

Publish subject to technical corrections (27 Nov 2015) by Dr. Jack Middelburg

Comments to the Author:

Dear Dr. Kawamura:

Thank you for submitting this paper to Biogeosciences. I have read your revised paper, your rebuttal and the reviews, and I am happy to inform you that your paper is now accepted for publication. However, while reading I identified a few minor corrections that need attention.

Response: We thank you for the useful comments and corrections. We have inserted the changes in the revised MS.

Line 67: I suggest changing to (C10-C18) in lipopolysaccharides (LPS) as constituents.....

Details of lipid A fraction can be reserved to discussion.

Response: Corrected. Please see lines 66-67 in the revised MS.

Line 113/114. However, no study which focuses on the transport..... using organic markers is available from Sapporo.

Response: Corrected. Please see lines 113-114 in the revised MS.

Line 155: snow samples can be estimated from:

Response: Corrected. Please see line 155 in the revised MS.

Line 191. Delete manuscript in preparation. All scientists have multiple manuscript in preparation, some do not make it: forward citations should always be avoided.

Response: Following the comment, we removed the phrase "*manuscript in preparation*". Please see line 191 in the revised MS.

Line 205: which are more common....

Response: Corrected. Please see line 205 in the revised MS.

Line 222: difference is much larger for....

Response: Corrected. Please see lines 221-222 in the revised MS.

Line 233: Use italic for species.

Response: Corrected. Please see line 233 in the revised MS.

Line 262: transport pathways from....

Response: Corrected. Please see line 262 in the revised MS.

Line 268: can trigger and cause allergies...

Response: Corrected. Please see line 268 in the revised MS.

Line 270: primarily by the Limulus...

Response: Corrected. Please see line 270 in the revised MS.

Line 278: in snow to vary from and from 36 to 1100.....

Response: Corrected. Please see lines 278 and 279 in the revised MS.

Line 282: bacteria (GNB). Beta-hydroxy FAs (do not refer to as stated on page 3, see also above).

Response: Following the comment, we removed the phrase. Please see line 282 in the revised MS.

Line 317: Figure 4 summarizes the ...

Response: Corrected. Please see line 317 in the revised MS.

Line 325: reformulate, sentence is unclear

Response: As suggested, we rephrased the sentence as follows:

“However, without snow scavenging, ambient bacterial endotoxin levels may stay high and can be transported further long distances, which can cause severe impacts on human health over the North Pacific and possibly in North America.”

Please see lines 324-326 in the revised MS.

Line 382: check authors: it is Goossens H, Rijpstra, I.W.C.... (Irene is her first name).

Response: Corrected. Please see line 379 in the revised MS.

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 as 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 (HgCl₂) 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 °C 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 °C 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 °C 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 BF₃/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) (SUPELCOTM Analytical) at 70 °C 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). 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 $\mu\text{g kg}^{-1}$ 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 $\mu\text{g kg}^{-1}$ 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.

MS-Id Ref-No: bg-2015-387

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) 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 °C), 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.

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

3
4 **Poonam Tyagi^{1,2}, Shinya Yamamoto^{2,3}, Kimitaka Kawamura^{2,*}**

5
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11 **Key points:**

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

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18 **Short title:** *Hydroxy fatty acids in fresh snow*

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31 Abstract

32 Hydroxy fatty acids (FAs) in fresh snow from Sapporo, one of the heaviest
33 snowfall regions in the world, have been studied to ascertain the airborne bacterial endotoxin
34 concentrations and their biomass. The presence of β -hydroxy FAs (C₉-C₂₈), constituents of
35 Gram-negative bacteria (GNB), suggests long-range transport of soil microbes. Likewise, the
36 occurrence of α - and ω -hydroxy FAs (C₉-C₃₀ and C₉-C₂₈, respectively) in snow reveals their
37 contribution from epicuticular waxes and soil microorganisms. Estimated endotoxin and GNB
38 mass can aid in assessing their possible impacts on the diversity and functioning of aquatic
39 and terrestrial ecosystems, as well as lethal effects on pedestrians through dispersal of
40 microbes. Air mass back trajectories together with hydroxy FAs unveil their sources from
41 Siberia, Russian Far East and North China by the Asian monsoon. This study highlights the
42 role of fresh snow that reduces the human health risk of GNB and endotoxin by the
43 scavenging from air.

