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.

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Reviewer#2 (Comments to author):

Overall comment: The MS entitled "Hydroxy fatty acids in fresh snow samples from Northern Japan: long-range atmospheric transport of Gram-negative bacteria by Asian winter monsoon" by P. Tyagi et al. presents a very clear and concise description of the measurements of hydroxy fatty acids in fresh snow collected from Sapporo, Japan. The results are placed into the context of published work on microbial sources of these fatty acids which allows the authors to suggest snow fall as an efficient scavenger of the pathogenic microbial compounds. In addition, estimation of endotoxin and bacterial dry mass in snow allows the authors to suggest sources and removal pathways of airborne GNB. Overall, the manuscript is well written, and the dataset presented here is unique and the focus of this manuscript surely meets the scope of the journal. I recommend the manuscript to be accepted for suitable publication in Biogeosciences after addressing following minor comments.

Response: We thank the reviewer for appreciating our work and the encouragement towards publication. We have now carefully revised the MS based on the reviewer's suggestions/comments.

Comment. The statements concerning sources of α-hydroxy FAs are over amplified. Authors attributed the occurrence of α-hydroxy FAs as their source contribution from higher plants. What about the insitu atmospheric formation of hydroxy FAs for the Sapporo snow given the sampling location is far away from the attributed source locations, Siberia and Russian Fareast etc. Recent study by Feng et al., (2015) (doi: 10.5194/bg-12-4841-2015) had suggested that α-hydroxy FAs can be found in the hydrolysis products of leaf waxes and wood, and in microalgae and seagrasses. Therefore, I recommend source attribution of α-

hydroxy FAs to their origin either from the contribution from higher plant waxes or the possibility of formation in the atmosphere.

Response: *α-Hydroxy FAs, in particular high molecular weight ones, come from the epicuticular waxes of higher plants as well from algae (please see page 4, lines 74-77). 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, 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.* These points have been added in the revised MS. Please see lines 188-199.

Comment: Page 13378, Sapporo is an urban city. What is the possibility of having contribution from local air pollution? Snow sampling can be influenced by the local emissions as well.

Response: All the snow samples were collected at the roof top of the Institute of Low Temperature Sciences (ILTS), Hokkaido University after every fresh snow fall (see page 5, lines 115-117). As explained, fresh snow was collected after the removal of surface layer to exclude the possible contamination by dry deposition from local sources (Yamamoto et al., 2011). Moreover, Sapporo is known by heavy snowfall and extended winter (December-March, av. Temperature 2.1 ºC), due to which snow does not melt and prevents the particles uplifted by wind from the ground and local vegetation. Thus, we assumed that thick snow cover on the ground, height of sampling site (~15 m agl) and time (fresh snow fall) of sample collection altogether minimize the emission of terrestrial biomarkers from the local vegetation sources.

Comment: Mention which type of statistical analysis was performed in the caption of the table S1.

Response: Two-tailed unpaired *t* test was performed for the statistical analysis. We have modified the caption of Table S1 as *"Two-tailed unpaired t test to ascertain the statistical significance of ratio of relative abundances of even to odd carbon numbered hydroxy FAs in snow samples collected from the Sapporo between winter-2010 and 2011"*. Please see Table S1.

Comment: Page 13382, lines 16-18: authors found statistical significant differences in the concentrations of beta and omega hydroxy FAs between 2010 and 2011. Authors should discuss about the possible reasons for this observation.

Response: 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. The reasons have been discussed as follows:

"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." These sentences were added in the revised MS. Please see lines 222-227.

Short Comment: The paper of Tyagi et al. which is published for open discussion in Biogeosciences studied hydroxy fatty acids in fresh snow samples collected from Sapporo. This paper is within the scope of BG. However the manuscript can be refreshing if new scientific original findings addressed by the field observations are specified. I found that the text as well as the data presentation suffers from several major deficiencies. This paper is not written well. I found many mistakes in English Language that make it difficult to understand what authors want to explain. Many clarifications are not clear in the paper. The entire paper

needs to be carefully edited for grammar and typing errors as well as missing or extra words. There are numerous errors that significantly detract from the quality of the document. I found that authors did not use properly articles such as "the" and "a". Articles are missing in number of places in the manuscript. They did not use properly plural and singular forms in the entire paper. The uses of "comma" need to be corrected throughout the manuscript. The selection of scientific words in various place of the manuscript is not up to the standard of Biogeosciences.

Response: We thank the reviewer's constructive comments. We have now thoroughly revised the manuscript for clarity and grammatical corrections.

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.

