Arctic microbial and next-generation sequencing approach for bacteria in snow and frost flowers: selected identification, abundance and freezing nucleation

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Abstract. During the spring of 2009, as part of the Ocean–Atmosphere–Sea Ice–Snowpack (OASIS) campaign in Barrow, Alaska, USA, we examined the identity, population diversity, freezing nucleation ability of the microbial communities of five different snow types and frost flowers. In addition to the culturing and gene-sequence-based identification approach, we utilized a state-of-the-art genomic next-generation sequencing (NGS) technique to examine the diversity of bacterial communities in Arctic samples. Known phyla or candidate divisions were detected (11–18) with the majority of sequences (12.3–83.1 %) belonging to one of the five major phyla: Proteobacteria, Actinobacteria, Bacteroidetes, Firmicutes, and Cyanobacteria. The number of genera detected ranged from, 101–245. The highest number of cultivable bacteria was observed in frost flowers (FFs) and accumulated snow (AS) with 325 ± 35 and 314 ± 142 CFU m L^{-1}, respectively; and for cultivable fungi 5 ± 1 CFU m L^{-1} in windpack (WP) and blowing snow (BS). Morphology/elemental composition and ice-nucleating abilities of the identified taxa were obtained using high resolution electron microscopy with energy-dispersive X-ray spectroscopy and ice nucleation cold-plate, respectively. Freezing point temperatures for bacterial isolates ranged from -20.3 ± 1.5 to -15.7 ± 5.6 °C, and for melted snow samples from -9.5 ± 1.0 to -18.4 ± 0.1 °C. An isolate belonging to the genus *Bacillus* (96 % similarity) had ice nucleation activity of -6.8 ± 0.2 °C. Comparison with Montreal urban snow, revealed that a seemingly diverse community of bacteria exists in the Arctic with some taxa possibly originating from distinct ecological environments. We discuss the potential impact of snow microorganisms in the 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, 2009; Fujii et al., 2010; 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 t

of dust per hour to the Arctic (Rahn et al., 1977). Dust has also been shown to transport microorganisms (S. Zhang et al., 2007, 2008; X. F. Zhang, 2008). 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, 2003, 2007, 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., 2003; Uno et al., 2009). During long distance transportation, air masses may undergo chemical and physical transformation under extreme environmental conditions such as high levels of solar radiation, multiple freeze–thaw cycles, relatively acidic conditions, and predominantly inorganic salts (Jickells, 1999; Ariya et al., 2002, 2009; Cote et al., 2008). Little is known on the effects of the photochemical and aging processes of the chemical and biological composition of dust particles, or whether chemical properties and the genomic structure of microbial entities transported with dust are altered, or mutated during long distance transport (Smith et al., 2010).

Pure water droplets homogeneously freeze in the atmosphere at approximately $-38\,°C$. Entities such as particles including mineral dust, soot, and biological materials (DeMott et al., 2003; Möhler et al., 2007) may serve as ice nuclei (IN) which enhance freezing at much higher temperatures in a process known as heterogeneous nucleation (Pruppacher and Klett, 1997). Depending on the nature of the impurities, heterogeneous nucleation can occur over a wide range of temperatures. Although dust particles are generally assumed to be the most important global effect on ice nucleation, several strains of *Erwinia herbicola* (Lindow, 1978), *Pseudomonas fluorescens* (Maki and Willoughby, 1978), *Pseudomona.s viridflava* (Paulin, 1978), and *Xanthomonas campestris pathovar translucens* (Kim et al., 1987) are recognized amongst the most efficient IN in biological particles. Yet the global effect of the importance of biological ice nuclei is still a subject of debate. Some strains of *Pseudomonas syringae* can initiate water freezing at temperatures as high as $-2\,°C$ (Orser et al., 1985) which have also been detected in clouds and snow (Amato et al., 2005, 2007; Vaïtilingom et al., 2012; Lohmann and Feichter, 2005; Joly et al., 2013). Atmospheric microbial, besides being considered efficient ice nuclei (Constantinidou et al., 1990; Kieft and Ahmadjian, 1989; Möhler et al., 2007; Möhler et al., 2008; Pouleur, 1992; Ariya et al., 2014; Mortazavi et al., 2008) have also been suggested to act as cloud condensation nuclei (Bauer et al., 2003; Möhler et al., 2007).

Both natural (e.g. mineral dust, biogenic nucleators) and anthropogenic (e.g. soot) sources can contribute to precipitation in Arctic regions (Hansson et al., 1993; Hinkley, 1994). There is some evidence for the observed increase in the number of storms in certain areas of the globe which can al-

ter the transport and distribution of chemicals or biological entities (Wang et al., 2011; Zhang et al., 2007; Erel et al., 2006) with potential impacts on precipitation patterns (Sempere and Kawamura, 1994; Satsumabayashi et al., 2001). Although the pivotal role of dust in the atmospheric global circulation (Dunion and Velden, 2004; Wu, 2007), radiative budget (Sokolik and Toon, 1996; Kaufman et al., 2001), air pollution (Prospero, 1999; VanCuren, 2003) and cloud formation (Toon, 2003) has been documented, there is little known about how the newly introduced pool of transported microbial entities by dust to the Arctic impacts the change of the total Arctic microbial pool or affects the freezing and melting processes of snow and ice matrices in this region.

Several studies using standard microbiology techniques have shown that there is a diverse population of bacteria in the snow (Carpenter et al., 2000; Amato et al., 2007; Mortazavi et al., 2008; Amoroso et al., 2010; Moller et al., 2011; Liu et al., 2011; Harding et al., 2011). Recent developments in high-throughput sequencing (HTS) techniques (Loman et al., 2012a, b), such as next-generation sequencing (NGS), also allow for metagenomic investigations of microbial populations in environmental samples. The present study was performed as part of the international Ocean–Atmosphere–Sea Ice–Snowpack (OASIS) campaign (2009) in Barrow, Alaska. Five different types of Arctic snow: (i) accumulated snow, (ii) windpack, (iii) blowing snow, (iv) surface hoar snow, (v) fresh snow and frost flowers were used for this study (Fierz et al., 2009; Glossary of Meteorology, 2009). Frost flowers are dendritic shape clusters of ice crystals that form at the interface between warm ice surface and sufficiently cold atmospheric temperature and humidity (Obbard et al., 2009). The chemistry of frost flowers has garnered increased interest because these salty ice crystals have been shown to act as a source for the following: (i) sea-salt aerosol (Perovich and Richter-Menge, 1994), and (ii) BrO, which contributes to ozone depletion events (Kaleschke et al., 2004). Increased bacterial abundance have also been found in frost flowers (Bowman and Deming, 2010). Yet, further research is still required to better understand the mechanisms of physical, chemical and biological processes involving frost flowers.

