



Long-range transported bioaerosols captured in snow cover on Mount Tateyama, Japan: Impacts of Asian-dust events on airborne bacterial dynamics relating to ice-nucleation activities

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Abstract. The westerly wind travelling at high altitudes over East Asia transports aerosols from the Asian deserts and urban areas to downwind areas such as Japan. These long-range transported aerosols include not only mineral particles, but also microbial particles (bioaerosols), that impact the ice-cloud formation processes as ice nuclei. However, the detailed relations of airborne bacterial dynamics to ice nucleation in high-elevation aerosols have not been investigated. Here, we used the aerosol particles captured in the snow cover at the altitudes of 2,450 m on Mt. Tateyama to investigate the sequential changes of ice-nucleation activities and bacterial communities in aerosols and elucidate the relationships between the two processes. After stratification of the snow layers formed on the walls of a snow pit on Mt. Tateyama, snow samples, including aerosol particles, were collected from 70 layers at the lower (winter accumulation) and upper (spring accumulation) parts of the snow wall. The aerosols recorded in the lower parts mainly came from Siberia (Russia), North Asia, and the Sea of Japan, while those in the upper parts showed an increase the Asian-dust particles, which originate from the desert regions and industrial coasts of Asian. The snow samples exhibited high levels of ice nucleation corresponding to the increase of Asian dust particles. Amplicon sequencing analysis using 16S rRNA genes revealed that the bacterial communities in the snow samples predominately included plant associated and marine bacteria (phyla Proteobacteria) during winter; whereas, during spring, when dust events arrived frequently, the majority were terrestrial bacteria of phyla Actinobacteria and Firmicutes. The relative abundances of Firmicutes (Bacilli) showed a significant positive relationship to the ice nucleation in snow samples. Presumably, Asian dust events change the airborne bacterial communities over Mt. Tateyama and carry terrestrial bacterial populations, which possibly induce ice-nucleation activities, thereby indirectly effecting on climate changes.

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1 Introduction

The westerly wind transports mineral particles from the middle desert areas of the Asian continents, including the Gobi and Taklamakan Deserts, and mineral particles contaminated by anthropogenic pollutants at continental coasts are dispersed eastward over the Japanese Sea to Japan (Duce et al., 1980; Iwasaka et al., 1983; Watanabe et al., 2006; Huang et al., 2015ab). In addition to abiotic particles, the microbial fractions associated with mineral-dust particles, which are commonly called “bioaerosols” include viruses, bacteria, fungi, and pollen as well as plant and animal debris (Jones and Harrison, 2004; Jaenicke, 2005; Iwasaka et al., 2009; Pointing and Belnap, 2014). The airborne bacterial compositions at high altitudes above Asian dust deposition and areas of anthropogenic pollution, such as Beijing (Li et al., 2010), Osaka (Yamaguchi et al., 2012), the Noto Peninsula (Maki et al., 2010, 2013), and the North American mountains (Smith et al., 2012), vary significantly. Airborne bacteria at high altitudes over East Asia are the focus of this study, because of their impacts in atmospheric chemical reactions and cloud formation (Pratt et al., 2009; Morris et al., 2011; Hara et al., 2016ab). In Japan, airborne microbial abundances increase in response to atmospheric depressions, which travel from the Asian continent (Murata and Zhang, 2016).

Some of airborne microorganisms act as ice nuclei that are related to ice-cloud formation processes, indicating the possibility that wind-blown bioaerosol particles indirectly contribute to atmospheric radiation transfer and the geochemical cycle of atmospheric constituents (Möhler et al., 2007; Delort et al., 2010; Creamean et al., 2013; Joly et al., 2013; Pöschl and Shiraiwa, 2015). In particular, the ice-nucleating cell components of some bacterial species belonging to the phylum Proteobacteria and class Bacilli exhibit high nucleation activities, initiating ice formation at relatively warmer temperatures (from -5 °C to -2 °C) (Morris et al., 2004) than the inorganic ice-nuclei, such as potassium feldspar (approximately -8 °C) (Atkinson et al., 2013). Ice-nucleating bioaerosols are believed to activate ice-cloud formation more efficiently than inorganic substances (Hoose and Möhler, 2012; Murray et al., 2012), and contribute to rapid ice-cloud formation, even at low concentrations, in the clouds at temperatures between -8 °C and -3 °C (Hallett and Mossop, 1974). Airborne bacteria carried by westerly winds over East Asia area also found to initiate high levels of ice nucleation (Hara et al., 2016a, b). Culture-independent analyses for bacterial taxonomic composition demonstrated the high bacterial diversity in cloud waters suggesting that some unculturable bacterial populations, including the keystone bacteria, primarily influence ice-cloud formations in North American mountains (Bowers et al., 2009; Pratt et al., 2009). However, in East Asia, the influences of airborne bacterial dynamics on atmospheric ice-nucleation and precipitation are unclear.

During the winter and early spring, strong north-westerly winds carry heavy falls of snow to Mt. Tateyama (3,015 m above sea level), which faces the East Sea. The snowfall sometimes includes the natural and anthropogenic dust particles from Asian continent. Since ice-nucleating bacteria possibly contribute to ice-cloud formation and snow fall over Mt. Tateyama, the bacterial communities in the snow cover can be useful for investigating the impact of airborne bacteria on ice-cloud formation processes. The snow covers on Mt. Tateyama exhibit the deep depths ranging from 6 m to 10 m in the spring (Osada et al., 2004; Watanabe et al., 2011). The air temperature, which rarely exceeds freezing from November to April,



generally maintains the frozen condition of the snow cover until early April. Moreover, the snow cover located at the high altitude avoids windblown contamination by regional soil materials. Some previous researches demonstrated that the chemical compounds (Osada et al., 2004; Watanabe et al., 2011) and bioaerosols (Maki et al., 2011; Tanaka et al., 2011) from continental areas were found in the coloured (dirty) layers of snow cover on Mt. Tateyama. Therefore, snow samples
5 that include aerosols from continental areas can be obtained from the snow cover on Mt. Tateyama to analyse the airborne microbial communities relating to ice-nucleation at high altitudes.

This study investigated the ice-nucleation activities of intercontinentally transported aerosols in the snow cover, and identified the airborne bacterial changes relating to ice nucleation. Firstly, we dug a snow wall with a height of 734 cm in the snow cover on Mt. Tateyama in the early spring (April) and collected snow samples, including aerosols, from it. The snow
10 samples were used for estimating the concentrations of chemical components and aerosol particles, using chemical analyses and fluorescence microscopic observations, respectively. The ice-nucleation activities of aerosol particles in the snow samples were also evaluated by water-drop freezing assays. The bacterial community structures in snow samples were determined using MiSeq sequencing analysis using PCR-amplified bacterial 16S ribosomal RNA (rRNA) genes in order to investigate links between ice-nucleation activities and the vertical distribution of bacterial taxa in snow cover.

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2. Materials and Methods

2.1 Snow sampling

The snow samples were collected from the snow cover at Murododaira (36.57N, 137.60E; 2,450 m) on Mt. Tateyama on the 20th April 2013 (Fig. 1). We dug a snow pit from the top of the snow-cover to the ground's surface (743 cm vertical extent)
20 and carefully smoothed the snow wall in the pit to leave the stratigraphy of the snow layers undisturbed. After the surface snow was removed from the snow wall using a sterilized snow sampler (polycarbonate plates: 3 cm × 20 cm × 0.1 cm), a 10 mL sample of snow was collected from a depth of 10 cm from the snow-wall's surface using a new sterilized snow sampler. The snow samples were obtained from each 3 cm layer of the snow wall at lower heights from 164 cm to 200 cm (lower part) and upper heights from 560 cm to 743 cm (upper part). The upper parts frequently included the coloured layers (dirty
25 layers) with natural and/or anthropogenic dust particles, while the lower part mainly composed of white layers (non-coloured layers). A total of 70 snow samples were obtained for use in fluorescence-microscopic observations, water-drop freezing assays (ice-nucleation assay) and DNA sequencing analyses. Alternative snow samples were also collected from each 10 cm layer from the top to the bottom of the snow wall for chemical analyses. The snow samples were preserved at -30 °C, prior to their use in each experiment.



