MS-Ref-No: bg-2015-387

Reviewer #1 (Comments to Author):

Overall comment: Tyagi et al describe distributions and abundances of, β-, and ω–hydroxy fatty acids in snow samples from the city of Sapporo in northern Japan. Along with air mass back trajectories, they use the hydroxyl acids are indicators of long-range atmospheric transport of continental soil material from Siberia and North China. Scavenging of hydroxyl-acids by snowfall removes these components from the atmosphere. Since hydroxy fatty acids, especially the β-isomers, are components of the lipopolysaccharides (LPS) of Gram negative bacteria (GNB), the concentrations of these acids are used to estimate the amount of GNB endotoxin/LPS that might effectively be removed from the atmosphere by scavenging in snow. By and large, the manuscript is well written and for the most parts the conclusions re-supported by the data and its presentation (tables and figures). However, some revisions are needed that would improve the manuscript and clear up several questions.

Response: We thank the reviewer's encouragement towards publication of the MS. We have now carefully revised the MS by taking all suggestions/comments. Below here are the point-by-point responses to reviewer's comments

Comment: p 1337 line 4 – "these plant pathogenic bacteria" – this would read a bit better if it were "these bacteria, which are plant pathogens, can influence".

Response: The sentence has been rephrased in the introduction as follows:

"these bacteria, which are plant pathogens, can influence the regional as well as global climate through cloud aerosol interactions." Please see lines 64-65 in the revised MS.

Comment: p 13379 line 6 onwards in this paragraph. This is pretty much verbatim from the Yamamoto paper that is cited in the next paragraph. Perhaps a bit different wording is needed.

Response: These sentences were reworded for clarity in the revision as follows:

"The detailed description about snow collection and analytical protocol of lipid fraction analyses is similar to that described in Yamamoto et al. (2011). To avoid the contribution of

any possible impurities from the dry deposition of aerosols, 1-2 cm of surface snow cover were removed prior to sample collection. Thereafter, snow samples were collected into a cleaned glass jar (8 L) by using a stainless steel shovel. In each glass jar, mercuric chloride ($HgCl_2$) was added before sampling to prevent microbial activity. Soon after the collection, glass jars were tightened with a Teflon-lined screw cap and stored at -20 ºC until analysis."

Please see lines 117-124 in the revised MS.

Comment: p 13379 section 2.2. The protocol of Yamamoto et al. used weak acid hydrolysis. Is this adequate to get at the LPS-hydroxy acids since this analysis usually requires stronger acid and heating for some period of time? Otherwise it seems that the hydroxyl acids reported here are mostly free (unbound) ones. This might not make much of a difference, but it should be noted.

Response: We agree that extraction of LPS-hydroxy fatty acids (FAs) requires stronger acid and heating for some period of time. To extract LPS-bound hydroxy FAs, snow samples were saponified with KOH/methanol at 80 ºC for 2 h. Later, solvent was acidified with 6 M HCl (strong acid) and then derivatized to methyl esters. We believe that by using this technique, most of the LPS-bound fatty acids can be extracted from the snow melt water. To elaborate more on the extraction procedure of hydroxy FAs, we have made additional statements in section 2.2 as follows:

"*In brief, melted snow samples (0.5-1 L) were saponified with 1.0 M KOH in methanol at 80 ºC for 2 h. After saponification, neutral fraction was separated and remaining solution was acidified with 6 M HCl to form free carboxylic acids. Further, these acids were derivatized with BF_3/methanol to form their methyl esters. The hydroxy acid methyl esters were isolated on a silica gel column by eluting with methylene chloride/methanol (95:5). The hydroxy FA methyl esters were, then, derivatized to their trimethylsilyl (TMS) ethers with N,O-bis-(trimethylsilyl) trifluoroacetamide (BSTFA) (SUPELCO™ Analytical) at 70 ºC for 1 h.*" Please see lines 127-134 in the revised MS.

Comment: On the other hand, the manuscript later on used the hydroxyl-acid concentrations to estimate GNB endotoxin concentrations. Are the hydroxyl-acids in the mathematical expression for calculating endotoxin (p 13380 line12) "free", 'bound" or "total". Whichever is the case, this should be explained.

Response: Following the comment, we have added a sentence to explain the text as follows:

"β-Hydroxy FAs in the mathematical expression are the total (LPS-bound+free) hydroxy FAs for the carbon numbers from C_{10} to C_{18}." Please see lines 161-162 in the revised MS.

Comment: Are the estimated of endotoxin calculate here "lower limits" due to the specifics of the analytical protocol? p 13384 section 3.4. Hydroxy acids can derive from either plant waxes or soil GNB, as pointed out. How might these be distinguished, in order that the amount of GNB-derived endotoxin may be calculated?

Response: The estimated lower limits of endotoxin in Table 1 and Table 2 are calculated based on the minimum concentration of β-hydroxy FAs (C_{10}-C_{18}), which are specific to Gram-negative bacteria (GNB). As stated on page 3, β-hydroxy FAs (C_{10}-C_{18}) are the structural constituents of lipid A, which are present in the outer cell membrane of GNB. Thus, the endotoxin concentrations in snow samples were estimated based on the abundances of β-hydroxy FAs having carbon chain length from 10 to 18 (section 2.3). Being consistent with this study, Lee et al. (2004) also reported endotoxin concentration based on β-hydroxy FAs (C_{10}-C_{18}). These points have been added in the revised MS. Please see lines 279-286.

Comment: p 13384 line 13. At least the Wakeham et al. paper did not assay endotoxin LPS, at least not directly. Don't know about the other papers. Perhaps the text should simply read that hydroxyl-acids were assayed in these references.

Response: Following the comment, we have changed the sentence in section 3.4 as follows:

"The β-hydroxy FAs, marker for endotoxin/LPS, were assayed in various environmental samples such as dust (Andersson et al., 1999; Hines et al., 2000), aerosols (Lee et al., 2004, 2007; Walters et al., 1994), soils (Keinänen et al., 2003), sewage (Spaan et al., 2008) and marine dissolved organic matter (Wakeham, 1999)." Please see lines 271-274 in the revised MS.

Comment: p 13384 lines 18 and 24. Are the concentrations ng kg^{-1} and μg kg^{-1} for kg of unmelted snow, or kg of melt water?

Response: The concentrations of endotoxin and GNB dry cell mass in snow samples are given in ng kg^{-1} and μg kg^{-1} of the melt water, respectively. We have now made the changes in the mathematical expression as follows: *Endotoxin (LPS, ng kg^{-1} of melt water) (i.e., in mg kg^{-1} of melt water)*, respectively.

Please see lines 157 and 168 in the revised MS.

