zhang 2016). The microbial compositions in the atmosphere also vary significantly in correspondence to Asian dust events in the downwind environments (the Noto Peninsula in Japan; Maki et al. 2010, 2013, the North American mountains in USA; Smith et al. 2012). However, the atmospheric dispersion of *Mycobacterium* species by Asian dust events has not been demonstrated by analyzing the bacterial communities in long-range transported aerosols.

For investigating the airborne bacterial communities, including nontuberculous mycobacteria transported from the Asian continent to Japan, sampling the snow cover in Japan, such as that on Mt. Tateyama (3,015 m above sea level) is suitable, because the aerosols carried by westerly winds are captured by heavy snowfall and deposited as snow cover in Japan. The snowfall sometimes includes natural desert dust and anthropogenic pollution from the Asian continent and forms dark-colored (dirty) layers of snow cover. The snow cover on Mt. Tateyama at depths ranging from 6 to 10 m in the spring generally remains frozen until early April (Osada et al. 2004; Watanabe et al. 2011). Some researchers have investigated the microbial compositions in the aerosols captured in such dirty layers to identify the airborne microorganisms associated with the natural desert dust and anthropogenic pollution transported from the Asian continent (Maki et al. 2011, 2018; Tanaka et al. 2011).

In this study, for investigating the airborne bacterial communities dispersed over East Asia, we collected aerosol samples at the seven sampling sites which were located from Asian-dust sources (natural desert dust and anthropogenic pollution) to the downwind areas. At the other sampling site at Mt. Tateyama in downwind area, snow samples were collected from snow cover which sequentially captured the aerosols transported from the Asian continent. Microbial densities in the samples were counted using the fluorescent microscopic observation, followed by the examination of bacterial taxonomic structures using the DNA-amplicon metagenome. Finally, the relative abundances of bacterial populations including *Mycobacterium* species were compared among the sampling sites to discuss the potential dispersion of NTM-PD via Asian dust.

## 2. Materials and methods

### 2.1. Sampling

Sample collection for Asian-natural dust and anthropogenic pollutants transported by westerly winds was performed through aerosol sampling at seven sites over East Asia and the snow sampling at the other site of Mt. Tateyama in Japan. The seven sites over East Asia covered

Asian desert dust sources (Tsogt-Ovoo in the Gob Desert of Mongolia), anthropogenic-pollutant sources (Erenhot and Beijing in China), and dust deposition area (Yongin in Korea, Yonago, Suzu and the Noto Peninsula in Japan), while the snow samples represented aerosols that were sequentially transported from the Asian continent to Mt. Tateyama.

Aerosol samples were collected on the Asian desert ground surfaces (Tsogt-Ovoo; 44.23° N, 105.17° E: 2 m height) and on the buildings of Asian continental inland cities (Erenhot; 43.67° N, 111.95° E: 5 m height, Beijing; 39.98° N, 116.38° E: 20 m height), Asian continental coastal cities (Yongin in Korea peninsula; 37.20° N, 127.16° E: 20 m height) and Japanese cities (Yonago; 35.43° N, 133.33° E: 20 m height, Suzu; 37.45° N, 137.36° E: 15 m height) (Fig. 1). Additionally, aerosol samples at the heights ranging from 2,000 m to 2,500 m over Noto Peninsula of Japan (from Uchinada [36.67° N, 136.64° E] to Hakui [36.92° N, 136.76° E]) were also obtained using helicopter sampling procedure (Maki et al. 2017). For sampling, sterilized filter folders with a polycarbonate filters (0.22 µm pore size; Whatman, Tokyo, Japan) were attached to one side of air tubes, which were placed vertically at the sampling site or attached to helicopter windows. After connecting the other side of the air tubes to an air pump (MAS-1, AS-ONE, Osaka, Japan), aerosols were collected through the polycarbonate filter for sampling periods of 1.0–96 h at heights of 2 and 20 m and 0.5–1.0 h at the higher altitudes, with an airflow rates of 5 L min<sup>-1</sup>. Finally, a total of 38, 17, 10, 41, 10, 53, and 17 samples was collected from Tsogt-Ovoo, Erenhot, Beijing, Yongin, Suzu, Yonago, and Noto peninsula, respectively (Table 1, Table S1).

The snow samples were collected from the snow cover at Murododaira (36.57° N, 137.60° E; 2,450 m) on Mt. Tateyama on April 20, 2013 (Fig. 1). First, snow wall was formed in the snow pit, which was dug from the top of the snow cover to the ground's surface (743 cm vertical extent), and then, 5 mL snow samples were collected from each 3 cm layer of the snow wall at “non-dust season layers” (at heights from 164 to 200 cm) and “dust season layers” (at heights from 560 to 743 cm). The dirty layers found in the snow wall included high concentrations of Ca, which is a tracer of mineral dusts from the Asian deserts (Suzuki and Tsunogai 1993). The deserts dust events forming four dirty layers have been confirmed using the depolarization rates of lidar measurements (Fig. S1). Finally, a total of 70 snow samples (Table S2) were obtained and reserved at -80 °C, prior to the experimental analyses.

### 2.2. Environmental factors

Backward trajectories were calculated using the National Oceanic and Atmospheric Administration Hybrid Single Particle Lagrangian Integrated Trajectory model (<https://www.arl.noaa.gov/HYSPLIT.php>) to track the air mass transport pathways towards Mt. Tateyama with an elevation of 2,500 m for the evaluation of long-range transport. The depolarization ratio measured by the lidar systems in nearby Toyama was used to identify the dust events from April to March 2013 (<https://www-lidar.nies.go.jp/>) (Shimizu et al. 2016). The dust-event transport over East Asia during the winter and spring seasons in 2013 was confirmed on the actual color images obtained using Moderate Resolution Imaging Spectroradiometer (MODIS) sensors onboard the satellites (<https://modis.gsfc.nasa.gov/about/>). The weather data observed at the meteorological observatories were defined by the World Meteorological Organization (WMO) (<https://public.wmo.int/en>) and plotted on the MODIS images.