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

2 Experimental methods

2.1 Study sites

Five different types of Arctic snow were studied: (i) accumulated snow, (ii) windpack, (iii) blowing snow, (iv) surface hoar snow, (v) fresh snow and frost flowers which were collected from 4–20 March 2009 during the OASIS campaign in Barrow, AK, USA. Detailed snow sampling procedures have been described elsewhere (Kos et al., 2014). Snow samples were collected from a field dedicated to snow research in the clean air sector at $71.31°$ N, $156.6°$ W, 400 m to the southeast of the Barrow Arctic Research Center (BARC), and frost flower samples were collected from sea ice at $71.36°$ N, $156.70°$ W. Vehicle access was restricted to the snow sampling area, and equipment was transported on foot with a hand-pulled sled to limit local pollution. Snow sampling devices were sterile and single-use. A sterile high-density polyethylene (HDPE) spoon (Fisher Scientific, Montreal, Canada) was used to collect the first 3 cm of the surface snow to fill the HDPE (Fisher Scientific, Montreal, Canada) sample containers ($220 \, mL^{-1}$). Similarly, a total of $900 \, mL^{-1}$ of frost flower samples were collected by carefully lifting the frost flower off the surface with a shovel to minimize (but not completely eliminate) brine content. Frost flower sampling was collected from a frost flower "field" on a flat area of thick sea ice, in a single location about 5 km northwest of Barrow on 20 March 2009 (also described by: Beine et al., 2012; Douglas et al., 2012). Snow temperature was measured using a long-stemmed thermometer (Fisher Scientific, Montreal, Canada), and the meteorological conditions were recorded (air temperature, wind direction, and cloud cover). Average snow temperature was at $-19°$C and air temperature was at $-21°$C. The frost flowers were characterized as "old frost flowers" having coatings from increased vapor phase deposition (Douglas et al., 2012). Samples were kept frozen ($-20°$C) until shipped out by airfreight (transit time 41 hours) in commercial coolers (Coleman). The maximum temperature upon arrival in the laboratory at McGill University in Montreal was $-5°$C. Arctic samples were stored in a freezer at $-20°$C (Viking) until analysis. Surface snow samples from heavy snowfall regions in the province of Quebec, Canada; Mont-Tremblant, the city of Montreal (urban snow) and its suburb, Pierrefonds, were also collected using similar techniques. A minimum of three samples for each snow type and frost flower were used for analysis.

2.2 Isolation of viable microorganisms and Drop-freezing assays

Snow and frost flower samples were melted directly by transferring from freezer to refrigerator at $4°$C, under sterile conditions, and these conditions were maintained at all times using sterile instruments and materials or certified sterile single-use supplies. Once melted, they were kept on ice for the IN experiment, or transferred to the laminar flow hood to culture the microorganisms. To grow the microorganisms, 1 milliliter of Arctic samples (snow and frost flower) were placed in standard 100×15 mm sterile plastic Petri dishes (Fisher Scientific, Montreal, Canada). The media used for bacteria were tryptic soy agar (TSA), and R2A agar, a low nutrient medium used to improve the recovery of stressed bacteria. For fungi, mycological agar (Rybnikar, 1986) at neutral pH, and sabouraud dextrose agar (SDA) at a low pH of approximately 5.6 (all media by Becton, Dickson and Co, Mississauga, Canada) were used. In a flask, agar was dissolved and heated in ultrapure Milli-Q water (18 ohms resistance) according to the manufacturer's recommendation. After boiling for 1 min or until the medium was completely dissolved, the flask was autoclaved at $121°$C for 15 min. Plates were incubated at $4°$C and were regularly checked for growth, and the colonies were counted.

Drop-freezing assays were done on: (i) viable isolated bacteria obtained from Arctic samples, and (ii) melted Arctic samples. Viable bacteria isolated from Arctic samples grown on Petri dishes were mixed with sterile ultrapure water (Millipore, Mississauga, Canada). The optical density of 1 at 600 nm was used to adjust the concentrations of bacteria to $10^8 \, cells \, mL^{-1}$. IN experiments were performed using a homemade copper cooling plate (cooling rate of $1°C \, min^{-1}$), a technique first described by Vali (1971). The copper plate was coated evenly with commercial Vaseline™ petroleum jelly. The samples were kept on ice and were loaded as 10 μL droplets. A minimum of 150 drops was used for each experiment. Tap water was also used as a control that showed IN activity between positive (*Pseudomonas syringae*) and negative controls (ultrapure water). The temperature of each frozen droplet was recorded. IN temperatures are a simple average of the temperatures at which a sample group of drops freezes; i.e., the sum of the freezing temperature of each drop in the ensemble divided by the total number of drops.

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

Part of each bacterial colony was picked by a sterile disposable inoculating loop (VWR, Mississauga, Canada) and mixed with Ready-Lyse™ Lysozyme (Epicentre Technologies, Madison, USA) and proteinase K in $1.5 \, mL^{-1}$ Eppendorf tube. DNA was extracted and purified equally well with either a DNAeasy kit (Qiagen , Toronto, Canada) or a Master Pure DNA purification kit (Epicentre Technologies, Madison, USA) according to the manufacturer's instructions. The conserved sequence of extracted DNA was amplified by polymerase chain reaction (PCR) (Techne Flexigene Thermal Cycler FFG02HSD) in a final volume of 25 μL using 16S universal primers 27F and 149R (Forward primer 5′-AGAGTTTGATCCTGGCTCAG-3′ and reverse primer 5′-ACGGCTACCTTGTTACGACTT-3′, Integrated DNA Technologies, Coralville, USA) yielding a prod-

uct of about 1465 bp. An Eppendorf™ tube containing all the ingredients, but DNA was used as control (blank) for every set of PCR. A typical PCR reaction for one tube contained 2.5 μL of 10X buffer, 1 μL of each primer of 2.5 μM, 0.6 μL of 10 mM dNTP, 1 μL of 0.1 μg μL^{-1} DNA, 0.1 μL of 5U / μL of Taq polymerase, 2.5 μL of 25 mM of MgCl$_2$, 0.8 μL of 1 M NaCl, and to 25 μL of nuclease free water (Promega, Madison, USA). PCR included 35 cycles of denaturing at 94 °C for 1 min, annealing at 55 °C for 1 min, and extending at 72 °C for 2 min, followed by a 7 min final extension at 72 and 4 °C forever. The PCR product was separated and analyzed in 1.2 % agarose gel electrophoresis stained with ethidium bromide.