2.3. Estimation of endotoxin levels and mass loading of GNB

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

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

In the above formula, the average molecular weight of endotoxin corresponds to 8000 as reported by Mielniczuk et al. (1993). 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}. We also estimated the mass loading of airborne GNB using the approach initially suggested by Balkwill et al. (1988) and later on by Lee et al. (2004), in which they used the chemical marker to bacterial mass conversion factor of 15 nmol of β-hydroxy FAs (C_{10}-C_{18}) per mg dry cell weight. Therefore, we have converted the sum of mass concentrations of β-hydroxy FAs from C_{10} to C_{18} (in nmol kg^{-1}) into equivalent dry cell weight of GNB (i.e., in mg kg^{-1} of melt water) by normalizing with 15.

3. Results and discussion

3.1. Air mass backward trajectory analysis

The air mass back-trajectories (AMBTs) provide a means to qualitatively assess the source regions of airborne pollutants over a receptor site. For this study, we have 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, 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

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. So far, no literature is available on endotoxin and GNB concentrations in snow samples from East Asia in order to make a comprehensive comparison with the present study.

Overall, the presence of endotoxin and GNB in snow affirms that biogenic particles of soil microbes and their potential health impact should not be overlooked. Routine and long-term measurements of airborne chemical markers (hydroxy FAs in this study) could aid the monitoring of the microbial content in long-range transported air masses. Further studies are required to examine their distributions in the atmospheric environment and health effects on human beings in the regional and global perspectives during long-range atmospheric transport.

4. Conclusions

Although low temperature is considered to be a limiting factor for bacterial activity in air/snow, some studies have shown that bacteria can be metabolically active even at subzero temperatures (Polymenakou, 2012 and references therein). Figure 4 summarized the whole idea, which was addressed in this study. We conclude that fresh snow in Japan acts as a filter, which aids in reducing the burden of pathogenic microbes from the atmosphere via wet scavenging of these particles.

Owing to prolonged winters and thus, snow fall in Sapporo, it is likely that ambient bacterial endotoxin (LPS) is largely scavenged from the atmosphere by snow, which

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

Author contribution

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

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References

Aggarwal, S. G. and Kawamura, K.: Molecular distributions and stable carbon isotopic compositions of dicarboxylic acids and related compounds in aerosols from Sapporo, Japan: Implications for photochemical aging during long‐range atmospheric transport, Journal of Geophysical Research: Atmospheres (1984–2012), 113, doi:10.1029/2007JD009365, 2008.

Andersson, A., Weiss, N., Rainey, F., and Salkinoja‐Salonen, M.: Dust‐borne bacteria in animal sheds, schools and children's day care centres, Journal of applied microbiology, 86, 622-634, 1999.

Balkwill, D. L., Leach, F. R., Wilson, J. T., McNabb, J. F., and White, D. C.: Equivalence of microbial biomass measures based on membrane lipid and cell wall components, adenosine triphosphate, and direct counts in subsurface aquifer sediments, Microbial Ecology, 16, 73-84, 1988.

Blokker, P., Schouten, S., de Leeuw, J. W., Damsté, J. S. S., and van den Ende, H.: Molecular structure of the resistant biopolymer in zygospore cell walls of Chlamydomonas monoica, Planta, 207, 539-543, 1999.

Cardoso, J. N. and Eglinton, G.: The use of hydroxyacids as geochemical indicators, Geochimica et Cosmochimica Acta, 47, 723-730, 1983.

Conte, M. H. and Weber, J.: Long‐range atmospheric transport of terrestrial biomarkers to the western North Atlantic, Global Biogeochemical Cycles, 16, doi:10.1029/2002GB001922, 2002.

Cranwell, P.: The stereochemistry of 2-and 3-hydroxy fatty acids in a recent lacustrine sediment, Geochimica et Cosmochimica Acta, 45, 547-552, 1981.

Draxler, R. and Rolph, G.: HYSPLIT (HYbrid Single-Particle Lagrangian Integrated Trajectory), NOAA Air Resources Laboratory, College Park, MD, Model access via NOAA ARL READY Website [Internet], 2013. ready. arl. noaa. gov/HYSPLIT. php, 2013.

Eglinton, G., Hunneman, D., and Douraghi-Zadeh, K.: Gas chromatographic—mass spectrometric studies of long chain hydroxy acids—II: The hydroxy acids and fatty acids of a 5000-year-old lacustrine sediment, Tetrahedron, 24, 5929-5941, 1968.

Feng, X., Gustafsson, Ö., Holmes, R. M., Vonk, J. E., van Dongen, B. E., Semiletov, I. P., Dudarev, O. V., Yunker, M. B., Macdonald, R. W., and Montlucon, D. B.: Multi-molecular tracers of terrestrial carbon transfer across the pan-Arctic: comparison of hydrolyzable components with plant wax lipids and lignin phenols, Biogeosciences, 12, 4841-4860, 2015.

Gagosian, R. B., Peltzer, E. T., and Merrill, J. T.: Long-range transport of terrestrially derived lipids in aerosols from the South Pacific, Nature, 325, 800–803, doi:810.1038/325800a325800, 1987.