For fluorescent observation of particles in snow samples, after the 500 µL solution of 70 samples of Mt. Tateyama was fixed with 1 % paraformaldehyde solution, particles in the sample solutions were stained with 0.5 µg mL<sup>-1</sup> DAPI (4',6-diamino-2-phenylindole) for 15 min and filtered through a 0.22 µm pore size polycarbonate filter (Millipore, Tokyo, Japan) (Russell et al. 1974). In contrast, the filter-collecting aerosol samples of Tsogt-Ovoo, Erenhot, Beijing, Yongin, Suzu, Yonago and Noto peninsula, were also stained with adding 1 %

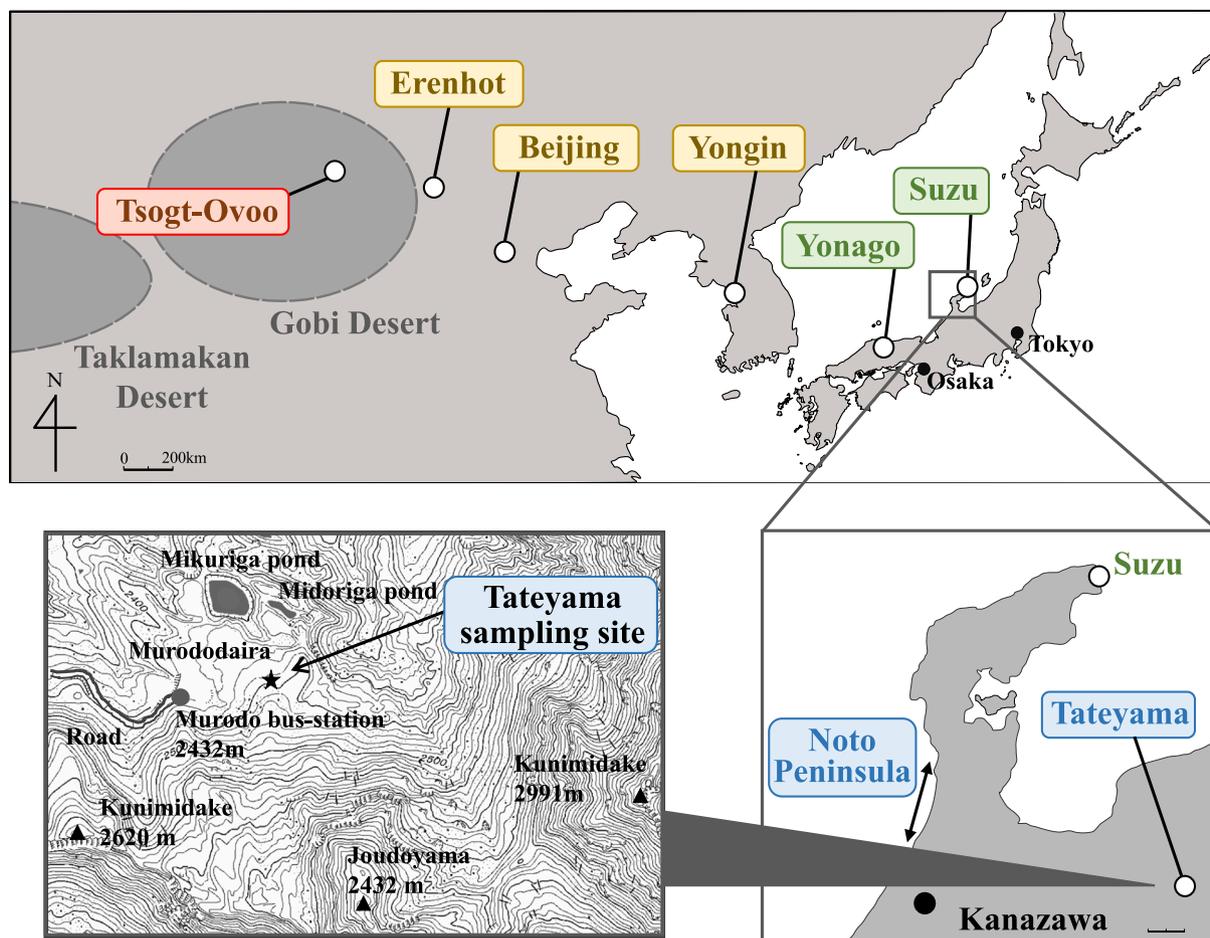


Fig. 1. Map of the sampling sites of aerosol samples and snow samples around East Asia.

paraformaldehyde and  $0.5 \mu\text{g mL}^{-1}$  DAPI solution directly to the filters. After the filter was placed on top of a drop of low-fluorescence immersion oil on a slide, another oil drop was used to cover along with a cover slide. Particles on the filter transects of slides were observed and counted on the filter transect using an epifluorescence microscope (Olympus, Tokyo, Japan) using a UV excitation system. The observed particles were classified into three categories: mineral particles with white auto-fluorescence; microbial particles (bacteria and fungi) with DAPI-stained blue fluorescence; and black carbon with black color. The detection limit of aerosols was  $1.32 \times 10^2$  particles/filter (from  $1.50 \times 10^2$  particles/ $\text{m}^3$  to  $7.00 \times 10^3$  particles/ $\text{m}^3$ ), which allowed the detection of at least one particle under every view for an area of  $1.0 \text{ mm} \times 1.0 \text{ mm}$ . Microbial particles were counted in the samples collected at every sampling site, and mineral particles and black carbon were means in only the snow samples of Mt. Tateyama.

### 2.3. High throughput sequencing of 16S rRNA genes in the samples

The particles in 5 mL melted snow samples of Mt. Tateyama were centrifuged at  $20,000 \times g$  for 10 min and collected as pellets. In contrast, the particles in filter-collecting aerosol samples of Tsogt-Ovoo, Erenhot, Beijing, Yongin, Suzu, Yonago, and Noto peninsula were suspended in 3 mL of sterile 0.6 % NaCl solution, before the particles were pelleted by centrifuging at  $20,000 \times g$  for 10 min. The pellets were resuspended into 500  $\mu\text{L}$  of sterile pure water and used for the extraction of genomic DNA (gDNA) using a phenol–chloroform method, which were combined with the microbial cell degradation by SDS, proteinase K, and lysozyme, as described previously (Maki et al. 2008). Fragments of 16S rRNA genes (approximately 290 bp) were amplified from the extracted gDNA using

polymerase chain reaction (PCR) using universal bacterial primers 515F and 806R for the V4 region (Caporaso et al. 2011). The first PCR fragments were amplified again using the second PCR primers, which targeted the additional sequences of first PCR primers and included eight tag nucleotides designed for sample identification barcoding. Thermal cycling conditions were employed from the previous investigation (Maki et al. 2017). The PCR amplicons were used for high-throughput sequencing on a MiSeq Genome Sequencer (Illumina, CA, USA). The paired-end sequences with area length of 250 bp were grouped based on the tag sequences for each sample. At the PCR analysis steps, negative controls (sterile pure water and unused filter) contained no fragments of 16S rRNA gene amplicons showing the absence of artificial contamination.

The forward and reverse paired-end reads in the raw sequencing database were merged using R package DADA2 v1.14 (Callahan et al. 2016). Primer sequences were removed using Cutadapt v2.8 (Martin 2011). The reads were uniformly trimmed to 185 bp (forward) and 130 bp (reverse) and then filtered by removing reads exceeding maximum expected errors ( $>2$  for forward reads and  $>5$  for reverse reads) or reads containing ambiguity N symbol. The reads were used to train the error model and then dereplicated to acquire unique sequences, which were used to infer amplicon sequence variants (ASVs) with the trained error model. Greengenes release 13.8 (McDonald et al. 2012) was used for determining taxonomic compositions. All sequences have been deposited in the DDBJ database (accession number of the submission is Tateyama; PRJEB24035, Tsogt-Ovoo; DRA005058, Erenhot and Beijing; PRJNA413598, Yongin and Yonago; PRJEB22232, Suzu; DRA014079, and Noto Peninsula; PRJEB17915).