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

2.4 454 Pyrosequencing

We opted to use a conventional technique in concentrating the bacteria in Arctic samples using filtration, sonication and precipitation using high-speed centrifuge. For DNA analysis, bath sonication is a method that has been used to dislodge adherent bacteria in environmental samples (Buesing and Gessner, 2002; Bopp et al., 2011; Joly et al., 2006; Kesberg and Schleheck, 2013) as well as in medically devised explanted prosthetic instruments studied in hundreds of patients (Piper et al., 2009; Sampedro et al., 2010; Tunney et al., 1999). The dislodge bacteria is viable and can be cultured (Trampuz et al., 2007; Vergidis et al., 2011; Piper et al., 2009; Sampedro et al., 2010; Tunney et al., 1999; Joly et al., 2006; Kesberg and Schleheck, 2013; Solon et al., 2011). Melted snow was passed through a 0.22 micron filter (Millipore, Mississauga, Canada). Filter was sonicated in 17 m L^{-1} of 1X Tris/Borate/EDTA (TBE) buffer in an ultrasound bath for 10 min. For removing the viable bacteria from surface by sonication, no major differences were reported for duration of exposure time at 5 or 10 min, and temperatures at 22 °C (room temperature) or 6 °C; though the latter only slightly improved bacterial viability (Monsen et al., 2009). In our experiment, all the above factors were considered for removing the bacteria from filters. All sequences obtained in this study have been submitted to NCBI/SRA under bioproject ID PR-JNA285322.

The liquid was collected in sterile 50 m L^{-1} polycarbonate centrifuge tubes (Nalgen. Rochester, NY, USA). The liquid was centrifuged for 15 min at 18 000 g, and the pellet was re-suspended in 200 ul of 1X TBE buffer (Moran et al., 2008; Gharaibeh et al., 2009; Gantner et al., 2011; Medinger et al., 2010). Ready-Lyse™ Lysozyme (Epicentre Technologies, Madison, USA) was used to lyse the cell, and DNA was extracted and purified using either a DNAeasy kit (Qiagen, Toronto, Canada) or a Master Pure DNA purification kit (Epicentre Technologies, Madison, USA) according to the manufacturer's instructions. A barcoded 16S rDNA tag was used to amplify three distinct regions (V1-V3) of the bacterial 16S rDNA gene ($\sim$ 500 bp). The forward primer consisted of 454 Life Science adaptor A, a unique 10-base barcode rapid library Multiplex identifier (MID), and the specific forward primer sequence: 5$'$-CCATCTCATCCCTGCGTGTCTCCGACTCAGACGAGT GCGTATTACCGCGGCTGCTGG-3$'$, and the reverse primer consisted of 454 Life Science adaptor B fused to the specific reverse primer sequence: 5$'$-CCTATCCCCTGTGTGCCTTGGCAGTCTCAGAGAGTT TGATCCTGGCTCAG-3$'$. Amplification was done in triplicate and performed in a 20 μL reaction volume containing 13.85 μL of RNase and DNase free water, 2 μL of 5 ng μL^{-1} of DNA template, 2 μL of 10X AccuPrime PCR buffer (Invitrogen, Burlington, Canada), 1 μL of 2 μM of each primer, and 0.15 μL of AccuPrime Taq DNA polymerase Hifi (Invitrogen, Burlington, Canada). Cycling conditions were performed at 95 °C for 2 min, followed by 30 cycles at 95 °C for 20 s, 56 °C (V1-V3 primer set), 72 °C for 5 min and 4 °C forever. PCR products were purified with AMPure XP beads (Agencourt, Beckman Coulter, Canada), and eluted in 20 μL of ultrapure water. The quality and size of the amplicons were assessed on a 2100 Bioanalyzer using a DNA 1000 kit (Agilent Technologies, Mississauga, Canada) and quantified with the PicoGreen Assay (Invitrogen, Burlington, Canada). The amplicons library was pooled in equimolar amounts. NGS sequencing was performed using 1/8 of sequencing plate of GS FLX Titanium (454/Roche, Mississauga, Canada) for reading. The pool was sequenced uni-directionally from adaptor A with the Genome Sequencer FLX Titanium (454/Roche, Mississauga, Canada) at McGill University and Génome Québec Innovation Centre, Montreal, Canada. The generated sequences from pyrosequencing was analyzed with software MOTHUR formatted version of the RDP classifier using a Bayesian method (Wang et al., 2007) with 1000 bootstrap replicates for preprocessing (quality-adjustment, barcode split), identification of operational taxonomic units (OTUs) defined at $\geq$ 97 % 16S rRNA sequence identity level, taxonomic assignment, community comparison, and statistical analysis (Schloss et al., 2009). Trimming was done by quality from the 3$'$ end. Sequences containing ambiguous bases, homopolymers longer than eight bases, or an average quality score below 20 over a 50 bp long window were excluded (Schloss et al., 2011). The diversity of the bacterial communities for four different snow types and frost flowers was estimated using the Simpson diversity index, species richness using a

rarefaction metric, and the nonparametric Chao index (Chao, 1984). Chao1 index is a good estimator for obtaining true species richness based on the observed species accumulation pattern wherein the number of singletons and doubletons were used.

2.5 Electron microscopy analysis

Analysis transmission electron microscopy (TEM) in conjunction with energy-dispersive X-ray spectroscopy (EDS) analyses were used on Arctic snow samples and frost flowers to detect microbial and chemical compounds. Samples were freeze-dried. A sample solution of 7 μL was put onto a 200 mesh carbon-coated copper grid for 1 min. It was negative stained with 2 % uranyle acetate for 30 s. Imaging was done under TEM (Hitachi H7500 operated at 100 keV and spot size 5), a Philips CM200 200 kV TEM equipped with Gatan Ultrascan 1000 2 k × 2 k CCD camera system (Model 895) and EDAX Genesis EDS Analysis System.

3 Results and discussion

Recent observations have indicated that snowpack is indeed a complex microhabitat that permits the growth of diverse microorganisms allowing for photo-chemical and biological reactions to occur (Amoroso et al., 2010). Nitrification (Amoroso et al., 2010), transformation of mercury (Moller et al., 2011) and other pollutants within the snowpack have been detected. Ammonia-oxidizing Betaproteobacteria are active nitrifiers in glacial ice microcosms (Miteva et al., 2007), and the presence of nifH genes has been previously suggested the potential for nitrogen fixation in supraglacial snow (Boyd et al., 2011). A clear understanding of the bacterial population and their interactions will be required to further reveal the role these play in altering the Arctic environment and climate.

In this study, the next-generation sequencing (NGS) technique in conjunction with a classical cultural method was used to identify and compare the bacterial community in different types of Arctic snow and frost flowers. Moreover, the Arctic microbial population was compared to urban snow from the cold North American city of Montreal. Using GS FLX Titanium (450/Roche), a total of 88 937 reads was made for all the samples with the average number of total reads being 17 787. The average read length for all the reading was a 373 base with an average read quality of 34. After trimming and passing through quality control, the final read length was recovered as follows: 319 ± 18 bases (urban snow, US), 299 ± 17 bases (blowing snow, BS), 401 ± 20 bases (surface hoar snow, SH), 385 ± 20 bases (windpack, WP), and 419 ± 20 bases (frost flowers, FFs).