Gagosian, R. B., Peltzer, E. T., and Zafiriou, O. C.: Atmospheric transport of continentally derived lipids to the tropical North Pacific., Nature, 291, 312-314, 1981.

Golokhvast, K. S.: Airborne biogenic particles in the snow of the cities of the russian far east as potential allergic compounds., Journal of Immunology Research, 2014. 141378, doi:141310.141155/142014/141378, 2014.

Goossens, H., Irene, W., Rijpstra, C., Düren, R., De Leeuw, J., and Schenck, P.: Bacterial contribution to sedimentary organic matter; a comparative study of lipid moieties in bacteria and recent sediments, Organic Geochemistry, 10, 683-696, 1986.

Gould, J. E.: Is rainwater safe to drink? A review of recent findings. , In: Proceedings of the Ninth International Rainwater Catchment Systems Conference., 1999. 1999.

Hines, C. J., Milton, D. K., Larsson, L., Petersen, M. R., Fisk, W. J., and Mendell, M. J.: Characterization and Variability of Endotoxin and 3-Hydroxy Fatty Acids in an Office Building During a Particle Intervention Study, Indoor Air, 10, 2-12, 2000.

Hines, C. J., Waters, M., Larsson, L., Petersen, M., Saraf, A., and Milton, D.: Characterization of endotoxin and 3‐hydroxy fatty acid levels in air and settled dust from commercial aircraft cabins, Indoor air, 13, 166-173, 2003.

Jacobs, R. R.: Airborne endotoxins: an association with occupational lung disease, Applied Industrial Hygiene, 4, 50-56, 1989.

Kawamura, K.: Land-derived lipid class compounds in the deep-sea sediments and marine aerosols from North Pacific, Biogeochemical Processes and Ocean Flux in the Western Pacific, Terra Scientific Publishing Company (TERRAPUB), Tokyo, 31-51, 1995.

Kawamura, K. and Gagosian, R.: Atmospheric transport of soil-derived dicarboxylic acids over the North Pacific Ocean, Naturwissenschaften, 77, 25-27, 1990.

Kawamura, K. and Ishiwatari, R.: Fatty acid geochemistry of a 200 m sediment core from Lake Biwa, Japan. Early diagenesis and paleoenvironmental information, Geochimica et Cosmochimica Acta, 48, 251-266, 1984.

Kawamura, K., Ishiwatari, R., and Ogura, K.: Early diagenesis of organic matter in the water column and sediments: microbial degradation and resynthesis of lipids in Lake Haruna, Organic Geochemistry, 11, 251-264, 1987.

Kawamura, K. and Kaplan, I.: Biogenic and anthropogenic organic compounds in rain and snow samples collected in southern California, Atmospheric Environment (1967), 20, 115-124, 1986.

Kawamura, K. and Kaplan, I. R.: Organic compounds in the rainwater of Los Angeles, Environmental science & technology, 17, 497-501, 1983.

Kawamura, K., Y. Ishimura, and Yamazaki, K.: Four years' observations of terrestrial lipid class compounds in marine aerosols from the western North Pacific, Global Biogeochemical Cycles, 17, 1003-1022, doi:1010.1029/2001GB001810, 2003.

Keinänen, M., Korhonen, L., Martikainen, P., Vartiainen, T., Miettinen, I., Lehtola, M., Nenonen, K., Pajunen, H., and Kontro, M.: Gas chromatographic–mass spectrometric detection of 2-and 3-hydroxy fatty acids as methyl esters from soil, sediment and biofilm, Journal of Chromatography B, 783, 443-451, 2003.

Larsson, L.: Determination of microbial chemical markers by gas chromatography‐mass spectrometry‐potential for diagnosis and studies on metabolism in situ, Apmis, 102, 161-169, 1994.

Lee, A. K. Y., Chan, C. K., Fang, M., and Lau, A. P. S.: The 3-hydroxy fatty acids as biomarkers for quantification and characterization of endotoxins and Gram-negative bacteria in atmospheric aerosols in Hong Kong, Atmospheric Environment, 38, 6307-6317, 2004.

Lee, A. K. Y., Lau, A. P., Cheng, J. Y., Fang, M., and Chan, C. K.: Source identification analysis for the airborne bacteria and fungi using a biomarker approach, Atmospheric Environment, 41, 2831-2843, 2007.

Lehninger, A. L.: Biochemistry: the molecular basis of cell structure and functions, Worth, New York, 1975. 659, 1975.

Lüderitz, O., Galanos, C., and Rietschel, E. T.: Endotoxins of gram-negative bacteria, Pharmacology & therapeutics, 15, 383-402, 1981.

Lye, D. J.: Health risks associated with consumption of untreated water from household roof catchment systems., Journal of American Water Resource Association 38, 1301-1306, 2002.

