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**Key Points:**
- First study on hydroxy fatty acids (FAs) in snow pit samples
- High concentrations of hydroxy FAs in snowpack are consistent with occurrence of abundant $Ca^{2+}$
- Below-cloud scavenging by snowflakes of transit dust particles and associated soil microbes in the East Asian outflow

**Supporting Information:**
- Supporting Information S1
- Supporting Information S2
- Supporting Information S3
- Supporting Information S4
- Supporting Information S5
- Supporting Information S6
- Supporting Information S7

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# Hydroxy fatty acids in snow pit samples from Mount Tateyama in central Japan: Implications for atmospheric transport of microorganisms and plant waxes associated with Asian dust

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**Abstract** We report here the source apportionment of atmospheric soil microorganisms and higher plant metabolites based on chemical markers (hydroxy fatty acids: FAs) in the snowpack samples collected from Mount Tateyama in central Japan during spring 2009 ($N = 6$) and 2011 ($N = 7$). A homologous series of $\beta$-hydroxy FAs ($C_9$–$C_{20}$), constituents of Gram-negative bacteria (GNB), in snowpacks clearly suggest a long-range atmospheric transport of dust-associated bacteria followed by scavenging by snowflakes. Similarly, higher atmospheric abundances of $\alpha$-($C_{16}$–$C_{32}$) and $\omega$-($C_9$–$C_{30}$)-hydroxy FAs in the snow layers containing Asian dust revealed contributions from soil microbes and higher plant epicuticular waxes. Moreover, covariation between the concentrations of hydroxy FAs and water-soluble $Ca^{2+}$ (dust tracer), together with calculated air mass backward trajectories, demonstrated their source regions such as the Taklamakan Desert, Gobi Desert, and Loess Plateau. A close match of molecular distributions of hydroxy FAs (with the predominance of $\omega$- and $\beta$-isomers) is noteworthy between snowpack (present study) and springtime aerosols from Chichijima Island in the western North Pacific (WNP). This observation suggests a "below-cloud scavenging" of transported dust particles and associated soil microbes in the East Asian outflow by snowflakes. These distributions are, however, contrary to those observed in the fresh snow samples from Sapporo, northern Japan (predominance of $\alpha$-hydroxy FAs), which could be explained by "in-cloud" microbial oxidation processes. This comparison, therefore, provides additional insights regarding the aeolian transport of soil microbes in the East Asian outflow to the WNP, which has not been available.

## 1. Introduction

Several billion tons of Asian dusts are annually transported to Japan from the Taklamakan and Gobi Deserts and Loess Plateau [*Yamaguchi et al.*, 2014]. These dust particles carry microorganisms to long distances, possibly affecting human health and ecosystems [*Griffin*, 2007]. Molecular and culture-based techniques have been employed to identify the bacterial communities in soil surfaces [*Hua et al.*, 2007] and snow layers in Japan [*Yamaguchi et al.*, 2014]. However, among culture-independent techniques, the chemical marker-based approach is considered as a suitable tool for the rapid and comparative analysis of bacterial dry biomass and their endotoxins in a variety of environmental samples. Furthermore, our previous studies showed that atmospheric hydroxy fatty acids (FAs) can be used as chemical markers to trace soil microbes and higher plant metabolites in aerosol and snow samples [*Tyagi et al.*, 2015a, 2015b]. These results indicate that higher atmospheric abundances of hydroxy FAs are associated with Asian dust episodes.

The $\alpha$-, $\beta$-, and $\omega$-isomers of hydroxy FAs ($<C_{18}$) have been studied in environmental samples such as sediments [*Kawamura and Ishiwatari*, 1981, 1982; *Ratledge and Wilkinson*, 1988; *Wakeham et al.*, 2003], marine aerosols [*Kawamura*, 1995; *Tyagi et al.*, 2015a], and snow [*Tyagi et al.*, 2015b] as tracers of microorganisms such as algae, fungi, bacteria, protozoa, and epicuticular waxes of higher plants. $\beta$-Hydroxy FAs from $C_{10}$ to $C_{18}$ have been used as chemical markers for Gram-negative bacteria (GNB) and their lipopolysaccharides (LPS) in previous studies of indoor aerosols [*Lee et al.*, 2004; *Sebastian et al.*, 2006], dust [*Reynolds et al.*, 2005; *Saraf et al.*, 1997], and snow [*Tyagi et al.*, 2015b]. Positional isomers of short-chain $\alpha$- and $\omega$-homologues

of hydroxy FAs have been used as potential tracers of soil microorganisms (e.g., bacteria, fungi, yeasts, and protozoa) [*Ratledge and Wilkinson*, 1988], whereas long-chain $\alpha$- and $\omega$-hydroxy FAs ($C_{16}$ to $C_{32}$) are used as a proxy for microalgae and cyanobacteria [*Matsumoto and Nagashima*, 1984; *Matsumoto et al*., 1984], sea grasses [*Volkman et al*., 1999], and plant waxes [*Rogge et al*., 1993; *Simoneit*, 1989].

More specifically, bound $\alpha$-hydroxy FAs from $C_{16}$ to $C_{18}$ are present in microorganisms as well as in higher plants, but free $\alpha$-isomers from $C_{22}$ to $C_{26}$ are present in higher plants only and can be decomposed [*Cranwell*, 1981]. Bound $\beta$-hydroxy FAs from $C_{22}$ to $C_{26}$ can be incorporated into higher plant-bound lipids after $\beta$-oxidation of long-chain FAs [*Cranwell*, 1981]. Moreover, these $\alpha$-, $\beta$-, and $\omega$-hydroxy FAs can also act as intermediate products of photochemical as well as microbial oxidation of long-chain monocarboxylic acids to dicarboxylic acids in sediments [*Kawamura and Ishiwatari*, 1981; *Volkman et al*., 1998; *Wakeham*, 1999]. Furthermore, specificity of hydroxylation of fatty acids depends on the type of bacteria involved [*Wakeham*, 1999]. As intermediate oxidation products, these hydroxy FAs can either incorporate into higher plant fatty acids as bound lipids or decompose or remain free before the deposition to the sediments [*Cranwell*, 1981].

High mountain sites provide a means to assess the sources and transport pathways of atmospheric aerosols. Mount Tateyama is located in central Japan and is a unique observation point for aerosol research because the mountain faces the Sea of Japan and northeast China. The Murodo-Daira site located near the summit of Mount Tateyama is usually covered by thick snow (5–8 m) from November to July, reaching $\geq$6 m in April. Because tourism-related activities are scarce from winter to early spring, this site is ideal for the study of long-range atmospheric transport of microorganisms without local emissions. The snowpack over Mount Tateyama is a receptor surface and a storage compartment for the deposition of Asian dust [*Kawamura et al*., 2012; *Mochizuki et al*., 2015]. The snow cover over Mount Tateyama contains deposited and scavenged Asian dust particles [*Osada et al*., 2004]. Although previous studies have been conducted to assess the atmospheric chemistry over Mount Tateyama [*Maki et al*., 2011; *Mochizuki et al*., 2015; *Osada et al*., 2004; *Watanabe et al*., 2010], detailed analysis of bacterial transport and associated endotoxins is not available.

Hydroxy FAs in the snowpack are important to evaluate the contribution of soil- and plant-associated microbes to long-range transported aerosols as they can act as cloud condensation nuclei (CCN) and ice nuclei, affecting the climate [*Amato et al*., 2007; *Bauer et al*., 2003; *Delort et al*., 2010; *Christner et al*., 2008]. Furthermore, Asian dust events can facilitate the dispersal of airborne bacteria and their metabolites over the Pacific Ocean, spreading the potential pathogens that are associated with human allergies and infections. As snow can scavenge the airborne particles in the free troposphere, hydroxy FAs in snowpack over Mount Tateyama can be employed as chemical markers to assess the presence, sources, and transport of microorganisms and plant metabolites. In this study, we measured hydroxy FAs in the Asian dust deposited in snow layers of Mount Tateyama. We analyzed the molecular distributions of hydroxy FAs and estimated the airborne LPS mass from GNB and bacterial dry mass to better understand a long-range atmospheric transport and scavenging of Asian dust-associated microorganisms and plant waxes.

## 2. Experimental Methods

### 2.1. Site Description and Snow Sample Collection

We collected snow samples from two snow pits that were dug at the Murodo-Daira site (36.58°N, 137.36°E, 2450 m above sea level (asl)) on the western plateau of Mount Tateyama (3015 m asl), Toyama prefecture, central Japan (Figure 1). This site faces the Sea of Japan and is located on the outflow pathway of Asian dust. On 18 April 2009 and 17 April 2011, two snow pit holes were dug to the depth of 6.0 and 6.5 m, respectively, and snowpack samples were collected using a precleaned shovel to avoid possible contamination. We found dust-containing snow layers in the snow pits; they showed a light yellow-brown color and were later characterized by high $Ca^{2+}$ concentrations, suggesting long-range transport of Asian dust to the sampling site [*Mochizuki et al*., 2015]. Previous studies [*Kawamura et al*., 2012; *Maki et al*., 2011; *Osada et al*., 2004; *Tanaka et al*., 2011] also suggested that snow samples collected from the same site are influenced by Asian dust.

Six and eight snow samples including dust layers were collected from the snow pits in 2009 and 2011, respectively, and placed in a precleaned glass jar (8 L), to which $HgCl_2$ was added prior to sample collection to


**Figure 1.** Map showing the geographical location of Mount Tateyama in central Japan. The inset shows the Murodo-Daira site on Mount Tateyama, where snowpack samples were collected.

prevent any microbial activity. The thickness of snow layer collected was approximately 10 cm in 2009 and 5–20 cm in 2011. The detailed description of snow sample collection and the results of non-sea-salt $Ca^{2+}$ (nss-$Ca^{2+}$) concentrations are given in our previous publication [*Mochizuki et al.*, 2015] and summarized in Table 1. Because we used a large aliquot of samples (i.e., almost all of the snowpack melt water) for the determination of hydroxy FAs ($\alpha$, $\beta$, and $\omega$), insufficient material remained to attempt to characterize other organic molecular markers in the snowpack samples from Mount Tateyama.

To better assess the source regions for hydroxy FAs in snowpack samples, two reference materials, namely, Chinese loess (CJ-1) and simulated Asian dust (CJ-2) samples were analyzed. CJ-1 is a representative dust standard from Chinese Loess, whereas CJ-2 corresponds to simulated Asian Dust from Tengger Desert, North China (Figure S4 in the supporting information). More detailed information about the chemical composition of CJ-1 and CJ-2 has been given in *Nishikawa et al.* [2000].

**Table 1.** Description of the Snow Layers and Measured nss-$Ca^{2+}$ Concentrations in the Snowpack Samples Collected From the Murodo-Daira of Mount Tateyama, Central Japan in 2009 and 2011

| Sampling Date | Sampling ID | Depth From Snow Surface in Centimeters | Snow Type | nss $Ca^{2+}$ ($\mu g\,kg^{-1}$) |
|---|---|---|---|---|
| 18-Apr-2009 | 1 | Surface snow layer | Granular snow | - |
| | 2 | 325–335 | Asian dust-containing snow | 3.0 |
| | 3 | 410–420 | Clean snow | 0.43 |
| | 4 | 520–530 | Asian dust-containing snow | 1.54 |
| | 4′ | 520–530 | Asian dust-containing snow, 4′ collected from different snow pit parallel to sample 4 | 1.82 |
| | 5 | 530–540 | Granular snow | 0.46 |
| Average | | | | 1.45 |
| 17-Apr-2011 | 6 | 115–125 | Granular snow with ice plate | 0.17 |
| | 7 | 169–178 | Dusty and granular snow | 0.57 |
| | 8 | 290–300 | Compacted snow layer | 0.12 |
| | 9 | 390–400 | Compacted snow layer | 0.12 |
| | 10 | 400–410 | Dusty and compacted snow | 0.97 |
| | 11 | 507–527 | Compacted snow with ice plate | - |
| | 12 | 542–548 | Dusty and compacted snow | 0.53 |
| | 13 | 630–635 | Granular snow | 0.18 |
| Average | | | | 0.38 |

### 2.2. Solvent Extraction, Derivatization, and Identification of Hydroxy Fatty Acids

The analytical protocol used for extracting total hydroxy FAs (lipopolysaccharides; LPS bound and free) from the snowmelt water is described in *Yamamoto et al*. [2011] and *Tyagi et al*. [2015b]. In brief, melt snow samples (0.2–0.4 L) were first concentrated using a rotary evaporator under vacuum (<20°C) and then saponified with 1.0 *M* KOH in methanol at 80°C for 2 h. After the saponification, neutral components were removed by extraction with hexane/methylene chloride (10:1) and the remaining solution was acidified with 6 *M* HCl to form free carboxylic acids. The free carboxylic acids were then extracted with methylene chloride and converted to fatty acid methyl esters (FAMEs) using 14% $BF_3$/methanol at 70°C for 2 h. FAMEs were extracted with hexane/methylene chloride (10:1).