The diversity of the bacterial communities for four different snow types and frost flowers was estimated using the Simpson diversity index, species richness using rarefaction metric, and the nonparametric Chao index (Chao, 1984). The Simpson diversity index which takes into account both species' richness, and an evenness of abundance among the species present reached a plateau after the sequencing of sampling of about 5000 for BS, 6000 for WP, 7000 for US, 8000 for SH, and 10 000 for FFs (Fig. A1a). The Chao index gave values between 1500 and 7500 with BS exhibiting the lowest richness (Fig. A1b). The richness in total bacterial communities of Arctic samples was estimated by rarefaction analysis. The shapes of the rarefaction curves did not reach asymptote, indicating that bacterial richness for most samples especially for urban snow, and windpack is not yet complete (Fig. A1c). Using the 3 % cut-off value in sequence differences for OTU, the estimates of the richness of total bacterial communities ranged from 1033 in BS, 1971 in WP, 1956 in SH, 1933 in US and 1605 in FFs (Fig. A1c). Based on these analyses, the order of the highest diversity of bacteria to the lowest was observed in windpack, surface hoar snow, urban snow, frost flowers, and blowing snow, under experimental conditions herein used.

In the next-generation sequencing part of this study, pyrosequencing was done only for bacteria and not fungi which was feasible under our existing facilities. However, high resolution electron microscopy (Fig. 1) further revealed the appearance of the existence of several biological materials, remnants of biological activities, and not only biological entities in their entirety. The individual sequences represented known phyla or candidate divisions as follows: 11 (urban snow), Arctic samples: 18 (WP), 16 (SH), 15 (BS), and 18 (FFs) (see also the Appendix Table A1a). The majority of sequences (12.3–83.1 %) belonged to one of the five major phyla: Proteobacteria, Actinobacteria, Bacteroidetes, Firmicutes, and Cyanobacteria. The major phyla for urban snow and Arctic samples were as follows: (i) urban snow – Proteobacteria (49.04 %), Bacteroidetes (47.5 %); (ii) windpack – Proteobacteria (66.1 %), Cyanobacteria (12.3 %); (iii) surface hoar snow – Proteobacteria (67 %), Firmicutes (13.6 %); (iv) blowing snow – Proteobacteria (83.1 %), Firmicutes (6 %), Actinobacteria (5.09 %) and (v) frost flowers – Proteobacteria (50.2 %), and Actinobacteria (32.8 %) (Appendix Table A1a). Proteobacteria was the most widely expressed phylum among all the Arctic samples tested with the greatest abundance observed in blowing snow.