The Chao1 which is an indicator of bacterial diversity (alpha di-

**Table 1**  
The information of samples collected from the eight sampling sites.

Sampling sites	Location	Altitude (m)	Country	Area	Environmental characteristics	Sampling periods	Sample condition	Sampling time (h)	Sampling volume (L)	Sample numbers
Tsogt-Ovoo	44.23° N, 105.17° E	2	Mongolia	Gobi Desert	Source of natural desert dust	16–17 Mar in 2014 8–10 Mar in 2015 26–30 Apr in 2015 1–4 May in 2017 3–5 May in 2018 1–3 May in 2019	Aerosol	1–24.2	30–726	38
Erenhot	43.67° N, 111.95° E	5	China	Continental city	Source of anthropogenic pollution	31 Mar – 30 April in 2016	Aerosol	1.9–23.7	57.5–713.5	17
Beijing	39.98° N, 116.38° E	20	China	Continental city	Source of anthropogenic pollution	30 Mar – 16 April in 2015	Aerosol	24	720	10
Yongin	37.20° N, 127.16° E	20	Korea	Continental city	Downwind area	10 Mar – 19 June in 2015	Aerosol	19–96	570–2880	41
Suzu	35.43° N, 133.33° E	15	Japan	Island city	Downwind area	20 May – 18 June in 2015	Aerosol	8	240	10
Yonago	37.45° N, 137.36° E	20	Japan	Island city	Downwind area	10 Mar – 18 May in 2015	Aerosol	19–27	570–810	53
Noto Peninsula	From 36.67° N, 136.64° E to 36.92° N, 136.76° E	2,000–2,500	Japan	High elevation	Downwind area	23–24 Mar in 2014 8, 16 Mar in 2015 16 Mar n 2016	Aerosol	0.5–1	56–64	17
Mt. Tateyama	36.57° N, 137.60° E	2,450	Japan	High elevation	Downwind area	15, 21 Jun in 2016 11, 30 Mar in 2017 7, 12 May in 2017 24, 29 Mar in 2018 16 May in 2018 16 April 2013 (1 Feb – 16 Apr in 2013) (before 31 Dec 2012)	Snow	–	–	70

versity) was calculated as follows:  $H' = -\sum_{i=1}^S P_i \log_2 P_i$

$$S_{Chao1} = S_{obs} + n_1^2 / 2n_2 \quad (1)$$

where  $S_{obs}$  is the number of ASVs,  $n_1$  is the number of an individual ASV, and  $n_2$  is the number of an ASV containing two reads (Caporaso et al. 2010).

Principal coordinate analysis (PCoA) with weighted UniFrac distances were used to cluster some samples according to the phylogenetic distances of their bacterial communities. UniFrac measured the difference of sequencing dataset between two samples depending on the branch lengths of two sequences in phylogenetic trees (Lozupone et al. 2011).

#### 2.4. Data processing

JMP version 16.1.0 (SAS Institute, Cary, NC, USA) was used for the statistical analyses of particle concentrations, the Chao 1, and the relative abundances of MiSeq sequencing data. The samples were separated into several groups depending on the collection sites or the survey series. Comparisons among several groups were performed using Steel-Dwass analysis with nonparametric statistics. Differences were considered significance in case of a  $p$ -value < 0.05. The relation between particle concentrations and bacterial relative abundances was analyzed statistically using the Pearson correlation coefficient.

### 3. Results

#### 3.1. Particle concentrations of microorganisms during Asian dust events

The aerosol samples collected from the Asian desert area (Tsogt-Ovoo) and the Asian continental cities (Erenhot, Beijing, Yongin) exhibited higher ranges of particle concentrations than those collected from the Japanese cities (Suzu, Yonago) and at the high altitudes (Noto Peninsula) (Fig. 2a). However, there were a few statistical differences between the two types of two samples (Table S3). In every sampling site for aerosol sampling, the particle concentrations of minerals and airborne microorganisms increased by up to 100 folds during Asian dust events. The air-mass trajectories came frequently from the desert area to Erenhot and Beijing during the sampling periods (Fig. S2). Additionally the air masses over Asian desert and the continental cities sometimes moved to the downwind areas, such as Yongin, Suzu, Yonago, and Noto-Peninsula, indicating the several occurrences of Asian dust event during the sampling periods.

The snow cover layers of Mt. Tateyama were mainly composed of compacted snow (rounded grains) which have not melted during winter, indicating that the aerosols in the snow samples would maintain the condition at deposition. The profiles of chemical compounds in the snow walls retained the variations corresponding to the snow layers (Fig. S3), implying that the snow layers would generally retain the original particles of the snowfall. The colored layers of the dust season layers (heights from 560 to 743 cm) in snow cover of Mt. Tateyama included relatively high concentrations of microbial particles as well as mineral particles in comparison to non-colored layers (Fig. 2b). On the contrary, black carbon increased in the lower parts (heights from 560 to 644 cm) of the dust season layers, which were accumulated during late winter. The colored layers at heights from 560 to 743 cm matched the occurrences of the natural desert dust and anthropogenic pollutants from March to April, which were detected by the Lidar data analysis (Fig. S1). Those from 560 to 644 cm indicated anthropogenic pollutants in February. The MODIS data indicated several series of Asian-dust events dispersed over East Asia during the winter and spring of 2013 (Fig. S4). The air-mass trajectories also supported that the aerosols captured in the colored layers were transported from the Asian continent to Japan (Fig. S5).

