Arctic Microbial and Next Generation Sequencing Approach for Bacteria in Snow and Frost Flowers: Selected Identification, Abundance and Freezing nucleation

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20 **Abstract**

21 During the spring of 2009, as part of the Ocean-Atmosphere-Sea Ice-Snowpack (OASIS)
22 campaign in Barrow, Alaska, USA, we examined the identity, population diversity, freezing
23 nucleation ability of the microbial communities of five different snow types and frost flowers. In
24 addition to the culturing and gene sequence-based identification approach, we utilized a state-of-
25 the-art genomic next-generation sequencing (NGS) technique to examine the diversity of
26 bacterial communities in Arctic samples. Known phyla or candidate divisions were detected (11-
27 18) with the majority of sequences (12.3 - 83.1%) belonging to one of the five major phyla:
28 Proteobacteria, Actinobacteria, Bacteroidetes, Firmicutes, and Cyanobacteria. The number of
29 genera detected ranged from, 101-245. The highest number of cultivable bacteria was observed
30 in frost flowers (FF) and accumulated snow (AS) with 325 ± 35 and 314 ± 142 CFU mL^{-1},
31 respectively; and for cultivable fungi 5 ± 1 CFU mL^{-1} in windpack (WP) and blowing snow (BS).
32 Morphology/elemental composition and ice-nucleating abilities of the identified taxa were
33 obtained using high resolution electron microscopy with energy-dispersive X-ray spectroscopy
34 and ice nucleation cold-plate, respectively. Freezing point temperatures for bacterial isolates
35 ranged from -20.3 ± 1.5 ℃ to -15.7 ± 5.6 ℃, and for melted snow samples from -9.5 ± 1.0 ℃ to -
36 18.4 ± 0.1 ℃. An isolate belonging to the genus Bacillus (96% similarity) had ice nucleation
37 activity of -6.8 ± 0.2 ℃. Comparison with Montreal urban snow, revealed that a seemingly
38 diverse community of bacteria exists in the Arctic with some taxa possibly originating from
39 distinct ecological environments. We discuss the potential impact of snow microorganisms in the
40 freezing and melting process of the snowpack in the Arctic.

1 Introduction

The snowpack has been shown to act as an important matrix for (photo) chemical and biological reactions of organic compounds (Ariya et al., 2011). Snow and ice provide large surface areas which consist of interstitial air, water and ice that may exchange chemical and biological matter with the atmospheric boundary layer. Trace gas exchange, scavenging, photolysis, adsorption (Kos et al., 2014), and more recently, biological transformations in snowpack have been considered (Amoroso et al., 2010; Fujii et al., 2010; Amoroso et al., 2009; Segawa et al., 2005). Yet, the role of biomolecules, including microorganisms, in oxidation, ice nucleation, gas-particle transfer and aerosol formation remains poorly understood.

Climate change has been linked to changes in snow and ice patterns in the Arctic, potentially impacting the Earth's albedo and atmospheric energy balance (Grenfell and Maykut, 1977; Grenfell and Perovich, 1984, 2004; Hanesiak, 2001). Atmospheric transport events such as dust storms initiated long distances away have been considered to influence the Arctic climate. Saharan dust, for instance, has been reported as a source of certain biological particles to reach the Arctic region (Barkan and Alpert, 2010). In 1976, an Asian dust storm was responsible for bringing as much as 4000 tons of dust per hour to the Arctic (Rahn et al., 1977). Dust has also been shown to transport microorganisms (Zhang et al., 2007; Zhang et al., 2008a; Zhang et al., 2008b). Bacteria and fungi have been detected in Asian dust (Choi et al., 1977; Yeo and Kim, 2002; Wu et al., 2004; Ho et al., 2005) and in African desert winds (Griffin et al., 2001; Griffin et al., 2003; Griffin et al., 2007; Griffin et al., 2006; Kellogg et al., 2004; Prospero et al., 2005) whereby some have been found to be viable (Griffin et al., 2001; Prospero et al., 2005). Recently, the increase in the number of storms has been associated with the efficient long-range transport of dust, microbial and other chemicals to the Arctic regions (Clarke et al., 2001; Grousset et al.,

64 2003; Uno et al., 2009). During long distance transportation, air masses may undergo chemical
65 and physical transformation under extreme environmental conditions such as high levels of solar
66 radiation, multiple freeze–thaw cycles, relatively acidic conditions, and predominantly inorganic
67 salts (Jickells, 1999; Ariya et al., 2002; Ariya et al., 2009; Cote et al., 2008). Little is known on
68 the effects of the photochemical and aging processes of the chemical and biological composition
69 of dust particles, or whether chemical properties and the genomic structure of microbial entities
70 transported with dust are altered, or mutated during long distance transport (Smith et al., 2010).
71 Pure water droplets homogeneously freeze in the atmosphere at approximately −38 °C.
72 Entities such as particles including mineral dust, soot, and biological materials (DeMott et al.,
73 2003; Möhler et al., 2007) may serve as ice nuclei (IN) which enhance freezing at much higher
74 temperatures in a process known as heterogeneous nucleation (Pruppacher and Klett, 1997).
75 Depending on the nature of the impurities, heterogeneous nucleation can occur over a wide range
76 in temperatures. Although dust particles are generally assumed to be the most important global
77 effect on ice nucleation, several strains of *Erwinia herbicola* (Lindow, 1978), *Pseudomonas*
78 *fluorescens* (Maki and Willoughby, 1978), *Pseudomona.s viridflava* (Paulin, 1978), and
79 *Xanthomonas campestris pathovar translucens* (Kim et al., 1987) are recognized amongst the
80 most efficient IN in biological particles. Yet the global effect of the importance of biological ice
81 nuclei is still a subject of debate. Some strains of *Pseudomonas syringae* can initiate water
82 freezing at temperatures as high as -2 °C (Orser et al., 1985) which have also been detected in
83 clouds and snow (Amato et al., 2005; Amato et al., 2007; Vaïtilingom et al., 2012; Lohmann and
84 Feichter, 2005; Joly et al., 2013). Atmospheric microbial, besides being considered efficient ice
85 nuclei (Constantinidou, 1990; Kieft and Ahmadjian, 1989; Möhler et al., 2007; Mohler et al.,

86 2008; Pouleur, 1992; Ariya et al., 2014; Mortazavi et al., 2008) have also been suggested to act
87 as cloud condensation nuclei (Bauer et al., 2003; Möhler et al., 2007).

88 Both natural (e.g. mineral dust, biogenic nucleators) and anthropogenic (e.g. soot)
89 sources can contribute to precipitation in Arctic regions (Hansson et al., 1993; Hinkley, 1994).
90 There is some evidence for the observed increase in the number of storms in certain areas of the
91 globe which can alter the transport and distribution of chemicals or biological entities (Wang et
92 al., 2011; Zhang et al., 2007; Erel et al., 2006) with potential impacts on precipitation patterns
93 (Sempere and Kawamura, 1994; Satsumabayashi et al., 2001). Although the pivotal role of dust
94 in the atmospheric global circulation (Dunion and Velden, 2004; Wu, 2007), radiative budget
95 (Sokolik and Toon, 1996; Kaufman et al., 2001), air pollution (Prospero, 1999; VanCuren, 2003)
96 and cloud formation (Toon, 2003) has been documented, there is little known about how the
97 newly introduced pool of transported microbial entities by dust to the Arctic impacts the change
98 of the total Arctic microbial pool or affects the freezing and melting processes of snow and ice
99 matrices in this region.

100 Several studies using standard microbiology techniques have shown that there is a diverse
101 population of bacteria in the snow (Carpenter et al., 2000; Amato et al., 2007; Mortazavi et al.,
102 2008; Amoroso et al., 2010; Moller et al., 2011; Liu et al., 2011; Harding et al., 2011). Recent
103 developments in high-throughput sequencing (HTS) techniques (Loman et al., 2012a; Loman et
104 al., 2012b), such as next-generation sequencing (NGS), also allow for metagenomic
105 investigations of microbial populations in environmental samples. The present study was
106 performed as part of the international Ocean-Atmosphere-Sea Ice-Snowpack (OASIS) campaign
107 (2009) in Barrow, Alaska. Five different types of Arctic snow: (i) accumulated snow, (ii)
108 windpack, (iii) blowing snow, (iv) surface hoar snow, (v) fresh snow and frost flowers were used

109 for this study (Fierz et al., 2009; Glossary of Meteorology, 2009). Frost flowers are dendritic
110 shape clusters of ice crystals that form at the interface between warm ice surface and sufficiently
111 cold atmospheric temperature and humidity (Obbard et al., 2009). The chemistry of frost flowers
112 has garnered increased interest, because these salty ice crystals have been shown to act as a
113 source for: (i) sea-salt aerosol (Perovich and Richter-Menge, 1994), and (ii) BrO, which
114 contributes to ozone depletion events (Kaleschke et al., 2004). Increased bacterial abundance
115 have also been found in frost flowers (Bowman and Deming, 2010). Yet, further research is still
116 required to better understand the mechanisms of physical, chemical and biological processes
117 involving frost flowers.

118 The aim of the study, in five different snow types and frost flowers in the Arctic, was to
119 evaluate the: i) identification and quantification of the number of viable bacterial and fungal
120 colonies, ii) determination of the ice nucleation (IN) property of: a) selected isolated bacteria and
121 b) melted samples, and iii) identification of the total bacterial pool using next-generation
122 sequencing. We herein provide further information on the biological composition of Arctic snow
123 and frost flowers at genomic level, shed light on the potential influence of atmospheric transport
124 on the change of microbial diversity, and discuss their potential roles in the freezing-melting
125 processes of ice-snow in the Arctic.

126

127 2 Experimental methods

128 2.1 Study sites

129 Five different types of Arctic snow were studied: (i) accumulated snow, (ii) windpack, (iii)
130 blowing snow, (iv) surface hoar snow, (v) fresh snow and frost flowers which were collected
131 from March 4 to March 20, 2009 during the OASIS campaign in Barrow, AK, USA. Detailed

132 snow sampling procedures have been described elsewhere (Kos et al., 2014). Snow samples were
133 collected from a field dedicated to snow research in the clean air sector at 71.31° N, 156.6° W,
134 400 m to the Southeast of the Barrow Arctic Research Center (BARC) and frost flower samples
135 were collected from sea ice at 71.36° N, 156.70° W. Vehicle access was restricted to the snow
136 sampling area, and equipment was transported on foot with a hand pulled sled to limit local
137 pollution. Snow sampling devices were sterile and single-use. A sterile high-density polyethylene
138 (HDPE) spoon (Fisher Scientific, Montreal, Canada) was used to collect the first 3 cm of the
139 surface snow to fill the HDPE (Fisher Scientific, Montreal, Canada) sample containers (220 mL).
140 Similarly, a total of 900 ml of frost flower samples were collected by carefully lifting the frost
141 flower off the surface with a shovel to minimize (but not completely eliminate) brine content.
142 Frost flower sampling was collected from a frost flower "field" on a flat area of thick sea ice, in a
143 single location about 5 km northwest of Barrow on Mar 20, 2009 (also described by: Beine et al.,
144 2012; Douglas et al., 2012). Snow temperature was measured using a long-stemmed
145 thermometer (Fisher Scientific, Montreal, Canada) and the meteorological conditions were
146 recorded (air temperature, wind direction, and cloud cover). Average snow temperature was at -
147 19 °C and air temperature was at -21 °C. The Frost flowers were characterized as "old frost
148 flowers" having coatings from increased vapour phase deposition (Douglas et al., 2012).
149 Samples were kept frozen (-20 °C) until shipped out by airfreight (transit time 41 hours) in
150 commercial coolers (Coleman). The maximum temperature upon arrival in the laboratory at
151 McGill University in Montreal was -5 °C. Arctic samples were stored in a freezer at -20 °C
152 (Viking) until analysis. Surface snow samples from heavy snowfall regions in the province of
153 Quebec, Canada: Mont-Tremblant, the city of Montreal (urban snow) and its suburb, Pierrefonds,

