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 ℃), 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.

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

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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.
17
18   **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

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

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.

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 °C until analysis.

2.2. Identification and quantification of hydroxy FAs

The analytical protocol used for assessing the atmospheric abundances of hydroxy FAs is described in Yamamoto et al. (2011). In brief, melted snow samples (0.5-1 L) were saponified with 1.0 M KOH in methanol at 80 ℃ 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[TM] Analytical) at 70 ℃ for 1 h. After the reaction, 50 μl of n-hexane solution containing 1.43 ng μl^{-1} of internal standard (C_{13} n–alkane/tridecane, Wako) was added to dilute the derivatives prior to GC/MS injection (Hewlett-Packard, Model 6890 GC coupled to Hewlett-Packard Model 5973 mass-selective detector, MSD). The GC was installed with a split/splitless injector and DB-5MS fused silica capillary column.

For the quantification of hydroxy FAs, the GC oven temperature was programmed from 50 °C (2 min) to 305 °C (15 min) at 5 °C min^{-1}. Data were acquired and processed with the Chemstation software. Structural identification and comparison of retention time of hydroxy FAs were performed using authentic TMS derivatives of n–C_{12} and 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 n–C_{22} ω-hydroxy FAs. The recoveries of authentic fatty acid standards were better than 92±4% with analytical error (average 4.1%) for acidic compounds (Yamamoto et al., 2011). Laboratory blanks showed no contamination of any target compounds. The results of n-alkanes, n-alkanols and n-alkanoic acids (terrestrial biomarkers) in snow samples are reported in Yamamoto et al. (2011), which revealed a long-range atmospheric transport of terrestrial 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

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   manuscript in preparation), possibly due to photochemical oxidation of higher molecular

193   weight fatty acids. Such a possibility of in situ formation of α-hydroxy FAs has also been

194   reported in the hydrolysis products of leaf waxes and wood, and in microalgae and sea grasses

195   (Feng et al., 2015). Further, microbial oxidation could also be a possible source of α-hydroxy

196   FAs (Eglinton et al., 1968) in the snow samples studied. Hence, we suggest that α-hydroxy

197   FAs cannot be employed as the tracers of plant waxes only, as they can come from

198   microbial/photochemical oxidation of higher molecular weight fatty acids during long-range

199   atmospheric transport.

200   A characteristic feature of our data is the predominance of C_{16} hydroxy FAs in

201 all the types of hydroxy FAs measured. However, significant shifts were observed in the

202 carbon numbers of the second most abundant β-hydroxy FAs (mostly C number >16) and ω-

203 hydroxy FAs (i.e., C number <16; see Tables 1 and 2). A likely explanation for this

204 observation is that β-hydroxy FAs above C_{16} were formed by β-oxidation of long chain FAs,

205 which is a more common in microorganisms as discussed previously. In contrast, ω-hydroxy

206 FAs below C_{16} are present in plants and microbes (Cardoso and Eglinton, 1983), in which ω-

207 oxidation of fatty acids is secondary choice for microbial oxidation.

208 **3.3. Molecular distributions**

209   Figure 2 presents molecular distributions of α-hydroxy (C_9 to C_{30}), β- and ω-

210 hydroxy FAs (C_9 to C_{28}) in snow samples from Sapporo during winter 2010 and 2011. Even

211 carbon number predominance is noteworthy for α-, β- and ω-hydroxy FAs. α-Hydroxy FAs

212 show molecular distributions with the order $C_{16} > C_{24} > C_{22}$ in both years (see Figure 2a).

213 Likewise, β-hydroxy FAs show the predominance of C_{16} followed by C_{18} or C_{20} and then by

214 C_{14} in both winters. However, we found the predominance of C_{20} β-hydroxy FAs over C_{16} in

215 one snow sample during 2010. Similarly, ω-hydroxy FAs showed dominance of C_{16} followed

216 by the others as $C_{14} > C_{12} \sim C_{22} \sim C_{24}$ during snowfall in both the years.

