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Microbes in High Arctic snow and implications for the cold biosphere

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Running title: Microbial communities in remote Arctic snow

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ABSTRACT

We applied molecular, microscopic, and culture techniques to characterize the microbial communities in snow and air at remote sites in the Canadian High Arctic (Ward Hunt Island, Ellesmere Island and Cornwallis Island, latitudes 74-83°N). *Bacteria* and *Eukarya* were prevalent in the snow and their SSU rRNA gene signatures indicated strong local aerial transport within the region over the preceding eight months of winter snowpack accumulation. Many of the operational taxonomic units (OTUs) were similar to previously reported SSU rRNA gene sequences from the Arctic Ocean, suggesting the importance of local aerial transport processes for marine microbiota. More than 47% of the cyanobacterial OTUs in the snow have been previously reported from microbial mats in the region, indicating that this group was also substantially derived from local sources. Viable cyanobacteria isolated from the snow indicated free exchange between the snow and adjacent mat communities. Other sequences were most similar to those reported from outside the Canadian Arctic but were from snow, lake and sea ice, glaciers and permafrost, alpine regions, Antarctica and other regions of the Arctic, supporting the concept of global distribution of microbial ecotypes throughout the cold biosphere.

INTRODUCTION

As a result of their microscopic size, resistance to environmental extremes and large populations, free-living microbes have high dispersal rates that favour cosmopolitanism (19). According to a longstanding conjecture (6), the same microbial species will occupy similar environments throughout the biosphere because there are no effective barriers to their planetary dispersal. Several studies based on morphological and small subunit (SSU) rRNA gene sequence analyses have suggested that microbial genera are globally distributed (17, 29, 50, 55). However, other recent studies that have examined specific groups in detail indicate geographical patterning of microbial communities (36, 44, 66). Although the differing levels of taxonomic resolution from different markers has clouded the interpretation of global distribution patterns, SSU rRNA gene sequences are the most commonly available and are useful in comparing geographic and environmental distribution patterns (29, 35).

Microbial transport occurs via flowing rivers, percolating groundwater, thermohaline and surface oceanic circulation, convection currents and wind in the atmosphere, animal migration and anthropogenic activities. Aerial transport has long been viewed as a major transport route enabling microbes to colonize remote habitats. Spore-formers such as Gram-positive bacteria and fungi, in particular, should be able to survive long range transport. In remote sites under background atmospheric conditions, bacterial and fungal cell concentrations can reach 10^4 and 10^3 cells/m^3 respectively (5). The presence of microbes in the atmosphere varies temporally, with higher concentrations during the summer months, and spatially, with higher concentrations in urban compared to rural areas. Viable microorganisms have been reported from the stratosphere, at altitudes as high as 77 km (24, 65). The relative contribution of bacteria compared to fungi and pollen grains increases with altitude likely due to their smaller cell size and consequent lower

sinking velocity (5). This suggests that bacteria are better dispersed compared to eukaryotes in the air since they remain for longer periods of time in the atmosphere. In addition to implications for dispersal, microbes in the atmosphere are potentially involved in cloud chemistry due to ice nucleation activity of some species and their potential to grow on simple compounds present in cloud droplets (9, 15, 52).

The cold biosphere, defined as the ensemble of habitats over planet Earth that experience prolonged cold and freezing, acts as a severe ecological filter for all immigrant and resident organisms. These ecosystems are mostly microbial, and dominated by cold-adapted (psychrophilic) and cold-tolerant (psychrotolerant) microbes. Surveys of glaciers, snow, lake-ice, sea-ice and atmospheric clouds have revealed the recurrent presence of a number of bacterial phyla: *Bacteriodetes* (previously referred to as the *Cytophaga-Flavobacteria-Bacteroides* or CFB cluster), *Actinobacteria*, *Firmicutes* and *Proteobacteria* including representatives from the *Alpha*, *Beta* and *Gammaproteobacteria* Classes (4). The cryosphere bacteria must contend with a severe combination of environmental stresses that include low nutrient concentrations, high solar UV-b radiation, freeze/thaw cycles, and limited liquid water. The bacterial activity reported in supercooled cloud droplets (52) suggests that cold-dwelling microbes are better adapted to atmospheric transport than other microbes, and the cryosphere therefore provides an attractive environment for evaluating microbial dispersal and biogeography. However, environmental filters take time to select adapted communities and in the poor growth environment of the snow more local transport processes would favour organisms already abundant in the local environment and mask the signal from global cryosphere microbes. There are three major biomes in the coastal Arctic that could contribute to local inocula in the snow: 1) cyanobacterial mats characteristic of Arctic freshwater environments; 2) marine microbes from the adjacent sea; and