Comment: A little background would be useful – any information about concentrations of endotoxin or GNB biomass in rainwater (presumably this also scavenges these components); what concentrations of airborne biogenic particles in snow might be causing the allergic reactions noted in Golokhvast et al, for comparison with the concentrations reported here?

Response: We agree with the comment. Indeed rain and snow potentially scavenge the airborne biogenic particles. Following the comment, we have made additional statements as follows:

"The airborne biogenic particles can be scavenged efficiently by both wet precipitation and snow fall. Therefore, we have looked for the literature describing the occurrence of GNB in rainwater for comparison with our study on Sapporo snow. Towards this, Gould (1999) and Lye (2002) have documented the presence of various GNB (e.g., Salmonella, Shigella, Vibrio, Legionella and Campylobacter spp.) species in rainwater. Likewise, Kawamura and Kaplan (1983) also reported the presence of β-hydroxy FAs in rain water samples collected from Los Angeles (USA) and attributed their sources as bacterial membrane." Please see lines 297-304 in the revised MS.

Although we mentioned in the text that "Golokhvast et al. (2014) have identified the airborne biogenic particles in melted snow using light microscope and electronic microscope attached with an energy-dispersive spectrometer (EDAX)", they never reported the quantification on the abundances of GNB in snow.

Hydroxy fatty acids in fresh snow samples from northern Japan: long-range atmospheric transport of Gram-negative bacteria by Asian winter monsoon

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Key points:

- Hydroxy fatty acids (FAs) in snow indicate contribution from soil microbes and higher plants.
- Air mass back-trajectories reveal their transport from Russia, Siberia and China.
- Fresh snow acts as filter to reduce β-hydroxy FAs and endotoxin from the atmosphere and their further transport.

Short title: *Hydroxy fatty acids in fresh snow*

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Abstract

Hydroxy fatty acids (FAs) in fresh snow from Sapporo, one of the heaviest snowfall regions in the world, have been studied to ascertain the airborne bacterial endotoxin concentrations and their biomass. The presence of β-hydroxy FAs (C_9-C_{28}), constituents of Gram-negative bacteria (GNB), suggests long-range transport of soil microbes. Likewise, the occurrence of α- and ω-hydroxy FAs (C_9-C_{30} and C_9-C_{28}, respectively) in snow reveals their contribution from epicuticular waxes and soil microorganisms. Estimated endotoxin and GNB mass can aid in assessing their possible impacts on the diversity and functioning of aquatic and terrestrial ecosystems, as well as lethal effects on pedestrians through dispersal of microbes. Air mass back trajectories together with hydroxy FAs unveil their sources from Siberia, Russian Far East and North China by the Asian monsoon. This study highlights the role of fresh snow that reduces the human health risk of GNB and endotoxin by the scavenging from air.

Keywords

Hydroxy fatty acids, fresh snow, Gram-negative bacteria, endotoxin, long-range atmospheric transport.

1. Introduction

Lipid biomarkers from terrigenous plants, algae, fungi and soil microorganisms have been reported extensively in aerosols (Conte and Weber, 2002; Gagosian et al., 1987; Gagosian et al., 1981; Kawamura, 1995; Kawamura et al., 2003; Simoneit, 1977; Simoneit et al., 2004), sediments (Kawamura, 1995; Kawamura and Ishiwatari, 1984; Kawamura et al., 1987; Zhang et al., 2014), ice core (Sankelo et al., 2013) and rain/snow (Kawamura and Kaplan, 1986; Satsumabayashi et al., 2001; Yamamoto et al., 2011). These studies have utilized fatty acids as a proxy to assess the terrigenous contribution of higher plant waxes to various environmental samples owing to their abundant presence in biopolymers of plants and microorganisms. Similarly, certain hydroxy fatty acids (e.g., C_{10}-C_{18} β-hydroxy FAs) have been proposed as a tracer to understand the airborne bacterial transport (Tyagi et al., 2015).

Among the airborne soil microbes, the Gram-negative bacterium (GNB) is one of most extensively studied bacteria and is documented in aerosols, snow and rain samples (Morris et al., 2011). Owing to considerable ground based emissions of GNB and its ability to act as cloud condensation nuclei (CCN), these bacteria, which are plant pathogens, can influence the regional as well as global climate through cloud aerosol interactions (Morris et al., 2011 and references therein). In particular, GNB contains β-hydroxy FAs (C_{10}-C_{18}) in their lipid A fraction of lipopolysaccharides (LPS) as constituents of outer cell membrane (Westphal, 1975). Moreover, the environmental toxic effects of GNB are, in part, due to the presence of β-hydroxy FAs present in LPS (endotoxin) (Larsson, 1994; Saraf et al., 1997; Spaan et al., 2008).

Apart from β-hydroxy FAs, other positional isomers such as α-, ω- and (ω-1)-hydroxy FAs have also been documented in various environmental archives viz. aerosols (Kawamura, 1995; Tyagi et al., 2015) and sediments (Kawamura, 1995; Wakeham et al., 2003; Zhang et al., 2014). Short chain α-hydroxy FAs (C_{12}-C_{18}) are the constituent

biopolymers of fungi (Zelles, 1997), soil bacteria (Steinberger et al., 1999; Zelles and Bai, 1994) and protozoa (Ratledge and Wilkinson, 1988). In contrast, long chain α-hydroxy FAs (C_{16}-C_{26}) are abundant in plants, microalgae and cyanobacteria (Matsumoto and Nagashima, 1984). Likewise, ω- and (ω-1)-hydroxy FAs are highly cross-linked constituents of the cell walls of algae (Blokker et al., 1999) and plant seeds, suberin and cutin in terrestrial higher plants (Molina et al., 2006). In addition, ω- and (ω-1)-hydroxy FAs are the intermediates in the oxidation of monocarboxylic acids to dicarboxylic acids in sediments and marine aerosols (Kawamura, 1995; Kawamura and Gagosian, 1990). Further, specificity of hydroxylation in FAs depends on the type of microorganisms involved (Wakeham, 1999).

These tracer compounds in snow samples may be important to better understand the contribution of plant and pathogenic bacteria to regional versus long-range atmospheric transport (Hines et al., 2003; Lee et al., 2004; Lee et al., 2007; Tyagi et al., 2015) as their presence in the atmosphere can affect the CCN and ice nuclei activity (Morris et al., 2008). To the best of our knowledge, our study is the first to report α, β- and ω-hydroxy FAs in snow samples. Snow efficiently scavenges airborne particles including soil microbes and higher plant metabolites in the free boundary layer of troposphere. Since hydroxy FAs from GNB and plants are inert in nature, they do not undergo chemical modification during snow accumulation. Therefore, hydroxy FAs in fresh snow can be used as a tracer to assess the sources and transport pathways of microorganisms and plant metabolites.