2.2 Environmental factors

The snow samples collected from each 10-cm layer were allowed to melt in a laboratory, and their chemical compositions (anions and cations) were measured using ion chromatography (Dionex, ICS-1600 Thermo Fisher Scientific, Yokohama, Japan) (Watanabe et al., 2012). The values of nss-Ca^{2+} and nss-SO_4^{2-} were calculated from the concentration ratio of Na^+ to Ca^{2+} and SO_4^{2-} , respectively. The accuracy of the measured values was around 5%.

The concentrations of formaldehyde (HCHO) and acetaldehyde (CH_3CHO) were determined using high-performance liquid chromatographic analysis (HPLC, LC-2000Plus, JASCO, Tokyo, Japan) with a fluorescent derivatizing reagent, such as 1, 3-cyclohexanedione (Iwama et al., 2011; Watanabe et al., 2012). The detection limits of HCHO and CH_3CHO were 0.05 and 0.01 $\mu\text{mol kg}^{-1}$, respectively. The 500 μL solution of 70 snow samples collected from each 3-cm layer was fixed with a paraformaldehyde solution at a final concentration of 1 %. The samples were stained with DAPI (4',6-diamino-2-phenylindole) at a final concentration of 0.5 $\mu\text{g mL}^{-1}$ for 15 min and filtered through a 0.22 μm pore-size polycarbonate filter (Millipore, Tokyo, Japan) (Russell et al., 1974). After the filter was placed on a slide on top of a drop of low-fluorescence immersion oil, a drop of oil was added and then covered with a cover slide. Slides were examined using an epifluorescence microscope (Olympus, Tokyo, Japan) with UV excitation system. A filter transect was scanned, and the mineral particles (white particles), organic particles (yellow particles), bacterial cells (blue particles), and black carbon (black particles) on the filter transect were counted. In addition, the filter transect could be discriminated into yellow and white particles in two size categories of $<5 \mu\text{m}$ and $>5 \mu\text{m}$. The depolarization rates and spherical-particle rates measured by the light detection and ranging (LIDAR) system at Toyama, which is located at a distance of 50 km from Mt. Tateyama, were used for evaluating the occurrences of dust events and anthropogenic pollutants, respectively (<http://www-lidar.nies.go.jp/>).

2.3 Water-drop freezing assay

Melted snow samples (70 samples) of each 3-cm layers were passed through sterilized 0.22 μm pore size membrane filters (Millipore, Billerica, MA, USA) and the particulate matter was re-suspended in sterile nano-purewater at a 200-fold concentration. Concentrated samples were diluted to the lowest particulate densities of approximately 5.0×10^4 particles mL^{-1} (from 1.0 $\mu\text{g mL}^{-1}$ to 2.0 $\mu\text{g mL}^{-1}$) using the nano-purewater, and 50 μL of the liquid was aliquoted into each well of 24 wells in a sterile 96-well microplate. For the first assay, 96-wells microplates were placed onto an aluminum plate and the measured temperature was decreased from 0 $^\circ\text{C}$ to $-25 \text{ }^\circ\text{C}$ at a rate of 1.0 $^\circ\text{C min}^{-1}$. For each assay, wells of Arizona test dust (ATD: 2.0 $\mu\text{g mL}^{-1}$) and nano-purewater were prepared as positive and negative controls, respectively. Cumulative IN (Ice Nuclei) concentrations in 1 mL of melted snow at each temperature were calculated using the following equation (Vali, 1971).

$$\text{IN}_T = \frac{\Delta M_0 \ln(N_{\text{total}}) - \ln(N_{\text{unfrozen}})}{V}$$



N_{total} is the total number of tubes (24 wells), N_{unfrozen} is the number of wells still unfrozen (liquid) at each temperature, and V is the volume of melted snow (50 μL). In the present study, the measurable ice-nucleic concentrations in melted snow ranged between 1.74 IN L^{-1} and 49.7 IN L^{-1} .

2.4 High throughput sequencing of bacterial 16S rRNA genes in the snow samples

- 5 The particles in 5 mL melted snow samples collected from 70 layers were pelleted by centrifuging at 20,000 g for 10 minutes and re-suspended into 500 μL of nano-purewater. The re-suspending solutions were 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 rDNA (approximately 290 bp) were amplified from the extracted gDNA by PCR using universal bacterial primers 515F and 806R for the V4 region (Caporaso et al., 2011).
- 10 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., 2016). 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 (nano-
- 15 purewater) contained no fragments of 16S rDNA amplicons showing the absence of artificial contamination. The forward and reverse paired-end reads in the raw sequencing database were merged using USEARCH v.9.0.2132 (Edgar, 2013). After the irregular merged reads (lengths outside 200-500 bp range or exceeding 6 homopolymers) were removed by Mothur v1.36.1 (Schloss et al., 2009), sequences with low Q-scores (>1 expected error) and singleton reads were removed. These sequences were clustered *de novo* (with a minimum identity of 97 %) into operational taxonomic units (OTUs). The representative OTU sequences were identified using the RDP classifier (Wang et al., 2007) implemented in QIIME v9.1.1 (Caporaso et al., 2010). 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 PRJEB24035).

2.5 Quantitative real-time PCR (qRT-PCR)

- A qRT-PCR analysis was employed, following the method of previous workers (Kobayashi et al., 2015a), for investigating
- 25 the relative abundance of bacteria through amplification of their 16S rRNA gene. Standard curves were obtained using ABI 7500 system (ABI, CA, USA), and calibrated by the several dilutions of purified bacterial amplicons. All the standard curves obtained in this way met the required standards of efficiency ($R > 0.99$, $E > 90\%$). Reactions were performed in a 20- μL reaction mixture containing 10 μL of TaqMan Gene Expression Master Mix, 0.8 μL of Primer F20 (10 $\text{pmol } \mu\text{L}^{-1}$), 0.8 μL of Primer R20 (10 $\text{pmol } \mu\text{L}^{-1}$), 0.4 μL of TaqMan probe (10 $\text{pmol } \mu\text{L}^{-1}$), and 2 μL of DNA template (10 ng mL^{-1}), and 6.0 μL
- 30 of nano-purewater. Amplification consisted of initial denaturation at 95 $^{\circ}\text{C}$ for 5 min, then 50 cycles at 95 $^{\circ}\text{C}$ for 15 s, followed by annealing and extension at 59 $^{\circ}\text{C}$ for 60 s. All reaction steps were performed using the ABI 7500 system.



3. Results

3.1 Chemical analysis of snow samples obtained from snow layers

The majority of snow samples recovered from Mt. Tateyama, using the method outlined in Section 2.1, were collected from the snow wall, of which almost layers were composed of compacted snow or solid-type snow. The chemical compounds in the snow samples retained the variations corresponding to the snow layers, meaning that the snow layers would generally retain their original chemical and isotopic composition from the time of the snowfall (Fig. 2). Some coloured layers (dirty layers) that appeared brown-yellow or dark brown could be observed in four parts of the snow wall, at the heights ranging from 599 cm to 620 cm, from 626 cm to 644 cm, from 650 cm to 662 cm, and from 716 cm to 734 cm. The solutions of snow samples from the dirty layers contained higher concentrations of nss-Ca^{2+} , which is a tracer of dust mineral particles from Asian deserts, than non-coloured layers in the lower parts of snow wall. The nss-Ca^{2+} peaks at each of the four dirty layers had maximum concentrations of $25.4 \mu\text{eq L}^{-1}$, $47.9 \mu\text{eq L}^{-1}$, $25.4 \mu\text{eq L}^{-1}$, and $31.4 \mu\text{eq L}^{-1}$, respectively (Fig. 2). The deporalization ratio of LIDAR measurements also indicated the four series of Asian-dust events that occurred from 12 to 21 February, from 26 February to 13 March, from 18 to 26 March and from 4 to 19 April in 2013 over the northwest seashore of Japanese main-island (Fig. 3, Fig. S1). The air back-trajectory analyses indicated that, during these periods, air masses came from the desert areas of the Asian continent (Fig. S2). In addition, the concentrations of NO_3^- , SO_4^{2-} and acetaldehyde, which are originated from the anthropogenic pollutions in the continental coastal area, also trended to increase in the snow samples collected from the the dirty layers.