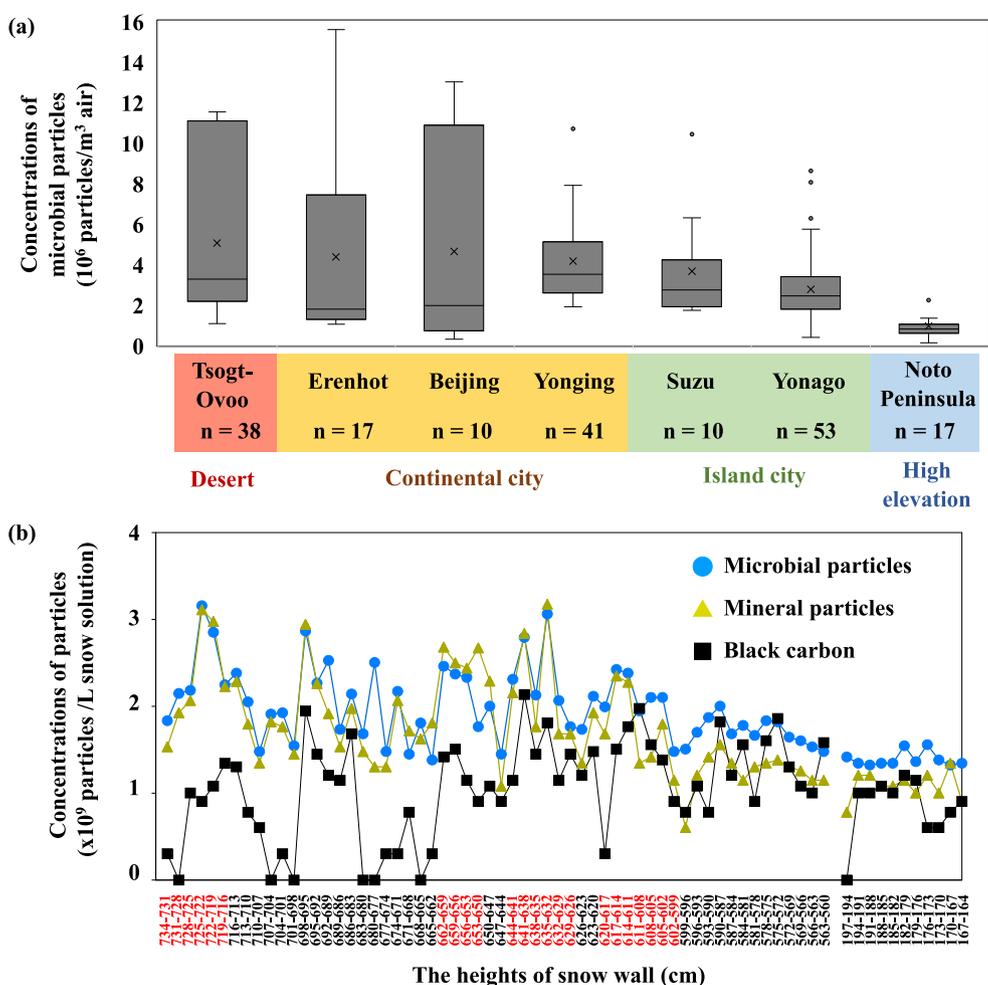


Fig. 2. (a) Concentrations of DAPI-stained microbial particles at the eight sampling sites in Asian desert (Tsogt-Ovoo), Asian continental cities (Erenhot, Beijing, Yongin), Japanese cities (Suzu, Yonago), and high elevation over Japan (Noto Peninsula). The error bars were obtained from the sample numbers which were collected at each sampling site. (b) Vertical concentration profile of DAPI-stained particles, such as mineral particle (yellow triangles), microbial particle (blue circles), and black carbon (black squares), in snow samples collected from Mt. Tateyama.

### 3.2. Analyses of bacterial community structures

To analyze bacterial compositions in the snow and aerosol samples, we obtained a total of 19,225,745 merged paired-end sequences with a median length of 292 bp remaining. The sequences of 16S rRNA gene were divided into 3,423 phylotypes (sequences with > 97 % similarity). At the PCR analysis steps, the absence of 16S rRNA gene amplicons from negative controls (no template and template from unused filters) demonstrated the avoidance for artificial contamination during the experimental processes. Phylogenetic assignment of sequences resulted in an overall diversity comprising 42 phyla, (and candidate divisions), 108 classes (and class-level candidate taxa), and 501 families (and family-level candidate taxa). Most of the phylotypes recovered from the aerosol and snow samples were related to the phyla Actinobacteria, Firmicutes, Bacteroidetes and Proteobacteria, which are typically well represented in 16S rRNA gene sequencing database generated from terrestrial, marine, freshwater, and phyllospheric environments (Fig. 3).

Alpha diversities (Chao I) were higher in the samples of the continental inland cities (Erenhot, Beijing) than those of the Asian desert (Tsogt-Ovoo) and the downwind areas (Yongin, Yonago, Suzu, Tateyama, Noto Peninsula) (Fig. 4). In particular, the aerosol samples of Erenhot and Noto Peninsula exhibited significantly high and low of alpha diversities, respectively, in comparison to the other samples excepting for Beijing (Steel-Dwass analysis:  $P < 0.05$ , Table S4). On the PCoA showing beta diversity, the samples collected at high altitudes of Mt. Tateyama and Noto Peninsula formed the cluster, which overlapped some area among those of Asian continent and Japan (Fig. 5). The aerosol samples of Asian desert (Tsogt-Ovoo) and the continental inland

cities (Erenhot, Beijing) indicated the different cluster from the continental peninsula cities (Yongin) and the Japanese cities (Yonago, Suzu).

The Firmicutes sequences tended to have increasing relative abundances from the Asian-dust sources (Asian desert: Tsogt-Ovoo, the Continental inland cities: Erenhot, Beijing) to the downwind areas (the Japanese cities: Yonago, Suzu, the high elevation: Noto Peninsula, Mt. Tateyama) (Fig. 3). The Actinobacteria sequences presented greater relative abundances in the samples from Tsogt-Ovoo, Erenhot, and the Noto Peninsula than in those from other sites. The Actinobacteria sequences in the snow samples of Mt. Tateyama exhibited the increase trend of relative abundances at the lower parts of snow wall (heights from 560 to 644 cm and from 164 to 197 cm), which would be formed with anthropogenic pollutants during winter season (Fig. 6).

### 3.3. Relative abundance of *Mycobacterium* species in the aerosol samples

The sequences of the phyla Actinobacteria and the order Corynebacteriales exhibited the decrease trends of relative abundances in the samples from Asian desert to Japan (Fig. 7). The aerosol samples of Tsogt-Ovoo and Erenhot indicated significant higher relative abundances of Actinobacteria sequences than those of other sampling sites (Steel-Dwass analysis:  $P < 0.05$ , Table S5). In contrast, the relative abundances of the *Mycobacterium* sequences in Corynebacteriales were higher in this order: high altitudes (Mt. Tateyama, Noto Peninsula) > Asian continental cities (Erenhot, Beijing, Yongin) = Japanese cities (Suzu, Yonago) cities > Asian desert (Tsogt-Ovoo). The samples of Tsogt-Ovoo showed significant low relative abundances in comparison to those of Erenhot and Mt. Tateyama (Steel-Dwass analysis:  $P < 0.05$ ,