436 Matsumoto, G. I. and Nagashima, H.: Occurrence of 3-hydroxy acids in microalgae and
437 cyanobacteria and their geochemical significance, Geochimica et Cosmochimica
438 Acta, 48, 1683-1687, 1984.
439 Mielniczuk, Z., Mielniczuk, E., and Larsson, L.: Gas chromatography-mass spectrometry
440 methods for analysis of 2- and 3-hydroxylated fatty acids: Application for
441 endotoxin measurement, Journal of Microbiological Methods, 17, 91-102, 1993.
442 Milton, D. K.: Bacterial endotoxins: a review of health effects and potential impact in the
443 indoor environment, in:, Indoor Air and Human Health, edited by: Gammage, R. B.
444 and Berven, B. A., Lewis Publishers, CRC Press, Florida, 2nd Ed., 179-195, 1996.
445 Molina, I., Bonaventure, G., Ohlrogge, J., and Pollard, M.: The lipid polyester composition of
446 Arabidopsis thaliana and Brassica napus seeds, Phytochemistry, 67, 2597-2610,
447 2006.
448 Morris, C., Sands, D., Bardin, M., Jaenicke, R., Vogel, B., Leyronas, C., Ariya, P., and
449 Psenner, R.: Microbiology and atmospheric processes: an upcoming era of research
450 on bio-meteorology, Biogeosciences Discussions, 5, 191-212, 2008.
451 Morris, C., Sands, D., Bardin, M., Jaenicke, R., Vogel, B., Leyronas, C., Ariya, P., and
452 Psenner, R.: Microbiology and atmospheric processes: research challenges
453 concerning the impact of airborne micro-organisms on the atmosphere and climate,
454 Biogeosciences, 8, 17-25, 2011.
455 Pavuluri, C. M., Kawamura, K., Uchida, M., Kondo, M., and Fu, P.: Enhanced modern carbon
456 and biogenic organic tracers in Northeast Asian aerosols during spring/summer,
457 Journal of Geophysical Research: Atmospheres, 118, 2362-2371, 2013.
458 Polymenakou, P. N.: Atmosphere: a source of pathogenic or beneficial microbes?,
459 Atmosphere, 3, 87-102, 2012.
460 Ratledge, C. and Wilkinson, S.: Fatty acids, related and derived lipids, Microbial lipids, 1, 23-
461 52, 1988.
462 Rietschel, E. T., Wollenweber, H. W., Brade, H., Zähringer, U., Lindner, B., Seydel, U.,
463 Bradaczek, H., Barnickel, G., Labischinski, H., and Giesbrecht, P. (Eds.): Structure
464 and conformation of lipid A component of lipopolysaccharides, Elsevier Science,
465 Amsterdam, pp. 187–220, 1984.
466 Sankelo, P., Kawamura, K., Seki, O., Shibata, H., and Bendle §, J.: n-Alkanes in fresh snow in
467 Hokkaido, Japan: implications for ice core studies, Arctic, Antarctic, and Alpine
468 Research, 45, 119-131, 2013.
469 Saraf, A., Larsson, L., Burge, H., and Milton, D.: Quantification of ergosterol and 3-hydroxy
470 fatty acids in settled house dust by gas chromatography-mass spectrometry:
471 comparison with fungal culture and determination of endotoxin by a Limulus
472 amebocyte lysate assay, Applied and Environmental Microbiology, 63, 2554-2559,
473 1997.
474 Satsumabayashi, H., Nishizawa, H., Yokouchi, Y., and Ueda, H.: Pinonaldehyde and some
475 other organics in rain and snow in central Japan, Chemosphere, 45, 887-891, 2001.
476 Simoneit, B. R.: Biogenic lipids in particulates from the lower atmosphere over the eastern
477 Atlantic, Nature, 267, 682-685, 1977.
478 Simoneit, B. R., Kobayashi, M., Mochida, M., Kawamura, K., Lee, M., Lim, H. J., Turpin, B.
479 J., and Komazaki, Y.: Composition and major sources of organic compounds of
480 aerosol particulate matter sampled during the ACE‐Asia campaign, Journal of
481 Geophysical Research: Atmospheres, 109, 1-22, doi:10.1029/2004JD004598, 2004.
482 Spaan, S., Smit, L., Eduard, W., Larsson, L., Arts, H., Wouters, I. M., and Heederik, D.:
483 Endotoxin exposure in sewage treatment workers: investigation of exposure
484 variability and comparison of analytical techniques, Ann Agric Environ Med, 15,
485 251-261, 2008.

Steinberger, Y., Zelles, L., Bai, Q. Y., von Lützow, M., and Munch, J. C.: Phospholipid fatty acid profiles as indicators for the microbial community structure in soils along a climatic transect in the Judean Desert, Biology and Fertility of Soils, 28, 292-300, 1999.

Stodola, F. H., Deinema, M. H., & Spencer, J. : Extracellular lipids of yeasts, Bacteriological Reviews, 31, 194-213, 1967.