FAMEs containing hydroxy FAs were separated on silica gel column and then converted to their trimethylsilyl (TMS) ethers with a mixture of 50 μL N,O-*bis*-(trimethylsilyl) trifluoroacetamide (BSTFA) and pyridine (SUPELCO$^{TM}$ Analytical) at 80°C for 1 h. After the reaction, 50 μL of *n*-hexane solution containing 1.43 ng $\mu L^{-1}$ of internal standard ($C_{13}$ *n*-tridecane, Wako) were added to dilute the derivatives prior to GC/MS (gas chromatography/mass spectrometry) injection (Agilent Technologies, Model 7890 GC coupled to Hewlett-Packard Model 5975 C inert XL EI/CI mass-selective detector, MSD with the Triple-Axis Detector). The GC was installed with a split/splitless injector and HP-5 fused silica column (Agilent 19091J-202; 325°C; 19 m × 200 μm, 0.5 μm film thickness).

The GC oven temperature was programmed from 50°C (2 min) to 120°C (15°C $min^{-1}$), then to 305°C (15 min) at 5°C $min^{-1}$. Helium was used as a carrier gas at a flow rate of 1 ml $min^{-1}$. Data were acquired and processed with Chemstation software. Identification of hydroxy FAs was performed by comparing retention times and mass spectra with FAME/TMS derivatives of authentic *n*-$C_{12}$ and *n*-$C_{16}$ $\alpha$-hydroxy FAs; *n*-$C_{12}$, *n*-$C_{14}$, *n*-$C_{15}$, and *n*-$C_{16}$ $\beta$-hydroxy FAs; and *n*-$C_{16}$, *n*-$C_{20}$, and *n*-$C_{22}$ $\omega$-hydroxy FAs. These derivatives were also used as external standards. The recoveries of authentic $\beta$- and $\omega$-hydroxy FA standards were better than 60% and 70%, respectively. We extracted organic-free deionized water as a blank sample to check the laboratory contamination during the analysis of real snow samples. We detected no target compounds in the blank.

### 2.3. LPS Mass and Bacterial Biomass

The LPS of GNB contains lipid A (an acylated diglucosamine head) fraction, which is present in the outer cell membrane of bacteria [*Pomorska et al*., 2007] and $\beta$-hydroxy fatty acids (FAs) with C-numbers from 10 to 18. Thus, LPS mass can be estimated by a measurement technique based on a specific LPS molecule such as $\beta$-hydroxy FAs quantified by GC/MS [*Pomorska et al*., 2007; *Reynolds et al*., 2005; *Tyagi et al*., 2015b]. LPS concentrations can be calculated using the mathematical expression as follows:

LPS mass from GNB, ng $kg^{-1}$ of melt water = [$\Sigma\beta$-hydroxy $C_{10}$–$C_{18}$ FAs (nmol $kg^{-1}$)/4] × 8000.

In the above equation, the number "8000" corresponds to average molecular weight of environmental LPS [*Laitinen et al*., 2001]. In addition, $\beta$-hydroxy FAs represent total hydroxy FAs that are LPS-bound and free with C-numbers of 10 to 18. In the present study, LPS mass from GNB is expressed in μg $kg^{-1}$ of snowmelt water. Furthermore, we used the marker to microbial mass conversion factor of 15 nmol of $\beta$-hydroxy FAs ($C_{10}$–$C_{18}$) per milligram dry bacterial cell weight to estimate the biomass of GNB. This approach was first employed by *Balkwill et al*. [1988], and later by *Lee et al*. [2004] and *Tyagi et al*. [2015b]. Therefore, we have converted the sum of mass concentrations of $\beta$-hydroxy $C_{10}$–$C_{18}$ FAs (nmol $kg^{-1}$ of melt water) into equivalent dry cell weight of GNB (μg $kg^{-1}$ of melt water) by normalizing to 15 nmol.

### 2.4. Air Mass Back Trajectory (AMBT) Analysis

To assess the probable source regions of Asian dust and its transport pathway to the Murodo-Daira sampling site on Mount Tateyama, we computed air mass back trajectories at an arrival height of using the computer based Hybrid Single Particle Lagrangian Integrated Trajectory (HYSPLIT, version 4) [*Draxler and Rolph*, 2013]. This HYSPLIT model used archived meteorological data sets (National Centers for Environmental Prediction/National Center for Atmospheric Research Reanalysis) from the National Oceanic and Atmospheric Administration Air Resources Laboratory as an input file. Backward trajectories were computed for air masses arriving at an altitude of 100 m above ground level for the previous 5 day period (Figure 2).

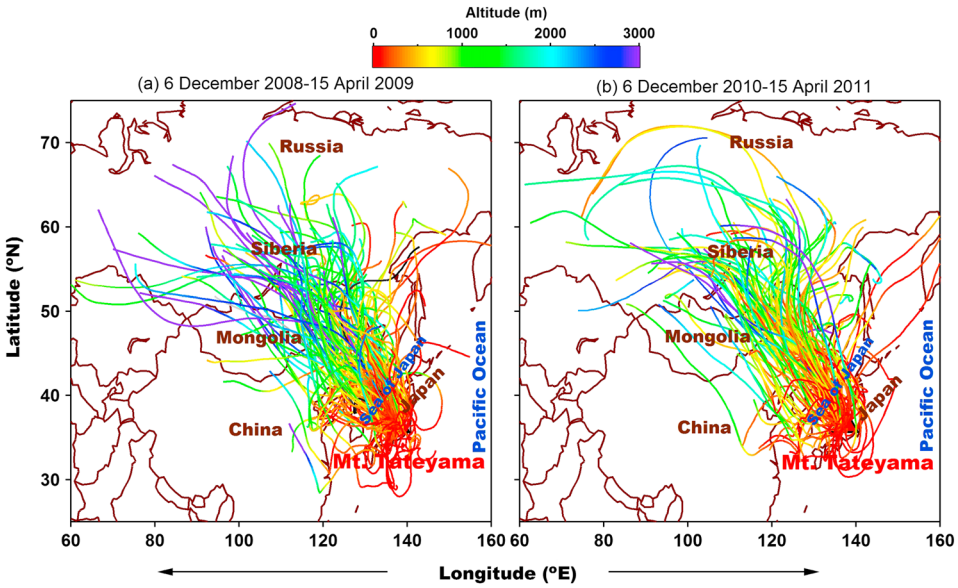


**Figure 2.** Five day backward trajectories of air masses arriving at the height of 100 m above ground level over the Murodo-Daira site near Mount Tateyama, central Japan. The color scale indicates the heights of the air mass parcels arriving from the source regions in the Asian continent.

## 3. Results and Discussion

### 3.1. Hydroxy Fatty Acids

A statistical summary of hydroxy FAs in the snowpack samples from spring 2009 and 2011 is presented in Tables 2 and 3, respectively. We found a series of saturated $\alpha$-hydroxy FAs, for which C-chain length of these organic compounds varied from 16 to 30 in 2009 and from 16 to 32 in 2011. In contrast, we found short-chain homologues of $\beta$-hydroxy FAs, ranging from C-number 9 to 20, in both the years. Interestingly, a wide carbon chain length of $\omega$-hydroxy FAs was found with C-numbers from 9 to 30 in both 2009 and 2011. All these hydroxy FAs showed strong even to odd carbon numbered predominance for the snowpack samples collected in April 2009 and April 2011, indicating their source as terrestrial lipids from vascular plants/soil microbes [*Gagosian and Peltzer*, 1986].

The even carbon predominance of fatty acids is a characteristic feature of biological processes involved in the synthesis of lipid compounds in higher plants and soil microorganisms [*Simoneit*, 1985; *Xie et al.*, 2003]. The wind abrasion of epicuticular waxes of higher plants contributes to even C-predominance of fatty acids ranging from $C_{22}$ to $C_{32}$ with characteristic peaks of either $C_{24}$ or $C_{26}$. Alternately, lipids from soil microbes mostly contribute to lower homologues of fatty acids, typically ranging from $C_{10}$ or $C_{12}$ to $C_{22}$ [*Xie et al.*, 2003]. We, therefore, estimated the even to odd carbon abundance ratio of hydroxy FAs in snow pit samples that ranged from 2.6 to 5.1 ($3.9 \pm 1.0$) for $\alpha$-hydroxy FAs, from 1.3 to 14.1 ($4.7 \pm 4.8$) for $\beta$-hydroxy FAs, from 2.6 to 59.3 ($16 \pm 22$) for $\omega$-hydroxy FAs in 2009. The abundance ratios varied from 1.4 to 6.2 ($2.6 \pm 1.6$) for $\alpha$-hydroxy FAs, from 0.6 to 6.8 ($2.2 \pm 2.0$) for $\beta$-hydroxy FAs, from 4.1 to 17.1 ($7.21 \pm 4.8$) for $\omega$-hydroxy FAs in 2011. These entire ratios indicate a significant contribution of lipids from plant waxes/soil microbes.

#### 3.1.1. Concentrations of Hydroxy FAs in Different Snowpack

In 2009, the median concentration of total hydroxy FAs (1.13 $\mu g\,kg^{-1}$) is comparable to that observed in 2011 (0.96 $\mu g\,kg^{-1}$). A similar feature was obtained in the median concentrations of total $\alpha$-, total $\beta$-, and total $\omega$-hydroxy FAs, which are consistent between 2009 (0.22 $\mu g\,kg^{-1}$, 0.53 $\mu g\,kg^{-1}$, and 0.47 $\mu g\,kg^{-1}$, respectively) and 2011 (0.14 $\mu g\,kg^{-1}$, 0.57 $\mu g\,kg^{-1}$, and 0.32 $\mu g\,kg^{-1}$, respectively). The overall median concentrations of hydroxy FAs in snowpack samples are overlapped between 2009 and 2011, suggesting their similar provenance (or source region, as also supported by the AMBTs). However, median concentration of water-soluble non-sea-salt $Ca^{2+}$ (a proxy for mineral dust) in 2009 (1.5 $\mu g\,kg^{-1}$) was 7 times higher than that found in the snowpack samples of 2011 (0.19 $\mu g\,kg^{-1}$) (see Table 1 for the ranges and mean). We found a significant correlation between nss-$Ca^{2+}$ and total $\beta$-hydroxy FAs for the snowpack samples collected in