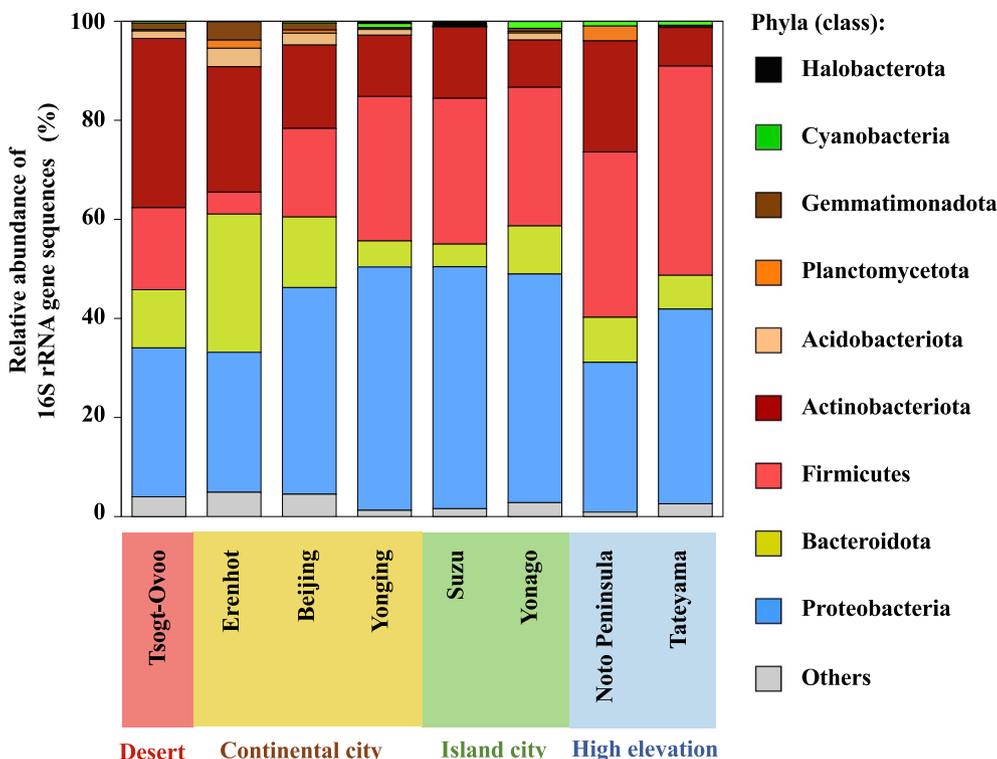


Fig. 3. The average relative abundance of bacterial compositions in the partial sequences obtained in the MiSeq sequencing database (approximately 250 bp). Obtained from the samples collected from eight sampling sites in Asian desert (Tsogt-Ovoo), Asian continental cities (Erenhot, Beijing, Yongin), Japanese cities (Suzu, Yonago), and high elevation over Japan (Mt. Tateyama, Noto Peninsula).

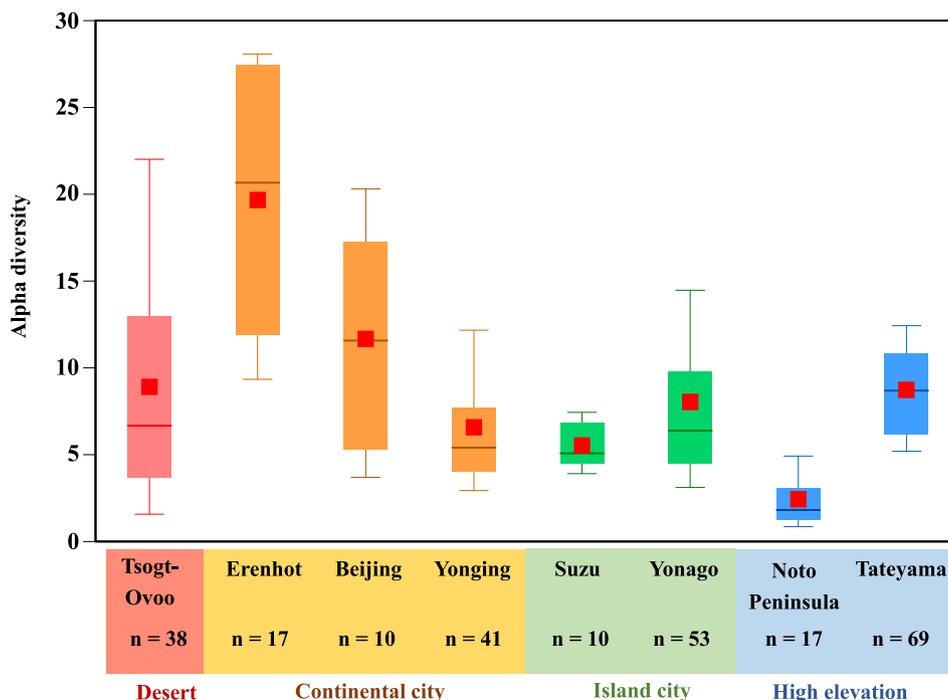


Fig. 4. Bacterial diversity observed in the snow samples collected from the samples of eight sampling sites in Asian desert (Tsogt-Ovoo), Asian continental cities (Erenhot, Beijing, Yongin), Japanese cities (Suzu, Yonago), and high elevation over Japan (Mt. Tateyama, Noto Peninsula). The species were binned at the 97 % sequence similarity level. Asterisk indicates statistically difference from other samples (Steel-Dwass analysis;  $p < 0.05$ ).

Table S5).

The *Mycobacterium* sequences in Actinobacteria were detected at a relative abundance of up to 6.5 % in the snow samples of Mt. Tateyama (The bottom graph in Fig. 6b). The snow samples collected from the

dirty layers at lower parts of snow cover (heights from 560 to 644 cm) included higher rates of *Mycobacterium* sequences than the other parts of snow wall (heights from 164 to 197 cm and from 644 to 731 cm). The order Corynebacteriales containing *Mycobacterium* species also showed

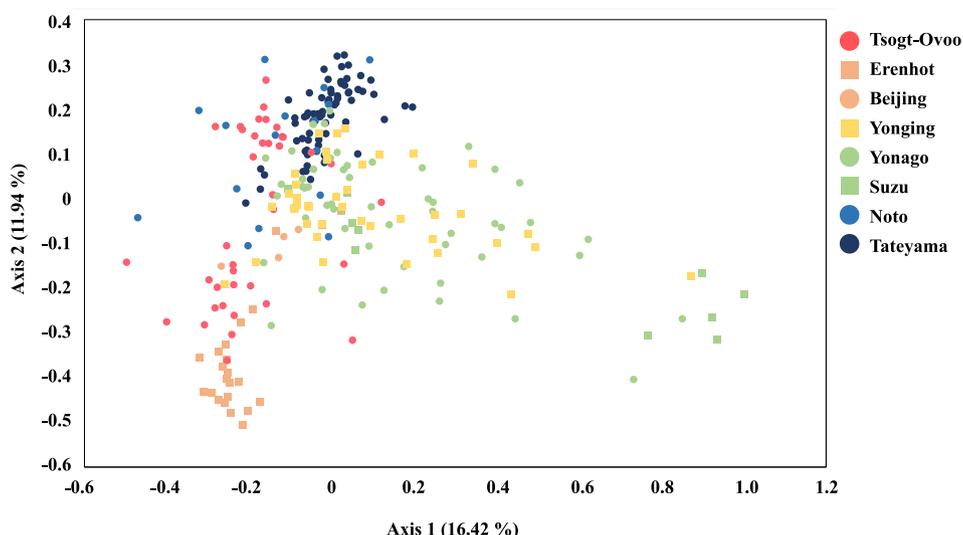


Fig. 5. Principal coordinates analysis of Bray Curtis distance matrix displaying phylogenetic clustering of the samples collected from eight sampling sites in Asian desert (Tsogt-Ovoo), Asian continental cities (Erenhot, Beijing, Yonging), Japanese cities (Suzu, Yonago), and high elevation over Japan (Mt. Tateyama, Noto Peninsula).