3) halotolerant sea ice flora of the Arctic Ocean. Given the distinct phylogenetic signatures of most freshwater and marine microorganisms (23, 33, 34), marine microbes in snow would indicate local transport.

The objectives of this research were to investigate the diversity and likely source environments of microbes in the snow and air in the High Arctic Canada. We sampled two polar desert sites, including at the remote, northern limit of the North American Arctic, and compared our SSU rRNA gene results with those from temperate and cold biosphere sites elsewhere including Antarctica.

MATERIALS AND METHODS

Study sites. Samples were collected from two locations in the Canadian High Arctic: the northern coast of Ellesmere Island in the vicinity of Ward Hunt Island (83° 05'N 74° 09' W), and 1000 km to the south, at Char Lake (74°42'N 94°53'W) in the Resolute Bay region of Cornwallis Island (Fig. 1). Ellesmere Island sampling was over a 50-km transect extending inland from Ward Hunt Island (near sea level) to the Disraeli Glacier (850 m above sea level). Ward Hunt Island is near the northern terrestrial limit of Canada (Cape Aldrich on Ellesmere Island, at 83° 07' 00" N 69° 35' 00" W; 61 km E of Ward Hunt Island), and is surrounded by the Ward Hunt Ice Shelf (WHIS). Microbial mats of the WHIS, which subsist under the snow during winter and then grow in summer in elongated meltwater ponds, have been investigated previously (8, 29, 42). WHIS lies at the seaward end of Disraeli Fjord, which is 5 km wide and extends 30 km inland (30). Disraeli Glacier is one of three glaciers that extend as floating ice tongues into the head of Disraeli Fjord. Snow was also sampled on the ice cover of Lake A, a meromictic lake on the northern coast of Ellesmere Island, 15 km west of Disraeli Fjord (further information in 40) and

of Ward Hunt Lake (details in 7). Snow samples from Char Lake were collected from the frozen lake surface at two sites.

Meteorological data. Automated meteorological stations were situated on the north shore of Ward Hunt Island (83° 05.550' N; 74° 07.816' W) and on the eastern shore of Lake A, Ellesmere Island (83° 00.136' N; 75° 23.377' W) and provided continuous year-round data (details in 64). Snow depth was measured with a Sonic SR50 sensor (Campbell Scientific Canada Corp.). Hourly averages of wind direction and speed measurements were measured with a R.M. Young Co. wind sensor (Model 05103) at a height of 10 m (Ward Hunt Island) or 3 m (Lake A).

Snow sampling. Snow samples for the molecular analysis were collected May-June 2008 from the surface of Lake A (LA) at three sites, Quttinirpaaq Lagoon (QL), Ward Hunt Lake (WHL), Disraeli Fjord (DF) at three sites, Char Lake (CL) at two sites, Disraeli Glacier (DG) and on Ward Hunt Ice Shelf (WHIS) (Fig. 1). Each site was sampled in duplicate, spaced 50 m apart. There was no discoloration of the snow indicating snow algae or sediment patches at any of the sites, and sampling was at random over the visually homogeneous surface. The operator wore sterile gloves and used an ethanol-flame sterilised shovel to transfer snow from throughout the snow profile (up to 88 cm deep) into ethanol-flame sterilized polyethylene boxes, and the samples were then allowed to thaw over 1-2 days. Sixty liters of packed snow per sample resulted in 12 to 30 L of snowmelt. Melting snow samples were frequently mixed to maintain a homogeneous water temperature ≤4°C. Twelve liters of snowmelt sample were filtered through a 0.2-µm pore-size Sterivex filter unit (small fraction, Millipore) after prefiltration through a 47-mm diameter, 3-µm pore-size polycarbonate filter (large fraction). The filters were then stored in lysis buffer (50 mM tris, 40 mM EDTA, and 750 mM sucrose) at -20°C until further analysis at

Université Laval. Autoclave-sterilised Milli-Q water was treated in tandem with the snow samples as a negative control field blank.