**Table 2.** Statistical Concentrations of $\alpha$-, $\beta$-, and $\omega$-Hydroxy FAs in Snowpack Samples Collected in 2009 From the Murodo-Daira, Mount Tateyama, Japan[a] 18 April 2009 (in $\mu g\ kg^{-1}$; $N = 6$)

| Carbon Chain Length | $\alpha$-Hydroxy FAs | | $\beta$-Hydroxy FAs | | $\omega$-Hydroxy FAs | | $\Sigma$ ($\alpha$, $\beta$, $\omega$) Hydroxy FAs | |
|---|---|---|---|---|---|---|---|---|
| | Av. ± SE | Min-Max (Median) | Av. ± SE | Min-Max (Median) | Av. ± SE | Min-Max (Median) | Av. ± SE | Min-Max (Median) |
| $C_9$ | | | 0.16 ± 0.04 | 0.07–0.32 (0.12) | 0.11 ± 0.09 | 0.00–0.29 (0.04) | 0.59 ± 0.25 | 0.34–0.84 (0.59) |
| $C_{10}$ | | | 0.36 ± 0.25 | 0.06–1.12 (0.12) | 0.14 ± 0.1 | 0.00–0.28 (0.14) | 0.86 ± 0.57 | 0.29–1.43 (0.86) |
| $C_{11}$ | | | 0.04 ± 0.03 | 0.00–0.1 (0.02) | 0.12 ± 0.1 | 0.00–0.26 (0.13) | 0.2 ± 0.07 | 0.13–0.27 (0.2) |
| $C_{12}$ | | | 0.15 ± 0.08 | 0.02–0.54 (0.07) | 0.78 ± 0.66 | 0.02–4.12 (0.08) | 2.82 ± 1.9 | 0.92–4.73 (2.82) |
| $C_{13}$ | | | 0.02 ± 0.00 | 0.00–0.04 (0.01) | 0.04 ± 0.03 | 0.00–0.16 (0.01) | 0.16 ± 0.06 | 0.1–0.22 (0.16) |
| $C_{14}$ | | | 0.13 ± 0.05 | 0.03–0.38 (0.06) | 0.53 ± 0.43 | 0.02–2.67 (0.1) | 1.98 ± 1.2 | 0.78–3.18 (1.98) |
| $C_{15}$ | | | 0.02 ± 0.01 | 0.00–0.08 (0.02) | 0.59 ± 0.04 | 0.00–0.27 (0.01) | 0.26 ± 0.09 | 0.17–0.35 (0.26) |
| $C_{16}$ | 0.09 ± 0.02 | 0.07–0.12 (0.01) | 0.15 ± 0.09 | 0.02–0.61 (0.06) | 1.34 ± 1.15 | 0.04–7.1 (0.11) | 3.06 ± 2.52 | 0.2–8.08 (0.9) |
| $C_{17}$ | | | 0.02 ± 0.01 | 0.00–0.08 (0.01) | 0.03 ± 0.02 | 0.00–0.14 (0.01) | 0.11 ± 0.06 | 0.00–0.2 (0.12) |
| $C_{18}$ | 0.04 ± 0.01 | 0.02–0.06 (0.02) | 0.08 ± 0.05 | 0.00–0.36 (0.03) | 0.1 ± 0.08 | 0.00–0.4 (0.1) | 0.39 ± 0.14 | 0.11–0.53 (0.52) |
| $C_{19}$ | 0.01 ± 0.00 | 0.00–0.01 (0.01) | 0.02 ± 0.02 | 0.00–0.08 (0.01) | 0.07 ± 0.06 | 0.00–0.19 (0.01) | 0.1 ± 0.05 | 0.02–0.2 (0.09) |
| $C_{20}$ | 0.03 ± 0.01 | 0.00–0.07 (0.02) | 0.14 ± 0.1 | 0.01–0.45 (0.05) | 0.18 ± 0.14 | 0.01–0.76 (0.04) | 0.54 ± 0.22 | 0.15–0.91 (0.57) |
| $C_{21}$ | 0.01 ± 0.00 | 0.00–0.02 (0.01) | | | 0.18 ± 0.17 | 0.00–0.8 (0.01) | 0.49 ± 0.43 | 0.06–0.92 (0.49) |
| $C_{22}$ | 0.05 ± 0.01 | 0.01–0.1 (0.05) | | | 0.38 ± 0.34 | 0.01–2.08 (0.02) | 1.29 ± 0.98 | 0.31–2.26 (1.29) |
| $C_{23}$ | 0.03 ± 0.01 | 0.01–0.1 (0.01) | | | 0.42 ± 0.41 | 0.00–2.1 (0.01) | 1.15 ± 0.97 | 0.18–2.11 (1.15) |
| $C_{24}$ | 0.13 ± 0.07 | 0.02–0.4 (0.01) | | | 0.23 ± 0.21 | 0.01–1.05 (0.02) | 0.95 ± 0.17 | 0.78–1.12 (0.95) |
| $C_{25}$ | 0.03 ± 0.01 | 0.00–0.01 (0.02) | | | 0.56 ± 0.55 | 0.00–2.2 (0.01) | 1.22 ± 1.04 | 0.18–2.26 (1.22) |
| $C_{26}$ | 0.03 ± 0.01 | 0.01–0.07 (0.02) | | | 0.2 ± 0.2 | 0.00–0.6 (0.00) | 0.41 ± 0.2 | 0.2–0.61 (0.41) |
| $C_{27}$ | 0.01 ± 0.00 | 0.00–0.04 (0.01) | | | 0.38 ± 0.37 | 0.00–0.76 (0.38) | 0.41 ± 0.35 | 0.07–0.76 (0.41) |
| $C_{28}$ | 0.03 ± 0.01 | 0.00–0.07 (0.02) | | | 0.08 ± 0.07 | 0.00–0.24 (0.01) | 0.18 ± 0.07 | 0.11–0.25 (0.18) |
| $C_{29}$ | 0.02 ± 0.00 | 0.00–0.02 (0.01) | | | 0.06 ± 0.05 | 0.00–0.11 (0.06) | 0.08 ± 0.04 | 0.05–0.12 (0.08) |
| $C_{30}$ | 0.02 ± 0.00 | 0.00–0.02 (0.01) | | | 0.04 ± 0.03 | 0.01–0.08 (0.43) | 0.07 ± 0.02 | 0.05–0.09 (0.07) |
| Total | 0.4 ± 0.2 | 0.1–1.2 (0.22) | 1.04 ± 0.6 | 0.1–3.8 (0.51) | 4.96 ± 4.3 | 0.16–26.8 (0.47) | 1.77 ± 0.46 | 0.13–9.18 (1.07) |

[a]Av. (average), SE (standard error) = $\sigma$/SQRT ($N$), where $N$ is total number of samples.

2011 ($R^2 = 0.73$; regression: $0.050x + 32.1$). However, no such relation was observed in 2009 possibly because only four samples are available for nss-$Ca^{2+}$ data. As reported in previous studies [*Mori et al*., 2002; *Nishikawa et al*., 2000; *Osada et al*., 2004], water-soluble $Ca^{2+}$ is a major ionic species detected in Asian and Chinese loess dust samples, and thus, we infer that hydroxy FAs in snowpack samples are strongly influenced by the atmospheric input of Asian dust.

Asian dust is a significant source of atmospheric water-soluble organic nitrogen (WSON) [*Shi et al*., 2010; *Mochizuki et al*., 2015]. In a previous study, we observed a moderately significant linear correlation between WSON and nss-$Ca^{2+}$in the snowpack samples from Mount Tateyama in spring 2009 and 2011 [*Mochizuki et al*., 2015], suggesting their probable source from Asian dust. However, WSON undergo intense oxidation during transport before reaching to Mount Tateyama. This process is inferred to explain the occurrence of a relatively lower abundance of WSON observed over Mount Tateyama compared to that found in Asian dust [*Mochizuki et al*., 2015]. Likewise, water-soluble nss-$Ca^{2+}$ concentration in aerosols is highly influenced by the aging processes (or chemical reactions), unlike lipid biomarker compounds, via the reactive uptake of acidic species such as anthropogenic $HNO_3$ and $H_2SO_4$ on atmospheric mineral dust during long-range transport [*Sullivan et al*., 2007; *Kim and Kim*, 2008].

To investigate the effect of aging processes during transport, we have examined the linear relationship between water-soluble nss-$Ca^{2+}$ and $NO_3^-$ in snowpack samples from spring 2009 and 2011. We found a good correlation between nss-$Ca^{2+}$ and $NO_3^-$ with different slopes (Figure 3), indicating various sources and/or different processing (i.e., extent of aging). In addition, the mass contribution of individual water-soluble ion to $\Sigma$WSIC ($Na^+ + NH_4^+ + K^+ + Ca^{2+} + Mg^{2+} + F^- + NO_3^- + SO_4^{2-}$) in snowpack samples collected during spring 2009 and 2011 differ significantly (Figure 4). That is, spring 2009 samples have more contributions from the Asian dust (as supported by the higher abundances of $Ca^{2+}$ and $Na^+$) compared to spring 2011 samples, which are more influenced by anthropogenic emissions. As mentioned earlier, $\beta$-hydroxy FAs can be derived from bound and/or free lipids due to the analytical protocol used. Although they could be originated from similar dust source regions in East Asia, it is not likely to expect a statistical correlation of these organic

**Table 3.** Statistical Concentrations of $\alpha$-, $\beta$-, and $\omega$-Hydroxy FAs in Snowpack Samples Collected in 2011 at the Murodo-Daira, Mount Tateyama, Japan[a] 17 April 2011 (in μg $kg^{-1}$; $N = 8$)

| Carbon Chain Length | $\alpha$-Hydroxy FAs | | $\beta$-Hydroxy FAs | | $\omega$-Hydroxy FAs | | Σ ($\alpha$, $\beta$, $\omega$) Hydroxy FAs | |
|---|---|---|---|---|---|---|---|---|
| | Av. ± SE | Min-Max (Median) | Av. ± SE | Min-Max (Median) | Av. ± SE | Min-Max (Median) | Av. ± SE | Min-Max (Median) |
| $C_9$ | | | 0.15 ± 0.02 | 0.08–0.3 (0.13) | 0.01 ± 0.00 | 0.00–0.01 (0.01) | 0.61 ± 0.59 | 0.02–1.21 (0.61) |
| $C_{10}$ | | | 0.16 ± 0.04 | 0.07–0.39 (0.12) | 0.04 ± 0.02 | 0.00–0.12 (0.01) | 0.72 ± 0.54 | 0.19–1.26 (0.72) |
| $C_{11}$ | | | 0.03 ± 0.01 | 0.01–0.06 (0.03) | 0.00 ± 0.00 | 0.00–0.00 (0.00) | 0.14 ± 0.13 | 0.00–0.27 (0.14) |
| $C_{12}$ | | | 0.08 ± 0.02 | 0.01–0.19 (0.04) | 0.06 ± 0.02 | 0.02–0.19 (0.04) | 0.57 ± 0.05 | 0.51–0.62 (0.57) |
| $C_{13}$ | | | 0.01 ± 0.00 | 0.00–0.03 (0.01) | 0.01 ± 0.00 | 0.00–0.01 (0.00) | 0.08 ± 0.03 | 0.05–0.1 (0.08) |
| $C_{14}$ | | | 0.04 ± 0.01 | 0.00–0.08 (0.03) | 0.06 ± 0.02 | 0.01–0.2 (0.04) | 0.42 ± 0.12 | 0.3–0.54 (0.42) |
| $C_{15}$ | | | 0.01 ± 0.00 | 0.00–0.03 (0.01) | 0.00 ± 0.00 | 0.00–0.01 (0.00) | 0.05 ± 0.02 | 0.04–0.07 (0.05) |
| $C_{16}$ | 0.08 ± 0.02 | 0.05–0.1 (0.08) | 0.07 ± 0.01 | 0.02–0.14 (0.06) | 0.09 ± 0.02 | 0.000.23 (0.04) | 0.5 ± 0.17 | 0.16–0.75 (0.5) |
| $C_{17}$ | 0.04 ± 0.00 | 0.00–0.04 (0.04) | 0.01 ± 0.00 | 0.00–0.02 (0.01) | 0.00 ± 0.00 | 0.00–0.01 (0.00) | 0.05 ± 0.01 | 0.04–0.07 (0.05) |
| $C_{18}$ | 0.03 ± 0.01 | 0.02–0.04 (0.03) | 0.03 ± 0.00 | 0.01–0.06 (0.02) | 0.01 ± 0.00 | 0.00–0.03 (0.01) | 0.15 ± 0.05 | 0.07–0.25 (0.15) |
| $C_{19}$ | | | 0.01 ± 0.00 | 0.00–0.01 (0.01) | 0.01 ± 0.00 | 0.00–0.02 (0.00) | 0.03 ± 0.01 | 0.00–0.04 (0.03) |
| $C_{20}$ | 0.02 ± 0.01 | 0.01–0.03 (0.02) | 0.02 ± 0.00 | 0.01–0.04 (0.01) | 0.02 ± 0.00 | 0.00–0.05 (0.01) | 0.13 ± 0.04 | 0.05–0.19 (0.13) |
| $C_{21}$ | 0.02 ± 0.00 | 0.01–0.02 (0.02) | | | 0.01 ± 0.00 | 0.00–0.05 (0.00) | 0.08 ± 0.03 | 0.04–0.11 (0.08) |
| $C_{22}$ | 0.02 ± 0.00 | 0.00–0.05 (0.01) | | | 0.03 ± 0.01 | 0.01–0.1 (0.02) | 0.24 ± 0.06 | 0.18–0.3 (0.24) |
| $C_{23}$ | 0.02 ± 0.00 | 0.00–0.05 (0.02) | | | 0.01 ± 0.00 | 0.00–0.03 (0.01) | 0.11 ± 0.05 | 0.07–0.16 (0.11) |
| $C_{24}$ | 0.06 ± 0.01 | 0.02–0.14 (0.04) | | | 0.02 ± 0.00 | 0.00–0.06 (0.01) | 0.32 ± 0.16 | 0.17–0.48 (0.32) |
| $C_{25}$ | 0.03 ± 0.00 | 0.00–0.06 (0.02) | | | 0.01 ± 0.00 | 0.00–0.01 (0.01) | 0.13 ± 0.11 | 0.02–0.23 (0.13) |
| $C_{26}$ | 0.02 ± 0.00 | 0.01–0.06 (0.02) | | | 0.02 ± 0.1 | 0.00–0.06 (0.02) | 0.16 ± 0.05 | 0.11–0.21 (0.16) |
| $C_{27}$ | 0.02 ± 0.00 | 0.01–0.03 (0.02) | | | 0.01 ± 0.00 | 0.00–0.01 (0.01) | 0.05 ± 0.04 | 0.02–0.09 (0.05) |
| $C_{28}$ | 0.02 ± 0.00 | 0.01–0.03 (0.02) | | | 0.02 ± 0.01 | 0.00–0.03 (0.01) | 0.06 ± 0.00 | 0.05–0.06 (0.06) |
| $C_{29}$ | 0.01 ± 0.00 | 0.00–0.01 (0.01) | | | 0.01 ± 0.00 | 0.00–0.01 (0.01) | 0.02 ± 0.00 | 0.00–0.02 (0.02) |
| $C_{30}$ | 0.01 ± 0.00 | 0.00–0.02 (0.01) | | | 0.00 ± 0.00 | 0.00–0.01 (0.00) | 0.03 ± 0.00 | 0.00–0.03 (0.03) |
| $C_{31}$ | 0.01 ± 0.00 | 0.00–0.02 (0.01) | | | | | 0.02 ± 0.00 | 0.00–0.02 (0.02) |
| $C_{32}$ | 0.02 ± 0.00 | 0.01–0.03 (0.02) | | | | | 0.05 ± 0.00 | 0.00–0.05 (0.05) |
| Total | 0.31 ± 0.08 | 0.06–1.2 (0.21) | 0.62 ± 0.08 | 0.3–1.01 (0.57) | 0.42 ± 0.13 | 0.11–1.17 (0.32) | 0.44 ± 0.09 | 0.02–1.49 (0.26) |