44

45 Keywords

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

48

49

50 1. Introduction

51 Lipid biomarkers from terrigenous plants, algae, fungi and soil microorganisms
52 have been reported extensively in aerosols (Conte and Weber, 2002; Gagosian et al., 1987;
53 Gagosian et al., 1981; Kawamura, 1995; Kawamura et al., 2003; Simoneit, 1977; Simoneit et
54 al., 2004), sediments (Kawamura, 1995; Kawamura and Ishiwatari, 1984; Kawamura et al.,
55 1987; Zhang et al., 2014), ice core (Sankelo et al., 2013) and rain/snow (Kawamura and
56 Kaplan, 1986; Satsumabayashi et al., 2001; Yamamoto et al., 2011). These studies have
57 utilized fatty acids as a proxy to assess the terrigenous contribution of higher plant waxes to
58 various environmental samples owing to their abundant presence in biopolymers of plants and
59 microorganisms. Similarly, certain hydroxy fatty acids (e.g., C₁₀-C₁₈ β -hydroxy FAs) have
60 been proposed as a tracer to understand the airborne bacterial transport (Tyagi et al., 2015).

61 Among the airborne soil microbes, the Gram-negative bacterium (GNB) is one
62 of most extensively studied bacteria and is documented in aerosols, snow and rain samples
63 (Morris et al., 2011). Owing to considerable ground based emissions of GNB and its ability to
64 act as cloud condensation nuclei (CCN), these bacteria, which are plant pathogens, can
65 influence the regional as well as global climate through cloud aerosol interactions (Morris et
66 al., 2011 and references therein). In particular, GNB contains β -hydroxy FAs (C₁₀-C₁₈) in
67 lipopolysaccharides (LPS) as constituents of outer cell membrane (Westphal, 1975).
68 Moreover, the environmental toxic effects of GNB are, in part, due to the presence of β -
69 hydroxy FAs present in LPS (endotoxin) (Larsson, 1994; Saraf et al., 1997; Spaan et al.,
70 2008).

71 Apart from β -hydroxy FAs, other positional isomers such as α -, ω - and (ω -1)-
72 hydroxy FAs have also been documented in various environmental archives viz. aerosols
73 (Kawamura, 1995; Tyagi et al., 2015) and sediments (Kawamura, 1995; Wakeham et al.,
74 2003; Zhang et al., 2014). Short chain α -hydroxy FAs (C₁₂-C₁₈) are the constituent

75 biopolymers of fungi (Zelles, 1997), soil bacteria (Steinberger et al., 1999; Zelles and Bai,
76 1994) and protozoa (Ratledge and Wilkinson, 1988). In contrast, long chain α -hydroxy FAs
77 (C_{16} - C_{26}) are abundant in plants, microalgae and cyanobacteria (Matsumoto and Nagashima,
78 1984). Likewise, ω - and (ω -1)-hydroxy FAs are highly cross-linked constituents of the cell
79 walls of algae (Blokker et al., 1999) and plant seeds, suberin and cutin in terrestrial higher
80 plants (Molina et al., 2006). In addition, ω - and (ω -1)-hydroxy FAs are the intermediates in
81 the oxidation of monocarboxylic acids to dicarboxylic acids in sediments and marine aerosols
82 (Kawamura, 1995; Kawamura and Gagosian, 1990). Further, specificity of hydroxylation in
83 FAs depends on the type of microorganisms involved (Wakeham, 1999).

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

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

100 2. Experimental methods

101 2.1. Site description and sample collection

102 Sapporo (43.07 °N, 141.36 °E) is the capital of Hokkaido, whose population is
103 1.9 million (June, 2013). Sapporo receives cold and dry air masses with heavy snowfall
104 during the Asian winter monsoon. The average temperature of Sapporo in winter goes up to ~
105 2 °C (Yamamoto et al., 2011). Snow cover over the ground and fallen leaves of deciduous
106 plants suppresses the suspension of soil particles during winter whereas the emissions of plant
107 biomarkers from local vegetation are minimal. During winter season, Asian monsoon affects
108 the regional climate, air quality and human health in Japan, delivering anthropogenic aerosols
109 and dust from China and Siberia (Yamamoto et al., 2011). Several studies have examined the
110 chemical and isotopic composition of ambient aerosols in various types of air masses in
111 Sapporo (Aggarwal and Kawamura, 2008; Pavuluri et al., 2013; Yamamoto et al., 2011) to
112 better understand the impacts of anthropogenic and biogenic contributions from Siberia, North
113 China and surrounding oceans. However, no study which focuses on the transport of
114 microorganisms using organic markers is available from Sapporo.