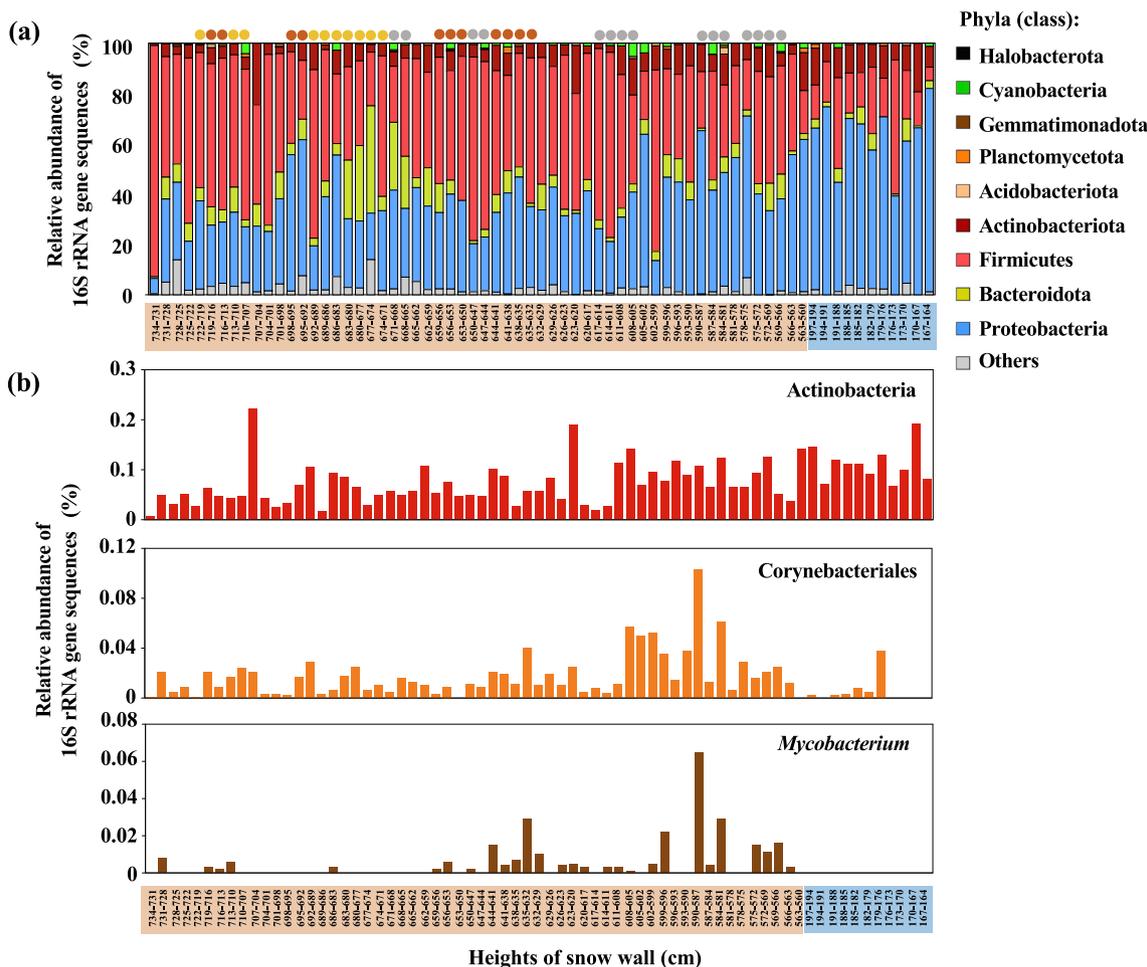
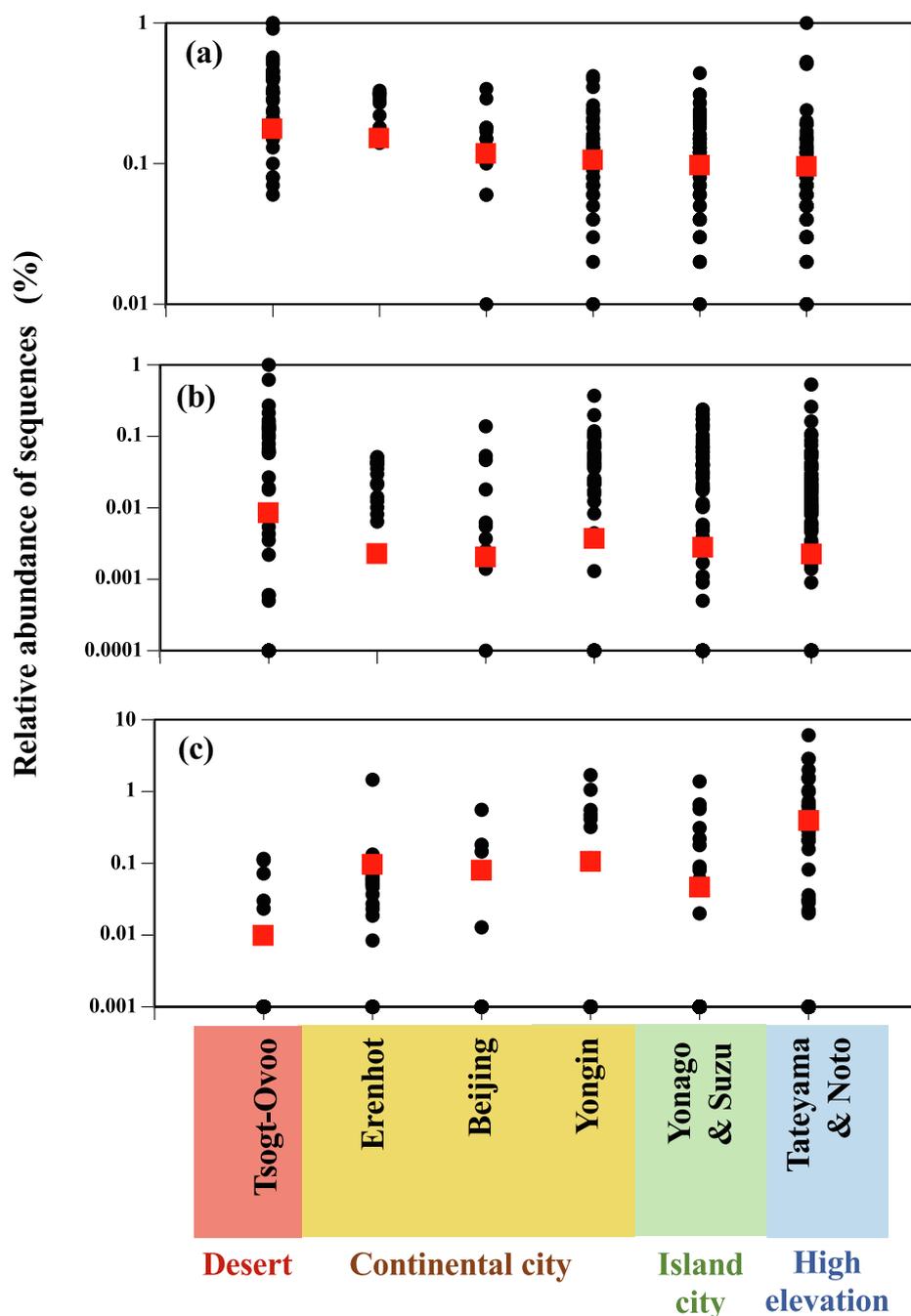