For microscopic analysis of the snow, samples were collected on top of the ice cover of Lake A, Ward Hunt Lake and Char Lake sites in July 2009 (snow depth ranging between 3 and 10 cm). Ten sites per lake were chosen in order to assess the horizontal patchiness. The snow was collected into wide mouth 4 L Nalgene bottles with a small, ethanol-flame sterilised shovel. After melting in darkness, conductivity of the meltwater was measured with a Water Analyser probe (Oakton, USA). A 50 mL subsample of snowmelt was fixed in glutaraldehyde (final concentration: 1%) for 1 hour at 4ºC. The samples were then passed through 0.2-µm black 25-mm-diameter polycarbonate filters (Poretics) and stained with 4',6-diamidino-2-phenylindole (DAPI, Invitrogen Inc.; 5 µg/mL final concentration) for 15 to 20 mins (47). Filters were mounted onto slides with nonfluorescent mounting oil (Immersol 518M). The slides were stored in the dark at 4°C and subsequently transferred to a -20°C freezer until enumeration by epifluorescence microscopy. Counts were made at 1000X magnification using an Olympus BX51 fluorescence microscope.

Air sampling. Arctic air samples were taken at Ward Hunt Island (WHI) in July 2009 using a Burkard multi-vial cyclone air sampler (Burkard Manufacturing Co., Rickmansworth, UK) installed 1 m above bare ground, and 100 m from the automated meteorological station. The operator wore sterile gloves, a clean hat, mask and laboratory coat to avoid contamination. Prior to the sampling, the sampler was disinfected with 70% ethanol and purged for 24 h. During the sampling period, airborne cells were collected in tubes under a 16.5 L/min airflow for 72 h. After sampling, lysis buffer was added to the tubes, which were stored at -20°C for subsequent DNA analysis.

SSU rRNA gene clone libraries. Nucleic acids were extracted following a standard salt protocol (1). Briefly, the microbial cells in lysis buffer were digested with lyzozyme (1 mg/mL, final concentration), proteinase K (0.2 mg/mL) and sodium dodecyl sulphate (0.01%). DNA was separated from the other organic phases by centrifugation in a supersaturated NaCl solution (3 M). The DNA was then precipitated with 70% ethanol and dissolved in 1X TE buffer (Tris-HCl 1 mM, EDTA 0.1 mM). The DNA concentration in the extracts was quantified with a fluorescence technique using picogreen and the Turner BioSystems TBS-380 fluorometer following the manufacturer's recommendations. Sites were selected to build clone libraries according to the DNA concentration and the localisation. 16S rRNA gene was amplified by PCR using the universal primer 1492R (5'-GGTTACCTTGTTACGACTT-3') and the *Bacteria*-specific primer 8F (5'-AGAGTTTGATCCTGGCTCAG-3') (20). Since cyanobacteria are poorly recovered using these primers they were specifically targeted using the forward primer 27F1 (5'-AGAGTTTGATCCTGGCTCAG-3') and the *cyanobacteria*-specific primer 809R (5'-GCTTCGGCACGGCTCGGGTCGATA-3') (28). The 18S rRNA gene was amplified with the eukaryote-specific primers NSF 4/18 (5'-CTGGTTGATYCTGCCAGT-3') and EukB (5'-TGATCCTTCTGCAGGTTCACCTAC-3') (37). For bacteria, the initial denaturation step at 94°C for 3 mins was followed by 35 cycles of DNA denaturation at 94°C for 30 s, primer annealing at 55°C for 30 s, strand elongation at 72°C for 1 min and a final extension at 72°C for 5 mins. For the Disraeli Fjord C clone library, additional PCR products that had been amplified following an annealing temperature of 60°C were pooled. For Disraeli Fjord B, two clone libraries were made, one with an annealing temperature of 55°C and another with an annealing temperature of 60°C. For cyanobacteria, the initial denaturation step at 94°C for 4 mins was followed by 35 cycles of DNA denaturation at 94°C for 20 s, primer annealing at 55°C for 30 s,