115 In this study, eleven fresh snow samples were collected from the rooftop of the
116 Institute of Low Temperature Science (ILTS) building, Hokkaido University in Sapporo
117 during intensive snow fall periods (January-March) in 2010 and 2011. The detailed
118 description about snow collection and analytical protocol of lipid fraction analyses is similar
119 to that described in Yamamoto et al. (2011). To avoid the contribution of any possible
120 impurities from the dry deposition of aerosols, 1-2 cm of surface snow cover were removed
121 prior to sample collection. Thereafter, snow samples were collected into a cleaned glass jar (8
122 L) by using a stainless steel shovel. In each glass jar, mercuric chloride (HgCl₂) was added
123 before sampling to prevent microbial activity. Soon after the collection, glass jars were
124 tightened with a Teflon-lined screw cap and stored at -20 °C 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 °C 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 BF₃/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™ Analytical) at 70 °C for 1 h. After the reaction, 50
135 µl of n-hexane solution containing 1.43 ng µl⁻¹ of internal standard (C₁₃ 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-C₁₂ and
143 n-C₁₆ α-hydroxy FAs, n-C₁₂, n-C₁₄, n-C₁₅, and n-C₁₆ β-hydroxy FAs and n-C₁₆, n-C₂₀ and
144 n-C₂₂ 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 can be estimated from mathematical expression as below.

$$156$$

$$157 \text{ Endotoxins (LPS, ng kg}^{-1} \text{ of melt water)} = [(\sum \beta\text{-hydroxy FAs from } C_{10} \text{ to } C_{18}; \text{ nmol kg}^{-1})$$

$$158 \text{ x } 8000]/4$$

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

175 trajectory (HYSPLIT) model (Draxler and Rolph, 2013 and references therein). The
176 meteorological parameters (GDAS data sets) from NOAA air resources laboratory were used
177 as an input for the HYSPLIT model. Figure 1 shows the AMBT cluster at an arrival height of
178 500 m over Sapporo during sampling days of winter 2010 and 2011. In almost all snow-
179 sampling periods in Sapporo, the AMBTs show plausible influence of air masses from Russia
180 and Siberia via the long-range atmospheric transport.

181 **3.2. Concentrations of hydroxy fatty acids**

182 Homologues series of α -, β - and ω -hydroxy FAs were detected in fresh snow
183 samples collected from Sapporo. Their mass concentrations are summarized in Table 1 and
184 Table 2 for winter 2010 and 2011, respectively. Based on two-year seasonal data on hydroxy
185 FAs, we found that concentrations of α -hydroxy FAs are significantly higher than β - and ω -
186 hydroxy FAs. The predominance of α -hydroxy FAs can be explained by the α -oxidation
187 pathway of FAs, which generally occurs in plants, animals and bacteria (Cranwell, 1981 and
188 references therein) whereas β - and ω -oxidation is specific to bacteria (Lehninger, 1975). α -
189 Hydroxy FAs, in particular high molecular weight ones, come from the epicuticular waxes of
190 higher plants as well from algae. However, we also found higher abundance of α -hydroxy
191 FAs in the biomass burning aerosols collected over Mt. Tai, China (Tyagi et al., 2015),
192 possibly due to photochemical oxidation of higher molecular weight fatty acids. Such a
193 possibility of in situ formation of α -hydroxy FAs has also been reported in the hydrolysis
194 products of leaf waxes and wood, and in microalgae and sea grasses (Feng et al., 2015).
195 Further, microbial oxidation could also be a possible source of α -hydroxy FAs (Eglinton et al.,
196 1968) in the snow samples studied. Hence, we suggest that α -hydroxy FAs cannot be
197 employed as the tracers of plant waxes only, as they can come from microbial/photochemical
198 oxidation of higher molecular weight fatty acids during long-range atmospheric transport.

199 A characteristic feature of our data is the predominance of C₁₆ hydroxy FAs in
200 all the types of hydroxy FAs measured. However, significant shifts were observed in the
201 carbon numbers of the second most abundant β-hydroxy FAs (mostly C number >16) and ω-
202 hydroxy FAs (i.e., C number <16; see Tables 1 and 2). A likely explanation for this
203 observation is that β-hydroxy FAs above C₁₆ were formed by β-oxidation of long chain FAs,
204 which are more common in microorganisms as discussed previously. In contrast, ω-hydroxy
205 FAs below C₁₆ are present in plants and microbes (Cardoso and Eglinton, 1983), in which ω-
206 oxidation of fatty acids is secondary choice for microbial oxidation.