At the genus level, sequences represented 134 different genera for urban snow; Arctic samples: 245 for windpack, 139 for surface hoar snow, 101 for blowing snow, and 158 for frost flowers. The distribution of bacterial genera observed at greater than 1 % and the number of occurrence for each percentage observed for any genus in total bacteria is shown in Fig. 2. The name of corresponding genera for each percentage (> 1 %) is listed in the Appendix Table A2. The top four genera with the highest percentage detected for each sample were as follows: urban snow (US) – *Flavobacterium* (40 %), *Polaromonas* (11.2 %), *Variovorax* (6.6 %) and *Sandarakinorhabdus* (6 %); windpack (WP) – *Methylobacterium* (9 %),
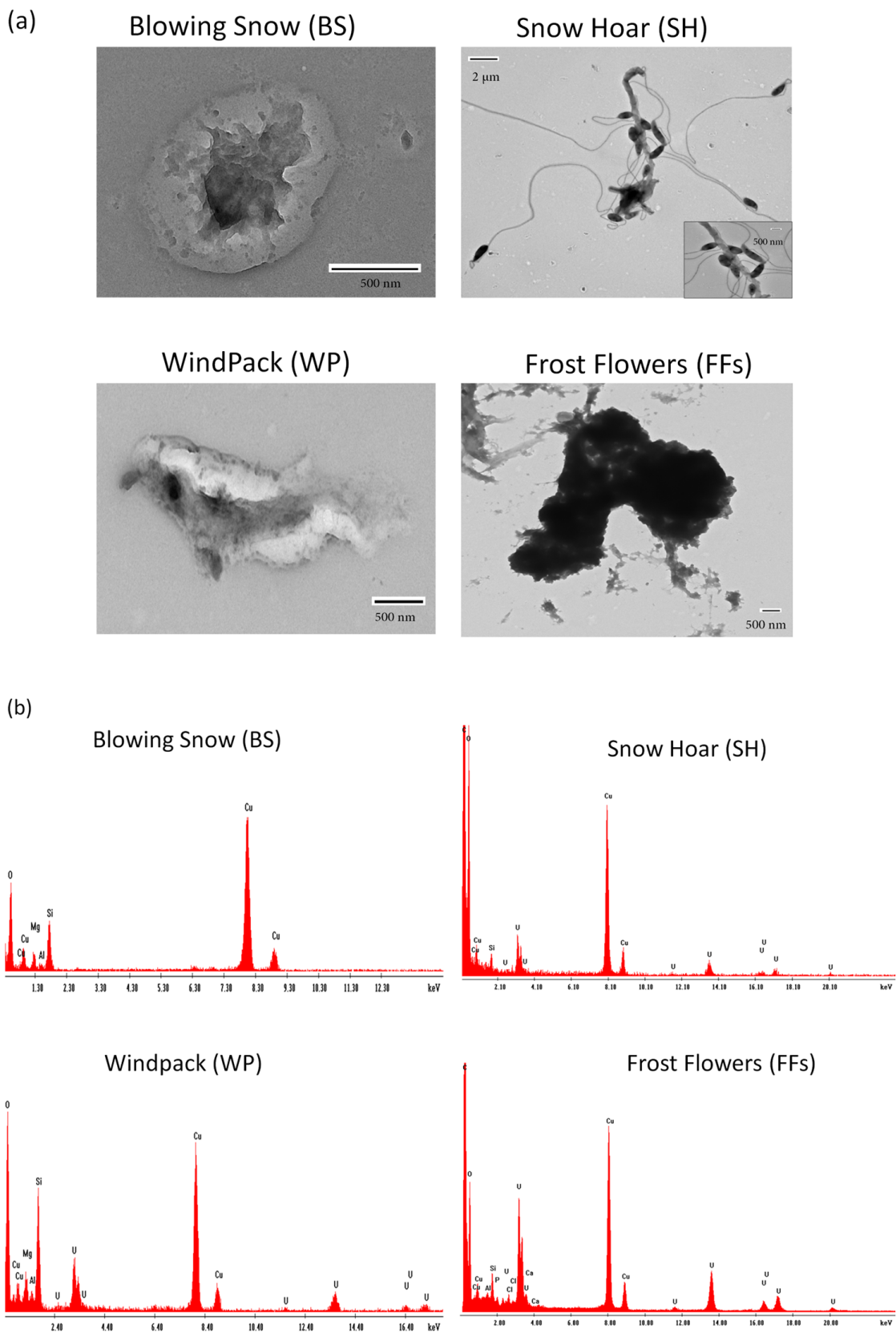
Figure 1. Analysis of snow-associated microorganisms by transmission electron microscopy (TEM) in conjunction with energy-dispersive X-ray spectroscopy (EDS). Microorganisms **(a)** and chemicals **(b)** were detected in selected Arctic samples: blowing snow, BS; surface hoar snow, SH; windpack, WP, and frost flowers, FFs.
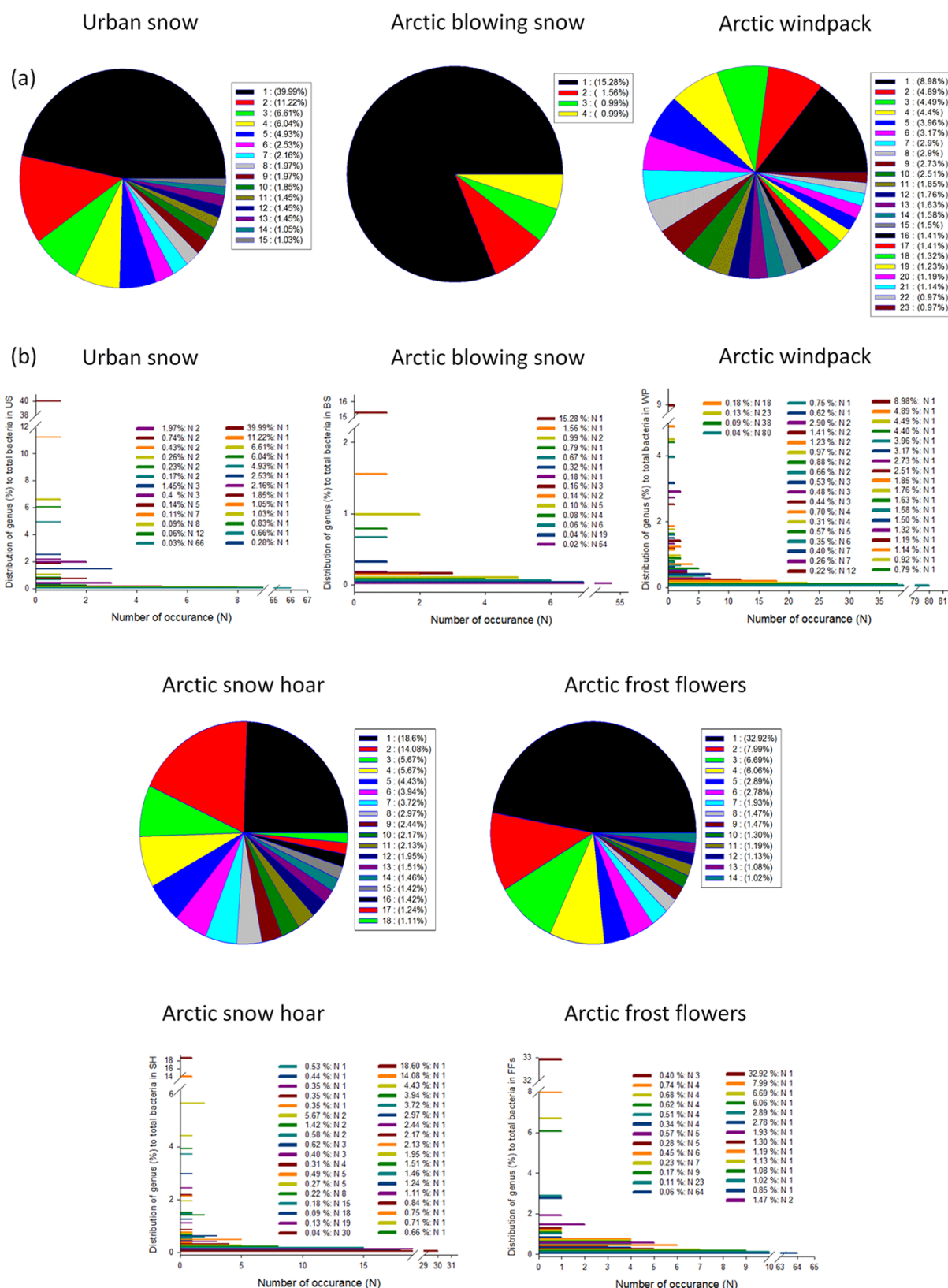
Figure 2. Bacterial community composition in Arctic samples and urban snow at genera level as detected by Roche 454 GS-FLX Titanium. **(a)**: distribution of bacterial genus observed at greater than 1 %. **(b)**: number of occurrence for each percentage observed for any genus in total bacteria. (The name of corresponding genera for each percentage (>1 %) is listed in the Appendix Table A2).

Sphingomonas (4.9 %), *Lamprocystis* (4.5 %), and *Roseateles* (4.4 %); surface hoar snow (SH) – *Roseateles* (18.6 %), *Methylobacterium* (14.1 %), *Bacillus* (5.7 %), and *Streptococcus* (5.7 %); blowing snow (BS) – *Methylobacterium* (15.3 %), *Bradyrhizobium* (1.6 %), *Bacillus* (1 %), *Sphingomonas* (1 %); and for frost flowers (FFs) – *Propionibacterineae* (32.9 %), *Roseateles* (8 %), *Staphylococcus* (6.7 %), and *Candidatus Pelagibacter* (6.1 %) (Appendix Table A1b).