154 were also collected using similar techniques. Minimum of three samples for each snow types and
155 frost flowers were used for analysis.
156
157 **2.2 Isolation of viable microorganisms and Drop-freezing assays**
158 Snow and frost flower samples were melted directly by transferring from freezer to refrigerator
159 at 4 °C, under sterile conditions and these conditions were maintained at all times using sterile
160 instruments and materials or certified sterile single-use supplies. Once melted, they were kept on
161 ice for the IN experiment, or transferred to the laminar flow hood to culture the microorganisms.
162 To grow the microorganisms, one milliliter of Arctic samples (snow and frost flower) were
163 placed in standard 100 × 15 mm sterile plastic Petri dishes (Fisher Scientific, Montreal, Canada).
164 The media used for bacteria were tryptic soy agar (TSA), and R2A agar, a low nutrient medium
165 used to improve the recovery of stressed bacteria. For fungi, mycological agar (Rybnikar, 1986)
166 at neutral pH, and sabouraud dextrose agar (SDA) at a low pH of approximately 5.6 (all media
167 by Becton, Dickson and Co, Mississauga, Canada) were used. In a flask, agar was dissolved and
168 heated in ultrapure Milli-Q water (18 ohms resistance) according to the manufacturer's
169 recommendation. After boiling for one minute or until the medium was completely dissolved, the
170 flask was autoclaved at 121 °C for 15 minutes. Plates were incubated at 4 °C and were regularly
171 checked for growth and the colonies were counted.
172 Drop-freezing assays were done on: i) viable isolated bacteria obtained from Arctic
173 samples, and ii) melted Arctic samples. Viable bacteria isolated from Arctic samples grown on
174 Petri dishes were mixed with sterile ultrapure water (Millipore, Mississauga, Canada). The
175 optical density of 1 at 600 nm was used to adjust the concentrations of bacteria to 10^8 cells ml^{-1}.
176 IN experiments were performed using a homemade copper cooling plate (cooling rate of 1°C

177 min^{-1}), a technique first described by Vali (1971). The copper plate was coated evenly with
178 commercial Vaseline™ petroleum jelly. The samples were kept on ice and were loaded as 10 µl
179 droplets. A minimum of 150 drops was used for each experiment. Tap water was also used as a
180 control that showed IN activity between positive (*Pseudomonas syringae*) and negative controls
181 (ultrapure water). The temperature of each frozen droplet was recorded. IN temperatures are a
182 simple average of the temperatures at which a sample group of drops freezes, i.e., the sum of the
183 freezing temperature of each drop in the ensemble divided by the total number of drops.
184

185 **2.3 Bacterial DNA isolation, amplification of 16S rDNA, sequencing and identification**

186 Part of each bacterial colony was picked by a sterile disposable inoculating loop (VWR,
187 Mississauga, Canada) and mixed with Ready-Lyse™ Lysozyme (Epicentre Technologies,
188 Madison, USA) and proteinase K in 1.5 ml eppendorf tube. DNA was extracted and purified
189 equally well with either a DNAeasy kit (Qiagen , Toronto, Canada) or a Master Pure DNA
190 purification kit (Epicentre Technologies, Madison, USA) according to the manufacturer's
191 instructions. The conserved sequence of extracted DNA was amplified by PCR (Techne
192 Flexigene Thermal Cycler FFG02HSD) in a final volume of 25 µl using 16S universal primers
193 27F and 149R (Forward primer 5'-AGAGTTTGATCCTGGCTCAG-3' and reverse primer 5'-
194 ACGGCTACCTTGTTACGACTT-3', Integrated DNA Technologies, Coralville, USA) yielding a
195 product of about 1465 bp. An Eppendorf™ tube containing all the ingredients but DNA was used
196 as control (blank) for every set of PCR. A typical PCR reaction for one tube contained 2.5 µl of
197 10X buffer, 1 µl of each primer of 2.5 µM, 0.6 µl of 10mM dNTP, 1 µl of 0.1 µg/ µl DNA, 0.1 µl
198 of 5U/ µl of Taq polymerase, 2.5 µl of 25mM of $MgCl_2$, 0.8 µl of 1M NaCl, and to 25 µl of
199 nuclease free water (Promega, Madison, USA). PCR included 35 cycles of denaturing at 94 °C

200 for 1 minute, annealing at 55 °C for 1 minute, and extending at 72 °C for 2 minutes, followed by
201 a 7 minutes. final extension at 72 °C and 4 °C forever. The PCR product was separated and
202 analyzed in 1.2% agarose gel electrophoresis stained with ethidium bromide.

203 The PCR product of 16S rDNA genes obtained from the cultured bacterial colonies were
204 purified using QIAquick PCR Purification Kit (Qiagen, Toronto, Canada), sequenced at McGill
205 University and at the Génome Québec Innovation Centre, Montreal, Canada. The 16S rDNA
206 sequences were aligned and compared with those available in the GenBank databases using the
207 BLASTN (Basic Local Alignment Search Tool for DNA/nucleic acid) through the NCBI
208 (National Center for Biotechnology Information server) to identify sequences that share regions
209 of homology with isolated sequences.

210

211 **2.4 454 Pyrosequencing**

212 We opted to use a conventional technique in concentrating the bacteria in Arctic samples using
213 filtration, sonication and precipitation using high-speed centrifuge. For DNA analysis, bath
214 sonication is a method that has been used to dislodge adherent bacteria in environmental samples
215 (Buesing and Gessner, 2002; Bopp et al., 2011; Joly et al., 2006; Kesberg and Schleheck, 2013)
216 as well as in medically devised explanted prosthetic instruments studied in hundreds of patients
217 (Piper et al., 2009; Sampedro et al., 2010; Tunney et al., 1999). The dislodge bacteria is viable
218 and can be cultured (Trampuz et al., 2007; Vergidis et al., 2011; Piper et al., 2009; Sampedro et
219 al., 2010; Tunney et al., 1999; Joly et al., 2006; Kesberg and Schleheck, 2013; Solon et al.,
220 2011). Melted snow was passed through 0.22 micron filter (Millipore, Mississauga, Canada).
221 Filter was sonicated in 17 ml of 1X TBE buffer in an ultrasound bath for 10 min. For removing
222 the viable bacteria from surface by sonication, no major differences were reported for duration of

223 exposure time at 5 or 10 min, and temperatures at 22 °C (room temperature) or 6 °C; though the
224 latter only slightly improved bacterial viability (Monsen et al., 2009). In our experiment, all the
225 above factors were considered for removing the bacteria from filters.
226 The liquid was collected in sterile 50 ml polycarbonate centrifuge tubes (Nalgen. Rochester, NY,
227 USA). The liquid was centrifuged for 15 min at 18,000 g, and the pellet was re-suspended in 200
228 ul of 1X TBE buffer (Moran et al., 2008; Gharaibeh et al., 2009; Gantner et al., 2011; Medinger
229 et al., 2010). Ready-Lyse™ Lysozyme (Epicentre Technologies, Madison, USA) was used to lyse
230 the cell, and DNA was extracted and purified using either a DNAeasy kit (Qiagen, Toronto,
231 Canada) or a Master Pure DNA purification kit (Epicentre Technologies, Madison, USA)
232 according to the manufacturer's instructions. A barcoded 16S rDNA tag was used to amplify
233 three distinct regions (V1-V3) of the bacterial 16S rDNA gene (~500 bp). The forward primer
234 consisted of 454 Life Science adaptor A, a unique 10 base barcode rapid library MID, and the
235 specific forward primer sequence: 5′-
236 CCATCTCATCCCTGCGTGTCTCCGACTCAGACGAGTGCGTATTACCGCGGCTGCTGG-
237 3′ and the reverse primer consisted of 454 Life Science adaptor B fused to the specific reverse
238 primer sequence: 5′-
239 CCTATCCCCTGTGTGCCTTGGCAGTCTCAGAGAGTTTGATCCTGGCTCAG-3′.
240 Amplification was done in triplicate and performed in a 20 μl reaction volume containing 13.85
241 μl of RNase and DNase free water, 2 μl of 5 ng/μl of DNA template, 2 μl of 10X AccuPrime
242 PCR buffer (Invitrogen, Burlington, Canada), 1 μl of 2 μM of each primer, and 0.15 μl of
243 AccuPrime Taq DNA polymerase Hifi (Invitrogen, Burlington, Canada). Cycling conditions were
244 performed at 95 °C for 2 minutes, followed by 30 cycles at 95 °C for 20 seconds, 56 °C (V1-V3
245 primer set), 72 °C for 5 minutes and 4 °C forever. PCR products were purified with AMPure XP

246 beads (Agencourt, Beckman Coulter, Canada), and eluted in 20 µl of ultrapure water. The quality
247 and size of the amplicons were assessed on a 2100 Bioanalyzer using a DNA 1000 kit (Agilent
248 Technologies, Mississauga, Canada) and quantified with the PicoGreen assay (Invitrogen,
249 Burlington, Canada). The amplicons library was pooled in equimolar amounts. NGS sequencing
250 was performed using 1/8 of sequencing plate of GS FLX Titanium (454/Roche, Mississauga,
251 Canada) for reading. The pool was sequenced uni-directionally from adaptor A with the Genome
252 Sequencer FLX Titanium (454/Roche, Mississauga, Canada) at McGill University and at the
253 Génome Québec Innovation Centre, Montreal, Canada. The generated sequences from
254 pyrosequencing was analyzed with software MOTHUR formatted version of the RDP classifier
255 using a Bayesian method (Wang et al., 2007) with 1000 bootstrap replicates for pre-processing
256 (quality-adjustment, barcode split), identification of operational taxonomic units (OTUs) defined
257 at ≥ 97 % 16S rRNA sequence identity level, taxonomic assignment, community comparison,
258 and statistical analysis (Schloss et al., 2009). Trimming was done by quality from the 3' end.
259 Sequences containing ambiguous bases, homopolymers longer than eight bases, or an average
260 quality score below 20 over a 50 bp long window were excluded (Schloss et al., 2011). The
261 diversity of the bacterial communities for four different snow types and frost flowers was
262 estimated using the Simpson Diversity Index, species richness using Rarefaction Metric, and the
263 nonparametric Chao index (Chao, 1984). Chao1 index is a good estimator for obtaining true
264 species richness based on the observed species accumulation pattern wherein the number of
265 singletons and doubletons were used.

266

267 **2.5 Electron microscopy analysis**

268 Analysis Transmission electron microscopy (TEM) in conjunction with energy-dispersive X-ray
269 spectroscopy (EDS) analyses were used on Arctic snow samples and frost flowers to detect
270 microbial and chemical compounds. Samples were freeze-dried. A sample solution of 7 μl was
271 put onto a 200 mesh carbon-coated copper grid for 1 min. It was negative stained with 2 %
272 uranyle acetate for 30 sec. Imaging was done under TEM (Hitachi H7500 operated at 100 keV
273 and spot size 5), a Philips CM200 200 kV TEM equipped with Gatan Ultrascan 1000 2k x 2k
274 CCD Camera System (Model 895) and EDAX Genesis EDS Analysis System.
275
276 **3 Results and discussion**
277 Recent observations have indicated that snowpack is indeed a complex microhabitat that permits
278 the growth of diverse microorganisms allowing for photo-chemical and biological reactions to
279 occur (Amoroso et al., 2010). Nitrification (Amoroso et al., 2010), transformation of mercury
280 (Moller et al., 2011) and other pollutants within the snowpack have been detected. Ammonia-
281 oxidizing Betaproteobacteria are active nitrifiers in glacial ice microcosms (Miteva et al., 2007),
282 and the presence of nifH genes has been previously suggested the potential for nitrogen fixation
283 in supraglacial snow (Boyd et al., 2011). A clear understanding of the bacterial population and
284 their interactions will be required to further reveal the role these play in altering the Arctic
285 environment and climate.
286 In this study, the next-generation sequencing (NGS) technique in conjunction with a
287 classical cultural method was used to identify and compare the bacterial community in different
288 types of Arctic snow and frost flowers. Moreover, the Arctic microbial population was compared
289 to urban snow from the cold North American City of Montreal. Using GS FLX Titanium
290 (450/Roche), a total of 88,937 reads was made for all the samples with the average number of

291 total reads being 17,787. The average read length for all the reading was 373 base with average
292 read quality of 34. After trimming and passing through quality control, the final read length was
293 recovered as: 319 ± 18 bases (urban snow, US), 299 ± 17 bases (blowing snow, BS), 401 ± 20
294 bases (surface hoar snow, SH), 385 ± 20 bases (windpack, WP), and 419 ± 20 bases (frost flower,
295 FF).