[a]Av. (average), SE (standard error) = $\sigma$/SQRT ($N$), where $N$ is total number of samples.

compounds with water-soluble nss-$Ca^{2+}$. Although the sample size of snowpacks ($N = 6$ and 8 for 2009 and 2011, respectively) analyzed here is rather small to infer statistical relation between these two chemical entities (i.e., $\beta$-hydroxy FAs and nss-$Ca^{2+}$), the occurrence of nss-$Ca^{2+}$ has been effectively used in the past studies from East Asia to ascertain the influence of Asian dust events [e.g., *Mori et al.*, 2002].

Interestingly, it is evident from Figure 4 that the reference materials from the source regions of mineral dust in East Asia (reference materials: CJ-1 and CJ-2) contain abundant quantities of water-soluble nss-$Ca^{2+}$. Therefore, high concentrations of water-soluble nss-$Ca^{2+}$ along with air mass back trajectories indicate significant contributions of Asian dust to snowpack samples from Mount Tateyama. However, compositional differences in the relative abundances of water-soluble inorganic ions to ΣWSIC between snowpack samples and reference materials (CJ-1 and CJ-2) could be explained by any of the following reasons: grain size fractionation, aging effects (or oxidation processes), increase in the contribution of sea salts, and anthropogenic sources during transport. This inference is supported by the relative increase in $Na^+$ (%, a major ion in sea salts), $SO_4^{2-}$ (%), and $NO_3^-$ (%) in

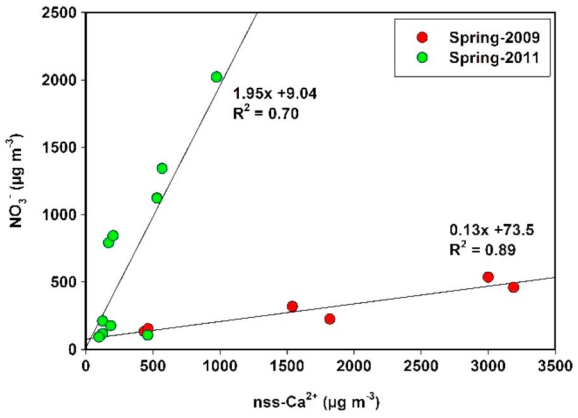


**Figure 3.** Linear regression analysis between concentrations of water-soluble non-sea-salt $Ca^{2+}$ and nitrate in the snowpack samples collected during Spring 2009 and 2011 over Mount Tateyama.

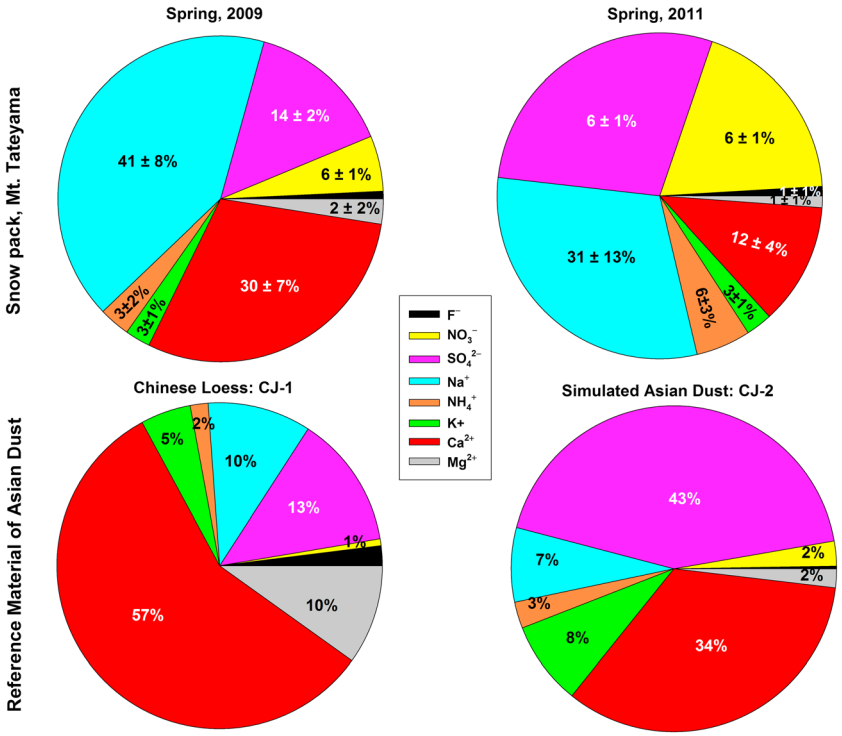


**Figure 4.** A comparison of fractional contributions of individual inorganic ions in the total mass concentrations for the snowpack samples collected during spring 2009 and 2011 with reference materials from Chinese loess (CJ-1 and CJ-2).

snowpack samples from Mount Tateyama compared to those documented for the reference materials, representing the transport induced alterations. Therefore, we compared the relative mass fraction of $K^+$, $Ca^{2+}$, and $Mg^{2+}$ in snowpack samples with reference materials by using ternary plots (Figure 5). A good agreement between snowpacks and reference materials (Figure 5) suggests the source of mineral dust in snowpacks from Mount Tateyama to be Asian dust. This observation clearly demonstrates an impact of Asian dust on the snowpack samples of Mount Tateyama.

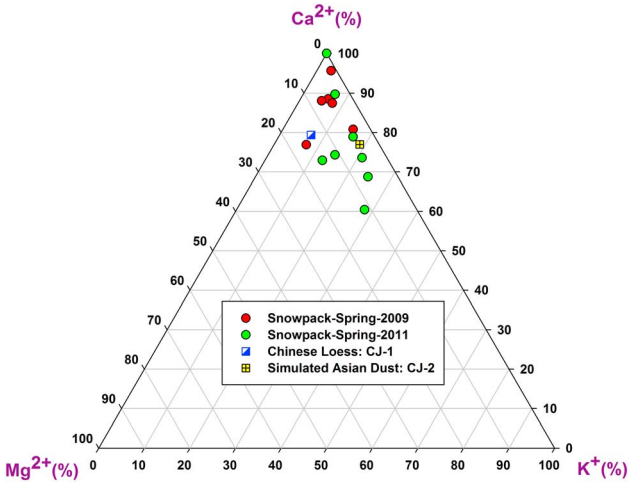


**Figure 5.** Ternary plots for the relative mass fractions (%) of $K^+$, $Ca^{2+}$, and $Mg^{2+}$ in snowpack samples collected in spring 2009 and 2011 along with two reference materials (CJ-1 and CJ-2).

Mass concentrations of total $\beta$- and $\omega$-hydroxy FAs in the dusty and nondusty layers (classified based on the visual appearance and nss-$Ca^{2+}$ concentration, see Table 1) of snowpacks collected in 2009 ($\beta$-isomers: 1.04 $\mu g\,kg^{-1}$; $\omega$-isomers: 4.96 $\mu g\,kg^{-1}$) and 2011 ($\beta$-isomers: 0.62 $\mu g\,kg^{-1}$; $\omega$-isomers: 0.42 $\mu g\,kg^{-1}$) were significantly higher than those reported in the fresh snow samples collected from Sapporo (northern Japan) during winter 2010 ($\beta$-isomers: 0.24 $\mu g\,kg^{-1}$; $\omega$-isomers: 0.64 $\mu g\,kg^{-1}$) and 2011 ($\beta$-isomers: 0.18 $\mu g\,kg^{-1}$; $\omega$-isomers: 0.15 $\mu g\,kg^{-1}$) [*Tyagi et al.*, 2015b]. This difference is due to a strong influence of the atmospheric input of Asian dust in the snowpack samples at the Murodo-Daira site. These hydroxy FAs (especially

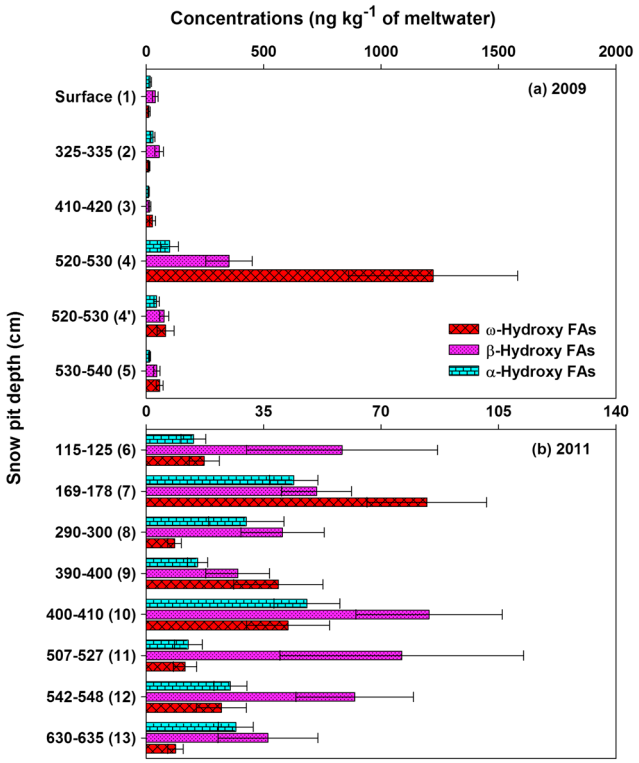


**Figure 6.** Bar graphs showing the mean concentrations of $\alpha$-, $\beta$-, and $\omega$-hydroxy FAs in the Asian dust-containing and underlying snow layers collected in spring of (a) 2009 and (b) 2011 from Mount Tateyama in Japan. Numbers in the brackets on the *y* axis are the sample IDs of the snow layers as given in Table 1.

$\beta$-isomers) from soil microbes such as bacteria can adhere to dust particles under strong wind conditions and can be carried long distances. Therefore, snowpacks collected in spring 2009 and 2011 from Mount Tateyama were strongly influenced by Asian dust, showing higher inputs of soil microorganisms transported from the Asian continent.