Fig. 6. (a) Vertical profiles for bacterial compositions at the class level of the partial sequences obtained in the MiSeq sequencing database (approximately 250 bp) from snow samples collected from Murodo-daira, Mt. Tateyama, in April 2013. (b) Vertical profiles for relative abundance of bacterial sequences belonging to the phyla Actinobacteria, the order Corynebacteriales and the genus *Mycobacterium* in the MiSeq sequencing database. Circles of yellow, grey and orange indicate the layers formed at the desert dust days, the anthropogenic pollutant days, and the both desert dust and anthropogenic pollutant days, respectively.



**Fig. 7.** Relative abundance of bacterial sequences belonging to the phyla Actinobacteria (a), the order Corynebacteriales (b), and the genus *Mycobacterium* (c) in the MiSeq sequencing database (approximately 250 bp) recovered from the samples collected from eight sampling sites in Asian desert (Tsogt-Ovoo), Asian continental cities (Erenhot, Beijing, Yongin), Japanese cities (Suzu, Yonago), and high elevation over Japan (Mt. Tateyama, Noto Peninsula). Red squares indicate the average relative abundance of each bacterial sequence. Asterisk indicates statistically difference from other samples (Steel-Dwass analysis;  $p < 0.05$ ).

the similar vertical patterns of relative abundances to the *Mycobacterium* sequences on the snow wall. The relative abundances of *Mycobacterium* and Corynebacteriales sequences in the snow samples of Mt. Tateyama exhibited positive relations to the black carbon densities in snow samples (Pearson correlation coefficient:  $P < 0.01$ ) (Table 2). These mean that the bacterial cells of *Mycobacterium* and the relatives were captured with anthropogenic particles in snow cover during the winter and early-spring seasons (Fig. S1). Additionally, in the aerosol samples collected at Noto Peninsula using a helicopter, the Corynebacteriales sequences suddenly increased to up to 53 % and the *Mycobacterium* sequences also appeared at relative abundances of up to 0.5 % in early spring, when air masses came from the Asian continental cities (Fig. S2).

#### 4. Discussion

At the sampling sites used in this survey, such as Tsogt-Ovoo, Erenhot, Beijing, Yongin, and Yonago, the aerosol concentrations of minerals and microorganisms reportedly increase by up to 100-folds during Asian dust events, potentially because of natural desert dust and/or anthropogenic pollution (Tang et al. 2017; Chen et al. 2022; Maki et al. 2014, 2016, 2019). The aerosols in the Asian desert (Tsogt-Ovoo) and the continental inland cities (Erenhot, Beijing) exhibited higher concentrations than those in the Japanese cities (Suzu, Yonago) (Fig. 2a). The colored layers capturing dust particles in the snow cover of Mt. Tateyama included higher concentrations of microbial particles than non-colored layers (Fig. 2b). During hazy days in the Asian continent, the airborne microorganisms associated with mineral particles and pollutant particles increased in the atmosphere of Beijing (Wei et al.

**Table 2**

Relatives of the bacterial relative abundances with particle concentrations in the snow samples of Mt. Tateyama.\*.

Bacterial categories	Fluorescent particle concentrations		
	Mineral particles	Microbial particles	Black carbon
Halobacterota	-0.04	0.03	-0.15
Cyanobacteria	-0.09	-0.14	0.19
Gemmatimonadota	0.14	0.09	0.14
Planctomycetota	-0.01	-0.02	0.05
Acidobacteriota	0.04	-0.13	0.15
Actinobacteriota	-0.35††	-0.29††	0.08
Firmicutes	0.44††	0.42††	-0.04
Bacteroidota	-0.02	-0.04	-0.38††
Proteobacteria	-0.40††	-0.37††	0.20
Others	0.08	0.04	-0.16
Actinobacteriota	-0.35††	-0.29††	0.08
Actinobacteria	-0.36††	-0.30††	0.10
Corynebacteriales	0.06	-0.09	0.24†
<i>Mycobacterium</i>	0.16	0.00	0.31††

Red cells indicate positive relations and blue cells indicate negative relations.

\* The marks †† and † indicate  $P < 0.01$  and  $P < 0.05$ , respectively, for the Pearson correlation coefficient.

2016). The microbial load transported to downwind areas by Asian dust are known to increase airborne microbial densities suddenly at the ground levels (Hara and Zhang 2012; Maki et al. 2019) and at high altitudes (Maki et al. 2013, 2017) in the western parts of Japan. Previous studies have thus suggested that airborne microorganisms associated with natural desert dust and anthropogenic pollution are dispersed over long distances in East Asia.

The bacterial communities in the snow and aerosol samples were composed of sequences of terrestrial, marine, freshwater, and phyllospheric bacteria belonging to the phyla Actinobacteria, Firmicutes, Bacteroidetes and Proteobacteria (Fig. 3). These bacterial populations are reportedly dominant in the aerosol samples collected around East Asia during the hazy days caused by natural desert dust and/or anthropogenic pollution (e.g., Jeon et al. 2011; Maki et al. 2008, 2016, 2019; Tanaka et al. 2011, Tang et al. 2016). The alpha diversities (Chao 1) of airborne bacterial communities were the highest in the continental inland cities (Erenhot, Beijing) among the sampling sites in this study (Fig. 4). The combination of anthropogenic pollution with natural desert dust in the continental inland cities (Erenhot, Beijing) likely led to the high diversity of airborne bacteria, which originated from terrestrial and phyllospheric sources. On the pCoA graph, the airborne bacterial compositions were separated into two groups: the desert-dust (Tsogt-Ovoo) and anthropogenic pollutant (Erenhot, Beijing) sources and the downwind cities (Yongin, Yonago, Suzu) (Fig. 5). Comparatively, the samples collected at high altitudes (Mt. Tateyama, Noto-Peninsula) formed a cluster at the common parts between the above-mentioned two groups. The common bacterial communities were likely transported from the Asian continent to Japan at high altitudes.