217   Table S1 describes the statistically significant differences in the ratios of even to

218 odd carbon numbers for α-, β-, and ω-hydroxy FAs in snow samples based on two-tailed

219 unpaired *t* test. No significant differences were observed between 2010 and 2011 for the ratios

220 of even to odd carbon number α-hydroxy FAs. In contrast, the difference is statistically

221 significant between 2010 and 2011 for β- and ω-hydroxy FAs. In fact, the difference is

222 extremely larger for ω-hydroxy FAs than that for β isomers. In 2010 winter, AMBTs show

223 atmospheric transport from the continents at 500, 1000 and 1500 m above ground, however, at

224 the same heights in 2011 winter, the air masses came from the oceans during one sample

225 collection. Higher plants in the continents contribute to higher abundances of hydroxy FAs

226 than the oceans, and thus explain higher abundances of β- and ω–hydroxy FAs in 2010 than

227 2011. On average, even carbon numbered α-, β- and ω-hydroxy FAs in their total mass

228 concentrations account for ~69, 68 and 84%, respectively. The even carbon number

229 predominance is also found in recent marine and lacustrine sediments (Cardoso and Eglinton,

230 1983; Goossens et al., 1986; Kawamura, 1995; Zhang et al., 2014).

231   Similar to our study, Volkman et al. (1980) documented the bimodal distribution

232 of α-hydroxy FAs with peaks at C_{16} and C_{24} in the intertidal sediments from Victoria,

233 Australia and attributed their contribution from sea grass (i.e., Zostera muelleri) detritus

234 owing to similar distribution pattern. However, it is noteworthy that our AMBTs show a

235 continental origin rather than the oceanic origin. Therefore, it is possible that waxes emitted

236 from continental grasses via wind abrasion can be transported to Sapporo through the

237 atmosphere. We speculate that α-hydroxy FAs (C_{16}-C_{28}) in Sapporo snow can be used as a

238 tracer of plant waxes. Likewise, higher plant derived cutin and suberin have been suggested as

239 a significant source of C_{16} to C_{22} α-, β- and ω-hydroxy FAs (Cardoso and Eglinton, 1983). In

240 a similar way, it has been proposed that hydroxy FAs (C_{20}-C_{30}) are principally derived from

241 terrestrial higher plants (Kawamura and Ishiwatari, 1984). Therefore, α-, β- and ω-hydroxy

242 FAs (C_{16}-C_{22}) in snow samples can be related to their sources from terrestrial higher plants

243 through long-range atmospheric transport.

244   Previous studies documented ubiquitous occurrence of these hydroxy FAs in soil

245 microbes such as yeast and fungi (Van Dyk et al., 1994 and references therein) and in the LPS

246 of GNB (Lee et al., 2007). In this regard, prior studies focussing on β-hydroxy FAs with the

247 predominance of C_{16} and C_{18}, suggested the contributions from yeast and fungi (Stodola et al.,

248 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

273　et al., 2004; Lee et al., 2007; Walters et al., 1994), soils (Keinänen et al., 2003), sewage

274　(Spaan et al., 2008) and marine dissolved organic matter (Wakeham, 1999).

275　　　　　As mentioned in section 2.3, we have estimated the abundances of endotoxin

276　and mass loading of GNB in fresh snow samples. This quantification is indeed crucial for

277　assessing a likely allergic impact of endotoxin globally via long-range atmospheric transport.

278　Here, we estimated the endotoxin concentrations in snow varied to be 424 to 1080 ng kg^{-1} (av.

279　789±237 ng kg^{-1}) in 2010 and 36 to 1100 ng kg^{-1} (av. 579±435 ng kg^{-1}) in 2011 samples. The

280　estimated lower limits of endotoxin in Table 1 and Table 2 are calculated based on the

281　minimum concentration of β-hydroxy FAs (C$_{10}$-C$_{18}$), which are specific to Gram-negative

282　bacteria (GNB). As stated on page 3, β-hydroxy FAs (C$_{10}$-C$_{18}$) are the structural constituents

283　of lipid A, which are present in the outer cell membrane of GNB. Thus, the endotoxin

284　concentrations in snow samples were estimated based on the abundances of β-hydroxy FAs

285　having carbon chain length from 10 to 18 (section 2.3). Being consistent with this study, Lee

286　et al. (2004) also reported endotoxin concentration based on β-hydroxy FAs (C$_{10}$-C$_{18}$).

287　Although relative abundances of endotoxin during winter 2010 (N = 5) are higher than those

288　of 2011 samples (N = 6), the two-tailed t-test revealed no significant differences (t = 0.96; df

289　= 9; P > 0.05) with regard to mean concentrations of the two years.