Szponar, B., Norin, E., Midtvedt, T., and Larsson, L.: Limitations in the use of 3-hydroxy fatty acid analysis to determine endotoxin in mammalian samples, Journal of Microbiological Methods, 50, 283-289, 2002.

Tyagi, P., Ishimura, Y., and Kawamura, K.: Hydroxy fatty acids in marine aerosols as microbial tracers: 4-year study on β-and ω-hydroxy fatty acids from remote Chichijima Island in the western North Pacific, Atmospheric Environment, 115, 89-100, 2015.

Van Dyk, M., Kock, J., and Botha, A.: Hydroxy long-chain fatty acids in fungi, World journal of microbiology & biotechnology, 10, 495-504, 1994.

Volkman, J., Johns, R., Gillan, F., Perry, G., and Bavor, H.: Microbial lipids of an intertidal sediment—I. Fatty acids and hydrocarbons, Geochimica et Cosmochimica Acta, 44, 1133-1143, 1980.

Wakeham, S. G.: Monocarboxylic, dicarboxylic and hydroxy acids released by sequential treatments of suspended particles and sediments of the Black Sea, Organic Geochemistry, 30, 1059-1074, 1999.

Wakeham, S. G., Pease, T. K., and Benner, R.: Hydroxy fatty acids in marine dissolved organic matter as indicators of bacterial membrane material, Organic Geochemistry, 34, 857-868, 2003.

Walters, M., Milton, D., Larsson, L., and Ford, T.: Airborne environmental endotoxin: a cross-validation of sampling and analysis techniques, Applied and environmental microbiology, 60, 996-1005, 1994.

Westphal, O.: Bacterial endotoxins (part 1 of 2), International Archives of Allergy and Immunology, 49, 1-21, 1975.

Wilkinson, S. G.: Gram-negative bacteria, in: Microbial Lipids, edited by: Ratledge, C. and Wilkinson, S. G., Vol. 1., Academic Press, New York, 1988. 299-488, 1988.

Yamamoto, S., Kawamura, K., and Seki, O.: Long-range atmospheric transport of terrestrial biomarkers by the Asian winter monsoon: Evidence from fresh snow from Sapporo, northern Japan, Atmospheric Environment, 45, 3553-3560, 2011.

Zelles, L.: Phospholipid fatty acid profiles in selected members of soil microbial communities, Chemosphere, 35, 275-294, 1997.

Zelles, L. and Bai, Q. Y.: Fatty acid patterns of phospholipids and lipopolysaccharids in environmental samples, Chemosphere, 28, 391-411, 1994.

Zhang, Z., Metzger, P., and Sachs, J. P.: Bound lipid biomarkers in sediments from El Junco Lake, Galápagos Islands, Organic Geochemistry, 75, 122-128, 2014.

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.

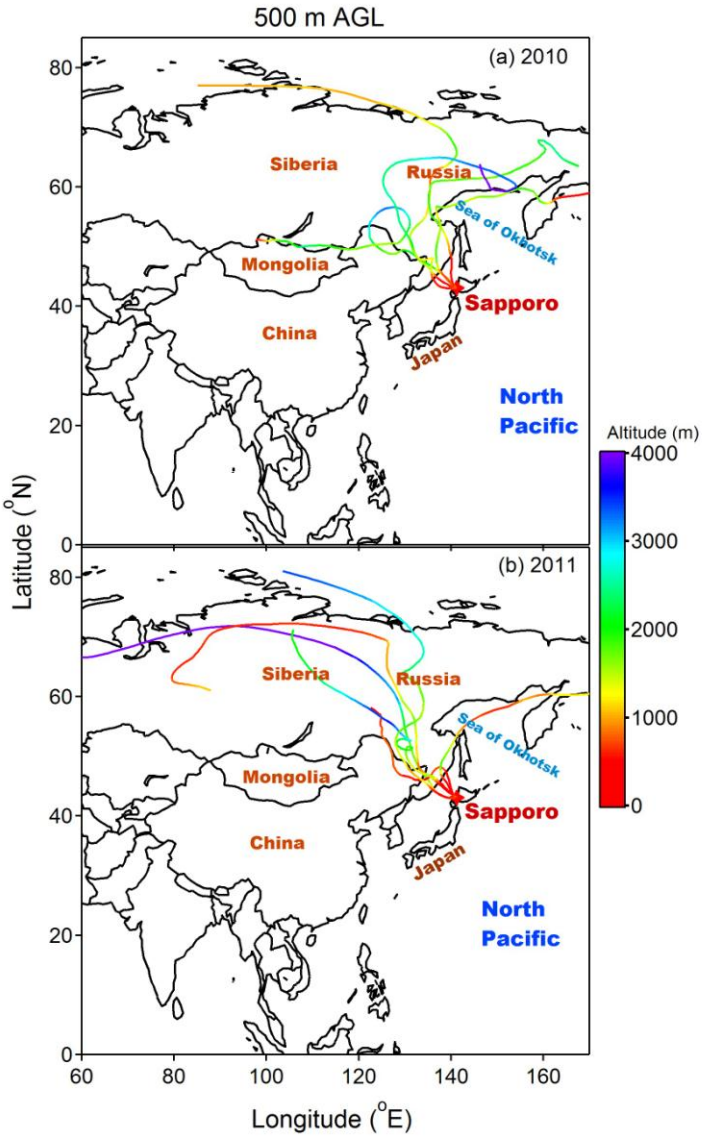
2010									
C-number	α-Hydroxy FAs			β-Hydroxy FAs			ω-Hydroxy FAs		
	range	mean±S.E.	median	range	mean±S.E.	median	range	mean±S.E.	median
C_9	b.d.-7.1	2.4±1.3	1.7	0.5-2.7	1.8±0.47	2	b.d.-1.7	0.97±0.4	1.4
C_{10}	b.d.-37.3	14.6±7.6	10.9	1.7-6.5	4.6±1.2	5.1	b.d.-5.1	1.7±1.1	0
C_{11}	b.d.-35.1	21±6.5	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).