$\alpha$-Hydroxy FAs measured in 2009 (0.4 $\mu g\,kg^{-1}$) and 2011 (0.23 $\mu g\,kg^{-1}$) were found to be less abundant than those detected in fresh snow from Sapporo (2010: 0.59 $\mu g\,kg^{-1}$ and 2011: 0.64 $\mu g\,kg^{-1}$). This difference could be explained by photochemical/microbial degradation of long-chain fatty acids present in cloud water via $\alpha$-oxidation to short-chain $\alpha$-hydroxy FAs as detected in fresh snow samples. *Cranwell* [1981] suggested that lower abundances of long-chain $\alpha$-hydroxy FAs from $C_{22}$ to $C_{26}$ (i.e., which are contributed from higher plants; allochthonous) as well as short-chain $\alpha$-hydroxy FAs from $C_{14}$ to $C_{18}$ (i.e., derived from marine biota; autochthonous) are due to their oxidation/degradation before deposition into pelagic sediments. Likewise, *Eglinton et al.* [1968] suggested that long-chain $\alpha$-hydroxy FAs are more susceptible to degradation during transport and have a wide variety of formation pathways such as photochemical/microbial oxidation of higher molecular weight fatty acids. Thus, $\alpha$-isomers of hydroxy FAs cannot be employed as specific tracers of higher plant metabolites for the snowpacks collected from Mount Tateyama.

### 3.1.2. Interannual Variability of Hydroxy Fatty Acids

Figure 6 shows the mean concentrations of $\alpha$-, $\beta$-, and $\omega$-hydroxy FAs measured in snow layers from the Murodo-Daira site on Mount Tateyama. In 2009, maximum mean concentrations of $\alpha$-, $\beta$-, and $\omega$-hydroxy FAs (1.09 $\mu g\,kg^{-1}$, 4.2 $\mu g\,kg^{-1}$, and 26.8 $\mu g\,kg^{-1}$, respectively) were detected in the snow layer of 520–530 cm in depth (sample no. 4). This layer was identified as a dusty layer with a high concentration of nss-$Ca^{2+}$, which is influenced by air masses derived from arid regions in western China, Mongolia, and Siberia (Figure 2a). We collected another dusty snow sample (sample no. 4′) parallel to the sample no. 4 at the same depth. This sample also showed higher mean concentrations of hydroxy FAs and nss-$Ca^{2+}$, evidently receiving air masses from the same regions in the Asian continent. We also observed significant concentrations of $\alpha$- and $\beta$-hydroxy FAs in sample no. 2 collected at the depth of 325–335 cm. However, this snow layer was clean as reported by *Mochizuki et al.* [2015] and also characterized by lower abundance of nss-$Ca^{2+}$. As the Asian dust is enriched with calcium minerals (see Figure 4 for nss-$Ca^{2+}$ content in CJ-1 and CJ-2), lower concentrations of nss-$Ca^{2+}$ and higher abundances of hydroxy FAs indicate a different source contribution of these lipid biomarkers than those originated from Chinese loess.

Although we did not observe any clear trend in the abundances of hydroxy FAs with depth, we found higher abundances of hydroxy FAs in sample nos. 4, 4′, and 5. These snow layers are also characterized by a dusty layer and high concentrations of nss-$Ca^{2+}$ (a dust tracer). This feature can be explained by the occurrence of Asian dust events, as suggested by previous studies [*Kawamura et al.*, 2012; *Mochizuki et al.*, 2015; *Tanaka et al.*, 2011] over Mount Tateyama. At the same sampling site, *Tanaka et al.* [2011] and *Maki et al.* [2011]

reported higher values of bacterial cell counts in the snow layers containing Asian dust than in other layers collected during the same period at the same sampling site. They also assigned the sources of bacteria in the snowpack layers to the Asian dust observed in the early spring of 2008 and 2009. All these observations suggest that hydroxy FAs are the constituents of soil microbes and their abundances in the snow layers over Mount Tateyama can be strongly influenced by a long-range atmospheric transport of Asian dust.

In the snowpack samples collected in April 2011, we detected high abundances of hydroxy FAs in sample nos. 7, 10, and 12 (Figure 6), which were also characterized by yellow/dusty color and high abundances of nss-$Ca^{2+}$. In our previous study [*Mochizuki et al*., 2015], we found high abundances of WSON in the dust-containing snow layers at the same sampling site, which clearly shows inputs from biological sources such as bacteria, algae, and plant debris from the terrestrial sources. There is also a possibility of photochemical production of these hydroxy FAs during the melting of surface snow at the Murodo-Daira site, when exposed to high solar radiation and warmer temperatures. However, hydroxy FAs found in underlying dusty snow layers should better reflect the atmospheric inputs of Asian dust-containing soil microorganisms and higher plant metabolites during the outflow of East Asian aerosols. Similar to 2009, we also observed dust events during 2010–2011 (Figure 2b), which explains high abundances of inorganic ions and hydroxy FAs in the snowpack layers.

Interestingly, we detected maximum concentrations of hydroxy FAs in the snowpack samples collected at the middle depths (such as sample nos. 4 and 10). The sunlit surface snow layer is usually exposed to fluctuations in the ambient insolation, temperature, and relative humidity and, thereby, has a possibility of intense photochemical oxidation of organic matter (here, hydroxy FAs) [*Grannas et al*., 2004]. Unlike surface snow, high concentrations of $C_{10}$–$C_{20}$ $\beta$-hydroxy FAs in the middle layers (or the intermediate depths) of the snowpack imply that these are bound cell membrane lipids of soil bacteria, which could survive even at low temperatures through the consumption of other organic matter within snow layers. Presumably, bacteria and other soil microorganisms can digest organic acids and survive for longer times in the middle snow layers by avoiding environmental stress factors [*Kawamura et al*., 2012]. This idea is supported by *Maki et al*. [2011], who reported the presence of viable halotrophic and oligotrophic bacteria associated with Kosa particles in snowpack samples collected at middle depths from Mount Tateyama. Thus, hydroxy FAs in the dusty snow layers serve as the best proxies for tracing dust- and plant-associated bacteria (such as *Pseudomonas syringae*, *Pseudomonas viridiflava*, *Pseudomonas fluorescens*, *Pantoea agglomerans*, and *Xanthomonas campestris*) [*Christner et al*., 2008].

### 3.2. Molecular Distributions

We detected a homologous series of saturated $\alpha$-($C_{16}$–$C_{32}$), $\beta$-($C_9$–$C_{20}$), and $\omega$-($C_9$–$C_{30}$)-hydroxy FAs in the snowpack samples collected from Mount Tateyama in 2009 and 2011 (Figures 7 and S1–S3). In the following sections, we compare the molecular distributions of $\alpha$-, $\beta$-, and $\omega$-hydroxy FAs with an emphasis on their even carbon predominance, peak maximum, and their possible sources associated with plant waxes/soil microbes. We also compare these results with those obtained in fresh snow samples from Sapporo, northern Japan [*Tyagi et al*., 2015b].

#### 3.2.1. $\alpha$-Hydroxy Fatty Acids

We observed a homologues series of saturated $\alpha$-hydroxy FAs from $C_{16}$ to $C_{30}$ (Figure S1a) and from $C_{16}$ to $C_{32}$ (Figure S1b) for the snowpack samples collected in 2009 and 2011, respectively. Although the molecular distributions of $\alpha$-hydroxy FAs were characterized by even C-predominance for the snowpack samples collected in 2009 and 2011, occurrence of odd carbon compounds is also possible (in lower abundances), perhaps due to the contribution from different source emissions (e.g., types of plant/microorganisms) or oxidation/degradation processes. In snowpack samples collected at different depths from the Murodo-Daira site on Mount Tateyama (Figure 7a), molecular distributions of $\alpha$-hydroxy FAs were dominated by $C_{24}$, which is found as a major component in epicuticular waxes of higher plants along with $C_{22}$, $C_{16}$, and $C_{18}$ homologues [*Volkman*, 2006]. These results are consistent with our previous study in fresh snow samples collected from Sapporo, northern Japan, where even C-number predominance of $\alpha$-hydroxy FAs was observed with maxima at $C_{16}$, $C_{24}$, and $C_{22}$ [*Tyagi et al*., 2015b].

Interestingly, we also found a predominance of odd C-numbered $C_{25}$, $C_{27}$, and $C_{23}$ $\alpha$-hydroxy FAs (Figure 7a, S1a, and S1b) in the Asian dust and snow layers containing ice plate (samples nos. 4, 7–9, and 13) from Mount Tateyama. These odd C-numbered species could be derived from photochemical/microbial oxidation of

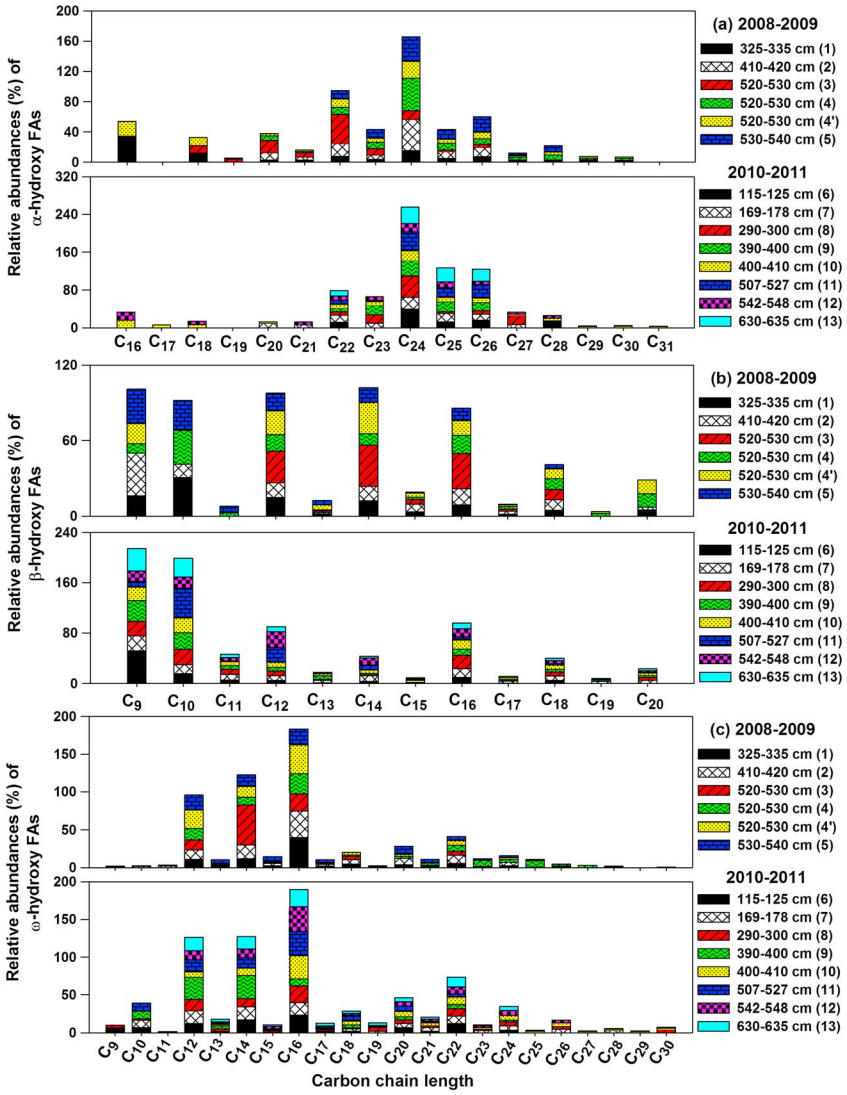


**Figure 7.** Molecular distribution of (a) $\alpha$-hydroxy FAs ($C_{16}$–$C_{32}$), (b) $\beta$-hydroxy FAs ($C_9$–$C_{20}$), and (c) $\omega$-hydroxy FAs ($C_9$–$C_{30}$) in the snowpack samples collected at different depths from the Murodo-Daira site on the western slope of Mount Tateyama in central Japan. The numbers in the parentheses indicate the sample IDs as given in Table 1.

monocarboxylic and dicarboxylic acids during the transport of polluted air masses from North China [*Cranwell*, 1981; *Osada et al*., 2009]. It has been reported that $\alpha$-oxidation is one of the degradation pathways of fatty acids in biological, geological, and atmospheric processes [*Stumpf and Barber*, 1960]. This oxidation pathway is utilized in yeasts to produce $\alpha$-hydroxy FAs as intermediates of fatty acid biosynthesis [*Fulco*, 1967]. Furthermore, $\alpha$-hydroxy FAs ($C_{16}$ to $C_{28}$) can be derived from higher plant waxes (cutin and suberin) [*Cardoso and Eglinton*, 1983] and sea grasses (*Zostera muelleri*) [*Volkman et al*., 1980], which contribute to the abundances in snowpacks from Mount Tateyama. Thus, $\alpha$-hydroxy FAs with a wide variation in chain length (C-numbers from 16 to 32) detected in the dust layers of snow may have mixed sources such as direct emissions of fatty acids from soil microbes and plant waxes associated with mineral dust during transport, as well as photochemical oxidation of fatty acids.