In contrast, the concentrations of formaldehyde photo-synthesized from plant products fluctuated in the range of $0.05 \mu\text{mol kg}^{-1}$ to $0.60 \mu\text{mol kg}^{-1}$, regardless of whether the samples were collected from non-coloured of dirty layers. Relatively constant high concentrations of around $0.45 \mu\text{mol kg}^{-1}$ were observed in samples from the lower parts (from 167 cm to 200 cm) of the snow wall. The concentrations of Na^+ , which primarily came from sea salt, increased in the snow samples in the two layers below the dirty layers (from 695 cm to 710 cm and from 584 cm to 589 cm) and in the low parts (from 167 cm to 200 cm) and ranged from $14.9 \mu\text{eq L}^{-1}$ to $28.9 \mu\text{eq L}^{-1}$. LIDAR measurements showed low ratios of deporalization and an attenuated backscatter coefficient before the middle of February 2013, indicating a lack of dust events during winter, when the snow cover from 167 cm to 200 cm formed (Fig. S1). The air back-trajectories before the middle of February 2013 frequently came from Siberia (Russia), North Asia, and the Sea of Japan, and remained over mountains and marine areas for longer periods, than those of April and March (Fig. S2).

Mineral particles, yellow particles and bacterial particles were observed in the solutions of snow samples under fluorescent microscopic observation, using the DAPI staining technique. The total densities of DAPI-fluorescent particles increased in the snow samples of the four dirty layers and exhibited the peaks ranging from 6.33×10^6 to 4.12×10^7 particles mL^{-1} (Fig. 4). In particular, yellow particles and bacterial particles in the snow samples from the two dirty layers had significantly higher densities, of the order of 10^7 particles mL^{-1} . Black particles were frequently detected, regardless of whether a sample had been collected from a dirty layer, and the densities fluctuated by approximately 1.00×10^5 particles mL^{-1} . Bacterial



densities determined using qRT-PCR gradually increased from the lower parts to the upper parts of the snow wall and exhibited some peaks of the order of 10^5 copies mL^{-1} in the dirty layers.

3.2 Ice-nucleation activities determined using water-drop freezing assay

Ice-nucleation assay using some of snow samples collected from the upper parts of the snow wall showed a higher freezing temperature of water drops than those from the lower parts of the snow wall (Fig. 5a). In particular, the snow samples of dirty layers (from 599 cm to 620 cm, from 626 cm to 644 cm, from 650 cm to 662 cm, and from 716 cm to 734 cm) indicated high freezing temperatures at more than -12 °C. The freezing temperatures detecting the half concentrations of ice-nuclei particles (IN-T50C) increased in the snow samples that include high amounts of fluorescent particles (Fig. 5b). Additionally, the freezing temperatures of snow samples increased significantly in relation to the higher densities of fluorescent particles ($P < 0.001$) (Table 1). This means that the snow samples including dust and microbial particles have high activities of ice nucleation.

3.3 Analyses of prokaryote community structures

For the analysis of bacterial compositions in the snow samples, we obtained a total of 17,676,926 reads. Following quality filtering 6,367,300 merged paired-end sequences with a median length of 292 bp remained. The sequences of 16S rRNA gene were divided into 1,451 phylotypes (sequences with $>97\%$ similarity). Phylogenetic assignment of sequences resulted in an overall diversity comprising 33 phyla, (and candidate divisions), 73 classes (and class-level candidate taxa), and 179 families (and family-level candidate taxa). Most of the phylotypes recovered from the air samples were related to the phyla Cyanobacteria, Actinobacteria, Firmicutes (Bacilli, Clostridia), Bacteroidetes and Proteobacteria (Alpha-, Beta- and Gamma-proteobacteria), which are typically well represented in 16S rRNA genes sequencing database generated from terrestrial, marine, freshwater, and phyllospheric environments (Fig. 6). For the PCR-analysis steps, negative controls (no template and template from unused filters) did not contain the amplicons of 16S rRNA genes demonstrating the absence of artificial contamination during the experimental processes.

The bacterial species numbers estimated by Chao 1 increased as the snow samples were collected from the upper parts of snow wall (Fig. 7). The Chao 1 values were higher in the snow samples of dirty layers than those of non-coloured layers ($P < 0.05$). On a non-metric multidimensional scaling plot with weighted UniFrac distances, the snow samples can be categorized into three clusters, corresponding to the heights of snow wall; the samples of lower parts (from 167 cm to 200 cm); the samples from 563 cm to 668 cm; and the samples from 671 cm to 734 cm (Fig. 8), indicating the variations of bacteria with respect to the height of the snow cover above the base. The snow samples from the three dirty layers (February, March) overlapped each other in the cluster of samples from 563 cm to 668 cm, while those of the other dirty layer (April) formed a different cluster of samples from 671 cm to 734 cm. The bacterial community structures in the clusters started at low parts of snow wall, moved to other areas on coordinate, and then returned to original areas again, resulting in a "rotation" on the



coordinate (indicated by arrows in Fig. 8). These results imply that the dust events changed the bacterial structures in the snow samples and that the temporary changes are different between February and April.

3.4 Distribution of prokaryote community structures in snow covers

Firmicutes (Bacilli) sequences, mainly belonging to the family Bacillaceae and Staphylococcaceae (>99.7 % similarity), dominated the snow samples of dirty layers, at relative abundances ranging from 25.0 % to 89.8 %. The relative abundances of Firmicutes (Bacilli) sequences showed positive relations to the white-particle densities in snow sample, as well as ice-nucleation activities ($P < 0.01$) (Table 2). The Alpha and Betaproteobacteria sequences maintained high relative abundances ranging from 32.0 % to 83.9 % in the lower parts of snow wall (lower than 590 cm: the winter season accumulation) and negatively related to the white-particle densities and the ice-nucleation activities. In Proteobacteria sequences, the relative abundances of Oxalobacteraceae and Sphingomonadaceae sequences predominantly increased in the snow samples from the lower parts and showed significant positive relations to the Na^+ concentrations ($P < 0.01$). The Gamma-proteobacteria sequences also did not relate to each environmental factor. The Bacteroidetes sequences increased to more than 20 % at the heights from 668 cm to 683 cm, which included non-coloured layers, and did not indicate any relationships with environmental factors. Actinobacteria sequences slightly increased to low relative abundances in the lower parts of snow wall and, in particular, Propionibacteriaceae sequences correlated positively to black-particle densities ($P < 0.05$). The concentrations of formaldehyde changed from $0.05 \mu\text{mol kg}^{-1}$ to $0.60 \mu\text{mol kg}^{-1}$ independent of the dirty layers, and maintained relatively high values at low layers from 164 cm to 200 cm. The relative abundances of Firmicutes sequences are positively related to the acetaldehyde concentrations, while they have negative relations to the formaldehyde concentrations. On the other hand, some members of Proteobacteria showed positive relations to the formaldehyde concentrations.