290　　　　　In this study, we estimated dry mass concentrations of GNB in snow samples to

291　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

292　airborne endotoxin is of crustal origin and thus can be transported long distances to the

293　outflow region. Since the AMBTs reveal the impact of long-range transport from Russia and

294　Siberia during the study period, we infer that estimated endotoxin concentrations and dry cell

295　weight of GNB over Sapporo are derived from those source regions. Recently, Golokhvast

296　(2014) documented the airborne biogenic particles in snow from Russian Far East that cause

297　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

323   can decrease their effect on human health via inhalation (Jacobs, 1989; Milton, 1996).

324   However, without snow scavenging, ambient bacterial endotoxin levels may stay high; having

325   an influence on human health as well can be transported to further long distances (North

326   Pacific). Overall, bacteria and their debris (biomass) can be evaluated in aerosols that are

327   scavenged by snow in free troposphere without prior culture by the determination of hydroxy

328   FAs for both LPS and GNB.

329

330   **Author contribution**

331   SY extracted the samples and conducted the experiments. PT prepared the

332   manuscript with contribution from KK.

333

334   **Acknowledgement**

338

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

C-number	2010								
	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) = σ/N$^{1/2}$, where σ refers to standard deviation of total samples (N).

540 **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

541 during winter 2011.

| C-number	2011								
	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).