The above idea on mixing of fatty acids with dust particles can be corroborated by the decreased relative abundances of mineral dust-derived $Ca^{2+}$ over $NO_3^-$ for the snowpack samples in 2011 compared to those of spring 2009 samples. Also, the percentage relative abundances of water-soluble inorganic ions are dominated by sulphate (in which the non-sea-salt fraction accounts for more than 90%) and sodium in spring 2011 samples than those in spring 2009 samples (i.e., $Na^+$ and $Ca^{2+}$ are the dominant water-soluble inorganic

species). Therefore, observed molecular distributions of $\alpha$-hydroxy FAs ($C_{16}$ to $C_{32}$) in spring 2011 snowpack samples are likely affected by an increased processing of mineral dust-containing soil microbes/plant waxes with anthropogenic pollutants during long-range atmospheric transport.

#### 3.2.2. $\beta$-Hydroxy Fatty Acids

Molecular distributions of $\beta$-hydroxy FAs are relatively simple with even C-numbered short-chain species ($C_{14}$, $C_{12}$, $C_{10}$, and $C_{16}$) being predominant (Figures 7b, S2a, and S2b). These results are consistent with our previous studies conducted in fresh snow and marine aerosols where similar C-number predominance was observed [*Tyagi et al*., 2015a, 2015b]. Odd C-numbered $\beta$-hydroxy FAs were present in very small amounts, with $C_9$ species being the most abundant in two snowpack samples collected in 2009 (sample nos. 1 and 4′) and all the samples collected in 2011. The relatively abundant presence of $C_9$ $\beta$-hydroxy FA can be explained by the formation either via photochemical oxidation of long-chain unsaturated fatty acids such as oleic acid or bacterial $\beta$-hydroxylation of $C_9$ fatty acid during long-range atmospheric transport [*Kawamura*, 1995; *Tyagi et al*., 2015a]. We also observed some interannual variability in the molecular distributions of $\beta$-hydroxy FAs. The snow layers collected in 2009 showed a predominance of $C_{14}$, $C_{12}$, $C_{10}$, $C_9$, and $C_{16}$ $\beta$-hydroxy FAs. However, in the snow layers collected during 2011, we observed the predominance of $C_9$ or $C_{10}$ $\beta$-hydroxy FA, except for sample no. 12 where $C_{12}$ was the dominant $\beta$-isomer. This difference in molecular compositions could be explained by a frequent influence of atmospheric inputs from Asian dust in 2009 compared to those in 2011. Moreover, snow layers collected in 2011 were mostly clean, having an ice plate (Table 1). This finding may suggest a possibility of photochemical degradation and bacterial modification of hydroxy FAs during refreezing of melt water in the snowpack samples collected in April 2011 [*Kawamura et al*., 2012].

Short-chain $\beta$-hydroxy FAs (typically $< C_{20}$) with a predominance of $C_{14}$ or $C_{12}$ are found in the LPS present in cell walls of GNB. *Goossens et al*. [1986] reported $C_{14}$, $C_{12}$, $C_{16}$, and $C_{18}$ $\beta$-hydroxy FAs in the GNB cultures. *Pomorska et al*. [2007] demonstrated that $\beta$-hydroxy FAs from $C_{14}$ to $C_{18}$ detected in aerosols collected from animal houses are most prevalent in the *Enterobacteriaceae* family of GNB. Furthermore, $\beta$-hydroxy FAs can be produced as intermediates by $\beta$-oxidation of fatty acids during the synthesis or degradation processes in microorganisms. $\beta$-Oxidation is more important than $\alpha$- and $\omega$-oxidation pathways [*Lehninger*, 1975; *Volkman et al*., 1998], which might have accounted for the greater abundances of $\beta$-isomers over $\alpha$- and $\omega$-isomers observed in the snowpack samples collected from Mount Tateyama. Furthermore, *Tanaka et al*. [2011] and *Maki et al*. [2011] evaluated bacterial diversity and abundances in the Asian dust-containing snow layers over Mount Tateyama and attributed their source regions as the Gobi and Taklamakan Deserts and Loess Plateau via atmospheric transport over the Korean Peninsula. Our results clearly show that $\beta$-hydroxy FAs (of GNB origin) are transported long distances in the atmosphere from the arid regions of the Asian continent and scavenged by snowflakes over Mount Tateyama.

In a previous study, *Zelles and Bai* [1994] documented a predominance of $\alpha$- and $\beta$-hydroxy FAs in grassland and aggregated soils, respectively, whereas $\omega$-hydroxy FAs dominate (70–80%) in forest soils. Furthermore, $\beta$-hydroxy FAs account for 70%, 27%, and 1% in aggregated-, grassland-, and forest-soil samples, respectively, with the peak centered at $C_{10}$, $C_{12}$, or $C_{14}$ species [*Zelles and Bai*, 1994]. This study emphasized that LPS-derived $\beta$-hydroxy FAs in soil samples mostly represent GNB species. Because the samples from Mount Tateyama are significantly influenced by the Asian dust outbreaks (as inferred from AMBTs and concurrent occurrence of abundant water-soluble nss-$Ca^{2+}$), we consider that measured $\beta$-hydroxy FAs are representative of soil GNB associated with mineral dust. In addition, similar molecular distributions of $\beta$-hydroxy FAs between the snowpack samples and reference materials (CJ-1 and CJ-2) with even C-predominance and higher abundances of $C_{10}$ or $C_{12}$ indicate the representative source signatures (Figure 8). We documented higher abundances of $\beta$-hydroxy FAs in the reference materials than those observed in snowpack samples from Mount Tateyama. Low concentrations of $C_{20}$ hydroxy FA in some snow samples could be explained by its oxidation to short-chain homologues within snow, or by temporal shifts in the source regions of dust in spring with varying contributions of loess deposits/dust from East Asian Deserts (Taklamakan, Tengger, and Gobi).

#### 3.2.3. $\omega$-Hydroxy Fatty Acids

We detected $\omega$-hydroxy FAs in the wide range of $C_9$ to $C_{30}$ in the snowpack samples (Figure 7c). Their relative abundances and molecular distributions are shown in Figures S3a and S3b, respectively. Short-chain $\omega$-hydroxy FAs from $C_9$ to $C_{19}$ were characterized by the predominance of even C-numbers with maxima

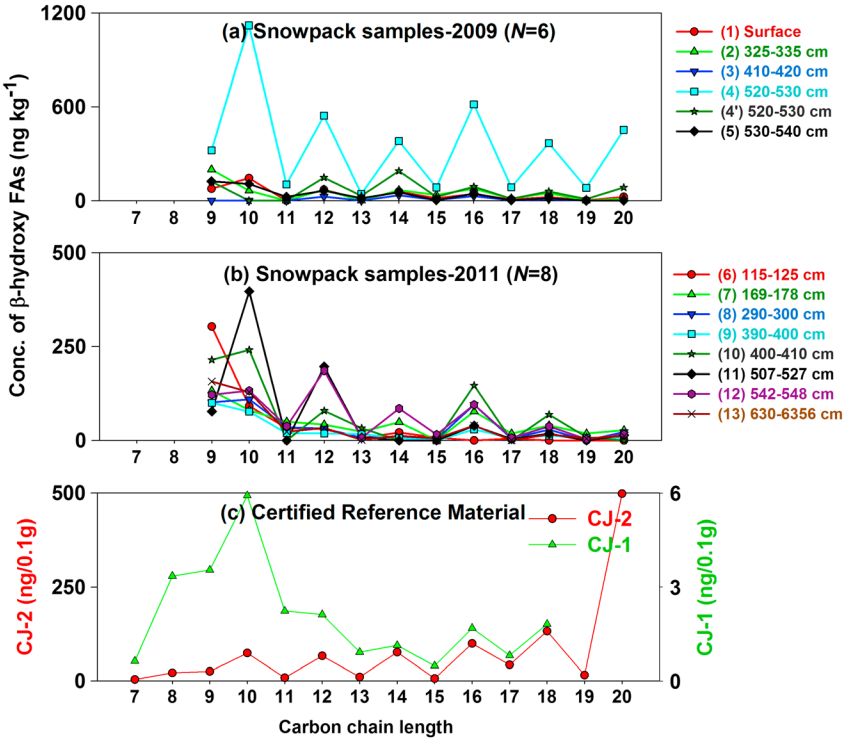


**Figure 8.** Comparison of molecular distributions of $C_9$–$C_{20}$ $\beta$-hydroxy FAs in the snowpack samples collected in (a) 2009, (b) 2011, with (c) those documented for reference materials of Chinese loess and Asian dust mineral standards (CJ-1 and CJ-2).

at $C_{16}$ followed by $C_{14}$, $C_{12}$, and $C_{10}$. In contrast, long-chain $\omega$-hydroxy FAs ($C_{20}$–$C_{30}$) showed a characteristic pattern of $C_{22} > C_{20} \geq C_{24} < C_{26}$. These results are consistent with the similar molecular distributions observed in our previous studies [*Tyagi et al.*, 2015a, 2015b]. Long-chain $\omega$-hydroxy FAs have been used as markers of higher plant waxes in sediments and aerosols [*Eglinton et al.*, 1968; *Kawamura et al.*, 2003].

$\omega$-Hydroxy FAs ($C_{20}$–$C_{26}$) have been detected as important constituents of suberin, which occurs in the cork layer of the woody parts of plants. Furthermore, $\omega$-hydroxy FAs are characterized by the dominance of $C_{16}$ and $C_{18}$ species in cutin, which makes part of the cuticle of leaves and fruits [*Eglinton et al.*, 1968]. Both suberin and cutin can contribute to short-chain $\omega$-hydroxy FAs, whereas the occurrence of long-chain isomers ($>C_{20}$) is considered to be characteristic of a suberin contribution [*Cardoso and Eglinton*, 1983]. Previous studies have reported that terminal oxidation of fatty acids by microorganisms can also produce $\omega$-hydroxy FAs as the intermediates [*Wakeham*, 1999, and references therein]. These hydroxy FAs can survive geological conditions due to their ubiquitous occurrence in woody parts; thus, we employ them as geochemical tracers of epicuticular waxes of higher plants and soil bacteria in the snowpack samples collected from Mount Tateyama.

### 3.3. Relative Abundances

In snowpack samples collected in spring 2009, we found high relative abundances of $\omega$-isomers ($45 \pm 25\%$) followed by $\beta$- ($35 \pm 19\%$) and $\alpha$-isomers ($20 \pm 14\%$). In contrast, snowpack samples collected in 2011 showed a predominance of $\beta$-isomers ($54 \pm 8\%$) over $\omega$- and $\alpha$-isomers ($30 \pm 16\%$ and $16 \pm 8\%$, respectively). However, the differences between 2009 and 2011 snowpack samples were not statistically significant (Table 4). This observation indicates their similar sources of soil microbes/plant waxes or their common long-range atmospheric transport over Mount Tateyama. The Asian dust can transport not only soil microorganisms but also epicuticular waxes derived from higher plants, fruits and leaves in East Asia, explaining high abundances of $\omega$-hydroxy FAs in the Asian dust-containing snow layers collected in this study.

We also compared the relative abundances of measured hydroxy FAs in snowpack samples collected from Mount Tateyama with those obtained in fresh snow from Sapporo, Japan [*Tyagi et al.*, 2015b] (Figure 9).