strand elongation at 72°C for 1 min and a final extension at 72°C for 7 mins. For eukaryotes, the initial denaturation step at 94°C for 3 mins was followed by 30 cycles of DNA denaturation at 94°C for 45 s, primer annealing at 55°C for 1 min, strand elongation at 72°C for 3 min and a final extension at 72°C for 10 mins. PCR products amplified from several DNA concentrations (ranging from 13-750 pg/µL in the PCR mix for the snow samples and 1-4 pg/µL for the air sample) were pooled, cleaned with the QIAGEN purification kit and cloned using the TA cloning kit (Invitrogen, California) or the Strataclone PCR cloning kit (Stratagene, California) following the manufacturers' directions. Positive clones were screened for Restriction Fragment Length Polymorphisms (RFLP) with HAE III (Gibco BRL, Maryland) for the universal bacteria and the eukaryotic sequences and with AluI and HpaII (Fermentas, New Hampshire) for the cyanobacterial sequences. Clones with the same RLFP pattern were considered members of the same phylotype. Multiple clones per RFLP pattern were sequenced to confirm that each pattern only represents one OTU. The 16S rRNA gene was sequenced in both directions using primers for the vectors M13 sites resulting in nearly full-length 16S rRNA sequences. Cyanobacterial and eukaryotic sequences were sequenced using the promoter T7 in the M13 vector and the internal primer 528F (16) respectively.

Isolation of cyanobacteria. Snow samples collected May 2008 were used to test for the presence of viable cyanobacteria using a method modified from Vézina and Vincent (61). Snow from the Ward Hunt Lake, Lake A and Char Lake sites were melted as above, and between 4 and 8 L were filtered onto 47-mm diameter, 0.2-µm pore-size polycarbonate filters. Isolates of cyanobacteria were obtained by incubating these filters in the liquid culture medium BG-11 (49) under a range of conditions (with and without nitrate, with and without pre-incubation in liquid media in the

field) to maximize the diversity of isolates. Cycloheximide (40 mg/L final concentration) was added to inhibit eukaryotic growth.

Ward Hunt Lake and Lake A cultures initially begun in the field were exposed to natural 24 h daylight at temperatures ranging between 3 and 10ºC for two weeks. For the second part of the field season the cultures were maintained at ~16ºC under natural light at Resolute Bay. Once back at Université Laval the cultures were transferred to a constant temperature growth cabinet and maintained at 10ºC and continuous irradiance under 50 µmol photons m^{-2} s^{-1} cool white fluorescent light.

Genomic DNA was extracted from unialgal cultures. Approximately 100 mg of cells were resuspended in 300 µL XS-buffer (1% potassium-ethyl-xanthogenate; 800 mM ammonium acetate; 20 mM EDTA; 1% sodium dodecyl sulphate; 100 mM Tris-HCl (pH 7.4); 57). The mixture was vortex-mixed and incubated for 2 h. The extracts were cooled on ice for 10 mins and cell debris was removed by centrifugation at 12 000 rpm for 10 mins. The supernatant was collected and DNA was precipitated overnight by addition of 1 volume of isopropanol and 1/10 volume of 4 M ammonium acetate at -20ºC. The precipitated DNA was pelleted by centrifugation at 12 000 rpm for 10 mins and washed with 70% ethanol. The extracted DNA was then resuspended in 100 µL of sterile water. PCR amplification was done as for the clone library analysis, and sequencing was done using the 809R internal primer (28).

Diversity and community composition. The SSU rRNA gene sequences from the snow and air samples were compared to those in the NCBI GenBank database using BLASTn (3) to identify closest matches and their source environments. Sequences were checked using the Chimera check

program at Ribosomal Data Project II (Michigan State University; http://wdcm.nig.ac.jp/RDP/cgis/chimera.cgi?su=SSU), and suspected chimeras were excluded from further analysis. The remaining sequences were manually trimmed and aligned using MUSCLE (www.ebi.ac.uk/Tools/muscle/index.html).