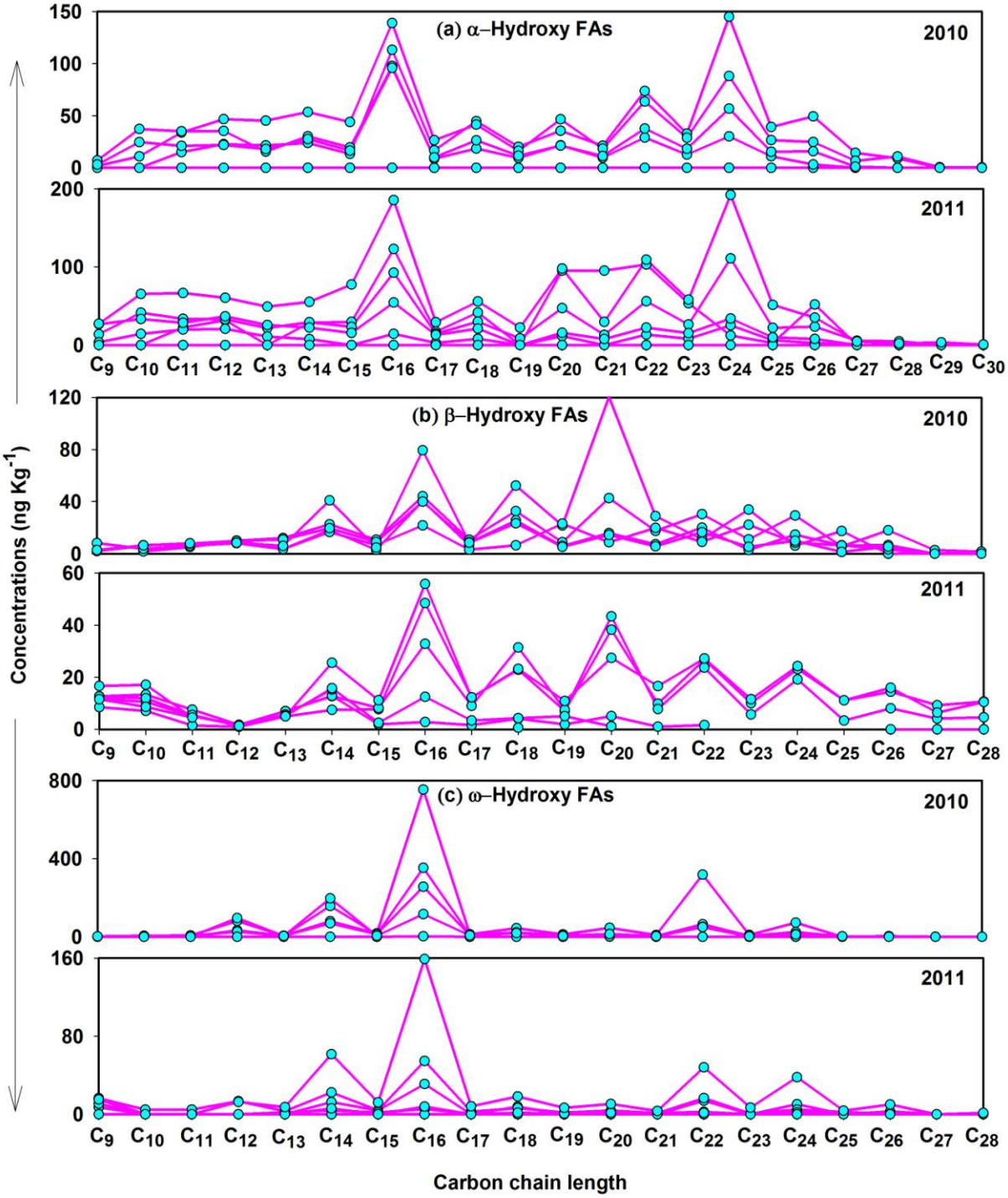
546 **Table 2.** Mass concentrations (in ng kg^{-1}) of α-, β- and ω-Hydroxy fatty acids (FAs) measured in snow samples (N=6) collected from Sapporo
547 during winter 2011.