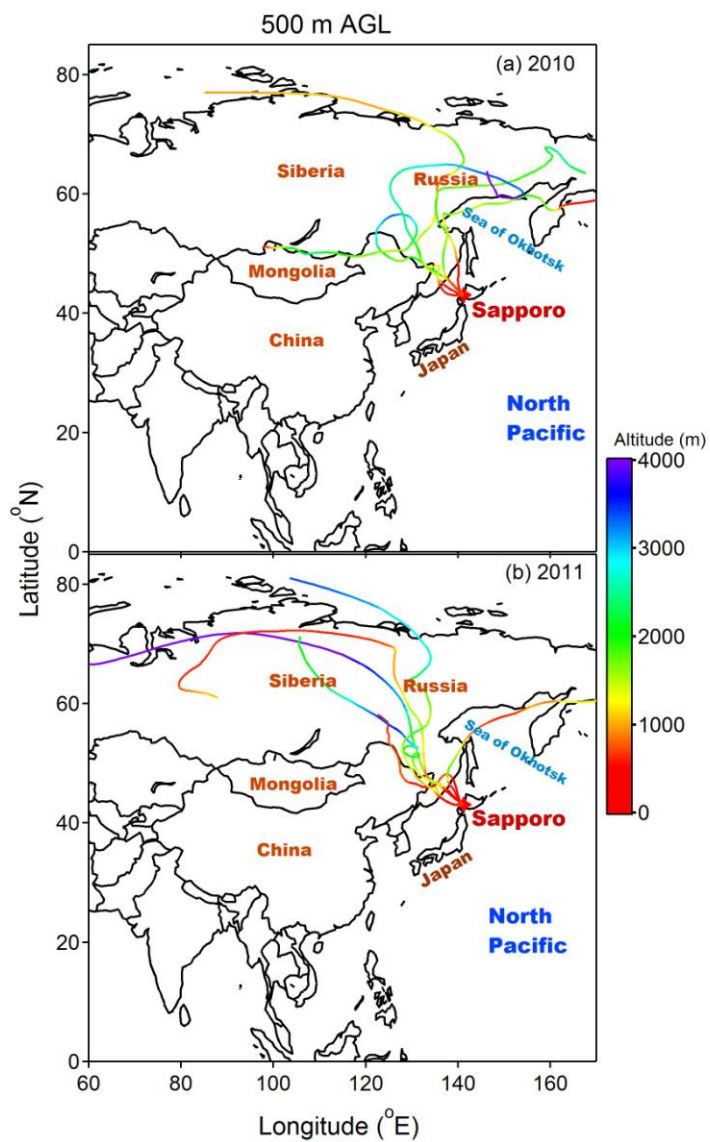
Figure. 1. Air mass back trajectory cluster at an arrival height of 500 m AGL (above ground level) for the sampling days in (a) winter 2010 and (b) winter 2011.
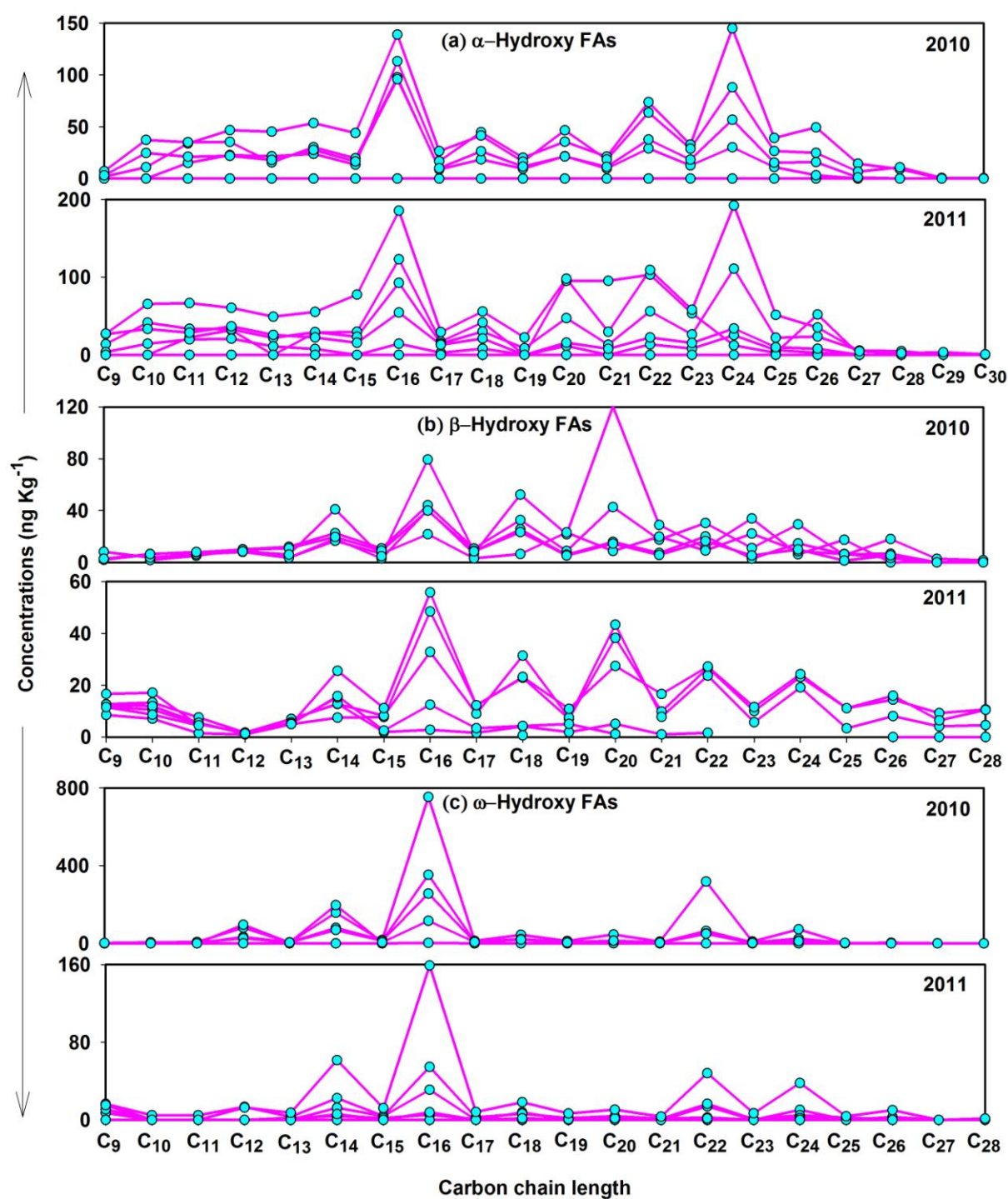
Figure. 2. Molecular distributions of (a) α-Hydroxy fatty acids (FAs) (C_9-C_{30}), (b) β-Hydroxy FAs (C_9-C_{28}) and, (c) ω-Hydroxy FAs (C_9-C_{28}) in the snow samples collected from Sapporo during winter 2010 and 2011.
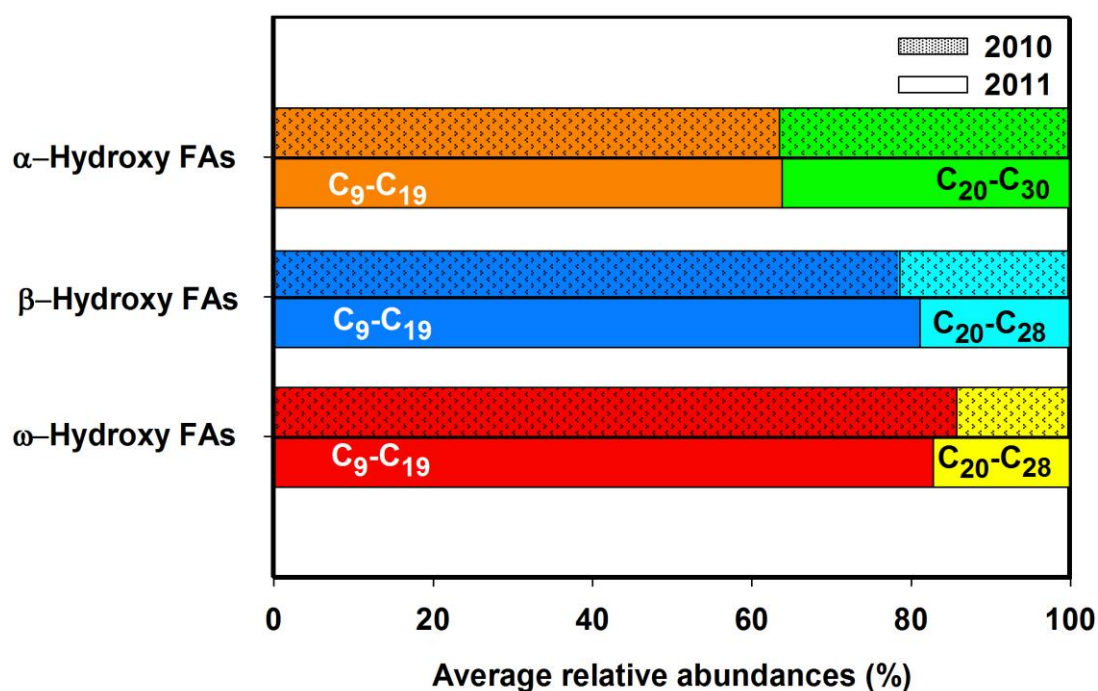
Figure. 3. Bar graph, showing the relative abundances of low molecular weight (C_9-C_{19}), and high molecular weight fatty acids (C_{20}-C_{30} for α-Hydroxy; C_{20}-C_{28} for β- and ω-Hydroxy) in their total mass for the snow samples collected during winter 2010 and 2011. The upper and lower horizontal bars for each type of hydroxy fatty acids indicate the data for 2010 and 2011, respectively.