**Table 4.** A Statistical Comparison of Mean Relative Abundances of Individual Type of Hydroxy FA in Their Total Mass Concentration and Also the Short-Chain $\omega$- or Long-Chain $\alpha$-Hydroxy FAs in Their Total $\omega$- and Total $\alpha$-Hydroxy FAs, respectively, Mass in Snowpack Samples Collected in April 2009 and 2011

| Compounds | 2009 | 2011 | *t* Score, df, *p* Value |
|---|---|---|---|
| $\alpha$-Hydroxy FAs | $20 \pm 14\%$ | $16 \pm 8\%$ | 0.65,12, >0.05 |
| $\beta$-Hydroxy FAs | $35 \pm 19\%$ | $54 \pm 18\%$ | 1.89, 12, >0.05 |
| $\omega$-hydroxy FAs | $45 \pm 25\%$ | $30 \pm 16\%$ | 1.38, 12, >0.05 |
| $\alpha$-$C_{20}$–$C_{32}$ hydroxy FAs/$\alpha$-$C_{16}$–$C_{32}$ hydroxy FAs | $85 \pm 20\%$ | $93 \pm 12\%$ | 1.0, 12, >0.05 |
| $\omega$-$C_9$–$C_{19}$ hydroxy FAs/$\omega$-$C_9$–$C_{30}$ hydroxy FAs | $78 \pm 12\%$ | $72 \pm 11\%$ | 0.98, 12, >0.05 |

For comparison, we classified each type of hydroxy FAs into short-chain ($C_{16}$–$C_{19}$ $\alpha$-hydroxy FAs and $C_9$–$C_{20}$ or $C_9$–$C_{19}$ $\beta$- or $\omega$-hydroxy FAs) and long-chain compounds ($C_{20}$–$C_{32}$ and $C_{20}$–$C_{30}$ $\alpha$- and $\omega$-hydroxy FAs) due to their differences in sources/formation pathways. It has been suggested that long-chain saturated hydroxy FAs mainly originate from higher plant waxes [*Cranwell*, 1981; *Rogge et al*., 1993], whereas their photodegradation/oxidation during transport and/or contribution from soil microbes account for the occurrence of corresponding short-chain compounds [*Cranwell*, 1981; *Rogge et al*., 1993; *Řezanka and Sigler*, 2009]. Therefore, higher abundances of long-chain $\alpha$-hydroxy FAs (>$C_{20}$; Figure 9) in snowpack samples could be explained by the contribution from epicuticular plant waxes, which are associated with atmospheric mineral dust and are scavenged by snowflakes during atmospheric transport as well as dry deposition over the snow field of Mount Tateyama. In contrast, the predominance of short-chain $\alpha$-hydroxy FAs (<$C_{20}$) in fresh snow [*Tyagi et al*., 2015b] could be due to either in-cloud $\alpha$-oxidation of long-chain fatty acids by airborne bacteria or contribution from soil microbes in cloud water (more detailed discussion in section 3.5).

Atmospheric mineral dust is a carrier of both plant waxes and soil microorganisms, which can act as important CCN [*Möhler et al*., 2007; *Bauer et al*., 2003; *Delort et al*., 2010]. Fatty acids and dicarboxylic acids in highly acidic cloud waters can serve as nutrients for airborne microorganisms and, thus, are subjected to the intense biological degradation [*Deguillaume et al*., 2008; *Vaïtilingom et al*., 2010; *Vaïtilingom et al*., 2013]. Because fresh snow samples were collected from Sapporo during nondust season (December–February) [*Tyagi et al*., 2015b], it is likely that cloud water may have more contribution from airborne plant waxes as CCN rather than dust particles. This idea is based on the predominance of $\omega$-hydroxy FAs (mostly derived from epicuticular plant waxes [*Kolattukudy*, 1980]) over $\beta$-hydroxy FAs (from soil microbes [*Zelles*, 1997]) in fresh snow from Sapporo and vice versa as observed in snowpack samples from Mount Tateyama. In a previous study, *Hamberg et al*. [1999] documented a preferential $\alpha$-oxidation of fatty acids in higher plants by plant pathogens. Further, microbe-mediated oxidation of organic matter in clouds dominates (90–99%) the free-radical reactions especially in nighttime; however, it also contributes significantly in daytime (2–37%) [*Vaïtilingom et al*., 2010]. All

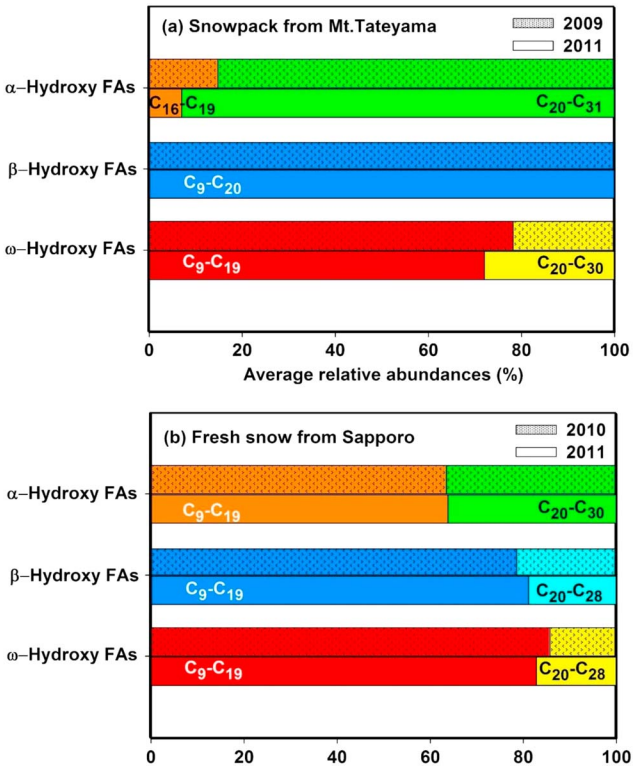


**Figure 9.** The relative abundances of short-chain (<$C_{20}$) and long-chain (>$C_{20}$) $\alpha$-,$\beta$-, and $\omega$-hydroxy FAs in their respective total mass concentration for (a) snowpack samples from Mount Tateyama (this study) and (b) fresh snow from Sapporo [*Tyagi et al*., 2015b].

these observations highlight a potential role of microbial oxidation processes in clouds and consequent shifts in overall relative abundances of $\alpha$-hydroxy FAs from long-chain to short-chain homologues in fresh snow.

The snowpack samples collected from Mount Tateyama in which we observed only short-chain $\beta$-hydroxy FAs (100%) are significantly influenced by Asian dust outbreaks. This observation clearly indicates a strong input of soil microorganisms associated with Asian dust to snowpack in Mount Tateyama. In contrast, the fresh snow samples from Sapporo are influenced by both plant waxes and soil microbes and, thus, explain the occurrence of both short- and long-chain $\beta$-hydroxy FAs (Figure 9). The relative abundances of $\omega$-hydroxy FAs were dominated by short-chain isomers in both fresh snow from Sapporo and snowpack samples from Mount Tateyama. This observation probably indicates that short-chain $\omega$-hydroxy FAs are also originated from higher plants. For instance, some studies documented the predominance of $C_{16}$ followed by $C_{18}$ and/or $C_{14}$ in higher plants [*Kawamura et al*., 2003; *Simoneit et al*., 2004]. Similarly, photochemical oxidation of long-chain $\omega$-hydroxy FAs during transport from Siberia and Russian Far East [*Tyagi et al*., 2015b] could be a potential source of short-chain homologues to both fresh snow (Sapporo) and snowpack (Mount Tateyama) samples. Moreover, based on the year-round observation from Gosan (Tyagi et al., under review in JGR, 2016), the marine aerosols in spring are influenced by Kosa events (dust storms in East Asia) and are characterized by the predominance of $\omega$-hydroxy FAs (~80–85%) followed by $\beta$-isomers (15–20%). Although the contribution of $\alpha$-isomers to total hydroxy FAs mass is lower (1–2%), distribution of long-chain $\alpha$-hydroxy FAs ($C_{21}$–$C_{34}$) in Gosan aerosols is in contrast to that observed in fresh snow [*Tyagi et al*., 2015b] and snowpack samples (where short-chain homologues dominate). Moreover, molecular distributions over Mount Tateyama are characterized by the predominance of $\beta$- and $\omega$-hydroxy FAs followed by $\alpha$-hydroxy FAs in the snowpack samples. Therefore, it is likely that short-chain $\alpha$-hydroxy FAs are produced via oxidation of other hydroxy FAs in aqueous medium.

### 3.4. LPS Mass and GNB Dry Mass in Snow Layers

Previous studies suggested that $\beta$-hydroxy FAs, as determined by GC/MS, can be used as molecular tracers for the presence/absence of airborne LPS, which are also known as pyrogens (fever-causing toxins) [*Maitra et al*., 1986; *Paba et al*., 2013; *Pomorska et al*., 2007; *Saraf et al*., 1997]. Human exposure to airborne LPS (inhalation) has been associated with a variety of clinical symptoms including respiratory disorders [*Mielniczuk et al*., 1993, and references therein]. Airborne LPS mass from GNB is usually measured by extracting the filter samples with water/buffer and analyzing with the Limulus Amebocyte Lysate (LAL) test. Although LAL-based assays of endotoxin/LPS from GNB [*Saraf et al*., 1999] have been employed in occupational environments, they are poorly reproducible and only limited to culturable soil GNB. In the literature, LAL assay is a widely adopted technique that essentially relies on clotting the enzyme activity through an artificial chromogenic substrate, leading to the formation of a gel clot [*Levin and Bang*, 1968; *Iwanaga et al*., 1978]. However, there has been substantial variability in the endotoxin levels with different GNB species, and thus, the LAL assay may not reflect all these differences. Moreover, this method is subject to strong interference from $\beta$-1-3 glucans from airborne molds and plant fragments present in organic dust. Thus, measurement techniques based on a detection of specific LPS molecules, i.e., homologous $\beta$-hydroxy FAs from $C_{10}$ to $C_{18}$, may present a more precise alternative to LAL test.

Unlike LAL assay, estimate of LPS mass from GNB using $\beta$-hydroxy FAs is free of interferences from $\beta$-1-3-glucans [*Saraf et al*., 1999]. Moreover, *Saraf et al*. [1999] observed significant linear correlations between LAL assays and the measured mass concentrations of $C_{10}$ to $C_{18}$ $\beta$-hydroxy FAs in 30 house dust samples. Therefore, a chemical marker-based assessment of "LPS mass of GNB" serves as a pseudo first-order approach and can be employed to both culturable and nonculturable soil GNB. Furthermore, this approach provides some information about the source of LPS due to a fact that molecular distributions of $\beta$-hydroxy FAs from different GNB species vary considerably [*Wilkinson*, 1988]. Although this approach is semiquantitative, in light of paucity of data sets related to quantification of LPS from GNB in the literature, the GC/MS-based quantification serves as a fast and reliable technique (in terms of reproducibility) owing to its large throughput of samples.

In a previous study, *Segawa et al*. [2005] documented the occurrence of two dominant GNB species (*Janthinobacterium lividum* and *Variovorax paradoxus*) in the dust-impacted layers of snow pit samples from Mount Tateyama, based on the analyses of 16S rRNA gene sequencing and real-time PCR. Moreover, their study observed that bacterial biomass component of both Gram-positive (e.g., *C. psychrophilum*) and

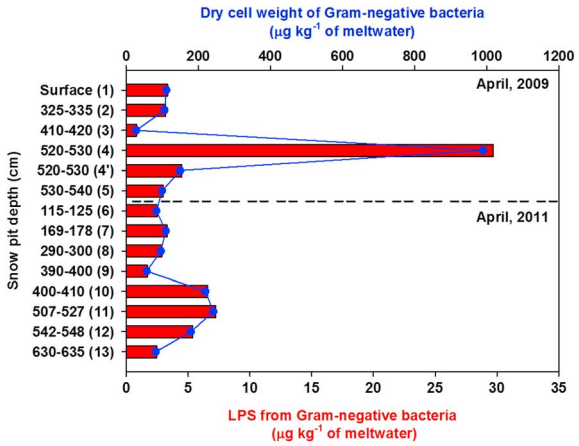


**Figure 10.** Bar graph representing airborne LPS mass (bottom *x* axis, red bars), and blue line shows dry cell weight of Gram-negative bacteria (GNB) (top *x* axis, blue circles) estimated in the snowpack samples deposited from winter to early spring of 2008–2009 and 2010–2011 at the Murodo-Daira site on Mount Tateyama in central Japan. Numbers in the parentheses on the left *y* axis represent the sample IDs as given in Table 1.