In this study, we determined hydroxy FAs in fresh snow samples collected from Sapporo, Japan, to evaluate the qualitative contribution from GNB and higher plant metabolites. Our results support the hypothesis that these hydroxy FAs are important tracers to better understand the contribution of microorganisms to the organic matter in snow. More importantly, we also discuss the possible transformations of these chemical markers during long-range atmospheric transport.

2. Experimental methods

2.1. Site description and sample collection

Sapporo (43.07 °N, 141.36 °E) is the capital of Hokkaido, whose population is 1.9 million (June, 2013). Sapporo receives cold and dry air masses with heavy snowfall during the Asian winter monsoon. The average temperature of Sapporo in winter goes up to ~ 2 °C (Yamamoto et al., 2011). Snow cover over the ground and fallen leaves of deciduous plants suppresses the suspension of soil particles during winter whereas the emissions of plant biomarkers from local vegetation are minimal. During winter season, Asian monsoon affects the regional climate, air quality and human health in Japan, delivering anthropogenic aerosols and dust from China and Siberia (Yamamoto et al., 2011). Several studies have examined the chemical and isotopic composition of ambient aerosols in various types of air masses in Sapporo (Aggarwal and Kawamura, 2008; Pavuluri et al., 2013; Yamamoto et al., 2011) to better understand the impacts of anthropogenic and biogenic contributions from Siberia, North China and surrounding oceans. However, no study is available from Sapporo, which focuses on the transport of microorganisms using organic markers.

In this study, eleven fresh snow samples were collected from the rooftop of the Institute of Low Temperature Science (ILTS) building, Hokkaido University in Sapporo during intensive snow fall periods (January-March) in 2010 and 2011. The detailed description about snow collection and analytical protocol of lipid fraction analyses is similar to that described in Yamamoto et al. (2011). To avoid the contribution of any possible impurities from the dry deposition of aerosols, 1-2 cm of surface snow cover were removed prior to sample collection. Thereafter, snow samples were collected into a cleaned glass jar (8 L) by using a stainless steel shovel. In each glass jar, mercuric chloride ($HgCl_2$) was added before sampling to prevent microbial activity. Soon after the collection, glass jars were tightened with a Teflon-lined screw cap and stored at -20 ºC until analysis.

125 **2.2. Identification and quantification of hydroxy FAs**

126 The analytical protocol used for assessing the atmospheric abundances of
127 hydroxy FAs is described in Yamamoto et al. (2011). In brief, melted snow samples (0.5-1 L)
128 were saponified with 1.0 M KOH in methanol at 80 ºC for 2 h. After saponification, neutral
129 fraction was separated and remaining solution was acidified with 6 M HCl to form free
130 carboxylic acids. Further, these acids were derivatized with BF_3/methanol to form their
131 methyl esters. The hydroxy acid methyl esters were isolated on a silica gel column by eluting
132 with methylene chloride/methanol (95:5). The hydroxy FA methyl esters were, then,
133 derivatized to their trimethylsilyl (TMS) ethers with N,O-bis-(trimethylsilyl)
134 trifluoroacetamide (BSTFA) (SUPELCO^{TM} Analytical) at 70 ºC for 1 h. After the reaction, 50
135 µl of n-hexane solution containing 1.43 ng μl^{-1} of internal standard (C_{13} n–alkane/tridecane,
136 Wako) was added to dilute the derivatives prior to GC/MS injection (Hewlett-Packard, Model
137 6890 GC coupled to Hewlett-Packard Model 5973 mass-selective detector, MSD). The GC
138 was installed with a split/splitless injector and DB-5MS fused silica capillary column.

139 For the quantification of hydroxy FAs, the GC oven temperature was
140 programmed from 50 °C (2 min) to 305 °C (15 min) at 5 °C min^{-1}. Data were acquired and
141 processed with the Chemstation software. Structural identification and comparison of
142 retention time of hydroxy FAs were performed using authentic TMS derivatives of n–C_{12} and
143 n–C_{16} α-hydroxy FAs, n–C_{12}, n–C_{14}, n–C_{15}, and n–C_{16} β-hydroxy FAs and n–C_{16}, n–C_{20} and
144 n–C_{22} ω-hydroxy FAs. The recoveries of authentic fatty acid standards were better than
145 92±4% with analytical error (average 4.1%) for acidic compounds (Yamamoto et al., 2011).
146 Laboratory blanks showed no contamination of any target compounds. The results of n-
147 alkanes, n-alkanols and n-alkanoic acids (terrestrial biomarkers) in snow samples are reported
148 in Yamamoto et al. (2011), which revealed a long-range atmospheric transport of terrestrial
149 organic materials from Northeast Asia to North Japan by the Asian winter monsoon.

150 **2.3. Estimation of endotoxin levels and mass loading of GNB**

151 Since the endotoxins from GNB contain β-hydroxy FAs from C_{10} to C_{18},
152 previous studies attempted to quantify atmospheric abundances of endotoxins using the
153 concentrations of ambient hydroxy FAs measured (Lee et al., 2004; Rietschel et al., 1984;
154 Wilkinson, 1988). According to these studies, concentrations of endotoxins in snow samples
155 were estimated by the mathematical expression as below.

156

157 Endotoxins (LPS, ng kg^{-1} of melt water) = [(Σ β-hydroxy FAs from C_{10} to C_{18}; nmol kg^{-1})
158 x 8000]/4

159

160 In the above formula, the average molecular weight of endotoxin corresponds to
161 8000 as reported by Mielniczuk et al. (1993). β-Hydroxy FAs in the mathematical expression
162 are the total (LPS-bound+free) hydroxy FAs for the carbon numbers from C_{10} to C_{18}. We also
163 estimated the mass loading of airborne GNB using the approach initially suggested by
164 Balkwill et al. (1988) and later on by Lee et al. (2004), in which they used the chemical
165 marker to bacterial mass conversion factor of 15 nmol of β-hydroxy FAs (C_{10}-C_{18}) per mg dry
166 cell weight. Therefore, we have converted the sum of mass concentrations of β-hydroxy FAs
167 from C_{10} to C_{18} (in nmol kg^{-1}) into equivalent dry cell weight of GNB (i.e., in mg kg^{-1} of
168 melt water) by normalizing with 15.