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4. Discussion

4.1 Vertical distributions of chemical compounds and particles in snow covers

The aerosols, which are transported from Asian continent to to Japan by the westerly wind, accumulate in the snow cover on Mt. Tateyama from fall to spring (Osada et al., 2004). Four dirty layers were found in the snow covers accumulated during spring (the upper parts of snow wall), and contained higher concentrations of nss-Ca^{2+} than non-coloured layers in the lower parts of snow wall (Fig. 2). Highly alkaline Ca is a tracer of mineral dusts from deserts and loess deposits in China (Suzuki and Tsunogai 1993). The depolarization rates of LIDAR measurements supported four series of dust events over Mt. Tateyama (Fig. 3), which correspond to the four dirty layers found in the snow wall. The distributions of particle densities in the snow samples demonstrated the preservation of the dust mineral particles in the snow covers of Mt. Tateyama (Fig. 4). The snow-cover layers were composed of compacted snow, indicating that the snow samples maintained the records on atmospheric aerosols that were represent during deposition, having avoided melting.

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Furthermore, the increases of NO_3^- and SO_4^{2-} concentrations in the dirty snow layers suggest the long-range transport of anthropogenic pollutants from Asian continental coastal areas. The chemical analyses in previous studies also detected anthropogenic pollutants in the snow cover of Mt. Tateyama. The snow samples of dirty layers significantly included high concentrations of acetaldehyde, which would have been synthesized from organic pollutants (Watanabe et al., 2012). The natural dust events from desert areas accumulate anthropogenic pollutants across the industrial areas during long-range transport processes (Huang et al., 2015a). The dirty layers in snow wall would be formed during the four series of dust events during spring (March and April) and include intercontinentally transported aerosols that originated from the desert areas and industrial coasts of the Asian continent.

In contrast, the concentrations of formaldehyde increased in the lower parts of snow wall. The air back-trajectories suggested that the aerosols recorded in the lower parts frequently came from the mountainous (Siberia of Russia, North Asia) and maritime (Japanese Sea) areas (Fig. S2). Airborne formaldehyde is not only synthesized by anthropogenic pollutants (Watanabe et al., 2012) but also photosynthesized from plant products, such as isoprene (Claeys et al., 2004; Guenther et al., 2006). Formaldehyde detected in the lower parts of the snow wall was possibly transported from the mountains. The level of Na^+ of marine origin also increased in the lower parts, suggesting the contamination by sea salts over the Japanese Sea. Consequently, we inferred that the aerosols recorded in the lower parts of snow wall mainly came from Siberia (Russia), North Asia, and the Japanese Sea, while those of the upper parts, which frequently included Asian dust particles, originate from the desert regions and industrial coasts of the Asian continent.

The microbial particles and yellow fluorescence particles increased in the dirty snow layer (Fig. 4). Asian dust events have been reported to carry the airborne microorganism associated with natural mineral particles (Hara and Zhang, 2012) and anthropogenic particles on hazy days (Wei et al., 2016), leading to an increase in the microbial biomasses in the downwind areas (Maki et al., 2014). The yellow fluorescence particles are organic materials that are interpreted to originate from dead microbial cells (Mostajir et al., 1995; Liu et al., 2014). Hara and Zhang (2012) reported that dust events in Kyushu, Japan, increased the ratio of damaged microbial cells in airborne microbial communities. Microbial cells transported by dust events would be exposed to environmental stressors throughout atmosphere, increasing the number of damaged and dead cells in the dirty layers.

4.2 Ice-nucleation activities of snow samples in snow covers

Ice-nucleation activities increased in the snow samples of the dirty layers and were positively related to the microbial cell densities in the snow samples (Table 1). Dust mineral particles without organic matters, such as ATD, showed lower temperatures (less than -15°C) for the initial freezing of water drops than snow samples of the dirty layers. Organic aerosols in the natural atmosphere are frequently reported to have higher activities of ice nucleation than inorganic particles (Hoose and Möhler, 2012; Murray et al., 2012). During the spring season, airborne microorganisms associated with Asian dust events possibly increase the ice-nucleation activities in the atmosphere and thus the amount of snow falling over Mt. Tateyama. For the investigation of the detailed characteristics of bacterial ice nucleation, a total 11 isolates were obtained



from the snow samples and their ice-nucleation activities were estimated using the water-drop freezing assay (Fig. S3). These bacterial isolates showed lower activities of ices nucleation than the snow samples of the dirty layers. In general, culturable microorganisms occupied 1%-10% of the environmental microbial communities (Olsen and Bakken 1987). Accordingly, the unculturable bacteria are expected to activate the majority of ice nucleation in the snow samples of dirty
5 layers.

4.3 Vertical distribution of bacterial compositions in snow covers

The bacterial populations in the snow samples (70 samples) mainly belonged to the phyla Cyanobacteria, Actinobacteria, Firmicutes (Bacilli), Bacteroidetes and Proteobacteria, showing higher diversity in the dirty snow layers than those of other snow layers (Fig. 6a). Asian dust events dynamically change bacterial community structures at high altitudes ranging from
10 200 m to 3,000 m above the ground (Jeon et al., 2011; Maki et al., 2013). The proportion of prokaryotes in snow samples varied from the low parts (winter) to the upper parts (spring) of the snow wall, and significantly differed between the dirty layers and the non-coloured layers (Fig. 7). The bacterial diversities of Chao 1 gradually increased towards the upper parts of the snow wall and were particularly high in the dirty layers (Fig. 7a). During the spring season, the variations in the taxonomic composition (terrestrial, marine or plant-associated bacteria) of the airborne bacterial populations that
15 accumulated on snow cover would correspond to the heterogeneous mixtures of dust events responsible for transporting the bacteria. Presumably, the airborne bacterial compositions in snow covers at Mt. Tateyama are influenced by the intercontinentally transported aerosols.

The relative abundances of Bacillaceae sequences showed a significant positive relationship with the ice-nucleation activities of the snow samples, while Proteobacteria showed a negative correlation to the ice-nucleation activities (Table 2). Some
20 isolates of Bacilli obtained from cloud waters were confirmed to activate ice nucleation and Bacilli members have been focused on ice nuclear agents (Matulova et al., 2014; Mortazavi et al., 2015). Several members of Proteobacteria isolated from plant bodies showed high activities of ice-nucleation activities in laboratory experiments (Morris et al., 2004). Ice-nucleating bacteria in Proteobacteria would be in a minority among the bacterial populations in atmosphere.

4.4 Bacterial compositions in the dirty snow layers

25 The Bacillaceae and Cytophagaceae sequences were significantly dominant in the snow samples collected from the dirty layers significantly (Fig. 6) and their relative abundances positively correlated to the white-particle densities and the acetaldehyde concentrations (Table 2). Airborne acetaldehyde was reported to be photo-synthesized from the pollutant organic materials, which are irradiated by sunlight in atmosphere (Watanabe et al., 2012). Bacillaceae members were the dominant populations at high altitudes above the Taklimakan Desert (Maki et al., 2008) and Asian downwind areas during
30 dust events (Korea: Jeon et al., 2011; Japan: Maki et al., 2014). The endospore forming bacteria, such as members of family Bacillaceae, could maintain their survival for long-distance transit, because of their resistance to UV irradiation and desiccations in the atmosphere (Kobayashi et al., 2015b). The Bacteroidetes sequences including Cytophagaceae were also



often detected from the aerosols sampled at high altitudes during Asian dust events (Maki et al, 2013, 2015). Since Cytophagaceae members trend to aggregate with the organic particles in terrestrial and aquatic environments (Newton et al., 2011), the bacterial cells involved in aggregations would be protected against atmospheric stressors. Long-range transportation would select the stressor-resisting bacteria among several bacterial communities associated with dust events.

5 4.5 Bacterial compositions in the non-coloured snow layers

The relative abundances of Actinobacteria sequences appeared randomly at low rates in the snow samples (Fig. 6). Actinobacteria group (in particular Propionibacteriaceae) have been primarily detected from anthropogenic particles collected in Beijing, China (Cao et al., 2014). Natural dust particles from Asian desert areas are mixed vertically with anthropogenic pollutants over the Asian continental coasts (Huang et al., 2015a). However, the non-spore forming bacteria, such as Propionibacteriaceae members, would be damaged by atmospheric stressors and few remaining populations could arrive in Japan continuously during the winter and spring seasons. Long-range transportation would select some bacterial populations among several terrestrial bacteria associated with dust particles that originated from the central desert area in Asia or agriculture field in continental anthropogenic areas.