296 The diversity of the bacterial communities for four different snow types and frost flowers
297 was estimated using the Simpson Diversity Index, species richness using Rarefaction Metric, and
298 the nonparametric Chao index (Chao, 1984). The Simpson Diversity Index which takes into
299 account both species' richness, and an evenness of abundance among the species present reached
300 a plateau after the sequencing of sampling of about 5000 for BS, 6000 for WP, 7000 for US,
301 8000 for SH, and 10,000 for FF (Appendix Fig. 1A). The Chao index gave values between 1500
302 and 7500 with BS exhibiting the lowest richness (Appendix Fig. 1B). The richness in total
303 bacterial communities of Arctic samples was estimated by rarefaction analysis. The shapes of the
304 rarefaction curves did not reach asymptote indicating that bacterial richness for most samples
305 especially for urban snow, and windpack is not yet complete (Appendix Fig. 1C). Using the 3%
306 cut-off value in sequence differences for OTU, the estimates of the richness of total bacterial
307 communities ranged from 1033 in BS, 1971 in WP, 1956 in SH, 1933 in US and 1605 in FF
308 (Appendix Fig. 1C). Based on these analyses, the order of the highest diversity of bacteria to the
309 lowest was observed in windpack, surface hoar snow, urban snow, frost flowers, and blowing
310 snow, under experimental conditions herein used.

311 In the next-generation sequencing part of this study, pyro-sequencing was done only for
312 bacteria and not fungi which was feasible under our existing facilities. However, high resolution
313 electron microscopy (Fig. 1) further revealed the appearance of the existence of several

314 biological materials, remnants of biological activities, and not only biological entities in their
315 entirety. The individual sequences represented known phyla or candidate divisions as: 11 (urban
316 snow), Arctic samples: 18 (WP), 16 (SH), 15 (BS), and 18 (FF) (see also the Appendix Table
317 A.1A). The majority of sequences (12.3 - 83.1%) belonged to one of the five major phyla:
318 Proteobacteria, Actinobacteria, Bacteroidetes, Firmicutes, and Cyanobacteria. The major phyla
319 for urban snow and Arctic samples were as follows: i) urban snow: Proteobacteria (49.04%),
320 Bacteriodetes (47.5%); ii) windpack: Proteobacteria (66.1%), Cyanobacteria (12.3%); iii) surface
321 hoar snow: Proteobacteria (67%), Firmicutes (13.6%); iv) blowing snow: Proteobacteria
322 (83.1%), Firmicutes (6%), Actinobacteria (5.09%) and v) frost flowers: Proteobacteria (50.2%),
323 and Actinobacteria (32.8%) (Appendix Table A.1A). Proteobacteria was the most widely
324 expressed phylum among all the Arctic samples tested with the greatest abundance observed in
325 blowing snow.

326 At the genus level, sequences represented 134 different genera for urban snow; Arctic
327 samples: 245 for windpack, 139 for surface hoar snow, 101 for blowing snow, and 158 for frost
328 flowers. The distribution of bacterial genera observed at greater than 1% and the number of
329 occurrence for each percentage observed for any genus in total bacteria is shown in Figure 2.
330 The name of corresponding genera for each percentage (>1%) is listed in the Appendix Table
331 A.2. The top four genera with the highest percentage detected for each sample were as follows:
332 urban snow, US: *Flavobacterium* (40%), *Polaromonas* (11.2%), *Variovorax* (6.6%) and
333 *Sandarakinorhabdus* (6%); windpack, WP: *Methylobacterium* (9%), *Sphingomonas* (4.9%),
334 *Lamprocystis* (4.5%), and *Roseateles* (4.4%); surface hoar snow, SH: *Roseateles* (18.6%),
335 *Methylobacterium* (14.1%), *Bacillus* (5.7%), and *Streptococcus* (5.7%); blowing snow, BS:
336 *Methylobacterium* (15.3%), *Bradyrhizobium* (1.6%), *Bacillus* (1%), *Sphingomonas* (1%); and for

337 frost flowers, FF: *Propionibacterineae* (32.9%), *Roseateles* (8%), *Staphylococcus* (6.7%), and
338 *Candidates Pelagibacter* (6.1%) (Appendix Table A.1&B).

339 Bhatia et al. (2006) compared bacterial communities from solid snow and snow melt
340 water from the high Arctic John Evans glacier with basal ice and sub-glacial communities of the
341 same glacier. Distinct bacterial communities were found in each one of these different
342 environments with very few common profiles. Similar to this study, our NGS analysis clearly
343 showed variation of distinct sets of microorganisms among different Arctic samples and urban
344 snow. Our observation also suggests the importance of the selective pressure of specific physical
345 and chemical characteristics of each snow type that may serve as a predictor of microbial
346 abundance and composition (Miteva, 2008). It may specifically favor the growth conditions for
347 microbial communities that originated from diverse sources. Interestingly, few Geobacter
348 bacteria (at 0.09%) were only detected in the windpack. Some of which have been suggested in
349 previous studies, to catalyze anaerobic U (IV) oxidation with nitrate serving as a potential
350 electron acceptor leading to the subsequent mobilization of uranium (Finneran et al., 2002).
351 Geobacter species have also shown to reduce soluble U(VI) to the less soluble U(IV) (Lovley,
352 1991). Arctic region is exposed to further uranium originating from radioactive waste due to
353 military activity, oil and gas, and uranium mining exploitation (Thomas et al., 1992; Dowdall et
354 al., 2004; Convey, 2010; Emmerson and Lahn, 2012). However, additional research is required
355 to evaluate the role of micro-organisms in chemical transformation of molecules in the Arctic
356 region.

357 Table 1 shows the analysis of NGS results encompassing bacteria at genus level that have
358 been previously detected: i) in Asian or African dust storms, ii) with antifreeze and/or ice
359 nucleation properties, and iii) in cold oceanic water. Note that the existence of these bacteria

360 does not ensure the expression of their property, and thus the existence of ice nucleating and
361 freezing bacteria does not reflect their expression in environmental matrices. The percentage of
362 bacteria (at genus level) that were previously observed in Asian or African dust samples were in
363 the range of 36-47% for all the snow categories and frost flower samples. Only a very small
364 percentage of identified bacteria with previously demonstrated antifreeze property (Ariya et al.,
365 2009; Yamashita et al., 2002), were detected in: windpack: 1%, surface hoar snow: 0.2%, and
366 frost flowers: 1%. Urban Montreal snow samples had the highest number (7%). 14-16% of
367 samples contained bacteria with ice nucleation properties, as shown in Table 1A . A very small
368 percentage of identified bacteria showed both ice nucleation and antifreeze properties. Some
369 bacteria such as *Pseudomonas fluorescens KUAF-68* and *Pseudomonas borealis DL7* have been
370 reported to have both antifreeze and ice nucleation activity (Kawahara et al., 2004; Wilson et al.,
371 2006). Having these two properties have been suggested to enhance the freeze tolerance survival
372 of bacteria by maintaining small ice crystals with ice recrystallization inhibition protecting
373 against freeze-thaw stress with antifreeze proteins (Xu et al., 1998), minimizing damage from
374 explosive ice crystal growth and stabilizing the outer membrane with the low thermal hysteresis
375 value (Xu et al., 1998), and minimizing the supercooling point with ice nucleation proteins
376 (Kawahara et al., 2004). In our study, under our experimental conditions, the highest percentage
377 was observed in urban snow samples (0.1%) and the lowest was observed in blowing snow
378 (0.03%). Only 0.01% of bacteria in windpack snow, surface hoar snow and frost flowers had
379 both ice nucleation and antifreeze properties. Similarly, Arctic samples showed a minute number
380 of bacteria that have been previously detected in cold oceanic water (0.01-0.04%); none were
381 detected in urban snow samples, under the experimental conditions in this work.

382 Some of the bacteria in Arctic samples have previously been identified in Asian or
383 African storms with ice nucleation (Kellogg et al., 2004; Griffin, 2007) or antifreeze properties
384 (Smith et al., 2013). Within these bacterial genera pool, 2-3% of Arctic snow samples and urban
385 snow showed ice nucleation properties with only 0.2% in frost flowers (Table 1B). Bacteria with
386 antifreeze properties were observed for only 0.4% in frost flowers and 1% in both blowing snow
387 and surface hoar snow. Higher numbers of such bacteria were observed for windpack (6%).
388 Interestingly, 13% of bacteria originating from dust storms in urban snow had antifreeze
389 properties. The possible introduction of antifreeze bacteria from the ocean into the air by
390 different mechanisms such as the bursting of frost flowers by wind and fresh snowfall may
391 further provide and facilitate infiltration into the snowpack (Rankin et al., 2002). The detection
392 of a high number of bacteria with a vast genetic diversity pool, using NGS analysis, further
393 illustrates that the snowpack is a heterogeneous soup of microbial entities. The chemical
394 environment of the snowpack is constantly evolving by novel streams of chemicals through fresh
395 precipitations, wind transportation and metabolic activity of microbial. On a speculative basis,
396 the increased incidence of dust storms, possibly due to climate change, the detection of specific
397 bacteria with possible mid-latitude desert origins into the Arctic environment may suggest a shift
398 in the balance of "native bacterial populations" in the Arctic, yet, there is no current evidence to
399 firmly support this hypothesis and further research is required. One may also speculate that it
400 might be conceivable to consider interactions among the heterogeneous population of microbial
401 in Arctic samples, including non-native taxa adaptation in the Arctic snow-ice genome. Though
402 the Arctic does not provide a native habitat for non-native bacteria or biological species
403 originating from elsewhere in the world, their entrance into the Arctic may affect certain bio-
404 chemical reactions, or alter the nutrient pool for the other native microbial entities. In turn, it

405 might affect the ratio and the survival rate of certain populations of microbial with freezing or
406 anti-freezing properties, impacting the melting of ice or snowpack in the Arctic region. Yet,
407 further studies are required to evaluate such speculations.

408 With Arctic regions currently warming at rapid rates (Hansen et al., 2006; Convey et al.,
409 2009), the interrelationship of ice/snow microbial, and increased water availability is yet to be
410 determined. Though fungi species and their spore are widespread in the atmosphere, little is
411 known about their role and presence in the Arctic. Interestingly, a few studies have shown that
412 fungi-like bacteria can be effective ice nucleators, capable of initiating ice nucleation at
413 temperatures as high as -2 °C (Kieft and Ahmadjian, 1989; Pouleur et al., 1992). Some fungi
414 have shown to exhibit an effect to prevent ice crystal expansion by synthesizing antifreeze
415 proteins permitting their growth at subzero temperatures (Tojo and Newsham, 2012; Hoshino et
416 al., 1998). Present study contribute also to fungal population in snow and frost flowers at an
417 Arctic site of Barrow, Alaska, USA.