169

170 **3. Results and discussion**

171 **3.1. Air mass backward trajectory analysis**

172 The air mass back-trajectories (AMBTs) provide a means to qualitatively assess
173 the source regions of airborne pollutants over a receptor site. For this study, we have
174 computed seven day isentropic AMBTs using hybrid single particle lagrangian integrated

trajectory (HYSPLIT) model (Draxler and Rolph, 2013 and references therein). The meteorological parameters (GDAS data sets) from NOAA air resources laboratory were used as an input for the HYSPLIT model. Figure 1 shows the AMBT cluster at an arrival height of 500 m over Sapporo during sampling days of winter 2010 and 2011. In almost all snow-sampling periods in Sapporo, the AMBTs show plausible influence of air masses from Russia and Siberia via the long-range atmospheric transport.

3.2. Concentrations of hydroxy fatty acids

Homologues series of α-, β- and ω-hydroxy FAs were detected in fresh snow samples collected from Sapporo. Their mass concentrations are summarized in Table 1 and Table 2 for winter 2010 and 2011, respectively. Based on two-year seasonal data on hydroxy FAs, we found that concentrations of α-hydroxy FAs are significantly higher than β- and ω-hydroxy FAs. The predominance of α-hydroxy FAs can be explained by the α-oxidation pathway of FAs, which generally occurs in plants, animals and bacteria (Cranwell, 1981 and references therein) whereas β- and ω-oxidation is specific to bacteria (Lehninger, 1975). α-Hydroxy FAs, in particular high molecular weight ones, come from the epicuticular waxes of higher plants as well from algae. However, we also found higher abundance of α-hydroxy FAs in the biomass burning aerosols collected over Mt. Tai, China (Tyagi et al., 2015, manuscript in preparation), possibly due to photochemical oxidation of higher molecular weight fatty acids. Such a possibility of in situ formation of α-hydroxy FAs has also been reported in the hydrolysis products of leaf waxes and wood, and in microalgae and sea grasses (Feng et al., 2015). Further, microbial oxidation could also be a possible source of α-hydroxy FAs (Eglinton et al., 1968) in the snow samples studied. Hence, we suggest that α-hydroxy FAs cannot be employed as the tracers of plant waxes only, as they can come from microbial/photochemical oxidation of higher molecular weight fatty acids during long-range atmospheric transport.

A characteristic feature of our data is the predominance of C_{16} hydroxy FAs in all the types of hydroxy FAs measured. However, significant shifts were observed in the carbon numbers of the second most abundant β-hydroxy FAs (mostly C number >16) and ω-hydroxy FAs (i.e., C number <16; see Tables 1 and 2). A likely explanation for this observation is that β-hydroxy FAs above C_{16} were formed by β-oxidation of long chain FAs, which is a more common in microorganisms as discussed previously. In contrast, ω-hydroxy FAs below C_{16} are present in plants and microbes (Cardoso and Eglinton, 1983), in which ω-oxidation of fatty acids is secondary choice for microbial oxidation.

3.3. Molecular distributions

Figure 2 presents molecular distributions of α-hydroxy (C_9 to C_{30}), β- and ω-hydroxy FAs (C_9 to C_{28}) in snow samples from Sapporo during winter 2010 and 2011. Even carbon number predominance is noteworthy for α-, β- and ω-hydroxy FAs. α-Hydroxy FAs show molecular distributions with the order $C_{16} > C_{24} > C_{22}$ in both years (see Figure 2a). Likewise, β-hydroxy FAs show the predominance of C_{16} followed by C_{18} or C_{20} and then by C_{14} in both winters. However, we found the predominance of C_{20} β-hydroxy FAs over C_{16} in one snow sample during 2010. Similarly, ω-hydroxy FAs showed dominance of C_{16} followed by the others as $C_{14} > C_{12} \sim C_{22} \sim C_{24}$ during snowfall in both the years.

Table S1 describes the statistically significant differences in the ratios of even to odd carbon numbers for α-, β-, and ω-hydroxy FAs in snow samples based on two-tailed unpaired *t* test. No significant differences were observed between 2010 and 2011 for the ratios of even to odd carbon number α-hydroxy FAs. In contrast, the difference is statistically significant between 2010 and 2011 for β- and ω-hydroxy FAs. In fact, the difference is extremely larger for ω-hydroxy FAs than that for β isomers. In 2010 winter, AMBTs show atmospheric transport from the continents at 500, 1000 and 1500 m above ground, however, at

the same heights in 2011 winter, the air masses came from the oceans during one sample collection. Higher plants in the continents contribute to higher abundances of hydroxy FAs than the oceans, and thus explain higher abundances of β- and ω–hydroxy FAs in 2010 than 2011. On average, even carbon numbered α-, β- and ω-hydroxy FAs in their total mass concentrations account for ~69, 68 and 84%, respectively. The even carbon number predominance is also found in recent marine and lacustrine sediments (Cardoso and Eglinton, 1983; Goossens et al., 1986; Kawamura, 1995; Zhang et al., 2014).

Similar to our study, Volkman et al. (1980) documented the bimodal distribution of α-hydroxy FAs with peaks at C_{16} and C_{24} in the intertidal sediments from Victoria, Australia and attributed their contribution from sea grass (i.e., Zostera muelleri) detritus owing to similar distribution pattern. However, it is noteworthy that our AMBTs show a continental origin rather than the oceanic origin. Therefore, it is possible that waxes emitted from continental grasses via wind abrasion can be transported to Sapporo through the atmosphere. We speculate that α-hydroxy FAs (C_{16}-C_{28}) in Sapporo snow can be used as a tracer of plant waxes. Likewise, higher plant derived cutin and suberin have been suggested as a significant source of C_{16} to C_{22} α-, β- and ω-hydroxy FAs (Cardoso and Eglinton, 1983). In a similar way, it has been proposed that hydroxy FAs (C_{20}-C_{30}) are principally derived from terrestrial higher plants (Kawamura and Ishiwatari, 1984). Therefore, α-, β- and ω-hydroxy FAs (C_{16}-C_{22}) in snow samples can be related to their sources from terrestrial higher plants through long-range atmospheric transport.

Previous studies documented ubiquitous occurrence of these hydroxy FAs in soil microbes such as yeast and fungi (Van Dyk et al., 1994 and references therein) and in the LPS of GNB (Lee et al., 2007). In this regard, prior studies focussing on β-hydroxy FAs with the predominance of C_{16} and C_{18}, suggested the contributions from yeast and fungi (Stodola et al., 1967; Van Dyk et al., 1994 and references therein). Molecular distributions of β-hydroxy FAs

249 show a predominance of C_{16} followed by C_{18} or C_{20} (see Figure 2b), suggesting that they
250 have been derived from soil microbes. Likewise, FAs $<C_{20}$ are derived from marine
251 phytoplankton (Kawamura, 1995 and references therein). β-Hydroxy FAs (C_{10}-C_{18}) have been
252 proposed as a biomarker for soil microbes as they are the constituents of LPS of GNB (Lee et
253 al., 2004; Szponar et al., 2002). Hence, it is likely that β-hydroxy FAs in snow samples may
254 have been significantly influenced by GNB and terrestrial higher plant metabolites.