The snow samples collected from the lower parts of snow wall (winter-season accumulation) predominantly contained Proteobacteria sequences (Fig. 6), which were related to the dominant bacterial populations in the phyllosphere (Redford et al., 2010; Fierer and Lennon, 2011) or freshwater environments (Nold and Zwart, 1998). Proteobacteria members were frequently detected from the air samples collected over mountains (Bowers et al., 2012) or over the Noto Peninsula during periods when north-western wind was blowing (Maki et al., 2010). These results suggest that some populations of Proteobacteria on Mt. Tateyama originated from the forest, river or lake areas of Siberia (Russia) or North Asia. The Alphaproteobacteria (in particular Sphingomonadaceae) (Cavicchioli et al., 2003), which predominately occupy marine bacterial communities, were also detected in the lower parts of snow wall (Fig. 6). The dust particles transported across the Sea of Japan would be mixed with marine bacterial populations as well as sea salts (Zhang et al., 2006), thereby contributing to the marine bacterial transportation to Mt. Tateyama. Marine bacteria are frequently prevalent in the troposphere when strong winds blow from sea areas (DeLeon-Rodriguez et al., 2013; Polymenakou et al., 2008; Maki et al., 2017).

25 5. Conclusion

The sequential changes of airborne bacteria from winter to spring have been investigated using aerosols that have been transported for long-distances and preserved in snow cover; their relations to ice-nucleation activities of snow samples were also evaluated. During the winter season, the northern-westerly wind would transport members of the phylum Proteobacteria, which are expected to originate from phyllosphere, freshwater in mountainous area, or marine environments. The intercontinental dust events during spring would carry the terrestrial bacteria of Bacilli (Bacillaceae) and Bacteroidetes (Cytophagaceae) from the Asian continent to Mt. Tateyama, while other terrestrial bacteria of Actinobacteria mainly disappear across the Sea of Japan. The Asian dust-associated bacteria, such as Bacilli, showed the positive relations to ice-



nucleation activities in snow samples. Since the ice-nucleation activities of bacterial isolates from snow samples are lower than those of snow samples, unculturable bacteria in the snow samples are expected to be responsible for high levels of ice-nucleation activities. Airborne microorganisms suspended over Japanese islands might act as ice nuclei supporting the heavy snow falling over Mt. Tateyama during spring season. In the future, the combination of sequencing analysis, with
5 physiological experiments targeting bacterial cultures, and metagenome analysis, targeting functional genes, would support to elucidate airborne bacterial influences on climate changes and human societies.

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Table 1. Relatives of the DAPI-fluorescent particles with ice-nucleation activities and some of chemical components

DAPI-fluorescent particles	Ice-nucleation activities**			Chemical components			
	Initial temp.	End temp.	IN-T50C	Formaldehyde	Acetaldehyde	Na ⁺	nss-Ca ²⁺
Yellow ≥5 μm	0.34†	0.48††	0.54††	-0.13	0.40††	-0.23	0.31†
Yellow <5 μm	0.47††	0.65††	0.69††	-0.06	0.42††	-0.34†	0.44††
White ≥5 μm	0.49††	0.59††	0.69††	-0.12	0.50††	-0.40††	0.39††
White <5 μm	0.38††	0.51††	0.57††	-0.05	0.39††	-0.34†	0.42††
Blue	0.43††	0.55††	0.62††	-0.04	0.35††	-0.41††	0.42††
Black	0.13	0.36††	0.32†	0.08	0.11	0.00	0.26

* The marks †† indicates P<0.001 and the mark † indicates P<0.005. Among the marked values, red cells indicate positive relations.

** Initial temp., End temp., and IN-T50C indicate the initial-freezing temperatures of water drops, the end-freezing temperature detecting 50% of IN particle concentrations, respectively.

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Table 2. Relatives of the relative abundances of representative bacterial categories with environmental factors.*

Bacterial categories	Fluorescent particle concentrations					Chemical components										IN-T50C ^{††}	Heights ^{***}
	Yellow µm	Yellow ≤5 µm	White µm	White µm	Blue µm	Black µm	Form- aldehyde	Acet- aldehyde	Na ⁺	nss-Ca ²⁺	NI ⁺	NI ⁺	NI ⁺	nss-SO ₄ ²⁻			
Cyanobacteria	0.03	-0.12	-0.10	-0.17	-0.15	0.19	-0.01	-0.10	-0.08	0.06	0.12	0.08	0.14	0.08	0.14	-0.10	0.06
Actinobacteria	-0.13	-0.23	-0.32††	-0.34††	-0.26	0.10	0.16	-0.36††	0.16	-0.07	0.00	-0.01	-0.34†	0.09	-0.24	0.35†	0.58††
Firmicutes (Bacilli)	0.27††	0.33††	0.43††	0.32††	0.36††	-0.01	-0.22††	0.43††	-0.44††	0.20	0.33††	0.19	0.32†	0.32†	0.35†	0.06	0.11
Firmicutes (Clostridia)	-0.03	0.16	0.12	0.14	0.13	-0.28††	0.07	-0.12	-0.03	-0.12	0.07	0.03	-0.05	-0.20	-0.18	0.24	0.24
Bacteroidetes	-0.05	0.00	-0.01	0.05	-0.05	-0.35††	-0.16	-0.09	-0.25†	-0.19	-0.22†	-0.20	-0.22	-0.18	-0.15	-0.57††	-0.75††
Alphaproteobacteria	-0.15	-0.27††	-0.33††	-0.28††	-0.25†	0.20	0.18	-0.27††	0.42††	-0.13	-0.24†	-0.18	-0.23	-0.18	-0.25	-0.75††	-0.75††
Betaproteobacteria	-0.26†	-0.42††	-0.44††	-0.42††	-0.41††	0.06	0.35††	-0.41††	0.54††	-0.31††	-0.31††	-0.18	-0.30†	-0.18	-0.25	-0.75††	-0.75††
Gammaaproteobacteria	0.06	0.05	0.15	0.05	0.07	0.07	0.03	0.05	-0.04	-0.01	0.19	0.16	0.13	0.02	0.02	0.22	0.22
Bacterial others	0.03	0.23†	0.09	0.31††	0.19	0.06	-0.04	0.29††	-0.10	0.47††	-0.03	0.08	0.02	0.02	0.07	0.16	0.16
Micrococcales	-0.23†	-0.34††	-0.37††	-0.35††	-0.29††	-0.05	0.21†	-0.43††	-0.30††	-0.29††	-0.29††	-0.29	-0.27	-0.27	-0.24	-0.64††	-0.64††
Propionibacteriaceae	0.05	0.05	-0.01	0.03	0.10	0.24†	-0.03	-0.05	-0.37††	0.21	0.24†	0.22	0.44††	0.18	0.29	0.35†	0.35†
Bacillaceae	0.16	0.17	0.31††	0.15	0.20	-0.16	-0.22††	0.49††	-0.31††	0.18	0.19	0.18	0.18	0.18	0.29	0.35†	0.35†
Aerococcales	0.09	0.14	0.06	0.13	0.21†	-0.20	0.00	-0.08	-0.14	0.01	0.16	0.04	0.12	0.02	0.02	0.22	0.22
Staphylococcales	0.15	0.20	0.18	0.21†	0.22†	0.15	-0.08	0.03	-0.20	0.04	0.17	0.02	0.17	0.13	0.13	0.29	0.29
Cytophagaceae	0.04	0.15	0.00	0.16	0.10	-0.27†	-0.21	-0.17	-0.26†	-0.18	-0.20	-0.18	-0.18	-0.16	-0.16	0.13	0.13
Chitinophagaceae	-0.18	-0.18	-0.11	-0.16	-0.17	-0.07	-0.23†	-0.06	-0.11	-0.21	-0.24†	-0.17	-0.21	-0.27	-0.27	0.06	0.06
Sphingomonadaceae	-0.26†	-0.37††	-0.38††	-0.36††	-0.34††	0.05	0.17	-0.33††	0.39††	-0.22	-0.27†	-0.26	-0.30†	-0.20	-0.20	-0.59††	-0.59††
Comamonadaceae	0.06	0.01	0.06	0.00	-0.02	0.06	0.07	0.00	0.05	-0.15	0.01	0.12	-0.02	-0.02	-0.09	0.16	0.16
Ovalobacteraceae	-0.34††	-0.45††	-0.51††	-0.41††	-0.42††	-0.02	0.33††	-0.42††	0.56††	-0.28	-0.34††	-0.26	-0.33†	-0.24	-0.24	-0.87††	-0.87††
Xanthomonadaceae	-0.10	0.01	0.07	0.07	0.09	-0.02	0.03	-0.01	-0.02	-0.05	0.08	0.00	0.04	0.03	0.03	0.06	0.06
Pseudomonadaceae	0.18	0.00	0.02	-0.08	-0.10	0.17	0.01	-0.05	0.10	-0.07	0.03	0.04	0.03	-0.08	-0.08	0.04	0.04