Operational taxonomic units (OTUs) were defined as ≥97%, ≥98% or ≥99% similarity for the bacterial (including cyanobacteria), eukaryotic and chloroplastic sequences respectively, and were determined using Mothur v.1.7.2 (http://www.mothur.org/wiki; 54). Thresholds for eukaryotic and chloroplastic sequences were set higher, because 18S rRNA gene and chloroplastic 16S rRNA gene are more conserved than the bacterial 16S rRNA gene, where the threshold is 97% based on DNA-DNA hybridization to differentiate species (51). This is based on empirical comparisons with classical taxonomy and 18S rRNA gene similarities (38). The same program was used to calculate the Shannon index and Chao1 richness. Equal numbers of sequences were randomly selected per site for inter-site diversity and richness comparison. Similarity between communities was assessed by Bray-Curtis cluster analyses using the program PAST v.1.90 (25).

Nucleotide sequence accession numbers. The nucleotide sequences data reported in the present study were submitted to GenBank under the accession numbers HQ230103 to HQ230240 and HQ529495 to HQ529499.

RESULTS

Snowpack characteristics. Snow sampling for molecular analyses in the Ward Hunt Island region was in late spring, at the time of maximum snow accumulation and immediately prior to

253 the rapid snowpack loss by melting (Fig. 2). At all sites, the snow was sampled to the depth of
254 hard ice or firn, which ranged from 10 cm on Char Lake to 85 cm on Disraeli Glacier. Wavelike
255 ridges of hard snow (termed 'kimugiyuk' or 'sastrugi', and normally formed perpendicular to the
256 direction of the wind) were observed at all sites except Disraeli Fjord. On Ward Hunt Lake and
257 Quttinirpaaq Lagoon, the snow cover was dense and uniform, and had a polystyrene-like texture,
258 with large crystals of snow on the surface. On Lake A, the snowpack had a dense layer at the
259 surface (5-10 cm thick), a loose intermediate layer with powdery to granular snow and another
260 dense layer at the bottom. At Disraeli Fjord A and B, the snow was wet, heavy and contained
261 cylindrical ice crystals at the surface and granular crystals at the base of the snow pits. At Disraeli
262 Fjord C and Disraeli Glacier, the snowpack was multi-layered. The snow on Char Lake showed
263 the onset of melt: it was wet and cohesive, and liquid water was discernable at the ice-snow
264 interface. In July 2009, conductivity of the melted snow ranged from 11.1-60.5 µS/cm for Char
265 Lake snow, 1.5-16.2 µS/cm for Lake A snow and 3.4-9.9 µS/cm for Ward Hunt Lake snow.
266
267 **Wind conditions.** For the snow accumulation period prior to sampling (September 2007 to June
268 2008)**,** the wind at Lake A came predominantly from the north-east quarter (35% of the hourly
269 mean samples). The wind came from the south-west, north-west and south-east quarters 29%,
270 17% and 19% of the time, respectively. There was a similar pattern for the same period 2008-
271 2009, with a predominance of wind from the north-east quarter (45% of the time). There was no
272 clear trend in wind direction on Ward Hunt Island. In the 2007-2008 snow accumulation period,
273 wind came from the north-west, south-west, south-east, and north-east quarters 32%, 28%, 28%
274 and 12 % of the time. For the same period over 2008-2009, wind came from the south-west,
275 north-west, south-east and north-east quarters 31%, 29%, 27% and 13% of the time. During the
276 air sampling, the meteorological station recorded winds from predominantly the north: 67% from