Gram-negative (*J. lividum*) increased significantly from March to April as a result of enhanced supply of mineral dust transported from Asian Deserts. Likewise, *Tanaka et al.* [2011] observed the presence of both Gram-negative (e.g., *Methylobacterium sp.*) and Gram-positive species (e.g., *Bacillus* sp.) in the snow pit samples collected in spring 2009 from Mount Tateyama. Interestingly, total bacterial cell counts reported by *Tanaka et al.* [2011] for the snow pit samples from Mount Tateyama in April 2009 were consistent with those collected in March 1998 by *Segawa et al.* [2005]. Therefore, we presume that snow pit samples analyzed for hydroxy FAs are significantly influenced by soil microorganisms associated with Asian dust storms.

In this study, we estimated the amount of "LPS from GNB" on a basis of $\beta$-hydroxy FAs determined in Asian dust-containing and underlying snow layers from the Murodo-Daira site of Mount Tateyama in 2009 and 2011 (Figure 10). We found very high mean concentrations of LPS mass from GNB in sample nos. 4 (29.7 $\mu g\,kg^{-1}$ in 2009), 10 (6.6 $\mu g\,kg^{-1}$ in 2011), 11 (7.3 $\mu g\,kg^{-1}$ in 2011), and 12 (5.4 $\mu g\,kg^{-1}$ in 2011), which are heavily influenced by dust with yellow to brown colors and high abundances of nss-$Ca^{2+}$. Although the mean value of LPS mass from GNB in the snowpack samples in 2009 (7.4 $\mu g\,kg^{-1}$) is almost twice higher than that reported in snow layers in 2011 (4.0 $\mu g\,kg^{-1}$), their median concentrations are consistent between 2009 (3.3 $\mu g\,kg^{-1}$) and 2011 (3.1 $\mu g\,kg^{-1}$). These values are also much higher than those reported in our previous study on fresh snow samples (~0.79 $\mu g\,kg^{-1}$ in 2010 and ~0.59 $\mu g\,kg^{-1}$ in 2011) from Sapporo [*Tyagi et al.*, 2015b]. Several studies were conducted on bacterial LPS mass using $\beta$-hydroxy FAs as biomarkers in the indoor environments [*Hines et al.*, 2003; *Pomorska et al.*, 2007; *Reynolds et al.*, 2005]; however, very few studies are available in relation to their atmospheric transport. Although there are not many studies on the measurement of LPS mass in snow and marine aerosols, this quantification is crucial for assessing a global allergic impact of LPS from GNB via long-range atmospheric transport.

The mass loading of airborne GNB was estimated using marker-to-microbial mass conversion factors as described in section 2.3 (Figure 10). We found that dry cell mass of GNB in snow layers varied from 28 to 990 $\mu g\,kg^{-1}$ (average 248 $\mu g\,kg^{-1}$) in 2009 samples and from 57 to 241 $\mu g\,kg^{-1}$ (average 134 $\mu g\,kg^{-1}$) in 2011. Similar to LPS mass, estimated concentrations of GNB dry cell mass are higher than those obtained in our earlier study conducted for fresh snow samples from Sapporo (average 26.3 $\mu g\,kg^{-1}$ in 2010 and 19.3 $\mu g\,kg^{-1}$ in 2011) [*Tyagi et al.*, 2015b]. *Golokhvast* [2014] reported that airborne biogenic particles, which could be scavenged by snow, can cause allergies to pedestrians in the Russian Far East. Additionally, *Lee et al.* [2007] showed a crustal origin for airborne endotoxin, bacterial and fungal markers. In our study, air masses associated with Asian dust might have been transported from the Gobi and Taklamakan Deserts and Loess Plateau in 2009 and 2011. Thus, source regions of these enhanced LPS and bacterial dry mass could be the arid regions of Mongolia, Siberia, northern China, and the Korean Peninsula during the study period.

Studies have shown that bacteria can be metabolically active even at subzero temperatures [*Amato et al.*, 2007; *Polymenakou*, 2012, and references therein]. Snow can efficiently scavenge these bacterial particles from the atmosphere and, hence, reduce the associated health burden. $\beta$-Hydroxy FAs, cell membrane constituents of GNB, can be used to quantify the dry cell weight in these snowpack samples. We estimated high abundances of LPS content and GNB dry cell mass in the Asian dust-containing snow layers, which clearly affirm their atmospheric transport along with the Asian dust particles. We emphasize that high abundances of LPS and dry cell mass of GNB in the Asian dust-containing snowpack samples confirm that bacterial

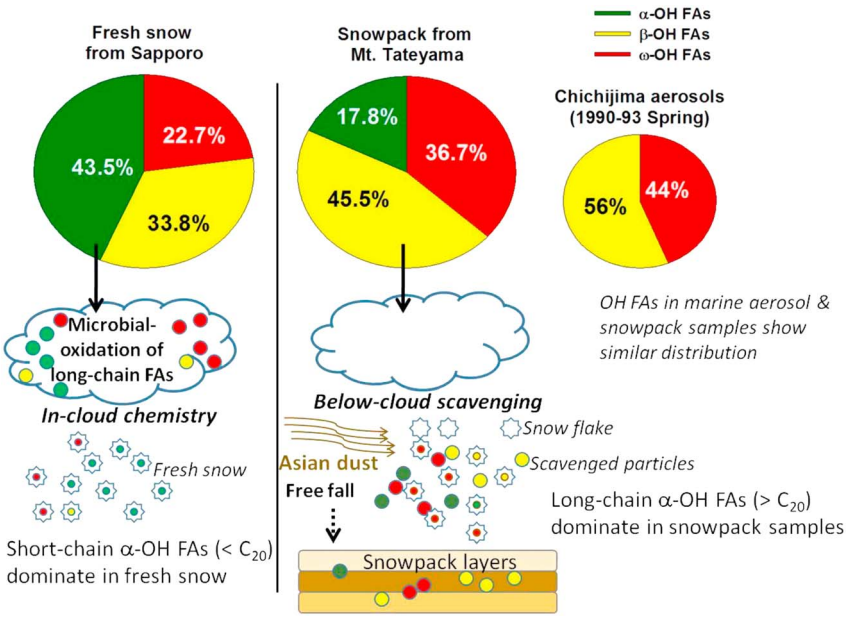


**Figure 11.** A conceptual diagram showing a comparison of relative abundances of hydroxy fatty acids (OH FAs) in snowpack samples collected from Mount Tateyama (central Japan) with fresh snow from Sapporo (northern Japan) [*Tyagi et al.*, 2015b] and aerosols from Chichijima (remote marine island in the western North Pacific) [*Tyagi et al.*, 2015a].

particles and the associated health impacts should be studied for long-range transported air masses on a global scale. Our study has used chemical markers (hydroxy FAs) for the assessment of biogenic particles in the snowpack, which could assist monitoring the microbial content during the Aeolian transport. The results presented here along with previous studies prove the robustness, sensitivity, and wide applicability of hydroxy FAs as tracers, which allow an application to long-range transport of airborne particles.

### 3.5. Inference on "Below-Cloud" and "in-Cloud Oxidation" Processes

A comparison of relative abundances of hydroxy FAs in snowpack samples collected from Mount Tateyama with fresh snow samples from Sapporo reveals that the former samples are characterized by the predominance of $\beta$- or $\omega$-hydroxy FAs, whereas $\alpha$-hydroxy FAs dominate in the latter samples (Figure 11). As mentioned earlier, short-chain ($C_9$–$C_{19}$) homologues of $\alpha$-hydroxy FAs contribute to fresh snow from Sapporo [*Tyagi et al.*, 2015b], whereas long-chain ($C_{20}$–$C_{32}$) homologues contribute mostly to snowpack samples from Mount Tateyama (present study). Furthermore, overall predominance of $\beta$- and/or $\omega$-hydroxy FAs in snowpack samples from Mount Tateyama (collected in spring 2009 and 2011) showed a good agreement with dust-laden air masses sampled over the remote marine island (Chichijima) in the western North Pacific [*Tyagi et al.*, 2015a]. Similarly, molecular distributions of these hydroxy FAs regarding the predominance of $\beta$- and/or $\omega$-hydroxy FAs together with occurrence of long-chain $\alpha$-hydroxy FAs ($C_{16}$–$C_{32}$) in snowpack samples from Mount Tateyama were also consistent with the dust events (Kosa) observed in Jeju Island (Gosan, South Korea; unpublished data). In addition, similar even C-predominance of $C_{10}$ followed by $C_{12}$ or $C_{16}$ $\beta$-hydroxy FAs in snowpack samples was observed in reference materials (CJ-1 and CJ-2) from the source regions of East Asian Deserts.

All these observations provide a hint for the below-cloud oxidation process of $\alpha$-hydroxy FAs. Several studies have documented the occurrence of GNB and soil microbes in cloud water (as CCN) and ice nuclei. Cloud waters are generally more acidic (pH ~ 2–4) and often contain high amount of oxygenated water-soluble species and free radicals. A close resemblance in the overall predominance of $\omega$- and $\beta$-hydroxy FAs between snowpack samples (present study) and marine aerosols conceivably indicates that Mount Tateyama represents "below-cloud scavenging" of transported dust particles and associated soil microbes by snow in the East Asian outflow during spring/winter. Moreover, these dust particles can be engulfed as ice nuclei and CCN to form snowflakes, which further scavenge atmospheric particles during snowfall.

As snow essentially comes from cloud water, the occurrence of both short- and long-chain $\alpha$-hydroxy FAs in fresh snow [*Tyagi et al*., 2015b] could be a result of direct emissions/in situ oxidation processes of microbes in cloud water. A close examination of results from *Tyagi et al*. [2015b] on the predominance of $\alpha$-hydroxy FAs followed by $\omega$-isomers together with similar even C-predominance of $C_{16}$ followed by $C_{22}$ or $C_{24}$ in fresh snow suggests an in-cloud oxidation of FAs. This inference can be further supported by higher relative abundances of short-chain homologues ($C_9$–$C_{20}$) accounting for ~65% of total ($C_9$–$C_{30}$) $\alpha$-hydroxy FAs in fresh snow [*Tyagi et al*., 2015b], suggesting their production through $\alpha$-oxidation. The microbial oxidation of fatty acids in cloud water could be a likely cause for the production of $\alpha$-hydroxy FAs (as discussed in section 3.2.1.) observed in fresh snow, being in contrast to those observed for dust-laden snowpack samples (this study) and aerosols [*Tyagi et al*., 2015a]. All these observations highlight a potential role of microbial oxidation processes in clouds. Therefore, comparative distributions of hydroxy FAs in snowpack samples from Mount Tateyama with fresh snow from Sapporo and marine aerosols from Chichijima provide additional insights toward the aeolian transport of soil microbes from East Asia to the western North Pacific.

## 4. Summary and Conclusions

Three homologous series of positional isomers of hydroxy FAs, i.e., $\alpha$-($C_{16}$–$C_{31}$), $\beta$-($C_9$–$C_{20}$), and $\omega$-($C_9$–$C_{30}$), were found in snowpack samples collected from snow pit sequences at the Murodo-Daira site in Mount Tateyama, central Japan. The molecular distributions of hydroxy FAs were characterized by an even C-number predominance, which is characteristic to lipid biomarkers from soil microorganisms and higher plant waxes. The occurrence of only short-chain homologues (i.e., specific to soil GNB) of $\beta$-hydroxy FAs and their significant linear relation with water-soluble nss-$Ca^{2+}$ indicate that snowpack samples are strongly influenced by soil microbes from Asian dust outbreaks. Moreover, air mass back trajectories and covariance of hydroxy FAs and nss-$Ca^{2+}$ in snowpack samples from Mount Tateyama suggest a long-range atmospheric transport of soil microbes and plant waxes originated from the Asian continent including Siberia, Mongolia, and Russian Far East. Overall, our study is one of the first attempt to employ hydroxy FAs as proxies to trace and quantify bacteria and their debris (biomass) as well as plant waxes in the Asian dust-containing and underlying snow layers in Mount Tateyama. Our results, along with other culture-dependent and culture-independent approaches, can provide a better understanding of long-range atmospheric transport of Asian dust-associated soil-borne microorganisms.

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