* The marks †† indicates P<0.01 and the mark † indicates P<0.05. Among the marked values, red cells indicate positive relations and blue cells indicate negative relations.
 ** The temperature indicating the 50% of concentrations of ice-nuclei particles.
 *** The heights (cm) of snow wall, from which snow samples were collected.

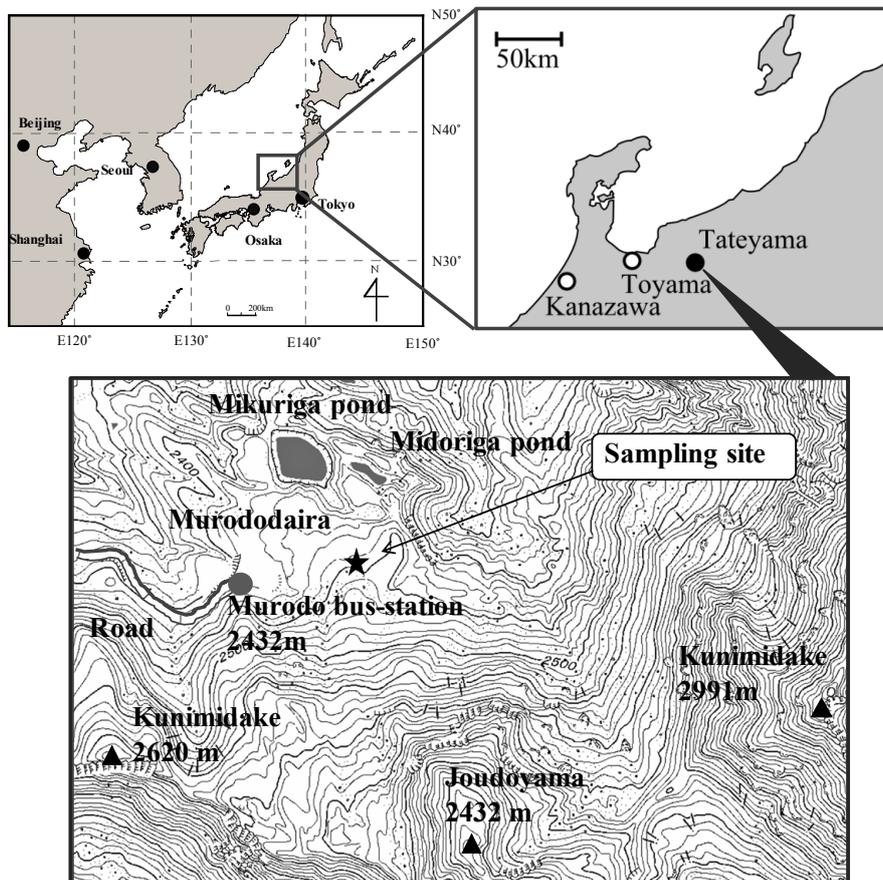


Figure 1: Map of Mt. Tateyama in Japan showing the sampling site: Murododaira (2,450 m, MR).

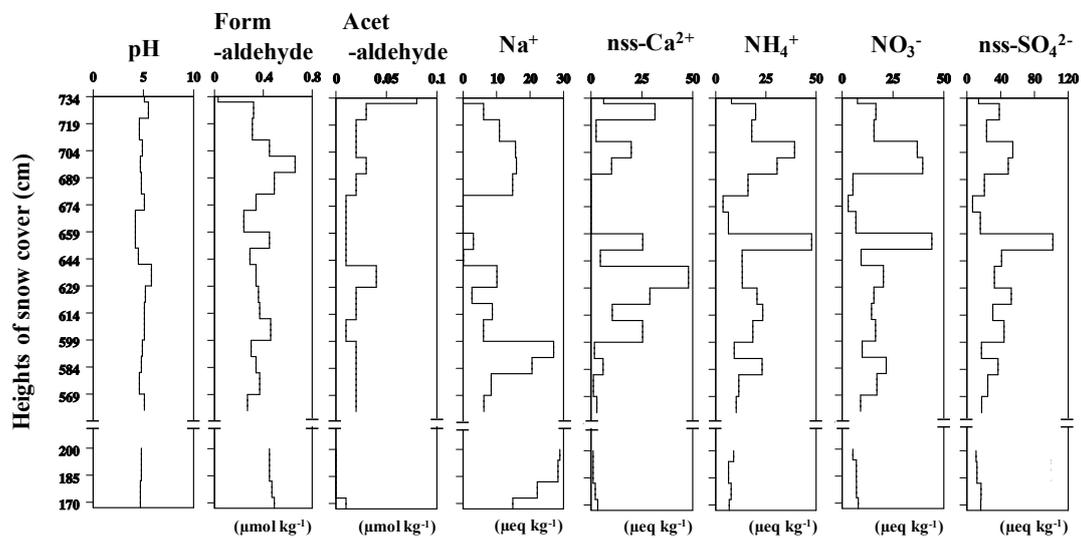


Figure 2: Vertical profiles for pH, formaldehyde, acetaldehyde and ionic concentration levels in snow samples collected from Murododaira, Mt. Tateyama, in April 2013.