The phyla Firmicutes and Actinobacteria, which include dominant terrestrial bacteria, were commonly detected at every sampling site (Fig. 3). In particular, the *Bacillus* sequences in Firmicutes was dominant in the samples collected at high altitudes. The members of the genus *Bacillus* are known to form endospores against atmospheric stressors to survive during long-range transport by westerly winds (Nicholson et al. 2000). Actinobacteria sequences also showed high relative abundances in the aerosol samples collected at Tsogt-Ovoo, Erenhot, and the Noto Peninsula (Fig. 7) and in the snow samples of Mt. Tateyama representing the Asian-dust particles (Fig. 6). Actinobacteria populations are dominant in the airborne bacterial communities on hazy anthropogenic pollution-related days in Beijing (Cao et al. 2014). The terrestrial bacteria belonging to Actinobacteria, which form biofilms to resist environmental stressors (Tong and Lighthart 1998), can be transported at high altitudes over Japan (Maki et al. 2017). The natural desert dust and anthropogenic pollution are thought to change the bacterial

compositions at high altitudes ranging from 200 to 3,000 m over East Asia (Jeon et al. 2011; Maki et al. 2013). Natural desert dust mixed with anthropogenic pollution and ocean sea spray can reportedly travel across the Japan Sea (e.g., Leaitch et al. 2009). The westerly winds over East Asia can thus induce substantial variations in the bacterial communities in the atmosphere of downwind areas under the influence of Asian dust events. In the regions affected by other kinds of desert dust, African dust (Prospero et al. 2005) and Australian dust (Close et al. 1978; Lim et al. 2011) have been reported to carry airborne bacteria to the downwind areas in Europe and Oceania, respectively.

The phyla Actinobacteria and the order Corynebacteriales had the highest relative abundances in the Asian desert samples (Tsogt-Ovoo). In contrast, the abundance of the genus *Mycobacterium* in Corynebacteriales was high in the samples collected at high altitudes (Mt. Tateyama, Noto Peninsula) and in Asian continental cities (Erenhot, Beijing, Yongin) (Fig. 7). Additionally, the *Mycobacterium* sequences presented high abundances in the colored snow layers on Mt. Tateyama (Fig. 6), which formed when air masses came from continental industrial areas, such as Erenhot and Beijing (Fig. S5). Anthropogenic pollutants without natural dust are frequently transported from the Asian continent to downwind areas, such as Japan, from winter to early spring (Huang et al. 2015a) because the frozen desert surfaces prohibit the transport of desert dust from the Gobi and Taklamakan deserts (Abulaiti et al. 2014). *Mycobacterium* sequences were positively related to the black carbon densities (Table 2), suggesting that these bacteria are transported with anthropogenic pollutants. The mineral particles and black carbon in the atmosphere are essential carriers of airborne microorganisms (Maki et al. 2008; Xie et al. 2018). Indeed, under fluorescent microscopic observation, the aggregation of microorganisms with black carbon was observed in aerosol samples collected at high altitudes over the Noto Peninsula using a helicopter (Fig. S6). The shade of coarse particles, such as black carbon and mineral particles, can prevent coexisting or mixed bacterial cells from being exposed to environmental stressors, such as UV radiation and desiccation (Tong and Lighthart 1998; Noda et al. 2022). Airborne *Mycobacterium* species are constantly suspended in the industrialized areas of the Asian continent, and can be transported to high altitudes by Asian dust events. *Mycobacterium* populations attached to black carbon are thus expected to have high abundances at high altitudes.

After deposition on ground-level environments in Japan, the relative abundances of *Mycobacterium* populations decreased (Fig. 7) because of atmospheric mixing with the local bacterial communities originating from Japanese seas and mountains. *M. intracellulare* is known to cause NTM-PD mainly in the southern and western parts of Japan, where anthropogenic dust is frequently transported from the Asian continent (Morimoto et al. 2017). The epidemiological investigations demonstrated that NTM-PD patients constantly appeared in Japan during the sampling periods from 2013 to 2018 (data not shown). The long-range transport of *Mycobacterium* populations likely to contribute to the potential dispersion of NTM-PD around East Asia.

There are some limitations of this study. First, the V3 region (ca. 450 bp) of 16S rRNA gene used in this study did not have a sufficient length to determine the species level of *Mycobacterium* sequences. Different regions, including V3 and V4 should be considered for identifying *Mycobacterium* species in the future. Second, the metagenomic analyses using the gDNA extracted directly from samples provided only a DNA sequencing database without information on bacterial viabilities. Further studies should combine the analyses of bacterial isolates to identify *Mycobacterium* dispersion directly causing NTM-PD. Finally, the differences between the snow and aerosol samples are suspected of causing the artificial effects on bacterial variations. Psychrophile microorganisms are known to grow gradually below freezing temperatures, and the melting process of snow samples may influence bacterial compositions (Srinivas et al. 2009). Moreover, microbial cells collected on the filter were likely damaged by desiccation, pump-absorption pressure, and sunlight irradiance during the sample filtering (Griffin

et al. 2011). However, these effects were expected to have minor contributions because the growth of psychrophile microorganisms was not confirmed, and the abundances of stressors-resistant bacteria was detected at similar levels in both aerosol and snow samples.

## 5. Conclusion

This is the first report of a global survey investigating the airborne bacterial communities transported by Asian dust performed using at eight sampling sites around East Asia. The bacterial communities at high altitudes over Japan exhibited common taxonomic compositions as those in the aerosol samples of Asia dust sources and deposition regions. The terrestrial bacteria belonging to the phyla Firmicutes and Actinobacteria were transported predominantly by anthropogenic and natural-desert dust because of their resistance to atmospheric stressors. In particular, *Mycobacterium* species in Actinobacteria were abundant in the samples at high altitudes and associated with anthropogenic particles, suggesting that the NTM-PD potentially dispersed from industrial areas to Japan owing to anthropogenic pollution. These results support the hypothesis that Asian dust events increase the NTM-PD by *M. intracellulare* mainly in the southern and western parts of Japan. However, the *Mycobacterium* sequences in this study could not be identified clearly as species level. In the future, molecular biological techniques, such as quantitative PCR or fluorescent *in situ* hybridization targeting *Mycobacterium* species could allow the elucidation of the long-range transport processes of *Mycobacterium* species that cause NTM-PD over East Asia. Additionally, isolates of *Mycobacterium* species are needed for evaluating their potential for NTM-PD.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Data availability

Data will be made available on request.

## Acknowledgments

The lidar data operated by AD-Net are accessible from <http://www.lidar.nies.go.jp>; last access. Backward trajectories can be calculated using the National Oceanic and Atmospheric Administration Hybrid Single Particle Lagrangian Integrated Trajectory model (<http://www.arl.noaa.gov/HYSPLIT.php>). Members of Fasmac Co., Ltd. helped with the MiSeq sequencing analyses. This work was supported by the Grant-in-Aid for Scientific Research (A) (21H04930, 17H01616) and (B) (20H04326, 18H03385) from the Japanese Society for the Promotion of Science (JSPS) and the Joint Research Program of Arid Land Research Center, Tottori University (28C2015).

## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.envint.2022.107471>.

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