255 Figure 3 depicts bar graphs, showing the relative abundances of α-, β- and ω-
256 hydroxy FAs in the snow samples from Sapporo during winter. We found that the proportions
257 of two classified groups (low molecular weight C_9-C_{19} and high molecular weight C_{20}-C_{30} or
258 C_{20}-C_{28}) of α-, β- and ω-hydroxy FAs are very similar between 2010 and 2011 (Figure 3).
259 This observation is perhaps related to their common sources/transport pathways of α-, β- and
260 ω-hydroxy FAs over Sapporo. This inference is further supported by the AMBTs computed at
261 arrival heights of 500, 1000 and 1500 m (see Figure 1 and Figure S1), indicating similar air
262 mass transport pathway from Russia and Siberia.

263 **3.4. Endotoxin potency of GNB-impact via Aeolian transport**

264 Endotoxin in GNB determines their viability and potentially causes pathological
265 effects on mammals (Lüderitz et al., 1981; Westphal, 1975). In particular, GNB contain LPS
266 in their outer membrane. When bacteria multiply, die and lyse, LPS are released from the
267 surface as a potential bacterial toxin, and therefore called as endotoxin (Westphal, 1975). In
268 addition to intact bacterial cells, this endotoxin can trigger to cause allergies, respiratory
269 problems and infections. Researchers have used LPS concentrations as a measure of GNB,
270 primarily by Limulus Amebocyte Lysate (LAL) Assay which has limited specificity (Saraf et
271 al., 1997). The β-hydroxy FAs, marker for endotoxin/LPS, were assayed in various
272 environmental samples such as dust (Andersson et al., 1999; Hines et al., 2000), aerosols (Lee

et al., 2004; Lee et al., 2007; Walters et al., 1994), soils (Keinänen et al., 2003), sewage (Spaan et al., 2008) and marine dissolved organic matter (Wakeham, 1999).

As mentioned in section 2.3, we have estimated the abundances of endotoxin and mass loading of GNB in fresh snow samples. This quantification is indeed crucial for assessing a likely allergic impact of endotoxin globally via long-range atmospheric transport. Here, we estimated the endotoxin concentrations in snow varied to be 424 to 1080 ng kg^{-1} (av. 789±237 ng kg^{-1}) in 2010 and 36 to 1100 ng kg^{-1} (av. 579±435 ng kg^{-1}) in 2011 samples. The estimated lower limits of endotoxin in Table 1 and Table 2 are calculated based on the minimum concentration of β-hydroxy FAs (C_{10}-C_{18}), which are specific to Gram-negative bacteria (GNB). As stated on page 3, β-hydroxy FAs (C_{10}-C_{18}) are the structural constituents of lipid A, which are present in the outer cell membrane of GNB. Thus, the endotoxin concentrations in snow samples were estimated based on the abundances of β-hydroxy FAs having carbon chain length from 10 to 18 (section 2.3). Being consistent with this study, Lee et al. (2004) also reported endotoxin concentration based on β-hydroxy FAs (C_{10}-C_{18}). Although relative abundances of endotoxin during winter 2010 (N = 5) are higher than those of 2011 samples (N = 6), the two-tailed t-test revealed no significant differences ($t = 0.96$; $df = 9$; $P > 0.05$) with regard to mean concentrations of the two years.

In this study, we estimated dry mass concentrations of GNB in snow samples to be 26.3±7.9 μg kg^{-1} in 2010 v.s. 19.3±1.4 μg kg^{-1} in 2011. Lee et al. (2007) reported that airborne endotoxin is of crustal origin and thus can be transported long distances to the outflow region. Since the AMBTs reveal the impact of long-range transport from Russia and Siberia during the study period, we infer that estimated endotoxin concentrations and dry cell weight of GNB over Sapporo are derived from those source regions. Recently, Golokhvast (2014) documented the airborne biogenic particles in snow from Russian Far East that cause allergy for the pedestrians. The airborne biogenic particles can be scavenged efficiently by

298 both wet precipitation and snow fall. Therefore, we have looked for the literature describing
299 the occurrence of GNB in rainwater for comparison with our study on Sapporo snow.
300 Towards this, Gould (1999) and Lye (2002) have documented the presence of various GNB
301 (e.g., Salmonella, Shigella, Vibrio, Legionella and Campylobacter spp.) species in rainwater.
302 Likewise, Kawamura and Kaplan (1983) also reported the presence of β-hydroxy FAs in rain
303 water samples collected from Los Angeles (USA) and attributed their sources as bacterial
304 membrane. So far, no literature is available on endotoxin and GNB concentrations in snow
305 samples from East Asia in order to make a comprehensive comparison with the present study.
306 Overall, the presence of endotoxin and GNB in snow affirms that biogenic
307 particles of soil microbes and their potential health impact should not be overlooked. Routine
308 and long-term measurements of airborne chemical markers (hydroxy FAs in this study) could
309 aid the monitoring of the microbial content in long-range transported air masses. Further
310 studies are required to examine their distributions in the atmospheric environment and health
311 effects on human beings in the regional and global perspectives during long-range
312 atmospheric transport.
313
314 **4. Conclusions**
315 Although low temperature is considered to be a limiting factor for bacterial
316 activity in air/snow, some studies have shown that bacteria can be metabolically active even at
317 subzero temperatures (Polymenakou, 2012 and references therein). Figure 4 summarized the
318 whole idea, which was addressed in this study. We conclude that fresh snow in Japan acts as a
319 filter, which aids in reducing the burden of pathogenic microbes from the atmosphere via wet
320 scavenging of these particles.
321 Owing to prolonged winters and thus, snow fall in Sapporo, it is likely that
322 ambient bacterial endotoxin (LPS) is largely scavenged from the atmosphere by snow, which

323 can decrease their effect on human health via inhalation (Jacobs, 1989; Milton, 1996).
324 However, without snow scavenging, ambient bacterial endotoxin levels may stay high; having
325 an influence on human health as well can be transported to further long distances (North
326 Pacific). Overall, bacteria and their debris (biomass) can be evaluated in aerosols that are
327 scavenged by snow in free troposphere without prior culture by the determination of hydroxy
328 FAs for both LPS and GNB.
329

330 **Author contribution**

331 SY extracted the samples and conducted the experiments. PT prepared the
332 manuscript with contribution from KK.
333

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338

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Table 1. Mass concentrations (in ng kg^{-1}) of α-, β- and ω-Hydroxy fatty acids (FAs) measured in snow samples (N=5) collected from Sapporo during winter 2010.