207 3.3. Molecular distributions

208 Figure 2 presents molecular distributions of α-hydroxy (C₉ to C₃₀), β- and ω-
209 hydroxy FAs (C₉ to C₂₈) in snow samples from Sapporo during winter 2010 and 2011. Even
210 carbon number predominance is noteworthy for α-, β- and ω-hydroxy FAs. α-Hydroxy FAs
211 show molecular distributions with the order C₁₆ > C₂₄ > C₂₂ in both years (see Figure 2a).
212 Likewise, β-hydroxy FAs show the predominance of C₁₆ followed by C₁₈ or C₂₀ and then by
213 C₁₄ in both winters. However, we found the predominance of C₂₀ β-hydroxy FAs over C₁₆ in
214 one snow sample during 2010. Similarly, ω-hydroxy FAs showed dominance of C₁₆ followed
215 by the others as C₁₄ > C₁₂ ~ C₂₂ ~ C₂₄ during snowfall in both the years.

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

223 heights in 2011 winter, the air masses came from the oceans during one sample collection.
224 Higher plants in the continents contribute to higher abundances of hydroxy FAs than the
225 oceans, and thus explain higher abundances of β - and ω -hydroxy FAs in 2010 than 2011. On
226 average, even carbon numbered α -, β - and ω -hydroxy FAs in their total mass concentrations
227 account for ~69, 68 and 84%, respectively. The even carbon number predominance is also
228 found in recent marine and lacustrine sediments (Cardoso and Eglinton, 1983; Goossens et al.,
229 1986; Kawamura, 1995; Zhang et al., 2014).

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

243 Previous studies documented ubiquitous occurrence of these hydroxy FAs in soil
244 microbes such as yeast and fungi (Van Dyk et al., 1994 and references therein) and in the LPS
245 of GNB (Lee et al., 2007). In this regard, prior studies focussing on β -hydroxy FAs with the
246 predominance of C_{16} and C_{18} , suggested the contributions from yeast and fungi (Stodola,
247 1967; Van Dyk et al., 1994 and references therein). Molecular distributions of β -hydroxy FAs

248 show a predominance of C₁₆ followed by C₁₈ or C₂₀ (see Figure 2b), suggesting that they
249 have been derived from soil microbes. Likewise, FAs <C₂₀ are derived from marine
250 phytoplankton (Kawamura, 1995 and references therein). β-Hydroxy FAs (C₁₀-C₁₈) have been
251 proposed as a biomarker for soil microbes as they are the constituents of LPS of GNB (Lee et
252 al., 2004; Szponar et al., 2002). Hence, it is likely that β-hydroxy FAs in snow samples may
253 have been significantly influenced by GNB and terrestrial higher plant metabolites.

254 Figure 3 depicts bar graphs, showing the relative abundances of α-, β- and ω-
255 hydroxy FAs in the snow samples from Sapporo during winter. We found that the proportions
256 of two classified groups (low molecular weight C₉-C₁₉ and high molecular weight C₂₀-C₃₀ or
257 C₂₀-C₂₈) of α-, β- and ω-hydroxy FAs are very similar between 2010 and 2011 (Figure 3).
258 This observation is perhaps related to their common sources/transport pathways of α-, β- and
259 ω-hydroxy FAs over Sapporo. This inference is further supported by the AMBTs computed at
260 arrival heights of 500, 1000 and 1500 m (see Figure 1 and Figure S1), indicating similar air
261 mass transport pathways from Russia and Siberia.

262 3.4. Endotoxin potency of GNB-impact via Aeolian transport

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

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

274 As mentioned in section 2.3, we have estimated the abundances of endotoxin
275 and mass loading of GNB in fresh snow samples. This quantification is indeed crucial for
276 assessing a likely allergic impact of endotoxin globally via long-range atmospheric transport.
277 Here, we estimated the endotoxin concentrations in snow to vary from 424 to 1080 ng kg⁻¹ (av.
278 789±237 ng kg⁻¹) in 2010 and from 36 to 1100 ng kg⁻¹ (av. 579±435 ng kg⁻¹) in 2011 samples.
279 The estimated lower limits of endotoxin in Table 1 and Table 2 are calculated based on the
280 minimum concentration of β-hydroxy FAs (C₁₀-C₁₈), which are specific to Gram-negative
281 bacteria (GNB). β-Hydroxy FAs (C₁₀-C₁₈) are the structural constituents of lipid A, which are
282 present in the outer cell membrane of GNB. Thus, the endotoxin concentrations in snow
283 samples were estimated based on the abundances of β-hydroxy FAs having carbon chain
284 length from 10 to 18 (section 2.3). Being consistent with this study, Lee et al. (2004) also
285 reported endotoxin concentration based on β-hydroxy FAs (C₁₀-C₁₈). Although relative
286 abundances of endotoxin during winter 2010 (N = 5) are higher than those of 2011 samples (N
287 = 6), the two-tailed t-test revealed no significant differences (t = 0.96; df = 9; P > 0.05) with
288 regard to mean concentrations of the two years.