	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

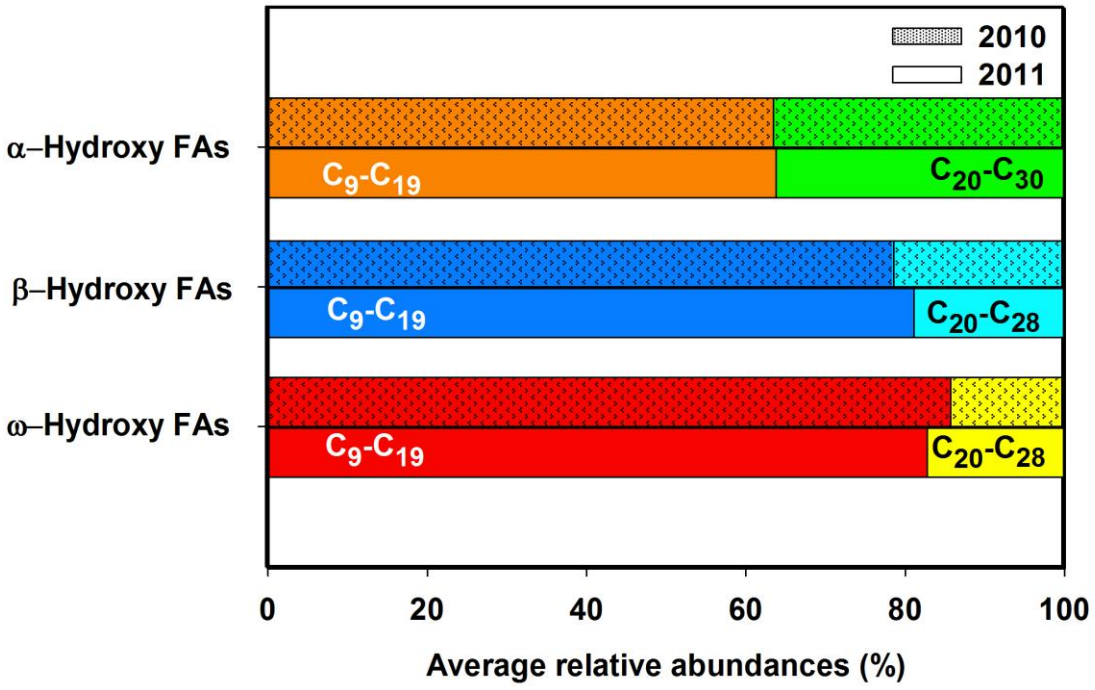
Note: b.d.= below detection limit ≤0.06 ng kg^{-1}. S.E. (Standard Error) = $\sigma/N^{1/2}$, where σ refers to standard deviation of total samples (N).



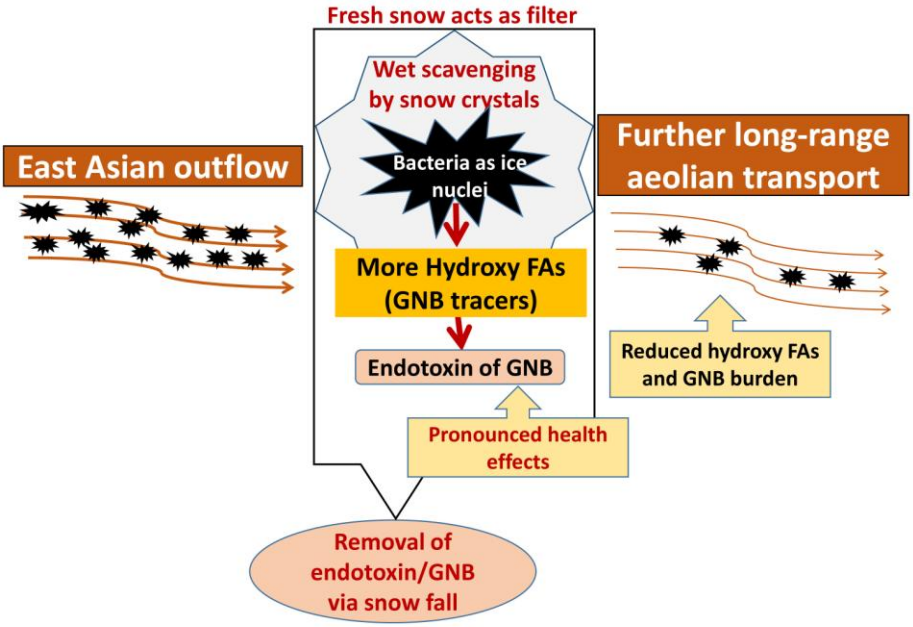
548 **Figure. 1.** Air mass back trajectory cluster at an arrival height of 500 m AGL (above ground
549 level) for the sampling days in (a) winter 2010 and (b) winter 2011.