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

Table 1 shows the analysis of NGS results encompassing bacteria at genus level that have been previously detected: (i) in Asian or African dust storms, (ii) with antifreeze and/or ice nucleation properties, and (iii) in cold oceanic water. Note that the existence of these bacteria does not ensure the expression of their property, and thus the existence of ice nucleating and freezing bacteria does not reflect their expression in environmental matrices. The percentage of bacteria (at genus level) that were previously observed in Asian or African dust samples were in the range of 36–47 % for all the snow categories and frost flower samples. Only a very small percentage of identified bacteria with previously demonstrated antifreeze property (Yamashita et al., 2002), were detected in: windpack – 1 %; surface hoar snow – 0.2 %; and frost flowers – 1 %. Urban Montreal snow samples had the highest number (7 %). 14–16 % of samples contained bacteria with ice nucleation properties, as shown in Table 1a. A very small percentage of identified bacteria showed both ice nucleation and antifreeze properties. Some bacteria such as *Pseudomonas fluorescens KUAF-68* and *Pseudomonas borealis DL7* have been

reported to have both antifreeze and ice nucleation activity (Kawahara et al., 2004; Wilson et al., 2006). Having these two properties has been suggested to enhance the freeze-tolerance survival of bacteria by maintaining small ice crystals with ice recrystallization inhibition protecting against freeze–thaw stress with antifreeze proteins (Xu et al., 1998), minimizing damage from explosive ice crystal growth and stabilizing the outer membrane with the low thermal hysteresis value (Xu et al., 1998), and minimizing the supercooling point with ice nucleation proteins (Kawahara et al., 2004). In our study, under our experimental conditions, the highest percentage was observed in urban snow samples (0.1 %), and the lowest was observed in blowing snow (0.03 %). Only 0.01 % of bacteria in windpack snow, surface hoar snow and frost flowers had both ice nucleation and antifreeze properties. Similarly, Arctic samples showed a minute number of bacteria that have been previously detected in cold oceanic water (0.01–0.04 %); none were detected in urban snow samples, under the experimental conditions in this work.

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

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 (FFs); 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.

(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 %
FFs	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 %
Ffs	0.4 %	0.2 %

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

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

Only a small fraction of a microbial community, especially from extreme environments such as the Arctic can be grown under laboratory conditions since many factors such as the composition of the medium that fully supports the basic needs of microorganisms for growth is not known. This notion was further confirmed as cultivable bacteria encompassing 0.1 to 3 % of the total bacteria, which was detected by NGS technique. Thus, the identified number of cultivated bacteria and fungi independent from different snow cate-

gories and frost flowers does not reflect the actual number of microbial and should be considered as the lower limit, and therefore more metagenomic analysis (such as NGS which was deployed in this study) is essential to decipher the complex pool of microorganisms in the Arctic. The cultivable bacteria might be representative of the active fraction of cultivable bacterial snow communities (Ellis et al., 2003; Frette et al., 2004), as was detected for bacteria living in different environmental samples such as soil, and marine samples (Pinhassi et al., 1997; Rehnstam et al., 1993). Table 2 contains the identified cultivable bacteria found in each category of Arctic samples (snow and frost flowers). Figure 3a (bacteria) and 3b (fungi) show the variability in numbers of colony-forming units (CFUs) within and between the different sample types using two different media (R2A and TSA) for bacteria and (SDA and mycological) for fungi. The average number of viable bacteria was higher than the number of fungi in Arctic samples. Overall, a higher number of CFUs was observed in the R2A medium with a more limited nutrient content than TSA, wherein most aerobic bacteria are able to grow. In the R2A plates, the highest number of bacteria was observed in frost flowers (FFs) and accumulated snow (AS) with 325 and $314\,CFU\,mL^{-1}$, respectively (Fig. 3a). However, the highest number of fungi grown in the mycological plate was observed in windpack (WP) and blowing snow (BS) with $5\,CFU\,mL^{-1}$ (Fig. 3b).

Both NGS and culture method analysis revealed a very high number of bacteria in frost flowers as compared to the other snow types that we tested. In recent years, special at-

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 (FFs); accession number, the nearest neighbor found in the database, 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
FFs	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
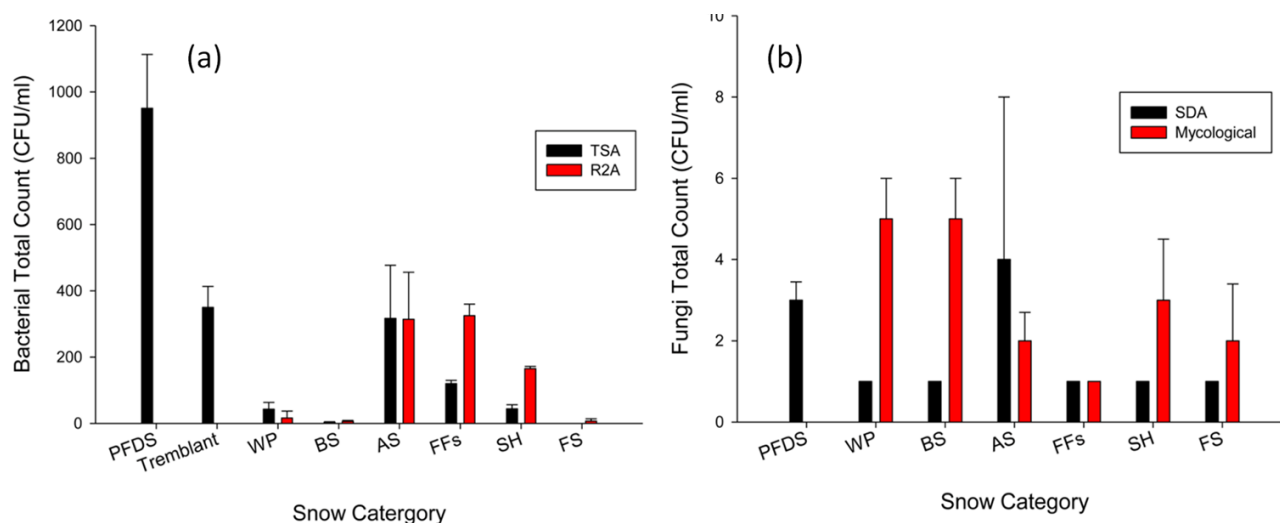
Figure 3. Concentration of viable cultivable bacteria and fungi in Arctic samples. Mean number of colony-forming units (CFUs) mL^{-1} of snow on two media for: **(a)** bacteria (TSA and R2A) and **(b)** fungi (SDA and mycological agar) in each Arctic snow categories: accumulated snow (AS), blowing snow (BS), fresh snow (FS), surface hoar snow (SH), windpack (WP), and frost flowers (FFs). 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.

tention has been focused on the role of frost flowers as a contributing factor to changing the chemistry of the atmosphere in the Arctic. Frost flowers are (i) an important source of sea-salt aerosol (Rankin et al., 2002; Perovich and Richter-Menge, 1994; Martin et al., 1995), (ii) a contributing factor in releasing the ozone-depleting molecule, bromine monoxide (BrO), as was detected by satellite (Kaleschke et al., 2004), and (iii) a source of sea ice bacteria (Collins et al., 2010). Moreover, with their physical structure and chemical composition, frost flowers might provide a habitat for microbiological bodies such as bacteria, as well as protective and favorable conditions for metabolic and photochemical reactions (Bowman and Deming, 2010). The observed simple organic compounds and increased concentrations of both formaldehyde (Barret et al., 2009), hydrogen peroxide (Beine and Anastasio, 2009) within frost flower, may suggest that selected bacterial strains can act as a substrate for the photolytic production of oxidants (Bowman and Deming, 2010), and simple organic compounds (Ariya et al., 2002). The regular release mechanism of bacteria through frost flower, such as those with high ice nucleation activity, into the atmosphere, with potential transportation, may provide an additional impact on bioaerosol lower tropospheric mixing ratios (Jayaweera and Flanagan, 1982).