289 In this study, we estimated dry mass concentrations of GNB in snow samples to
290 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
291 airborne endotoxin is of crustal origin and thus can be transported long distances to the
292 outflow region. Since the AMBTs reveal the impact of long-range transport from Russia and
293 Siberia during the study period, we infer that estimated endotoxin concentrations and dry cell
294 weight of GNB over Sapporo are derived from those source regions. Recently, Golokhvast
295 (2014) documented the airborne biogenic particles in snow from Russian Far East that cause
296 allergy for the pedestrians. The airborne biogenic particles can be scavenged efficiently by

297 both wet precipitation and snow fall. Therefore, we have looked for the literature describing
298 the occurrence of GNB in rainwater for comparison with our study on Sapporo snow.
299 Towards this, Gould (1999) and Lye (2002) have documented the presence of various GNB
300 (e.g., Salmonella, Shigella, Vibrio, Legionella and Campylobacter spp.) species in rainwater.
301 Likewise, Kawamura and Kaplan (1983) also reported the presence of β -hydroxy FAs in rain
302 water samples collected from Los Angeles (USA) and attributed their sources as bacterial
303 membrane. So far, no literature is available on endotoxin and GNB concentrations in snow
304 samples from East Asia in order to make a comprehensive comparison with the present study.

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

312

313 4. Conclusions

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

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

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

328

329 **Author contribution**

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

332

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337

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519

520 **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
 521 during winter 2010.

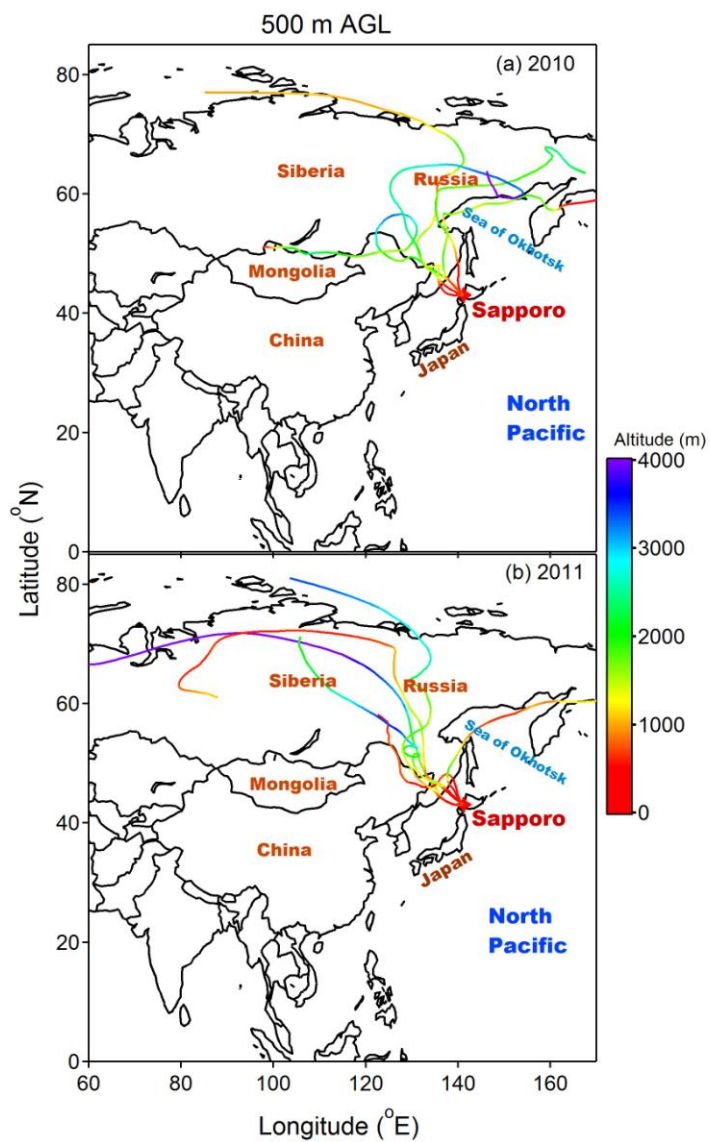
		2010								
522	C-number	α -Hydroxy FAs			β -Hydroxy FAs			ω -Hydroxy FAs		
		range	mean \pm S.E.	median	range	mean \pm S.E.	median	range	mean \pm S.E.	median
	C ₉	b.d.-7.1	2.4 \pm 1.3	1.7	0.5-2.7	1.8 \pm 0.47	2	b.d.-1.7	0.97 \pm 0.4	1.4
523	C ₁₀	b.d.-37.3	14.6 \pm 7.6	10.9	1.7-6.5	4.6 \pm 1.2	5.1	b.d.-5.1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

537 **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
 538 (N).