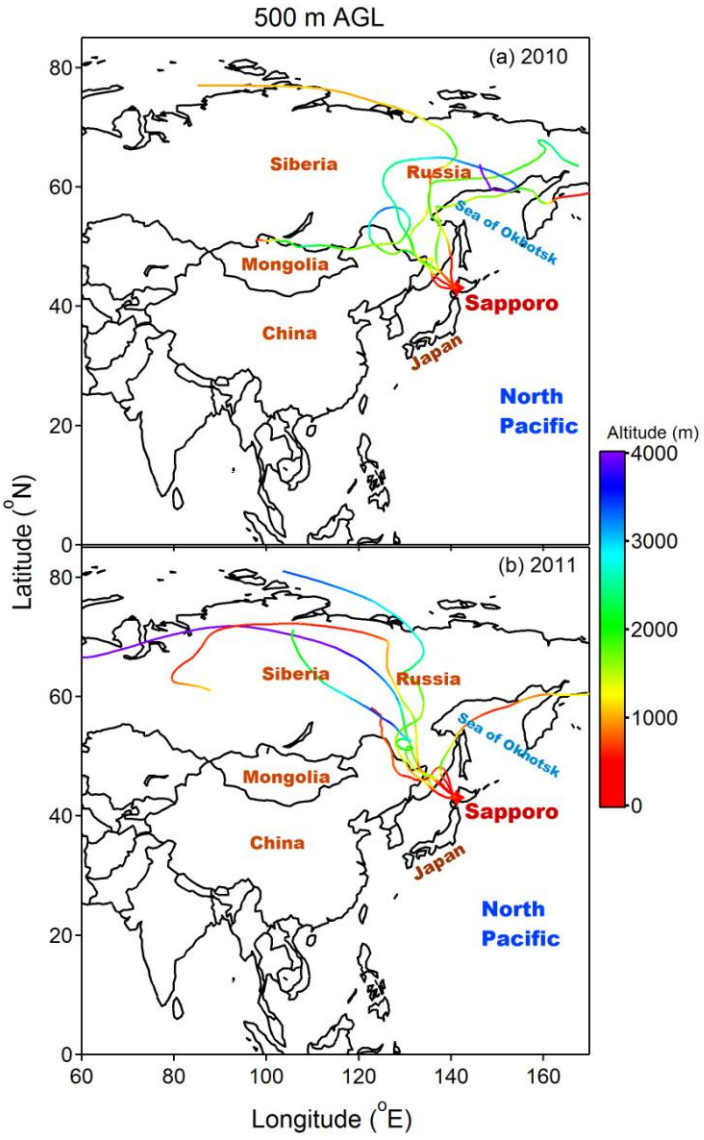
2010									
C-number	α-Hydroxy FAs			β-Hydroxy FAs			ω-Hydroxy FAs		
	range	mean±S.E.	median	range	mean±S.E.	median	range	mean±S.E.	median
C_9	b.d.-7.1	2.4±1.3	1.7	0.5-2.7	1.8±0.47	2	b.d.-1.7	0.97±0.4	1.4
C_{10}	b.d.-37.3	14.6±7.6	10.9	1.7-6.5	4.6±1.2	5.1	b.d.-5.1	1.7±1.1	0
C_{11}	b.d.-35.1	21±6.5	21.1	3.4-7.9	6.1±0.8	6.2	b.d.-6.4	2.2±1.4	0
C_{12}	b.d.-46.7	25.3±7.8	22.6	8-10.1	9.2±0.4	9.8	b.d.-95.6	47.2±17.8	32.7
C_{13}	b.d.-45.2	20±7.3	18	3.5-11.9	7.1±1.8	6	b.d.-5.1	3.7±0.9	4.4
C_{14}	b.d.-53.4	27.1±8.5	27.6	16.6-40.9	23.5±4.4	19.6	b.d.-196.7	101±34.7	79.8
C_{15}	b.d.-44	18.6±7.2	16.4	2.9-10.8	6.8±1.4	6.7	b.d.-17	9.6±3.1	12.8
C_{16}	b.d.-139	89.2±23.6	97.8	21.7-79.4	45.1±9.4	4.4	2.3-754.1	296±129	256.3
C_{17}	b.d.-26.5	12.4±4.4	10	3.1-10.7	7.5±1.3	8.4	b.d.-12.6	7.1±2	8.1
C_{18}	b.d.-44.7	26.2±8.1	26.3	23.4-52.3	33.5±6.6	29.1	b.d.-43.9	21.2±6.9	21
C_{19}	b.d.-20.1	11.5±3.4	11.5	5.3-21.7	10.4±3.8	7.3	b.d.-12.2	5.5±2	5.7
C_{20}	b.d.-46.6	25±7.8	21.5	14.4-120	48.3±25	29.2	0.2-45.6	17.2±7.6	13.5
C_{21}	b.d.-21.1	12.1±3.7	11.2	5.6-28.8	14.8±5.4	13	b.d.-8.7	3.6±1.4	3
C_{22}	b.d.-73.7	40.8±13.1	37.7	11.2-30.4	19.5±4.1	18.2	b.d.-318	96.4±56.5	50.7
C_{23}	b.d.-32.8	18.5±5.8	18.3	2.8-33.9	13.2±7.1	8.1	b.d.-9.2	3.8±1.6	3.6
C_{24}	b.d.-145	64±25	56.8	6.2-29	15±5.1	12.3	b.d.-72.4	24.1±12.7	13
C_{25}	b.d.-39.1	18.4±6.7	15.4	1.4-17.4	7.7±3.4	5.9	b.d.-2.6	1.02±0.5	1.2
C_{26}	b.d.-49.3	18.6±9	15.8	b.d.-18	7.5±3.8	6	b.d.-3.2	0.6±0.6	0
C_{27}	b.d.-14.4	4.4±2.8	1.1	b.d.-2.7	0.7±0.7	0	b.d.-0.2	0.03±0.03	0
C_{28}	b.d.-10.9	4±2.5	0	b.d.-1.6	0.3±0.3	0			
C_{29}	b.d.-0.54	0.1±0.1	0						
C_{30}	b.d.-0.32	0.06±0.06	0						
Total	432-774	593±88	582	70-379	247±52	252	2-1411	643±228	530

Note: b.d.= below detection limit ≤0.02 ng kg^{-1}; S.E. (Standard Error) = $\sigma/N^{1/2}$, where σ refers to standard deviation of total samples (N).