418 Only a small fraction of a microbial community, especially from extreme environments
419 such as the Arctic can be grown under laboratory conditions since many factors such as the
420 composition of the medium that fully supports the basic needs of microorganisms for growth is
421 not known. This notion was further confirmed as cultivable bacteria encompassing 0.1 to 3% of
422 the total bacteria, which was detected by NGS technique. Thus, the identified number of
423 cultivated bacteria and fungi isolate from different snow categories and frost flowers does not
424 reflect the actual number of microbial and should be considered as the lower limit, and therefore
425 more metagenomic analysis such as NGS which was deployed in this study, is essential to
426 decipher the complex pool of microorganisms in the Arctic. The cultivable bacteria might be
427 representative of the active fraction of cultivable bacterial snow communities (Ellis et al., 2003;

428 Frette et al., 2004), as was detected for bacteria living in different environmental samples such as
429 soil, and marine samples (Pinhassi et al., 1997; Rehnstam et al., 1993). Table 2 contains the
430 identified cultivable bacteria found in each category of Arctic samples (snow and frost flowers).
431 Figures 3A (bacteria) and 3B (fungi) show the variability in numbers of colony-forming units
432 (CFU) within and between the different sample types using two different media (R2A and TSA)
433 for bacteria and (SDA and mycological) for fungi. The average number of viable bacteria was
434 higher than the number of fungi in Arctic samples. Overall, a higher number of CFU was
435 observed in the R2A medium with a more limited nutrient content than TSA, wherein most
436 aerobic bacteria are able to grow. In the R2A plates, the highest number of bacteria was observed
437 in frost flowers (FF) and accumulated snow (AS) with 325 and 314 CFU mL^{-1}, respectively (Fig.
438 3A). However, the highest number of fungi grown in the mycological plate was observed in
439 windpack (WP) and blowing snow (BS) with 5 CFU mL^{-1} (Fig. 3B).

440 Both NGS and culture method analysis revealed a very high number of bacteria in frost
441 flowers as compared to the other snow types that we tested. In recent years, special attention has
442 been focused on the role of frost flowers as a contributing factor to changing the chemistry of the
443 atmosphere in the Arctic. Frost flowers are: i) an important source of sea-salt aerosol (Rankin et
444 al., 2002; Perovich and Richter-Menge, 1994; Martin et al., 1995), ii) a contributing factor in
445 releasing the ozone-depleting molecule, bromine monoxide (BrO), as was detected by satellite
446 (Kaleschke et al., 2004), and iii) as a source of sea ice bacteria (Collins et al., 2010). Moreover,
447 with their physical structure and chemical composition, frost flowers might provide a habitat for
448 microbiological bodies such as bacteria, as well as protective and favorable conditions for
449 metabolic and photochemical reactions (Bowman and Deming, 2010). The observed simple
450 organic compounds and increased concentrations of both formaldehyde (Barret et al., 2009),

451 hydrogen peroxide (Beine and Anastasio, 2009) within frost flower, may suggest that selected
452 bacterial strains can act as a substrate for the photolytic production of oxidants (Bowman and
453 Deming, 2010), and simple organic compounds (Ariya et al., 2002). The regular release
454 mechanism of bacteria through frost flower, such as those with high ice nucleation activity, into
455 the atmosphere, with potential transportation, may provide an additional impact on bioaerosol
456 lower tropospheric mixing ratios (Jayaweera and Flanagan, 1982).

457 As opposed to frost flowers, accumulated snow is characterized by several layers of
458 snowfall, which may have experienced repeated freeze-thaw cycles, and solar irradiation
459 exposure. Analysis by cultural method showed that the highest number of bacteria is present in
460 accumulated snow samples. Each fresh snowfall adds new nutrients and microorganisms to the
461 old pool of accumulated snow. With the detection of more than 100 organic species in the
462 aerosols at Alert in the Canadian high Arctic (February-June) (Fu et al., 2008), the snow layers
463 could be further enriched with nutrients by the air/snow exchange (Xie et al., 2007; Cincinelli et
464 al., 2005). Over time, bacterial populations in accumulated snow may increase by their
465 sustainability and slow growth at very low temperature (-2 °C to -35 °C) (Junge et al., 2004;
466 Gilichinsky et al., 1995; Panikov and Sizova, 2007; Bakermans et al., 2003).

467 Different types of Arctic snow and frost flower samples were tested for IN activities
468 using obtained cultured bacterial colonies as well as whole melted Arctic samples. Ultrapure
469 Milli-Q water (18 ohms resistance), tap water, and *P. syringae* mixed in ultrapure water were
470 used as controls. Tap water contains organic impurities that allow ice to nucleate at warmer
471 temperatures than ultrapure water. The individual average freezing temperature of bacterial
472 isolates from different types of Arctic samples is shown in Figure 4A. To explore the impact of
473 undetected biological and chemical contents of snow samples on IN activity, the IN activity of

474 each melted Arctic sample was directly measured and its IN activity was compared with the
475 corresponding freezing temperature of the average sum of the total individual isolated bacterial
476 colonies (Fig. 4B). The freezing temperatures of the average sum of the total individual (ASTI)
477 isolated bacterial colonies fall at intermediate values between sterile ultrapure water (-24.3 ± 1.2
478 °C) and tap water (-15.3 ± 0.9 °C). The highest and lowest ice nucleation activity of bacteria was
479 observed in fresh snow (ASTI: -15.7 ± 5.6 °C) and frost flowers (ASTI: -20.3 ± 1.5 °C)
480 respectively. The ice nucleation activity of fresh snow was comparable to tap water (-15.3 ± 0.9
481 °C) (Fig. 4B).

482 Many of the bacterial isolates in different categories of Arctic samples showed a
483 moderate IN activity at -15.9 ± 0.4 °C and -17.2 ± 0.8 °C in windpack (WP), -15.2 ± 1 °C and -
484 16.1 ± 1.4 °C in blowing snow (BS), -15.2 ± 0.6 °C, -12.9 ± 0.2 °C, and -17.3 ± 0.3 °C in
485 accumulated snow (AS), and -14.0 ± 0.4 °C , -13.7 ± 0.2 °C, and -6.8 ± 0.2 °C in fresh snow
486 (FS) (Fig. 4A). Interestingly, bacteria with a type 2 ice nucleation ability at -6.8 ± 0.2 °C was
487 isolated in fresh snow. This bacterium was identified with 96% similarity to the *Bacillus* species
488 (Table 3).

489 Among tested bacterial colonies, fresh snow showed the highest variation in ice
490 nucleation activities; higher variation was observed for accumulated snow as compared to frost
491 flowers. Different factors such as nutrient limitation and low temperature observed in the Arctic
492 might have further shifted the ice nucleation activity of bacteria to the higher temperature
493 (Nemecek-Marshall et al., 1993). Frost flower, a bridge between sea ice and the atmosphere and
494 linking biogenic to non-biogenic materials, had the lowest average ice nucleation activity. This
495 may be related to its salinity and ability to accumulate different chemicals, but further studies are
496 required to provide insight on the physical and chemical processes in frost flowers.

497 The observed range of IN activity in melted snow and frost flower samples was between -
498 9.5 ± 1.0 °C (FS) and -18.4 ± 0.1 °C (FF) (Fig. 4B). Interestingly, all the melted Arctic snow
499 samples and frost flowers showed ice nucleation activity at the range very close to the lowest
500 recorded ice nucleation activity of individual cultivable bacterial colonies. This observation may
501 indicate an additional role of other non-cultivable microbial and components in Arctic snow
502 samples and frost flowers which are important in increasing the ice nucleation temperature.
503 Culture-dependent methods selectively isolate a plate-growth-adapted subpopulation from the
504 microbial communities which may represent the majority of the total bacterial numbers in
505 samples (Pinhassi et al., 1997; Rehnstam et al., 1993), but not necessarily the total richness
506 (number of different species) of the bacterial population (Amann et al., 1995; Onstott et al.,
507 1998).

508 Table 3 enlists the identified cultivable bacteria with their ice nucleation activity in each
509 category of Arctic samples (snow and frost flowers). Isolated bacteria were identified belonging
510 to different genus, such as: *Afipia genosp*, *Bacillus*, *Paenibacillus*, *Microbacterium*, and *Kocuria*.
511 The highest IN activity corresponded to genus: *Bacillus* and *Paenibacillus* with -6.8 ± 0.2 °C and
512 -15.2 ± 1 °C respectively (Table 3). At the 16S rDNA gene level, they exhibited about 96% and
513 95% similarity to reported species of *Bacillus sp.*, *Bacillus megaterium*, *Bacillus flexus*, and
514 *Bacillus aryabhattai*; and *Paenibacillus amylolyticus*, *Paenibacillus xylanexedens*, and
515 *Paenibacillus tylopili* respectively.

516 The elemental composition of Arctic samples was determined using HR-TEM with EDS.
517 As shown in Figure 1B, the presence of elements such as Mg, Al, Cl, Ca, and U was detected.
518 The presence of Si and Al might be an indication of soil or through deposit of the dust
519 transported from soil (Sposito, 2008; Shridhar et al., 2010). The source of calcium might be

520 related to either marine or soil origins. Since phosphorous is the limiting macronutrient in marine
521 ecosystems (Toggweiler, 1999; Tyrrell, 1999), the positive observation of this element in frost
522 flower encourages further research in the role of frost flowers in Arctic ecosystem.

523 It is noteworthy that although we focused on biomolecules materials, as we can see from
524 HR-TEM/EDS analysis, the detection of inorganic matter in Arctic snow and frost flowers can
525 contribute to ice nucleation. Hence, to evaluate the snow freezing properties, the complex
526 chemical and bio-chemical pool of molecules and particles should be considered.

527

528 **4 Conclusions**

529 We herein examined the identity, population and ice nucleation ability of the microbial
530 communities of five different snow types and frost flowers during the spring 2009 campaign of
531 the Ocean-Atmosphere-Sea Ice-Snowpack (OASIS) program in Barrow, Alaska, USA. We used
532 the next-generation sequencing (NGS) technique to examine the true bacterial communities in
533 snow and frost flowers, in addition to conventional culture techniques. We gained further insight
534 into the wide range of taxa available in different types of snow and frost flowers. Arctic samples
535 and reference urban snow represented 11-18 known phyla or candidate divisions. The majority of
536 sequences (12.3 - 83.1%) belonged to one of the five major phyla: Proteobacteria,
537 Actinobacteria, Bacteroidetes, Firmicutes, and Cyanobacteria. At the genus level, 101-245
538 different genera were detected. A largely diverse community of bacteria exists in the Arctic with
539 many originating from remote ecological environments such as dust storms. This study revealed
540 that snow and frost flowers are rich media for the existence of microbial compounds. Biological
541 materials have been shown to act as reactive sites for (photochemical) reactions, and thus further
542 studies are required to decipher the complexity of the snow and frost flowers as a zone of

543 chemical pool. It is conceivable that changes on the ratio of antifreeze bacteria to ice nucleation
544 bacteria may have an impact on the melting and freezing processes of snowpack or frost flowers.
545 It is thus feasible that this shift in bacterial population could ultimately affect the snow melting-
546 freezing processes. Further studies are required to evaluate whether change of nucleation patterns
547 due to biological entities are indeed linked to climate change.
548

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Table Captions:

Table 1. Relative abundance of origin and physical properties of analyzed NGS bacteria. Bacteria in Arctic samples: blowing snow (BS), surface hoar snow (SH), windpack snow (WP), frost flowers (FF); and urban snow (US) were analyzed for their origin, and ice nucleation/melting properties as detected by Roche 454 GS-FLX Titanium; **A:** total bacteria in Arctic samples: i) previously observed in Asian/African (dust); ii) cold oceanic water, iii) with antifreeze (AF), iv) ice nucleation (IN), and v) IN and AF property; **B:** in subtotal of total Arctic bacterial pool originated from Asian/African dust: i) with AF, or ii) IN property.