As opposed to frost flowers, accumulated snow is characterized by several layers of snowfall, which may have experienced repeated freeze–thaw cycles, and solar irradiation exposure. Analysis by cultural method showed that the highest number of bacteria is present in accumulated snow samples. Each fresh snowfall adds new nutrients and microorganisms to the old pool of accumulated snow. With the detection of

more than 100 organic species in the aerosols at Alert in the Canadian High arctic (February–June) (Fu et al., 2008), the snow layers could be further enriched with nutrients by the air/snow exchange (Xie et al., 2007; Cincinelli et al., 2005). Over time, bacterial populations in accumulated snow may increase by their sustainability and slow growth at very low temperature (-2 to -35 °C) (Junge et al., 2004; Gilichinsky et al., 1995; Panikov and Sizova, 2007; Bakermans et al., 2003).

Different types of Arctic snow and frost flower samples were tested for IN activities using obtained cultured bacterial colonies as well as whole melted Arctic samples. Ultra-pure Milli-Q water (18 ohms resistance), tap water, and *P. syringae* mixed in ultrapure water were used as controls. Tap water contains organic impurities that allow ice to nucleate at warmer temperatures than ultrapure water. The individual average freezing temperature of bacterial isolates from different types of Arctic samples is shown in Fig. 4a. To explore the impact of undetected biological and chemical contents of snow samples on IN activity, the IN activity of each melted Arctic sample was directly measured and its IN activity was compared with the corresponding freezing temperature of the average sum of the total individual isolated bacterial colonies (Fig. 4b). The freezing temperatures of the average sum of the total individual (ASTI) isolated bacterial colonies fall at intermediate values between sterile ultrapure water (-24.3 ± 1.2 °C) and tap water (-15.3 ± 0.9 °C). The highest and lowest ice nucleation activity of bacteria was observed in fresh snow (ASTI: -15.7 ± 5.6 °C) and frost flowers (ASTI: -20.3 ± 1.5 °C), respectively. The ice nucle-
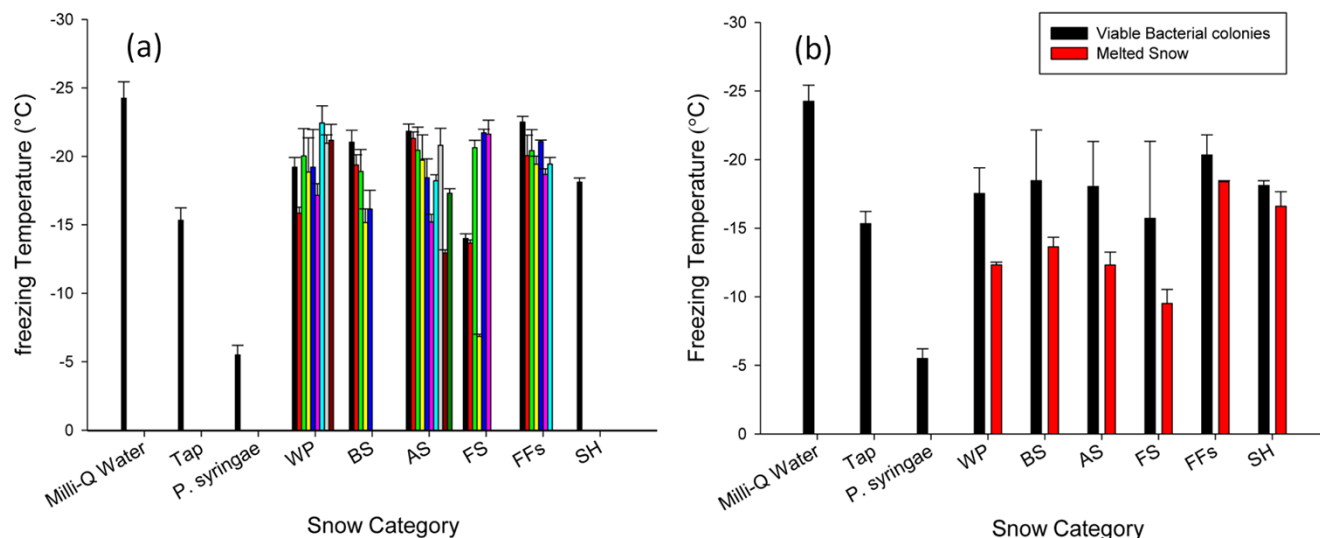
Figure 4. Ice nucleation activity of viable cultivable bacteria in Arctic samples. The average ice nucleation temperature of: **(a)** individual bacterial isolates and **(b)** total bacterial isolates and melted Arctic samples. Arctic snow categories: accumulated snow (AS), blowing snow (BS), fresh snow (FS), surface hoar snow (SH), windpack (WP), and frost flowers (FFs). 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.

ation activity of fresh snow was comparable to tap water ($-15.3 \pm 0.9\,°C$) (Fig. 4b).

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

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

The observed range of IN activity in melted snow and frost flower samples was between $-9.5 \pm 1.0\,°C$ (FS) and $-18.4 \pm 0.1\,°C$ (FFs) (Fig. 4b). Interestingly, all the melted Arctic snow samples and frost flowers showed ice nucleation activity at the range very close to the lowest recorded ice nu-

cleation activity of individual cultivable bacterial colonies. This observation may indicate an additional role of other non-cultivable microbial and components in Arctic snow samples and frost flowers which are important in increasing the ice nucleation temperature. Culture-dependent methods selectively isolate a plate-growth-adapted subpopulation from the microbial communities which may represent the majority of the total bacterial numbers in samples (Pinhassi et al., 1997; Rehnstam et al., 1993), but not necessarily the total richness (number of different species) of the bacterial population (Amann et al., 1995; Onstott et al., 1998).

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

The elemental composition of Arctic samples was determined using HR-TEM with EDS. As shown in Fig. 1b, the presence of elements such as Mg, Al, Cl, Ca, and U was detected. The presence of Si and Al might be an indication of soil or through deposit of the dust transported from soil (Sposito, 2008; Shridhar et al., 2010). The source of calcium might be related to either marine or soil origins. Since phos-

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 (FFs); 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
FFs	A	-20.0 ± 1.5	HQ425309.1	*Kocuria sp.*	98
			FR682683.1	*Kocuria rhizophila*	98

phorous is the limiting macronutrient in marine ecosystems (Toggweiler, 1999; Tyrrell, 1999), the positive observation of this element in frost flower encourages further research in the role of frost flowers in Arctic ecosystem.