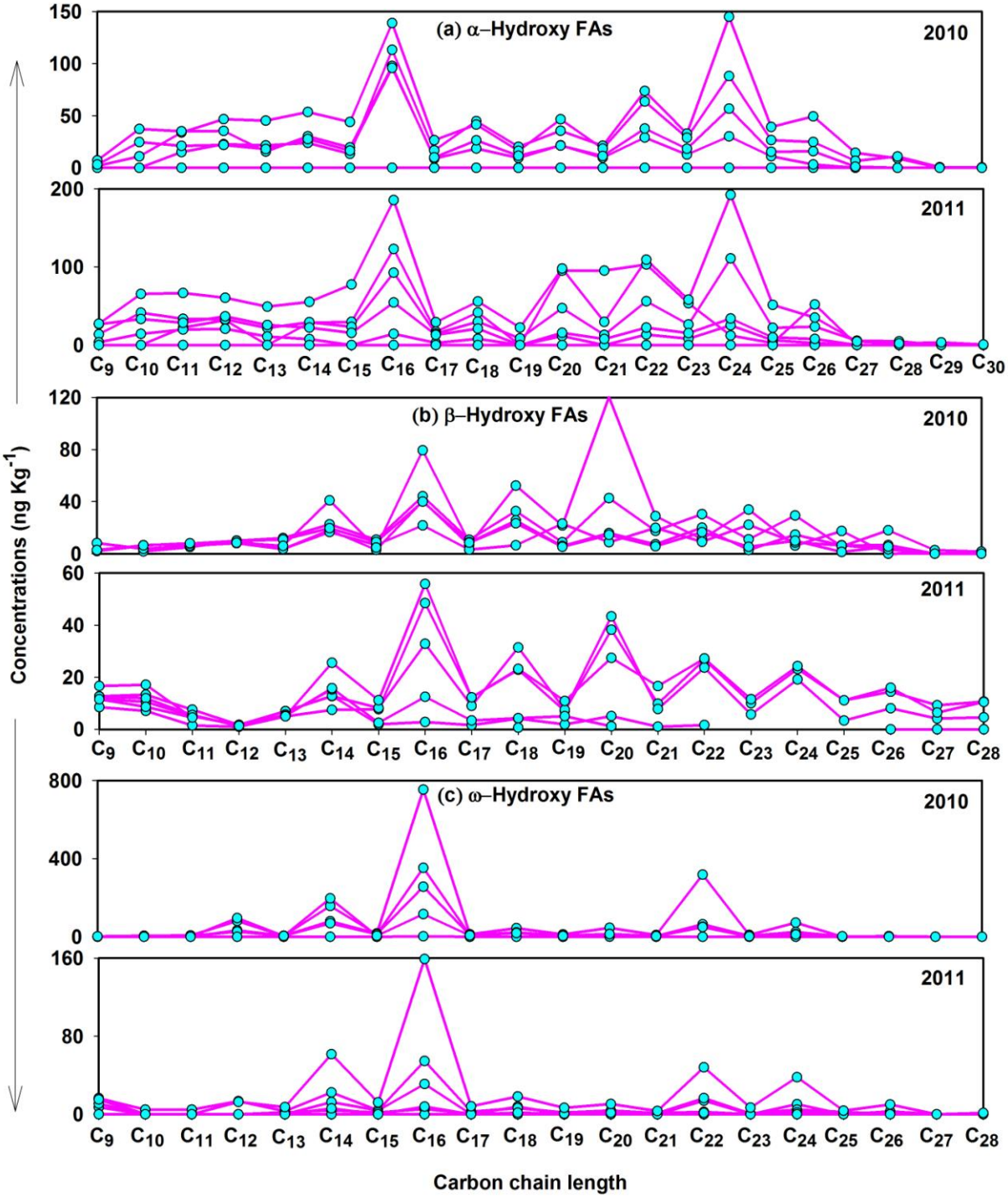
Table 2. Mass concentrations (in ng kg^{-1}) of α-, β- and ω-Hydroxy fatty acids (FAs) measured in snow samples (N=6) collected from Sapporo during winter 2011.

	2011								
C-number	α-Hydroxy FAs			β-Hydroxy FAs			ω-Hydroxy FAs		
	range	mean±S.E.	median	range	mean±S.E.	median	range	mean±S.E.	median
C_9	b.d.-27.2	14.2±5.7	13.8	1-8.5	5.1±1.3	6	b.d.-16.4	11.0±2.6	12.9
C_{10}	b.d.-65.4	30.9±11.2	33.3	1.7-12.7	8.1±1.8	8.8	b.d.-4.7	0.8±0.8	0
C_{11}	19.8-66.6	34.2±8.5	28.5	1.7-13.3	9.2±1.9	10.1	b.d.-4.7	0.8±0.8	0
C_{12}	20.7-60.4	36.5±6.6	32.9	1.3-15.3	8.7±2.2	8.8	b.d.-13.4	4.3±2.7	0
C_{13}	b.d.-49.2	21.5±8.2	21.8	4.5-15.8	9.1±2.1	8.6	b.d.-7.3	2.1±1.2	1
C_{14}	7.5-55.3	28.6±7.7	28.4	4.5-25.5	13.7±4	16.6	b.d.-61.5	17.7±9.3	9.1
C_{15}	b.d.-77.6	29.2±13.1	23.3	1.9-11.1	6.3±1.8	7.7	b.d.-12.1	4.0±2.2	3.9
C_{16}	14.3-186	94.0±29.3	92.5	2.8-55.8	30.5±10.2	32.8	b.d.-159	42.9±24.7	19.4
C_{17}	2.8-29.3	15.3±4.3	14.5	1.6-12.2	7.7±2.2	9	b.d.-8.2	1.9±1.3	0.3
C_{18}	8.0-55.8	31.3±8.2	29.9	0.6-31.4	14.4±5.3	13.6	b.d.-18.2	5.8±2.8	3.9
C_{19}	b.d.-22.4	6.2±4.4	0	1.9-10.9	6.5±1.5	7.1	b.d.-6.5	1.5±1.0	0.5
C_{20}	11.5-97.9	53.5±18.6	47.3	1.2-43.4	23±8.6	27.4	b.d.-10.5	3.3±1.5	2.3
C_{21}	b.d.-95.2	29.1±17.2	13	1.0-16.6	8.8±3.2	8.8	b.d.-3.4	1.0±0.5	0.6
C_{22}	13.4-109	60.8±19.9	56.1	1.6-27.2	19.8±6.1	25.2	b.d.-48.1	13.7±7.4	8.2
C_{23}	8.1-58.1	32.2±10.1	26.3	5.7-11.6	9.1±1.7	10	b.d.-6.8	1.2±1.1	0
C_{24}	12.3-92.2	74.9±34	34	19.1-24.3	22.2±1.6	23.1	b.d.-38	9.1±6.0	3.2
C_{25}	2.6-51.3	18.4±8.9	9.8	3.3-11.1	8.5±2.6	11.1	b.d.-3.7	1.0±0.6	0
C_{26}	2.6-52.0	24.2±9	23.5	b.d.-15.9	6.4±3.1	4	b.d.-10	2.2±1.6	0.1
C_{27}	b.d.-5.6	2±1.3	0	b.d.-9.2	3.3±1.6	2.1			
C_{28}	b.d.-4.8	1.4±0.9	0	b.d.-10.6	4.3±2.1	2.3	b.d.-1.4	0.2±0.2	0
C_{29}	b.d.-3.35	0.7±0.67	0						
C_{30}	b.d.-0.60	0.12±0.12	0						
Total	169-1279	639±187	651	6-354	179±64	170	27-422	149±73	102