Table 2. Identification of viable cultivable bacteria in Arctic samples. Some of the bacterial colonies were identified in each snow categories: accumulated snow (AS), blowing snow (BS), fresh snow (FS), windpack (WP), and frost flowers (FF); accession number, the nearest neighbor found in the data base, a unique identifier given to a DNA sequence; identified species; and % similarity, the ratio of identical query bases to known bases in the database.

Table 3. Ice nucleation of identified viable cultivable bacteria in Arctic sample. Ice nucleation temperature of identified bacterial isolates in each snow categories: blowing snow (BS), fresh snow (FS), windpack (WP), and frost flowers (FF); accession number, a unique identifier given to a DNA sequence; identified species; and % similarity, the ratio of identical query bases to known bases in the database.

<u>Figure Legends:</u>

Figure 1. Analysis of snow-associated microorganisms by transmission electron microscopy (TEM) in conjunction with energy-dispersive X-ray spectroscopy (EDS). Microorganisms **(A)** and chemicals **(B)** were detected in selected Arctic samples: blowing snow, BS; surface hoar snow, SH; windpack, WP, and frost flowers, FF.

Figure 2. Bacterial community composition in Arctic samples and urban snow at genera level as detected by Roche 454 GS-FLX Titanium. **A**: distribution of bacterial genus observed at greater than 1%. **B**: number of occurrence for each percentage observed for any genus in total bacteria.

Figure 3. Concentration of viable cultivable bacteria and fungi in Arctic samples. Mean number of colony-forming units (CFU) ml^{-1} of snow on two media for: **(A)** bacteria (TSA and R2A) and **(B)** fungi (SDA and mycological agar) in each Arctic snow categories: accumulated snow (AS), blowing snow (BS), fresh snow (FS), surface hoar snow (SH), windpack (WP), and frost flowers (FF). Samples from several urban sites collected in Quebec, Canada: Pierrefonds (suburb of Montreal; "PFDS"), and Mont-Tremblant (Laurentians; "Tremblant") are included for comparison. Error bars indicate standard deviation (SD) of the mean for three experiments.

934 **Figure 4**. Ice nucleation activity of viable cultivable bacteria in Arctic samples. The average
935 ice nucleation temperature of: **(A)** individual bacterial isolates and **(B)** total
936 bacterial isolates and melted Arctic samples. Arctic snow categories: accumulated
937 snow (AS), blowing snow (BS), fresh snow (FS), surface hoar snow (SH),
938 windpack (WP), and frost flowers (FF). Controls: ultrapure water (Milli-Q-water),
939 tap water, and a suspension of laboratory-grown *Pseudomonas syringae* (*P.*
940 *syringae*).
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Table 1. Relative abundance of origin and physical properties of analyzed NGS bacteria. Bacteria in Arctic samples: blowing snow (BS), surface hoar snow (SH), windpack snow (WP), frost flowers (FF); and urban snow (US) were analyzed for their origin, and ice nucleation/melting properties as detected by Roche 454 GS-FLX Titanium; **A:** total bacteria in Arctic samples: i) previously observed in Asian/African (dust); ii) cold oceanic water, iii) with antifreeze (AF), iv) ice nucleation (IN), and v) IN and AF property; **B:** in subtotal of total Arctic bacterial pool originated from Asian/African dust: i) with AF, or ii) IN property.

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A. **Total bacteria in Arctic Snow Categories**	**Dust**	**Cold Oceanic Water**	**AF**	**IN**	**IN & AF**
US	47%	0%	7%	15%	0.1%
BS	36%	0.01%	0.10%	15%	0.03%
SH	44%	0.04%	0.20%	16%	0.01%
WP	39%	0.01%	1%	16%	0.01%
FF	44%	0.01%	0.10%	14%	0.01%

B. **Subtotal of Arctic bacteria in Asian/African dust origin** **Snow Categories**	**AF**	**IN**
US	*13%*	2%
BS	*1%*	3%
SH	*1%*	3%
WP	*6%*	2%
FF	*0.40%*	0.2%

Table 2. Identification of viable cultivable bacteria in Arctic samples. Some of the bacterial colonies were identified in each snow categories: accumulated snow (AS), blowing snow (BS), fresh snow (FS), windpack (WP), and frost flowers (FF); accession number, the nearest neighbor found in the data base, a unique identifier given to a DNA sequence; identified species; and % similarity, the ratio of identical query bases to known bases in the database.

Snow Category	Bacterial Colony #	Accession #	Species	% Similarity
FF	1	GU975796.1	*Curtobacterium sp.D2.2*	97
		JF798380.1	*Curtobacterium luteum*	97
		FN178369.1	*Curtobacterium citreum*	97
		HM045842.1	*Bacillus sp. WJ18*	97
	2	EU196527.1	*Paracoccus sp. B10*	97
		DQ195864.1	*Rhodobacteraceae bacterium*	97
	3	HQ425309.1	*Kocuria sp. M1-36*	98
		FR682683.1	*Kocuria rhizophila*	98
	4	JN084144.1	*Curtobacterium oceanosedimentum*	93
		EF592577.1	*Flavobacterium oceanosedimentum*	93
		EU373393.1	*Bacillus subtilis*	93
FS	1	JN208198.1	*Bacillus sp. DG7*	96
		AB648987.1	*Bacillus megaterium*	96
		JN092792.1	*Bacillus flexus*	96
		HQ857752.1	*Bacillus aryabhattai*	96
	2	JN085952.1	*Microbacterium sp. ZL2*	98
		JF700471.1	*Microbacterium hydrocarbonoxydans*	98
		HQ113206.1	*Microbacterium oxydans*	98
AS	1	EU379295.1	*Micrococcus luteus*	92
	2	HE578790.1	*Micrococcus luteus*	95
		HM209728.1	*Micrococcus yunnanensis*	95
	3	EU584512.1	*Frigoribacterium sp. Everest-gws-26*	97
		AY599739.1	*Actinobacterium TB3-4-I*	97
	4	JF969180.1	*Bacterium REGD8*	95
		JF778689.1	*Sporosarcina sp. DRB20*	94
WP	1	JF728909.1	*Leifsonia sp. DAB_MOR27*	96
		DQ172984.2	*Bacterium TSBY-9*	96
		NR_042669.1	*Leifsonia kafniensis*	96
		NR_041548.1	*Microterricola viridarii*	96

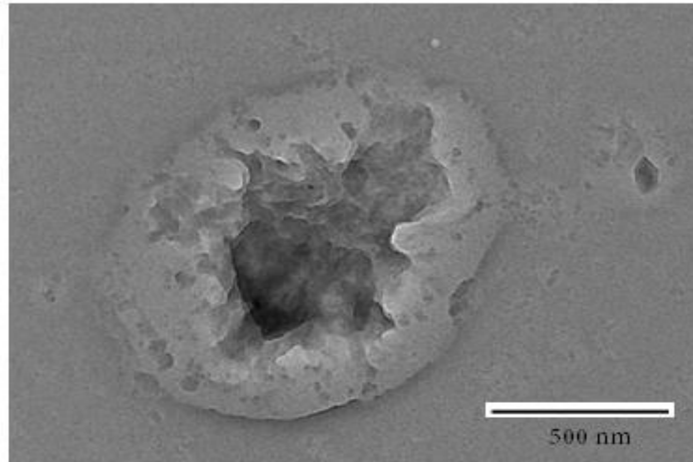
BS	1	EF540454.1	*Brevundimonas sp. d1M*	96
		JN020187.1	*Uncultured alpha proteobacterium clone cher4_1B_11*	96
		NR_037106.1	*Brevundimonas variabilis*	96
	2	FR691407.1	*Brevundimonas sp. R-36741*	96
		AB452982.1	*Alpha proteobacterium HIBAF003*	96
	3	JF778709.1	*Paenisporosarcina macmurdoensis*	99
		JF778708.1	*Sporosarcina sp. GRT2*	99
	4	JF969180.1	*Bacterium REGD8*	98
		HM224487.1	*Sporosarcina sp. TPD39*	98
		JF778709.1	*Paenisporosarcina macmurdoensis*	98
	5	JN082256.1	*Bacillus sp. cf30*	97
		JN092792.1	*Bacillus flexus*	97
		HQ143640.1	*Geobacillus stearothermophilus*	97
	6	FJ487574.1	*Paenibacillus amylolyticus*	95
		NR_044524.1	*Paenibacillus xylanexedens*	95
		HQ202814.1	*Paenibacillus tylopili*	95

Table 3. Ice nucleation of identified viable cultivable bacteria in Arctic sample. Ice nucleation temperature of identified bacterial isolates in each snow categories: blowing snow (BS), fresh snow (FS), windpack (WP), and frost flowers (FF); accession number, a unique identifier given to a DNA sequence; identified species; and % similarity, the ratio of identical query bases to known bases in the database.

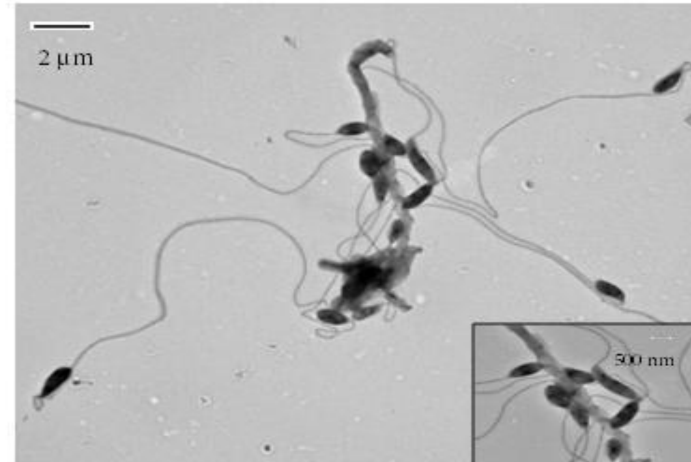
Snow Category	Bacterial Colony	Ice Nucleation Temp. (°C)	Accession #	Species	% Similarity
WP	A	- 18.9 ± 2.5	U87778.1	*Afipia genosp*	86
			JF799916.1	*Bradyrhizobium*	87
			FR691406.1	*Bosea*	87
BS	A	- 18.9 ± 1.6	JN082256.1	*Bacillus sp.*	97
			JN092792.1	*Bacillus flexus*	97
			HQ143640.1	*Geobacillus stearothermophilus*	97
	B	- 15.2 ± 1	JF343205.1	*Paenibacillus amylolyticus*	95
			NR_044524.1	*Paenibacillus xylanexedens*	95
			HQ202814.1	*Paenibacillus tylopili*	95
FS	A	- 6.8 ± 0.2	JN208198.1	*Bacillus sp.*	96
			AB648987.1	*Bacillus megaterium*	96
			JN092792.1	*Bacillus flexus*	96
			HQ857752.1	*Bacillus aryabhattai*	96
	B	- 21.6 ± 1	JN085952.1	*Microbacterium sp.*	98
			JF700471.1	*Microbacterium hydrocarbonoxydans*	98
			HQ113206.1	*Microbacterium oxydans*	98
FF	A	- 20.0 ± 1.5	HQ425309.1	*Kocuria sp.*	98
			FR682683.1	*Kocuria rhizophila*	98

(A)

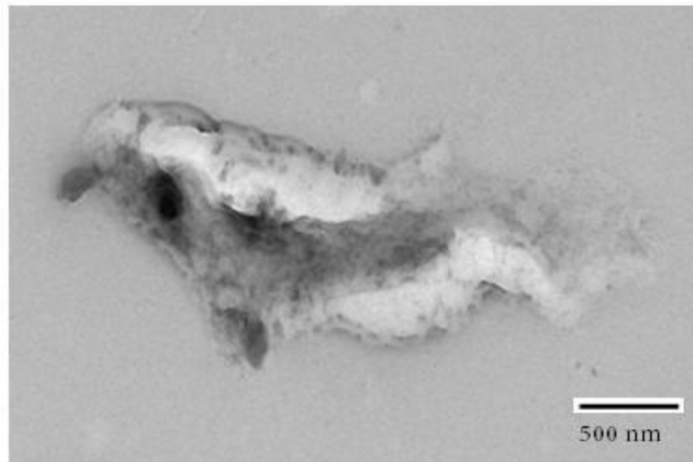
Blowing Snow (BS)



Snow Hoar (SH)



WindPack (WP)



Frost Flowers (FS)

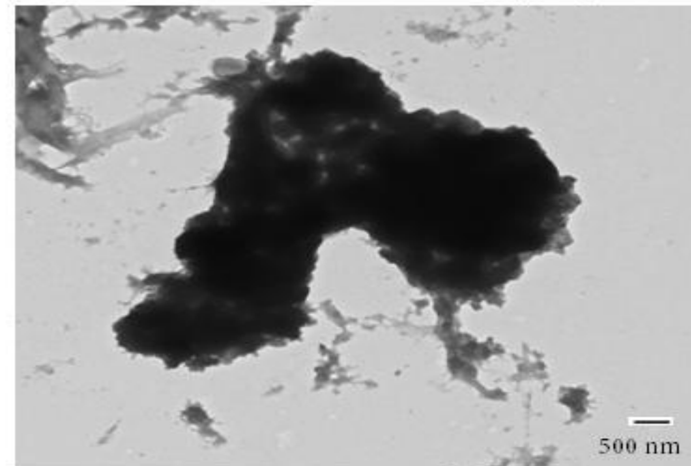


Figure 1A.