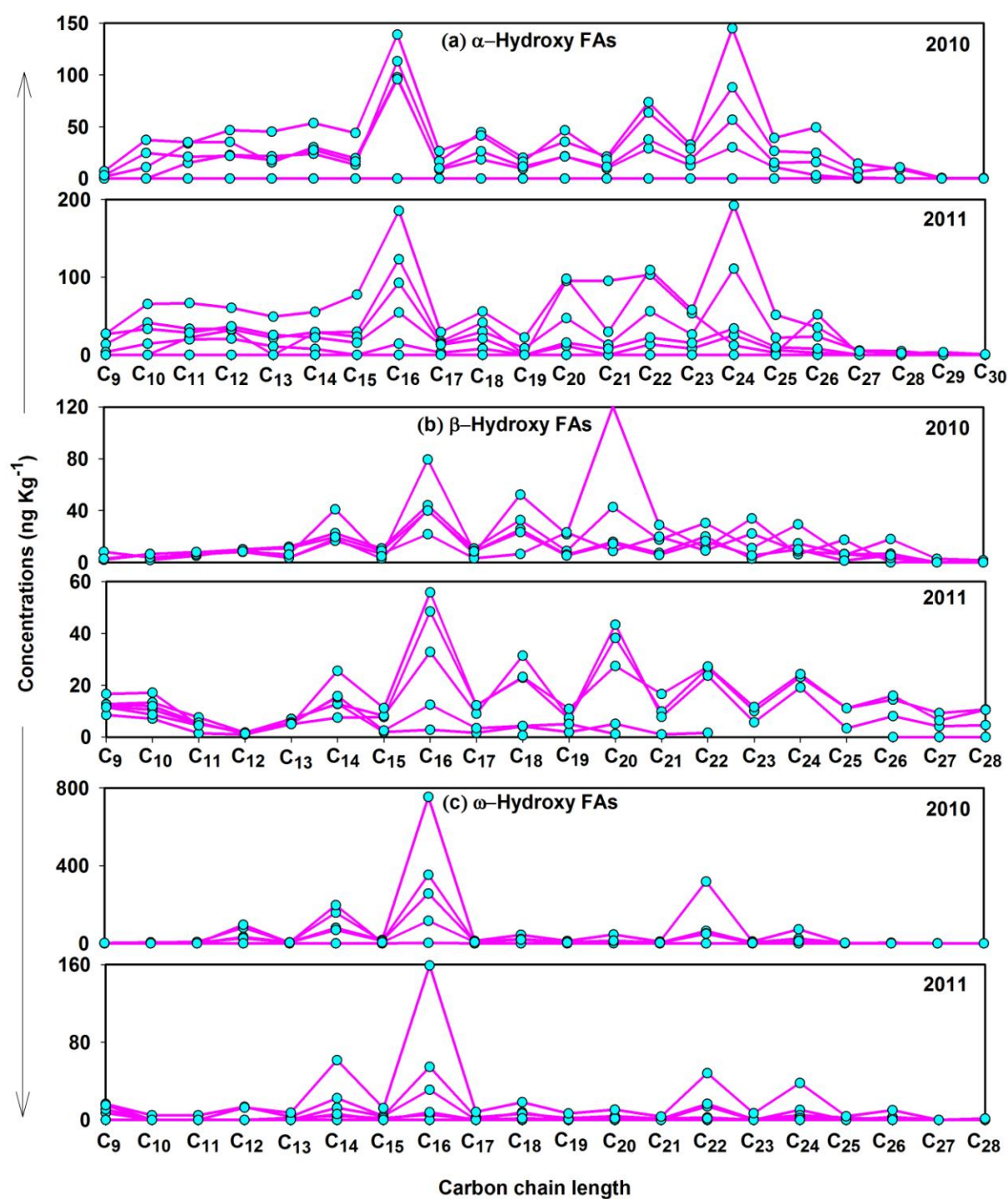
539 **Table 2.** Mass concentrations (in ng kg⁻¹) of α -, β - and ω -Hydroxy fatty acids (FAs) measured in snow samples (N=6) collected from Sapporo
 540 during winter 2011.