5

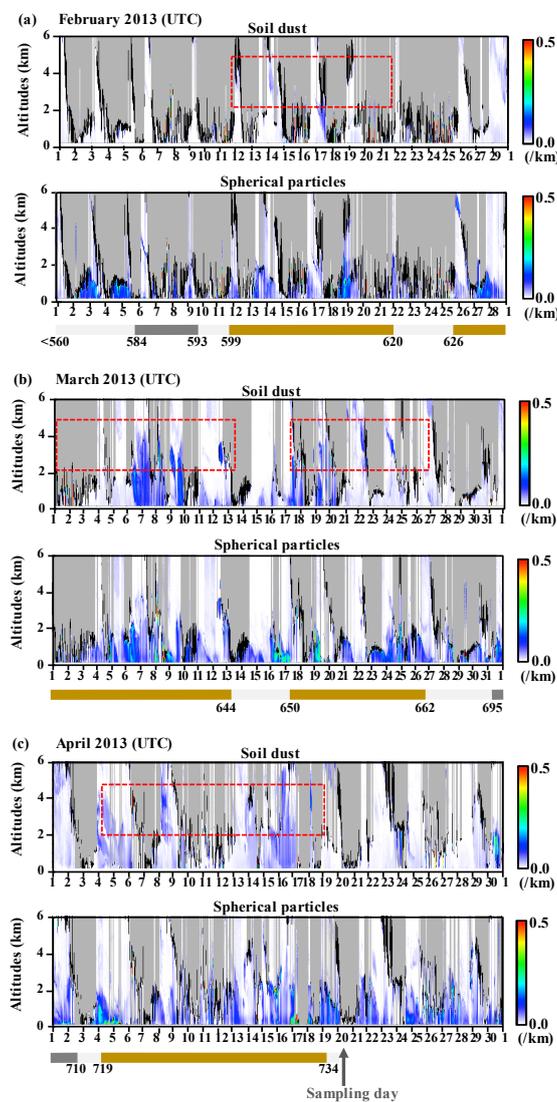
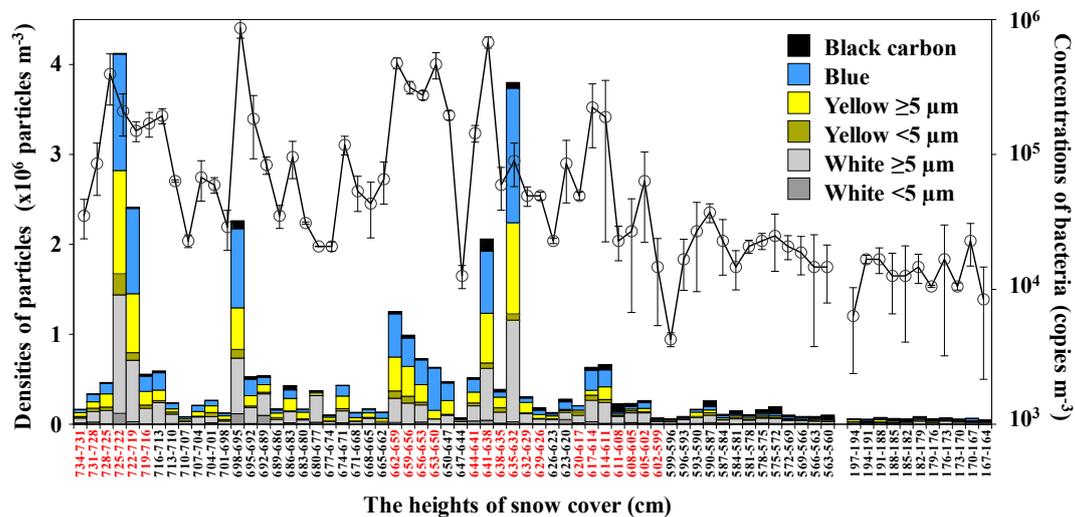
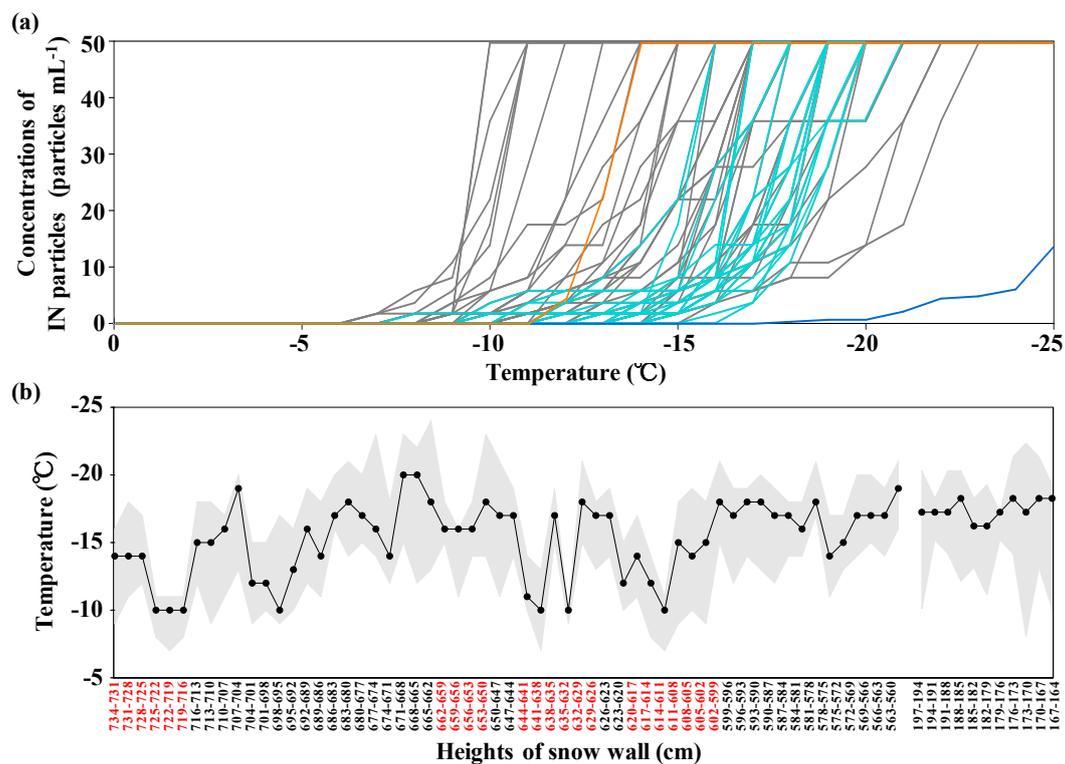


Figure 3: LIDAR observation of soil dust (depolarization ratio) and spherical particles (ratio of attenuated backscatter coefficient) in Toyama in February (a), March (b) and April (c) of 2013. Red dotted lines indicate the occurrences of Asian mineral dust events. Bars of light grey, dark grey and yellow below each graphics showed normal days, anthropogenic pollutant days and Asian mineral dust days, respectively, that were referred by the heights of snow wall of Murododaira, Mt. Tateyama, in April 2013.

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5 Figure 4: Vertical profiles for DAPI-stained particles densities (bars) and 16S rRNA genes copies determined by qRT-PCR (open circles), in snow samples collected from Murododaira, Mt. Tateyama, in April 2013. DAPI-stained particles were classified as white particles at the sizes of $\geq 5 \mu\text{m}$ (dark grey bars) and $< 5 \mu\text{m}$ (light grey bars), yellow fluorescent particles at the sizes of $\geq 5 \mu\text{m}$ (dark yellow bars), and $< 5 \mu\text{m}$ (light yellow bars), microbial particles (blue bars), or black carbon (black bars). Red letters of height numbers indicate the snow samples of dirty layers.



5 Figure 5: (a) Variations of ice-nuclei concentrations in the snow samples collected from the upper (grey lines) and lower (green lines) parts of the snow wall at Murododaira, Mt. Tateyama, in April 2013. Orange and blue lines indicate the experiments using ATD and nano-purewater, respectively. (b) Vertical profiles for the initial freezing temperatures of water drops (the top of the grey areas), the end-freezing temperature of water drops (the bottom of the grey areas), and the temperatures detecting 50 % of ice-nuclei concentrations (IN-T50C) (black circles), respectively, in the snow samples. Red letters of height numbers indicate the snow samples of dirty layers.