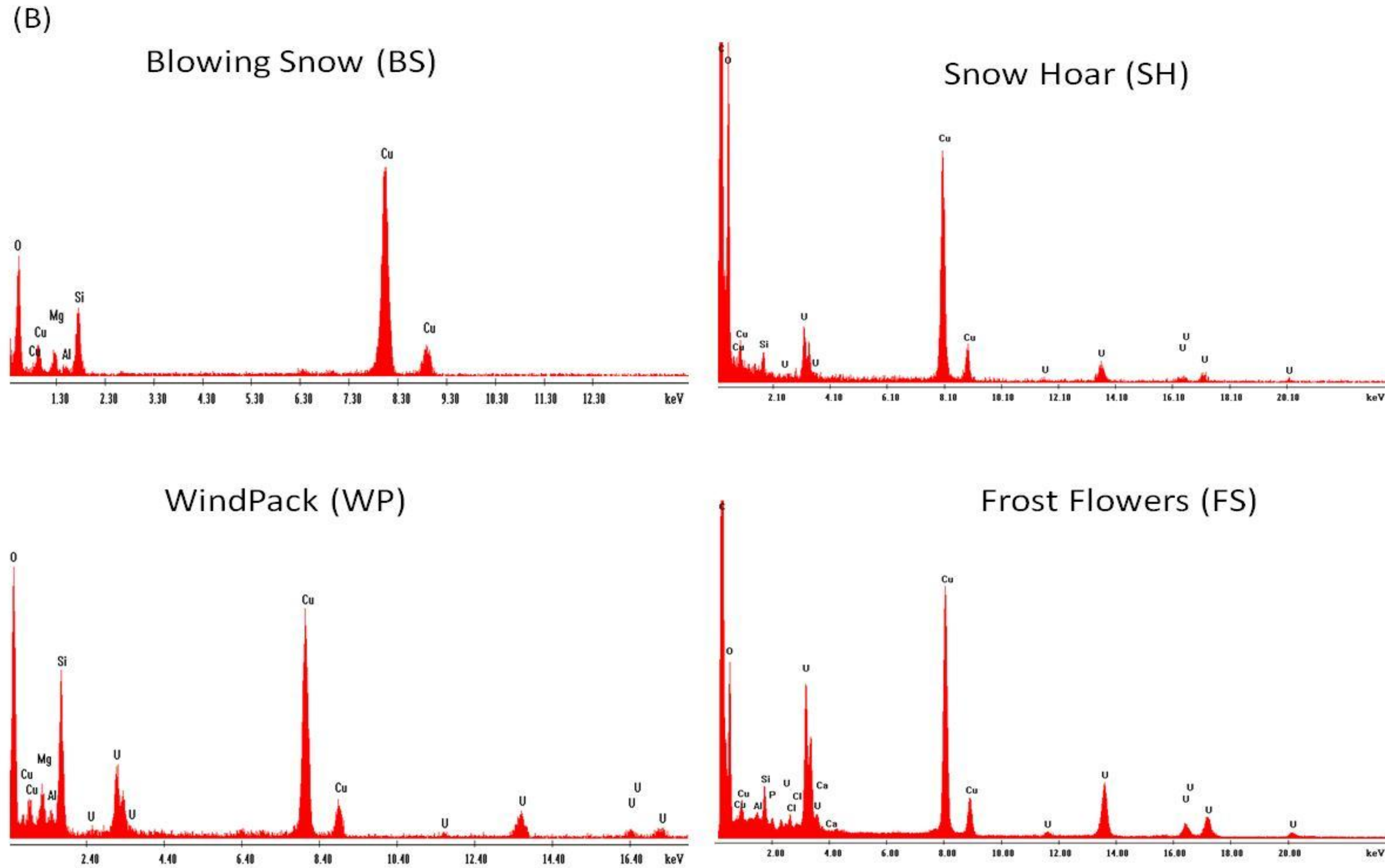
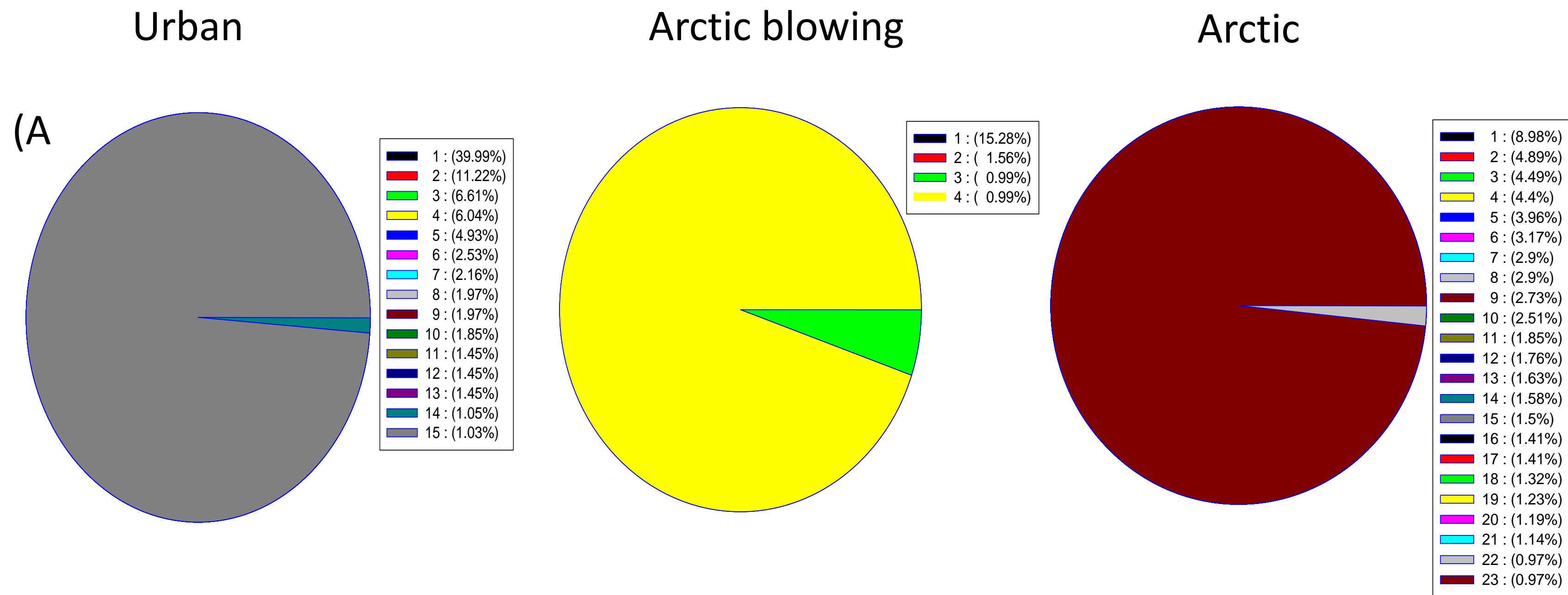
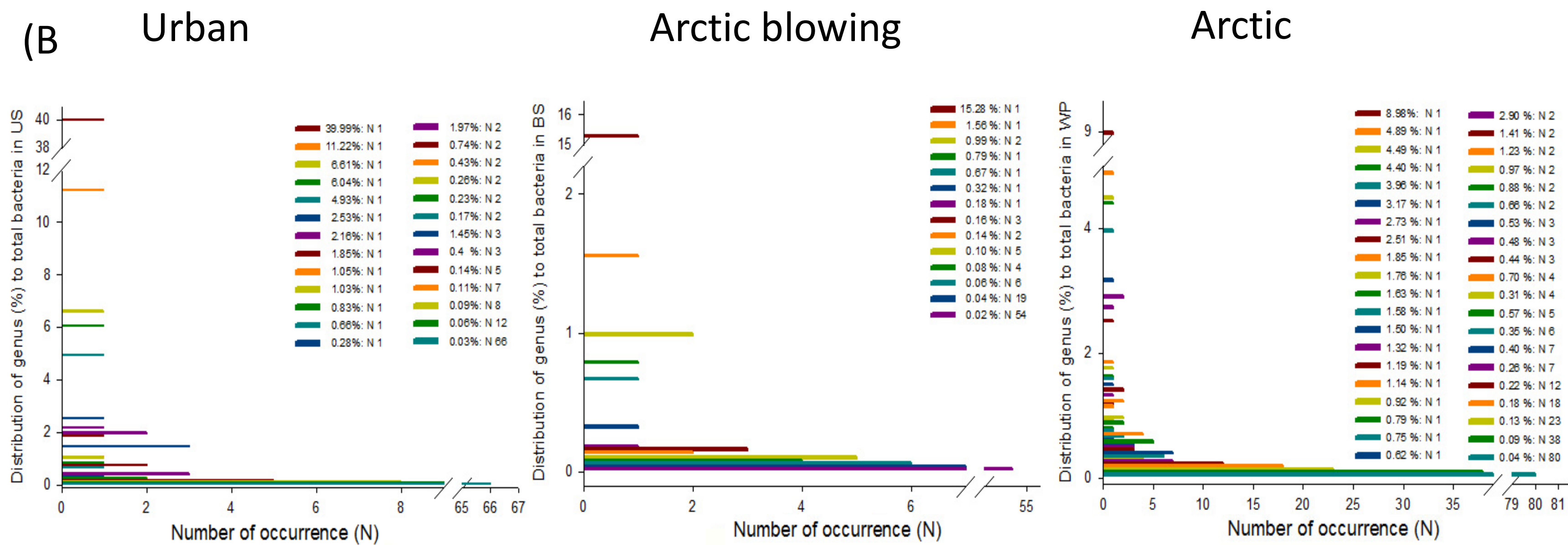


Figure 1B

Figure 1 Analysis of snow-associated microorganisms by transmission electron microscopy (TEM) in conjunction with energy-dispersive X-ray spectroscopy (EDS). Microorganisms **(A)** and chemicals **(B)** were detected in selected Arctic samples: blowing snow, BS; surface hoar snow, SH; windpack, WP, and frost flowers, FF.