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

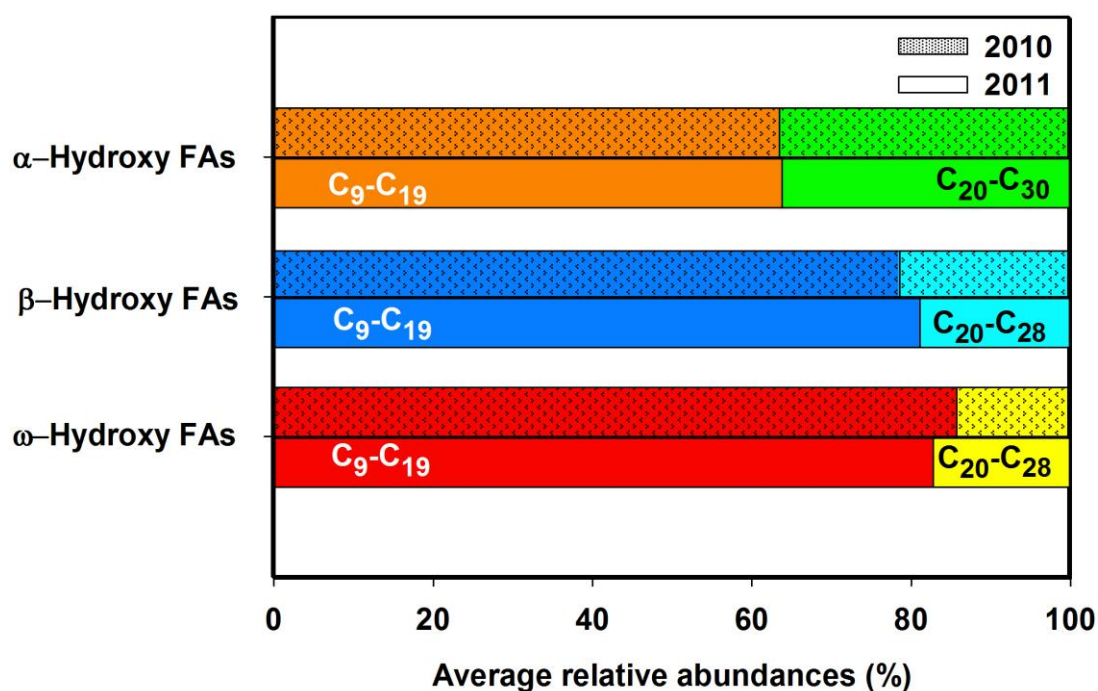
Note: b.d.= below detection limit ≤ 0.06 ng kg⁻¹. S.E. (Standard Error) = $\sigma/N^{1/2}$, where σ refers to standard deviation of total samples (N).



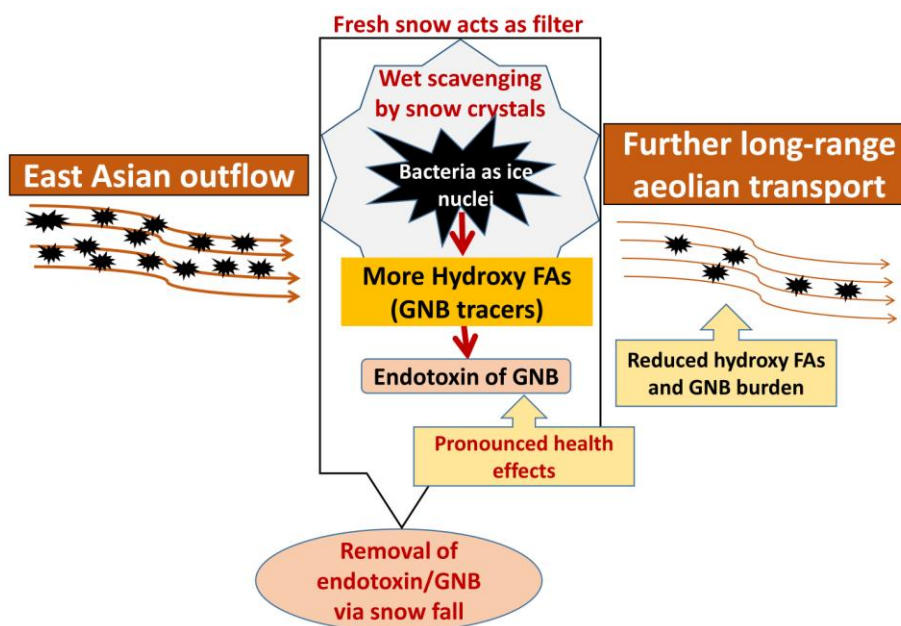
541 **Figure. 1.** Air mass back trajectory cluster at an arrival height of 500 m AGL (above ground
 542 level) for the sampling days in (a) winter 2010 and (b) winter 2011.