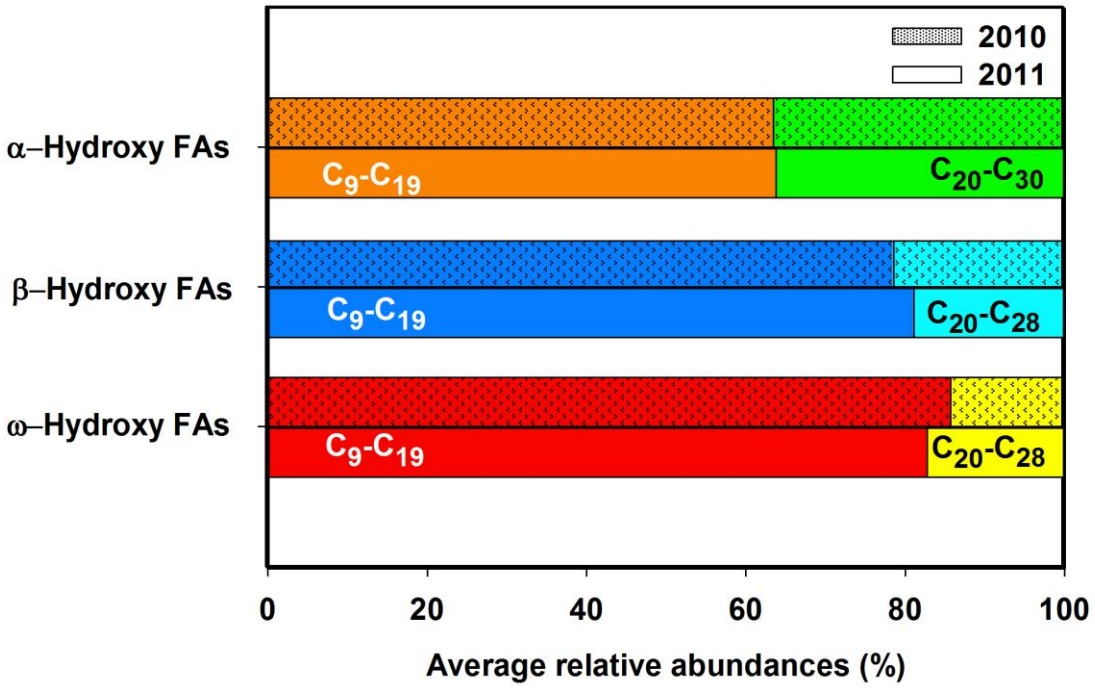
Note: b.d.= below detection limit ≤0.06 ng kg^{-1}. S.E. (Standard Error) = $\sigma/N^{1/2}$, where σ refers to standard deviation of total samples (N).



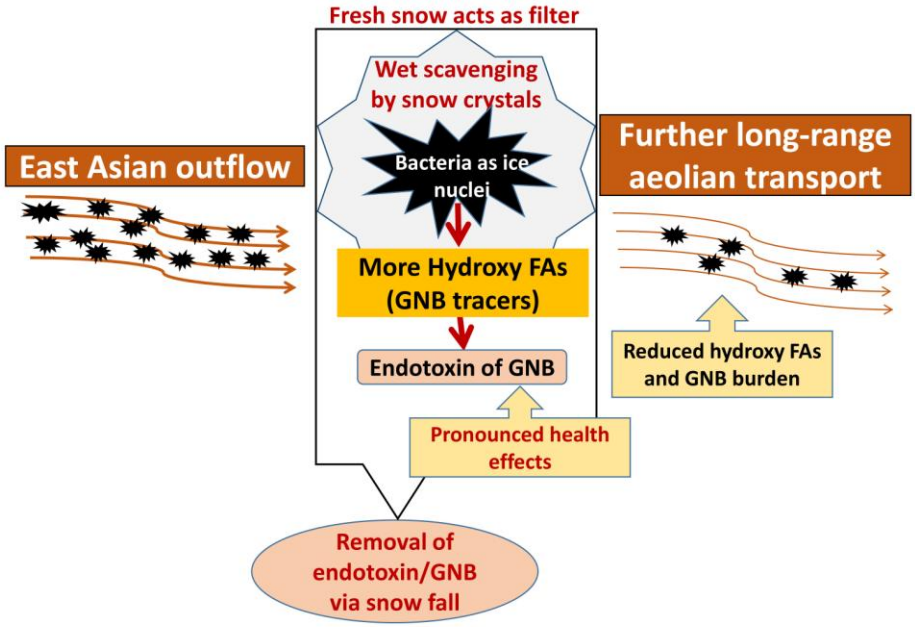
542 **Figure. 1.** Air mass back trajectory cluster at an arrival height of 500 m AGL (above ground
543 level) for the sampling days in (a) winter 2010 and (b) winter 2011.



544 **Figure. 2.** Molecular distributions of (a) α-Hydroxy fatty acids (FAs) (C_9-C_{30}), (b) β-Hydroxy
545 FAs (C_9-C_{28}) and, (c) ω-Hydroxy FAs (C_9-C_{28}) in the snow samples collected from Sapporo
546 during winter 2010 and 2011.



547 **Figure. 3.** Bar graph, showing the relative abundances of low molecular weight (C_9-C_{19}), and
548 high molecular weight fatty acids (C_{20}-C_{30} for α-Hydroxy; C_{20}-C_{28} for β- and ω-Hydroxy) in
549 their total mass for the snow samples collected during winter 2010 and 2011. The upper and
550 lower horizontal bars for each type of hydroxy fatty acids indicate the data for 2010 and 2011,
551 respectively.



552 **Figure 4**. Conceptual model to explain the scavenging of hydroxy fatty acids (FAs) by fresh
553 snow in the free troposphere. Snow fall in north Japan acts as a filter in reducing the hydroxy
554 FAs (tracers of Gram-negative bacteria; GNB), which in turn results in the removal of
555 endotoxin from the atmosphere and reduction in their health effects during long-range aeolian
556 dust transport.