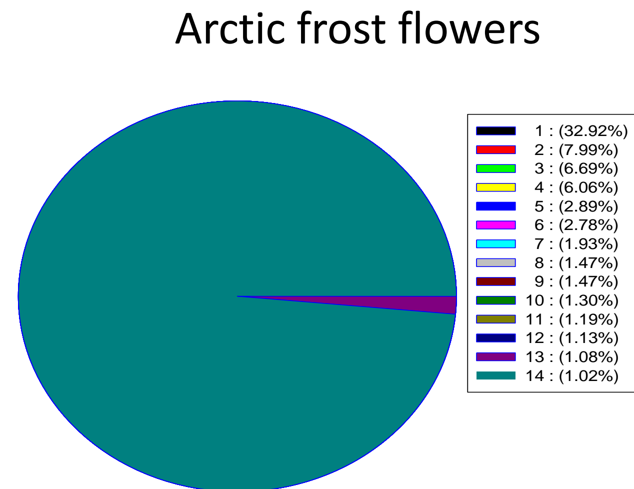


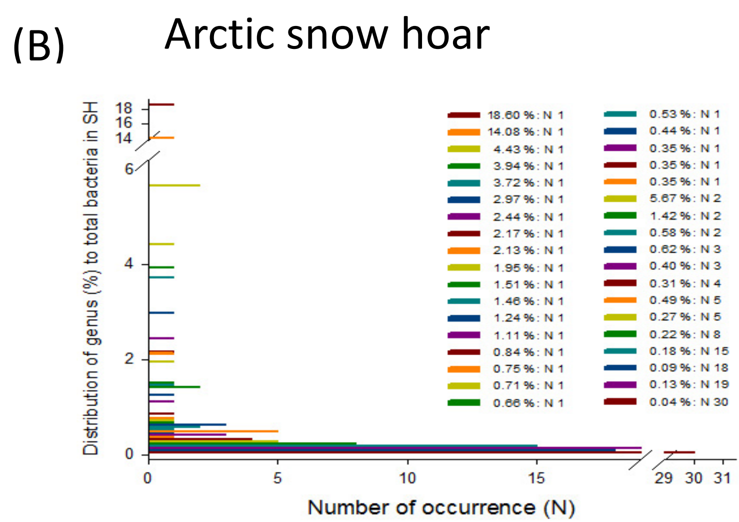
Urban
Arctic blowing
Arctic
(A
1 : (39.99%)
2 : (11.22%)
3 : (6.61%)
4 : (6.04%)
5 : (4.93%)
6 : (2.53%)
7 : (2.16%)
8 : (1.97%)
9 : (1.97%)
10 : (1.85%)
11 : (1.45%)
12 : (1.45%)
13 : (1.45%)
14 : (1.05%)
15 : (1.03%)
1 : (15.28%)
2 : (1.56%)
3 : (0.99%)
4 : (0.99%)
1 : (8.98%)
2 : (4.89%)
3 : (4.49%)
4 : (4.4%)
5 : (3.96%)
6 : (3.17%)
7 : (2.9%)
8 : (2.9%)
9 : (2.73%)
10 : (2.51%)
11 : (1.85%)
12 : (1.76%)
13 : (1.63%)
14 : (1.58%)
15 : (1.5%)
16 : (1.41%)
17 : (1.41%)
18 : (1.32%)
19 : (1.23%)
20 : (1.19%)
21 : (1.14%)
22 : (0.97%)
23 : (0.97%)



(B
Urban
Arctic blowing
Arctic
Distribution of genus (%) to total bacteria in US
Distribution of genus (%) to total bacteria in BS
Distribution of genus (%) to total bacteria in WP
Number of occurrence (N)
Number of occurrence (N)
Number of occurrence (N)

Figure 2. Bacterial community composition in Arctic samples and urban snow at genera level as detected by Roche 454 GS-FLX Titanium. **A**: Distribution of bacterial genus observed at greater than 1%. **B**: Number of occurrence for each percentage observed for any genus in total bacteria. (The name of corresponding genera for each percentage (>1%) is listed in the Appendix (A1) Table A.2).





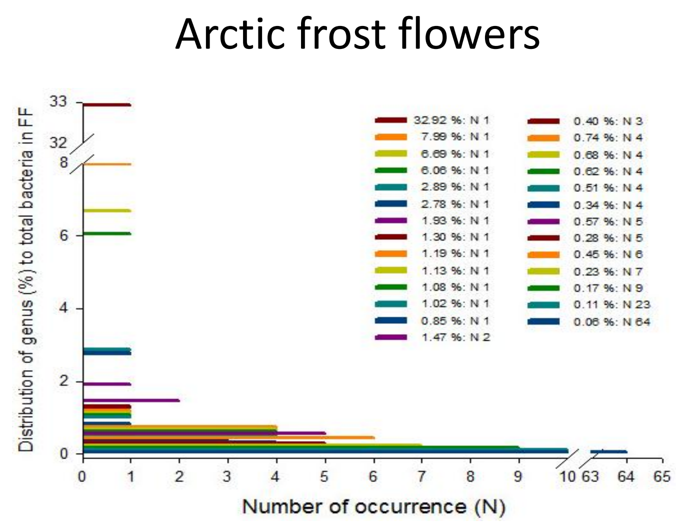


Figure 3

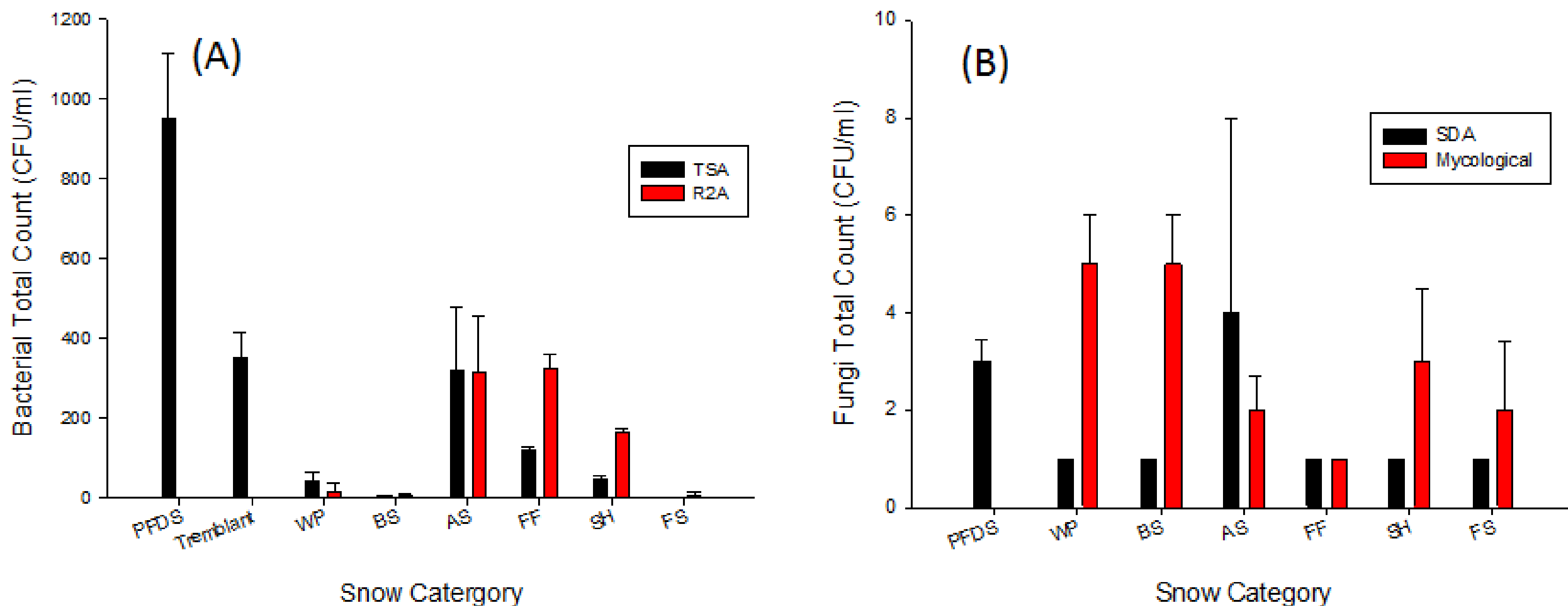


Figure 3. Concentration of viable cultivable bacteria and fungi in Arctic samples. Mean number of colony-forming units (CFU) ml^{-1} of snow on two media for: **(A)** bacteria (TSA and R2A) and **(B)** fungi (SDA and mycological agar) in each Arctic snow categories: accumulated snow (AS), blowing snow (BS), fresh snow (FS), surface hoar snow (SH), windpack (WP), and frost flowers (FF). Samples from several urban sites collected in Quebec, Canada: Pierrefonds (suburb of Montreal; "PFDS"), and Mont-Tremblant (Laurentians; "Tremblant") are included for comparison. Error bars indicate standard deviation (SD) of the mean for three experiments.

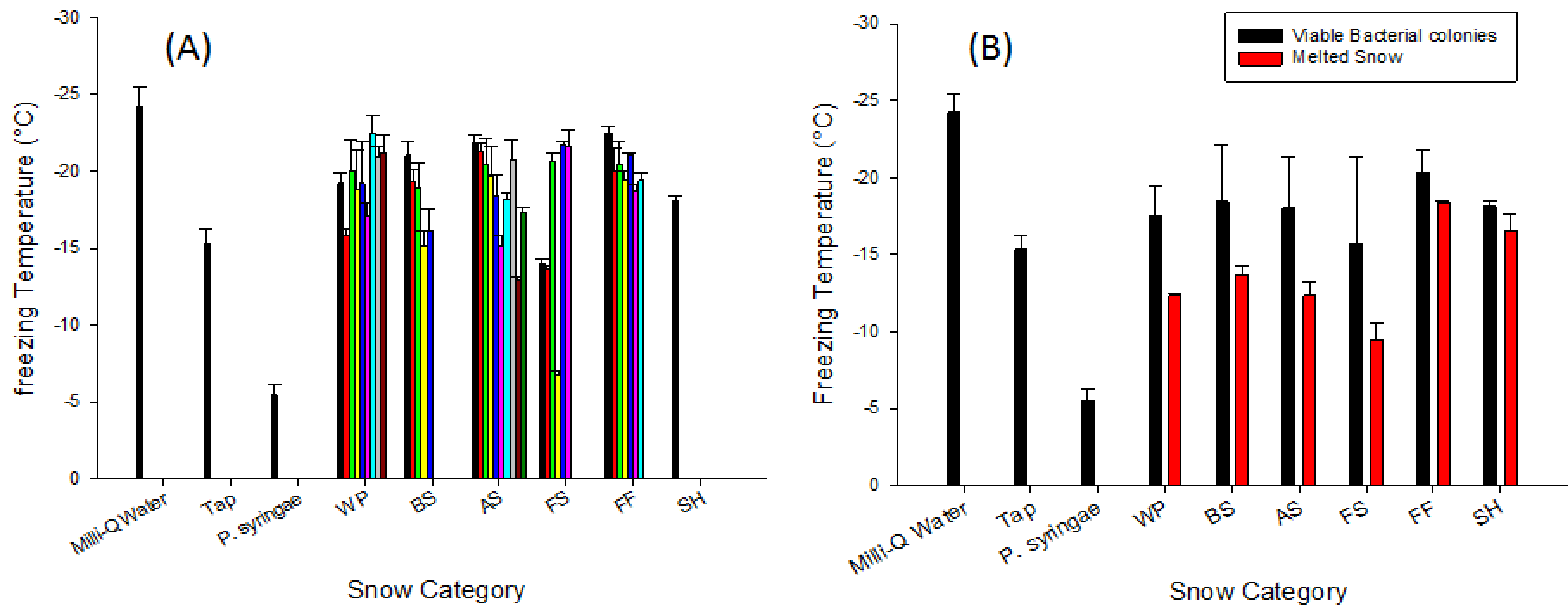


Figure 4. Ice nucleation activity of viable cultivable bacteria in Arctic samples. The average ice nucleation temperature of: **(A)** individual bacterial isolates and **(B)** total bacterial isolates and melted Arctic samples. Arctic snow categories: accumulated snow (AS), blowing snow (BS), fresh snow (FS), surface hoar snow (SH), windpack (WP), and frost flowers (FF). Controls: ultrapure water (Milli-Q-water), tap water, and a suspension of laboratory-grown *Pseudomonas syringae* (*P. syringae*). Error bars indicate standard deviation (SD) of the mean for three experiments.