543 **Figure 2.** Molecular distributions of (a) α -Hydroxy fatty acids (FAs) (C₉-C₃₀), (b) β -Hydroxy
 544 FAs (C₉-C₂₈) and, (c) ω -Hydroxy FAs (C₉-C₂₈) in the snow samples collected from Sapporo
 545 during winter 2010 and 2011.



546 **Figure. 3.** Bar graph, showing the relative abundances of low molecular weight (C₉-C₁₉), and
 547 high molecular weight fatty acids (C₂₀-C₃₀ for α-Hydroxy; C₂₀-C₂₈ for β- and ω-Hydroxy) in
 548 their total mass for the snow samples collected during winter 2010 and 2011. The upper and
 549 lower horizontal bars for each type of hydroxy fatty acids indicate the data for 2010 and 2011,
 550 respectively.



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

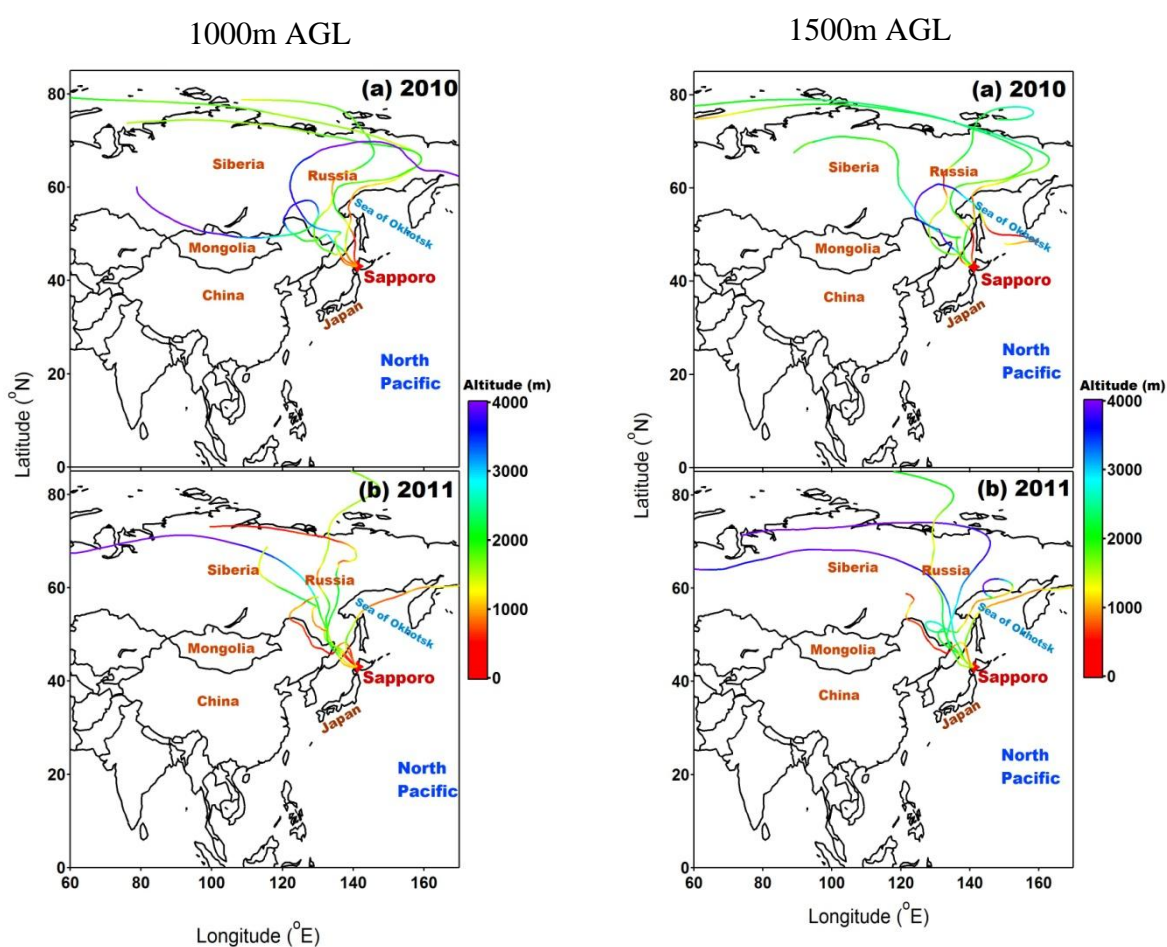


Figure S1. Air mass back trajectory cluster at an arrival heights of 1000 and 1500m AGL (above ground level) for the sampling days in (a) winter 2010 and (b) winter 2011.