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



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



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

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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Table S1: Two-tailed unpaired *t* test to ascertain the statistical significance of ratio of relative abundances of even to odd carbon numbered hydroxy FAs in snow samples collected from the Sapporo between winter-2010 and 2011.

even/odd	2010	2011	t-score, df, P-value
α-hydroxy FAs	2.4 ± 0.3	2.2 ± 0.3	1.4, 7, > 0.05
β-hydroxy FAs	2.9 ± 0.8	1.8 ± 0.6	2.5, 9, < 0.05
ω-hydroxy FAs	15.8 ± 4.5	3.2 ± 1.8	5.8, 7, < 0.05

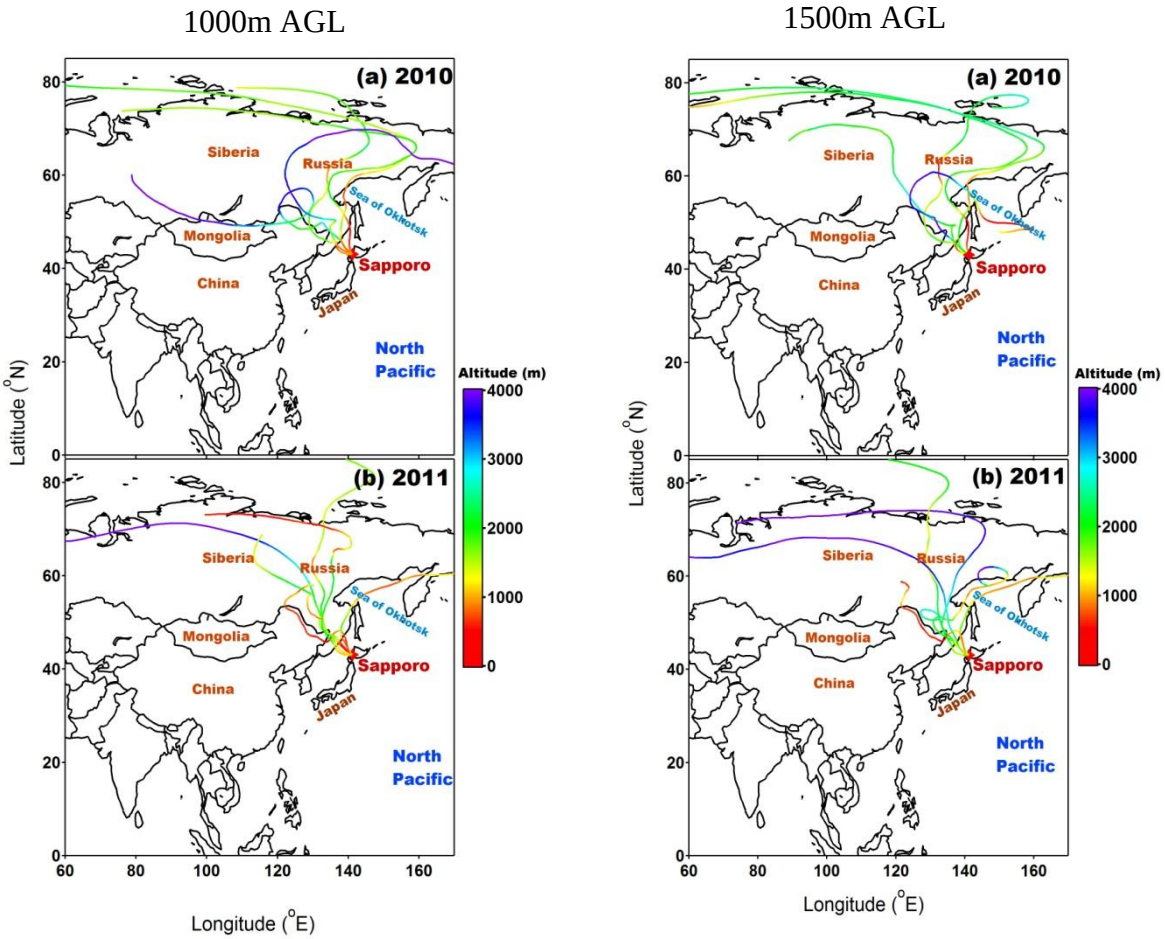

Figure S1. Air mass back trajectory cluster at an arrival heights of 1000 and 1500m AGL (above ground level) for the sampling days in (a) winter 2010 and (b) winter 2011.