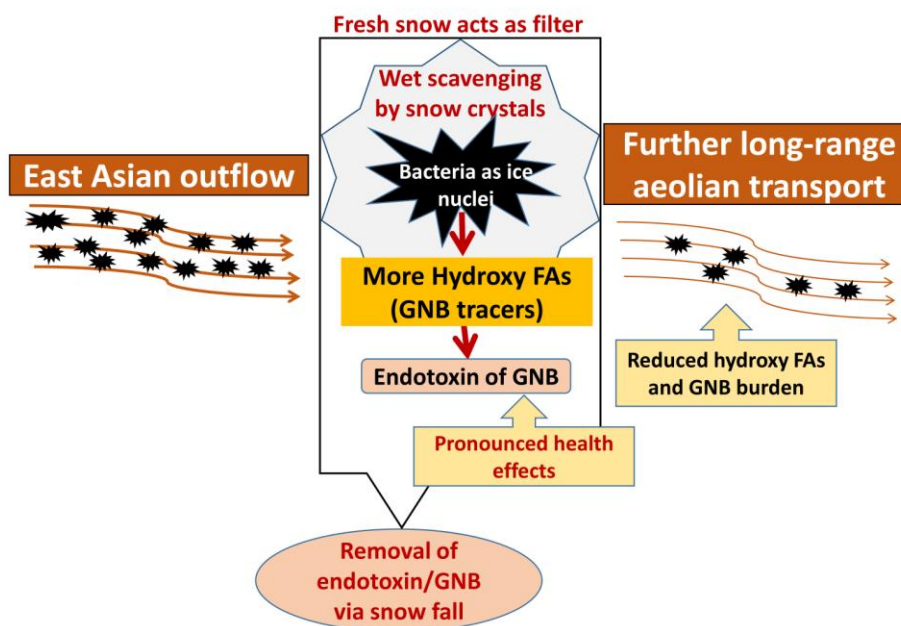
Figure 4. Conceptual model to explain the scavenging of hydroxy fatty acids (FAs) by fresh snow in the free troposphere. Snow fall in north Japan acts as a filter in reducing the hydroxy FAs (tracers of Gram-negative bacteria; GNB), which in turn results in the removal of endotoxin from the atmosphere and reduction in their health effects during long-range aeolian 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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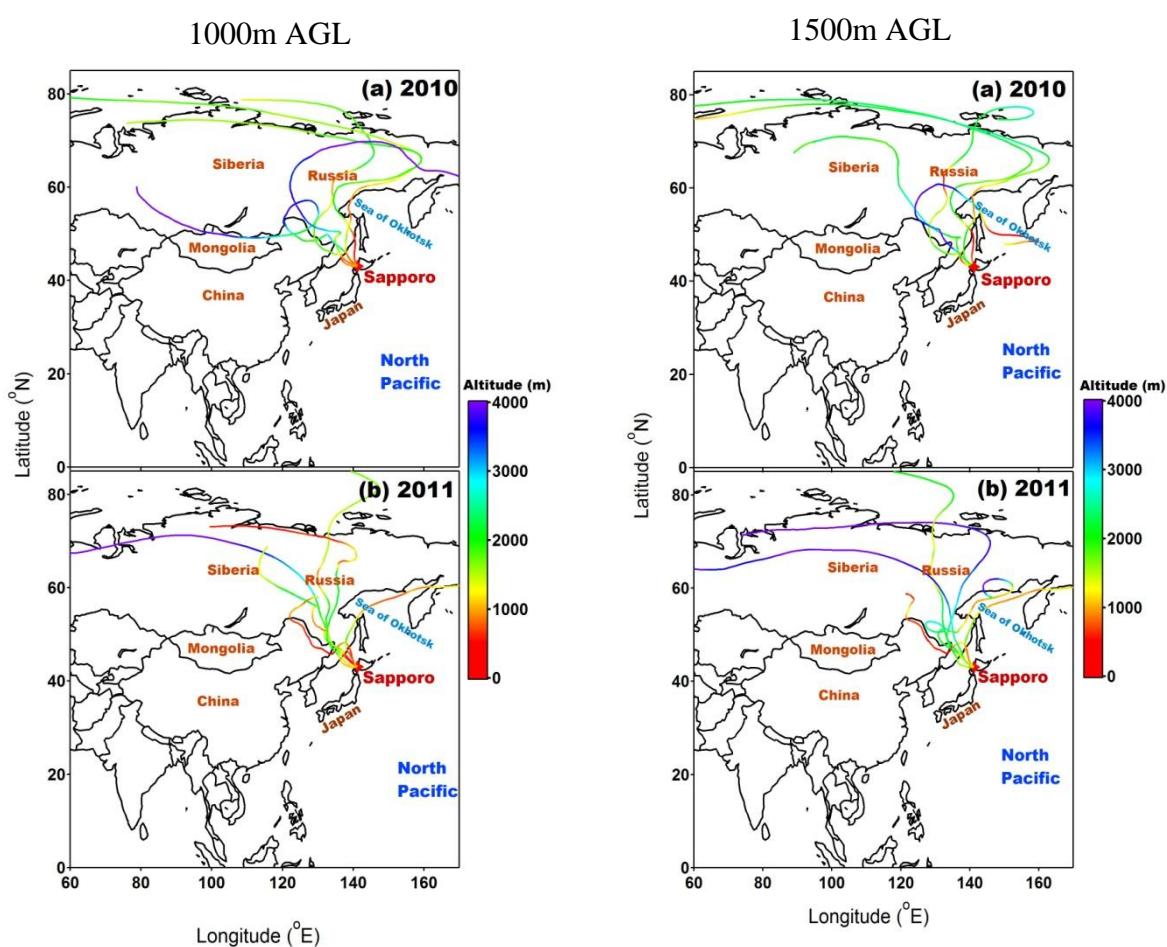| even/odd	2010	2011	t-score, df, P-value
α-hydroxy FAs	2.4 ± 0.3	2.2 ± 0.3	1.4, 7, > 0.05
β-hydroxy FAs	2.9 ± 0.8	1.8 ± 0.6	2.5, 9, < 0.05
ω-hydroxy FAs	15.8 ± 4.5	3.2 ± 1.8	5.8, 7, < 0.05

1000m AGL   1500m AGL

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.