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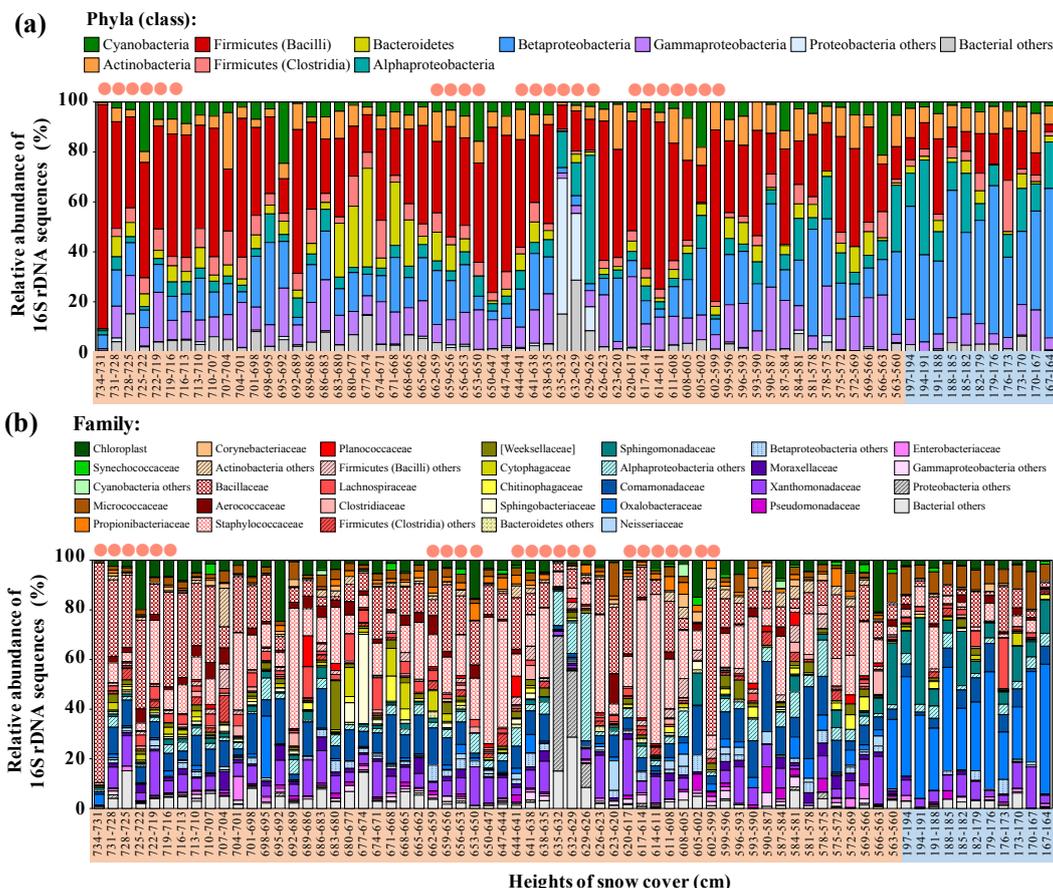


Figure 6: Vertical profiles for bacterial compositions at (a) the class level and (b) the family level of the partial sequences obtained in the MiSeq sequencing database (ca. 400 bp) from snow samples collected from Murododaira, Mt. Tateyama, in April 2013.

5 Orange circles indicate the snow samples of dirty layers.

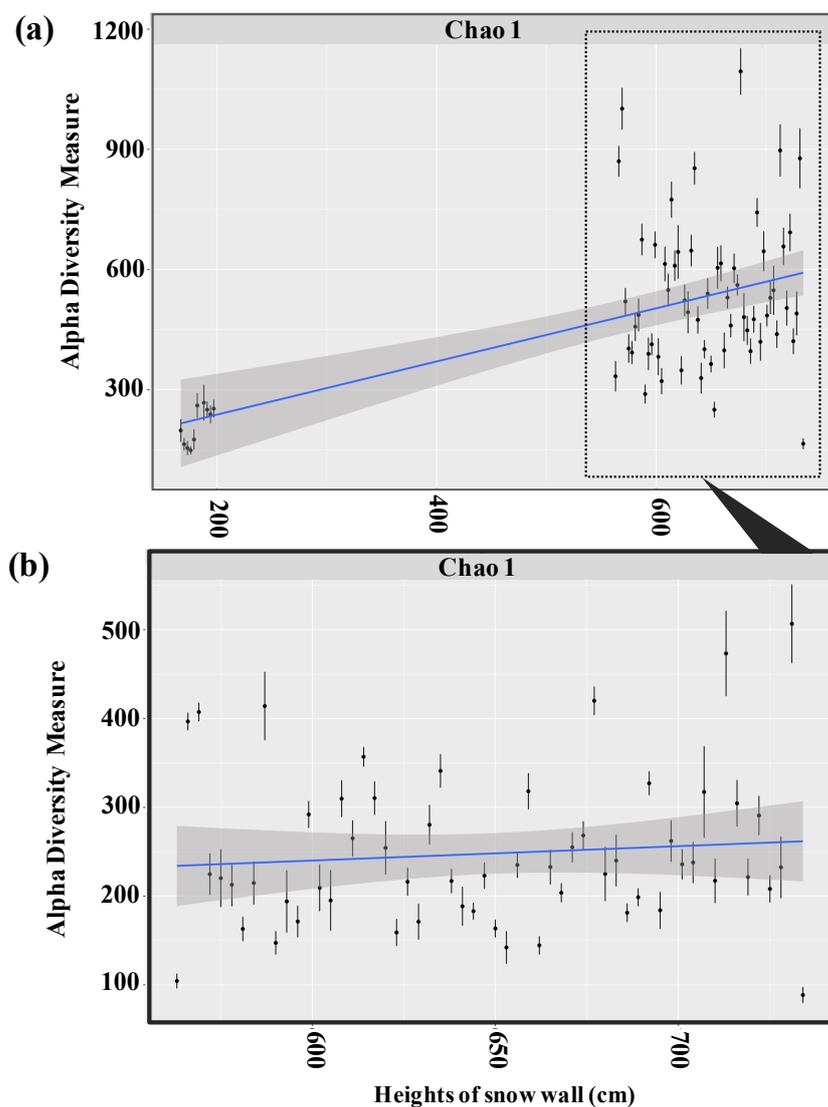


Figure 7: Changes in the bacterial diversity observed in the snow samples collected from snow wall at the heights from 164 cm to 734 cm (a) and from the partial heights from 560 cm to 734 cm (b) in Murododaira, Mt. Tateyama, in April 2013. Species were binned at the 97 % sequence similarity level.

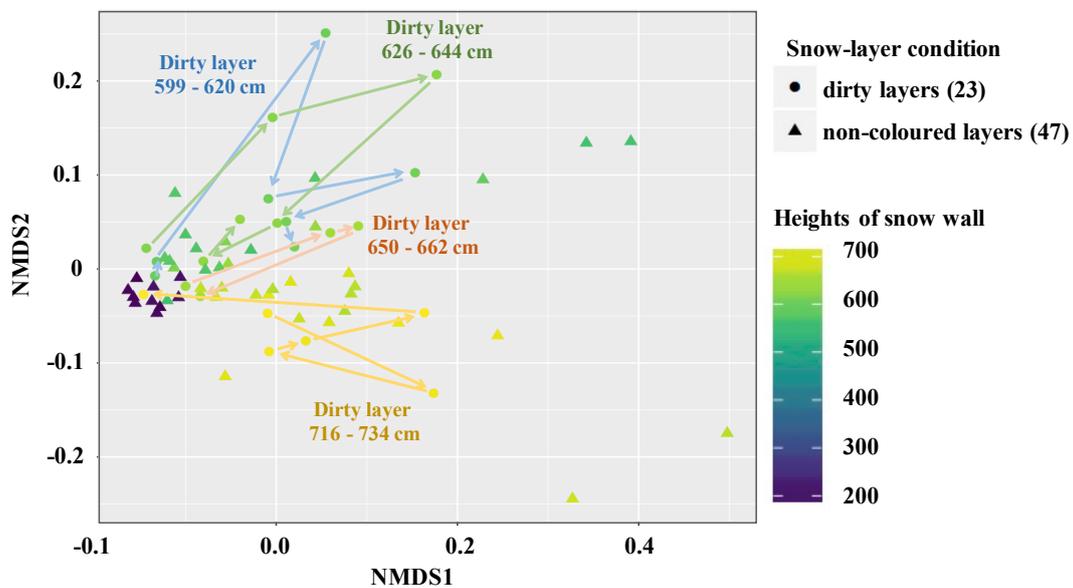


Figure 8: Principal coordinates analysis of Bray Curtis distance matrix displaying phylogenetic clustering by the snow samples collected from Murododaira, Mt. Tateyama, in April 2013. Arrows of four colours indicate the shifts of bacterial compositions in snow samples collected from the four dirty layers, respectively.