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

4 Conclusions

We herein examined the identity, population and ice nucleation ability of the microbial communities of five different snow types and frost flowers during the spring 2009 campaign of the Ocean–Atmosphere–Sea Ice–Snowpack (OASIS) program in Barrow, Alaska, USA. We used the next-generation sequencing (NGS) technique to examine the true bacterial communities in snow and frost flowers, in addition to conventional culture techniques. We gained further insight into the wide range of taxa available in different types of snow and frost flowers. Arctic samples and reference urban snow represented 11–18 known phyla or candidate divisions. The majority of sequences (12.3–83.1 %) belonged to one of the five major phyla: Proteobacteria, Actinobacteria, Bacteroidetes, Firmicutes, and Cyanobacteria. At the genus level, 101–245 different genera were detected. A largely diverse community of bacteria exists in the Arctic with many originating from remote ecological environments such as dust storms. This study revealed that snow and frost flowers are rich media for the existence of microbial compounds. Biological materials have been shown to act as reactive sites for (photochemical) reactions, and thus further studies are required to decipher the complexity of the snow and frost flowers as a zone of chemical pool. It is conceivable that changes on the ratio of antifreeze bacteria to ice nucleation bacteria may have an impact on the melting and freezing processes of snowpack or frost flowers. It is thus feasible that this shift in bacterial population could ultimately affect the snow melting-freezing processes. Further studies are required to evaluate whether change of nucleation patterns due to biological entities are indeed linked to climate change.

Appendix A

Table A1. 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 (FFs); and urban snow (US). The number in parenthesis shows the sequence percentage obtained from the overall bacterial pool.

(a) Phylum				
US	BS	SH	WP	FFs
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 %)	Gemmatimonadetes (0.05 %)
			Candidate_division_OP10 (0.04 %)	Lentisphaerae (0.05 %)
			Deinococcus-Thermus (0.04 %)	Spirochaetes (0.05 %)

(b) Genus				
US	BS	SH	WP	FFs
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 A2. Genus distribution (> 1 %) of Bacterial community in Arctic samples/urban snow by NGS. Windpack snow (WP), surface hoar snow (SH), urban snow (US), blowing snow (BS), and frost flowers (FFs).

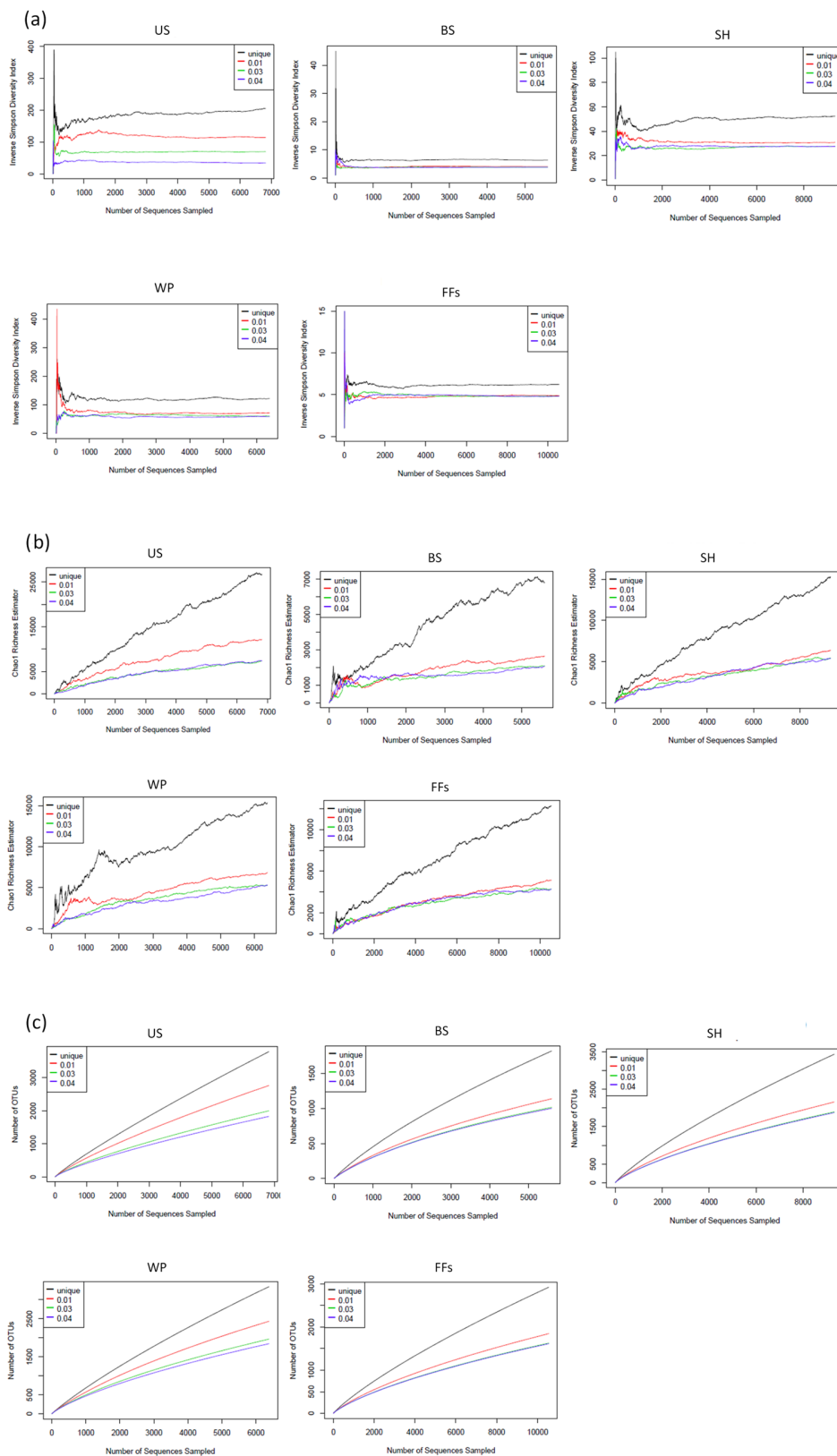|										Genus
Number of Occurrence (N)	WP	%	SH	%	US	%	BS	%	FFs	%
1	Methylobacterium	8.98	Roseateles	18.60	Flavobacterium	39.99	Methylobacterium	15.28	Propionibacterineae	32.92
2	Sphingomonas	4.89	Methylobacterium	14.08	Polaromonas	11.22	Bradyrhizobium	1.56	Roseateles	7.99
3	Lamprocystis	4.49	Bacillus	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 (FFs); 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.

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