APPENDIX (A1):

Table A.1. Relative abundance of bacterial taxa detected by Roche 454 GS-FLX Titanium using 16S rDNA gene. Detected phyla **(A)** and the top four genera with the highest percentage **(B)** in each Arctic sample: blowing snow (BS), surface hoar snow (SH), windpack snow (WP), frost flowers (FF); and urban snow (US). The number in parenthesis shows the sequence percentage obtained from the overall bacterial pool.

Table A.2 Genus distribution (>1%) of Bacterial comminity in Arctic samples/urban snow by NGS. Windpack snow (WP), surface hoar snow (SH), urban snow (US), blowing snow (BS), & frost flowers (FF).

Figure A.1. Bacterial diversity by 454 NGS analysis. The diversity of the bacterial communities for Arctic samples: blowing snow (BS), surface hoar snow (SH), windpack snow (WP), frost flowers (FF); and urban snow (US) was estimated using the Inverse Simpson Diversity Index **(A)**, species richness using the nonparametric Chao index **(B)**, and Rarefaction Metric (number of OTUs) **(C)** with 3% cut-off value in sequence differences for OTU.

Table A.1 Relative abundance of bacterial taxa detected by Roche 454 GS-FLX Titanium using 16S rDNA gene.Detected phyla **(A)** and the top four genera with the highest percentage **(B)** in each Arctic sample: blowing snow (BS), surface hoar snow (SH), windpack snow (WP), frost flowers (FF); and urban snow (US). The number in parenthesis shows the sequence percentage obtained from the overall bacterial

(A) Phylum				
US	**BS**	**SH**	**WP**	**FF**
Proteobacteria (49%)	Proteobacteria (83.1%)	Proteobacteria (67%)	Proteobacteria (60.5%)	Proteobacteria (50.2%)
Bacteroidetes (47.5%)	Firmicutes (5.9%)	Firmicutes (13.6%)	Cyanobacteria (12.3%)	Actinobacteria (32.7%)
Actinobacteria (2.5%)	Actinobacteria (5.1%)	Actinobacteria (8.8%)	Bacteroidetes (8.5%)	Firmicutes (7.8%)
Cyanobacteria (0.3%)	Cyanobacteria (2%)	Cyanobacteria (3.1%)	Planctomycetes (4.6%)	Bacteroidetes (2.9%)
Candidate_division_TM7 (0.2%)	Bacteroidetes (1.8%)	Bacteroidetes (2.2%)	Actinobacteria (4.3%)	Verrucomicrobia (2.2%)
Firmicutes (0.2%)	Acidobacteria (0.6%)	Verrucomicrobia (1.8%)	Firmicutes (3.4%)	Planctomycetes (1.9%)
Candidate_division_OP10 (0.1%)	Planctomycetes (0.3%)	Candidate_division_OD1 (1.3%)	Verrucomicrobia (2.6%)	Cyanobacteria (0.8%)
Acidobacteria (0.08%)	SM2F11 (0.3%)	Candidate_division_TM7 (0.8%)	Gemmatimonadetes (1.6%)	Deferribacteres (0.4%)
Planctomycetes (0.08%)	Verrucomicrobia (0.2%)	Planctomycetes (0.4%)	Chloroflexi (0.9%)	Candidate_division _OD1 (0.3%)
Chloroflexi (0.03%)	Candidate_division_OD1 (0.2%)	SM2F11 (0.3%)	Acidobacteria (0.6%)	SM2F11 (0.2%)
Verrucomicrobia (0.03%)	Gemmatimonadetes (0.2%)	Fusobacteria (0.2%)	Candidate_division_TM7 (0.2%)	BD1-5 (0.1%)
	WCHB1-60 (0.2%)	Synergistetes (0.2%)	Chlorobi (0.2%)	Candidate_division _OP3 (0.05%)
	Candidate_division_TM7 (0.1%)	Candidate_division_OP11 (0.1%)	Candidate_division_OD1 (0.1%)	Candidate_division _TM7 (0.05%)
	Chloroflexi (0.1%)	Acidobacteria (0.04%)	Candidate_division_OP11 (0.07%)	Chloroflexi (0.05%)
	Deferribacteres (0.1%)	Candidate_division_OP10 (0.04%)	Fibrobacteres (0.07%)	Fusobacteria (0.05%)
		Lentisphaerae (0.04%)	SM2F11 (0.07%)	Gemmatimonadete

				s (0.05%)
			Candidate_division_OP10 (0.04%)	Lentisphaerae (0.05%)
			Deinococcus-Thermus (0.04%)	Spirochaetes (0.05%)

(B) Genus				
US	**BS**	**SH**	**WP**	**FF**
Flavobacterium (40%)	*Methylobacterium* (15.3%)	*Roseateles* (18.6%)	*Methylobacterium* (9%)	*Propionibacterineae* (32.9%)
Polaromonas (11.2%)	*Bradyrhizobium* (1.6%)	*Methylobacterium* (14.1%)	*Sphingomonas* (4.9%)	*Roseateles* (8%)
Variovorax (6.6%)	*Bacillus* (1%)	*Bacillus* (5.7%)	*Lamprocystis* (4.5%)	*Staphylococcus* (6.7%)
Sandarakinorhabdus (6%)	*Sphingomonas* (1%)	*Streptococcus* (5.7%)	*Roseateles* (4.4%)	*Candidatus_Pelagibacter* (6.1%)

Table A.2 Genus distribution (>1%) of Bacterial comminity in Arctic samples/urban snow by NGS. Windpack snow (WP), surface hoar snow (SH), urban snow (US), blowing snow (BS), & frost flowers (FF).

Genus										
Number of Occurrence (N)	WP	%	SH	%	US	%	BS	%	FF	%
1	*Methylobacterium*	8.98	*Roseateles*	18.60	*Flavobacterium*	39.99	*Methylobacterium*	15.28	*Propionibacterineae*	32.92
2	*Sphingomonas*	4.89	*Methylobacterium*	14.08	*Polaromonas*	11.22	*Bradyrhizobium*	1.56	*Roseateles*	7.99
3	*Lamprocystis*	4.49	*Bacillus*	5.67	*Variovorax*	6.61	*Bacillus*	0.99	*Staphylococcus*	6.69
4	*Roseateles*	4.40	*Streptococcus*	5.67	*Sandarakinorhabdus*	6.04	*Sphingomonas*	0.99	*Candidatus_Pelagibacter*	6.06
5	*Flavobacterium*	3.96	*Propionibacterineae*	4.43	*Sphingomonas*	4.93			*Nitrospina*	2.89
6	*Bradyrhizobium*	3.17	*Candidatus_Pelagibacter*	3.94	*Brevundimonas*	2.53			*uncultured*	2.78
7	*Albidiferax*	2.90	*Bradyrhizobium*	3.72	*Janthinobacterium*	2.16			*Sphingopyxis*	1.93
8	*Rhodobacter*	2.90	*Azospirillum*	2.97	*Chryseobacterium*	1.97			*Acidovorax*	1.47
9	*uncultured*	2.73	*Micrococcineae*	2.44	*Pedobacter*	1.97			*Brevundimonas*	1.47
10	*Sandarakinorhabdus*	2.51	*Sphingomonas*	2.17	*Micrococcineae*	1.85			*uncultured_Verrucomicrobia _bacterium*	1.30
11	*Rhizobacter*	1.85	*uncultured*	2.13	*Dyadobacter*	1.45			*uncultured*	1.19
12	*Gemmatimonas*	1.76	*uncultured_alpha_ proteobacterium*	1.95	*Herbaspirillum*	1.45			*Streptococcus*	1.13
13	*Gemmata*	1.63	*Corynebacterineae*	1.51	*Pseudomonas*	1.45			*uncultured_alpha_ proteobacterium*	1.08
14	*Staphylococcus*	1.58	*Nitrospina*	1.46	*Epilithonimonas*	1.05			*uncultured_marine _bacterium*	1.02
15	*Micrococcineae*	1.50	*Staphylococcus*	1.42	*Rhizobium*	1.03				
16	*Propionibacterineae*	1.41	*uncultured_Verrucomicrobia _bacterium*	1.42						
17	*Roseomonas*	1.41	*uncultured_marine _bacterium*	1.24						

18	*Ideonella*	1.32	*Roseobacter_clade _DC5-80-3_lineage*	1.11						
19	*uncultured*	1.23								
20	*Isosphaera*	1.19								
21	*Pirellula*	1.14								
22	*Chlorochromatium*	0.97								
23	*Hydrogenophaga*	0.97								

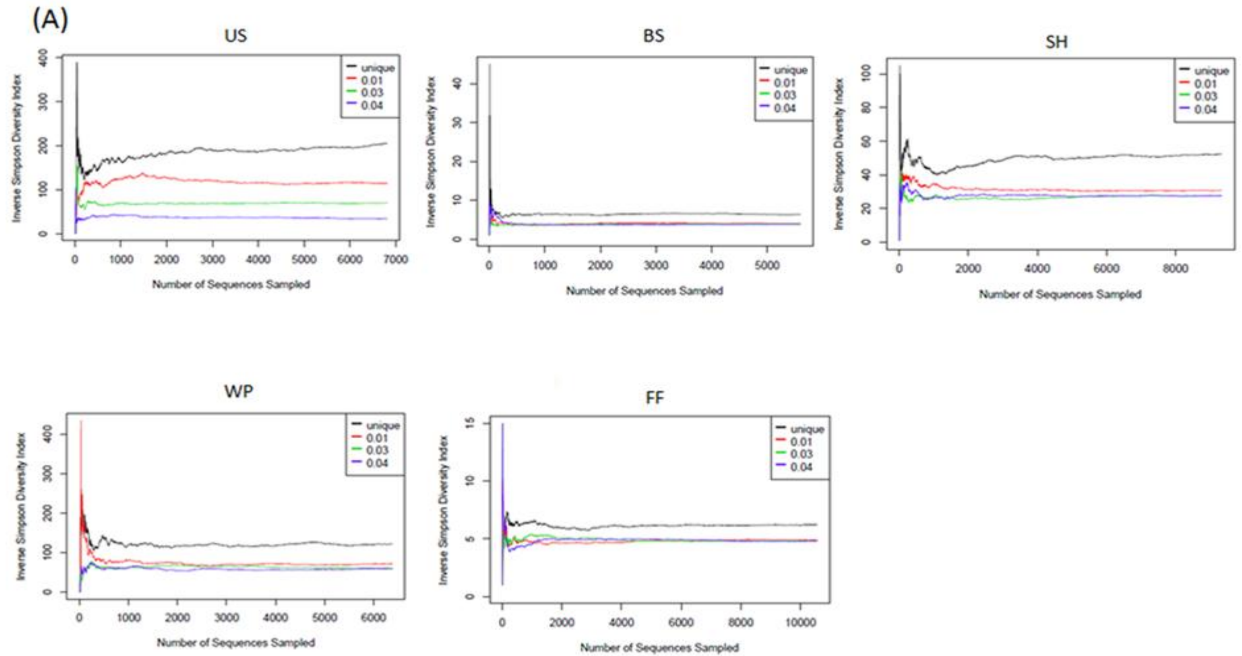


Figure A1. Bacterial diversity by 454 NGS analysis. The diversity of the bacterial communities for Arctic samples: blowing snow (BS), surface hoar snow (SH), windpack snow (WP), frost flowers (FF); and urban snow (US) was estimated using the Inverse Simpson Diversity Index **(A)**, species richness using the nonparametric Chao index **(B)**, and Rarefaction Metric (number of OTUs) **(C)** with 3% cut-off value in sequence differences for out.