277 the NE quarter and 18% of the time from the NW quarter, with the strongest winds coming from
278 the north-west (Fig. 3).
279
280 **Microbial DNA and cells.** DNA quantification and prokaryotes cell concentration estimated
281 from the DAPI stained cells indicated that the microbiota were non-homogeneously distributed in
282 the snowpack in both sampling years. In June 2008, relative extracted DNA concentrations varied
283 from undetectable to 47 pg/mL (all values are for the melted snow water; Fig. 4). Median
284 concentrations were 2.2 and 2.9 pg/mL for the small and large fractions respectively. These DNA
285 concentrations varied by up to 23-fold between sites distant by tens of meters and by up to 31-
286 fold between sites that were tens of kilometers distant. Prokaryotes cell concentrations in 2009
287 samples were also heterogeneous (Fig. 5). Consistent with the DNA results from the previous
288 year, cell concentrations were low for the Lake A and Char Lake samples. The medians between
289 the lakes differed by a factor of 1.2. Overall, the counts ranged from 0.02 to 2.07 x 10^3
290 prokaryotic cells/mL, with substantial variation at both small and large length scales. Higher but
291 similarly variable concentrations were recorded in WHL snow, with cell counts ranging from
292 1.52 to 53.2 x 10^3 cells/mL. Sites a few meters apart varied by up to a factor of 5 while those
293 distant by tens of meters varied by up to two orders of magnitude.
294
295 *Ochromonas*-like microbial eukaryotes were also identified in the DAPI slides from the three
296 sites, and as with the prokaryotes, the cells were patchy. The median cell concentrations varied
297 by a factor of 8.5 among sites (Fig. 5), and ranged from 0.01 to 1.73 x 10^3 cells/mL in Ward Hunt
298 Lake snow from 0.28 to 8.50 x 10^3 cells/mL in Lake A snow and from 0.02 to 2.11 x 10^3
299 cells/mL in Char Lake snow.
300

Bacteria in the snow. We recovered 285 16S rRNA gene clones from the bacterial libraries constructed from five of the sampling sites. No PCR-amplification was obtained using DNA extracts from the field blanks and these results therefore appear to be free of contamination. The bacterial phylotypes were distributed into 18 OTUs at the >97% similarity level (Suppl. Table 1). *Proteobacteria* was the dominant phylum among all OTUs, in particular the order *Burkholderiales* in the class *Betaproteobacteria* which represented 64% of bacterial clone sequences. Other OTUs belonged to the phyla *Bacteriodetes* and *Cyanobacteria*, and the *Deinococcus* group. All but one of the OTUs matched sequences that had been previously recovered from cold environments such as glaciers, alpine lakes, snow and cold soils. The exception was an OTU (represented by the clone DFCb18 in Suppl. Table 1) that had no >97% match in GenBank.

OTUs belonging to genera with known psychrophilic taxa were detected in many sites: *Polaromonas, Aquaspirillum Octadecabacter* and *Glaciecola*. However there were differences in clonal abundance and diversity among the sites (Table 1 and Suppl. Table 1). Lowest diversity was found in the Lake A, Char Lake A and B snow samples. Even though these sampling sites were a thousand kilometers apart, their microbial snow communities were highly similar as shown by the Bray-Curtis cluster analysis (Fig. 6). *Aquaspirillum arcticum* was the dominant taxon, representing 96% of the bacterial clone sequences in the Lake A snow sample, 89% in the Char Lake A and the only bacterial OTU in Char Lake B. Another *Aquaspirillum* was found in the Disraeli Fjord B snow sample but had no close match to cultivated strains in GenBank.

Higher diversity indices for bacteria were recorded in the Disraeli Fjord B and C samples (Table 1). These snow communities were dominated by sequences closest to marine groups that require

sea salts for growth. The Disraeli Fjord B bacterial community was dominated by *Glaciecola pallidula* (24%) and *Polaribacter irgensii* (26%), while the Disraeli Fjord C clone library was dominated by *Glaciecola psychrophila* (35%) and by *Colwellia piezophila* (28%).

The bacterial rarefaction curves (Suppl. Fig. 1), with the exception of DFBb, reached an asymptote indicating that there was a reasonable sampling of the species richness. The Chao1 nonparametric diversity estimator confirmed this finding by showing that 100% of the predicted number of OTUs was recovered except for DFBb for which the number of OTUs recovered corresponded to 86% of the predicted asymptote.

In addition to the bacterial 16S rRNA gene clones, 195 sequences were from the chloroplast 16S rRNA gene (Suppl. Table 2). Several of these showed matches to the algal phyla *Cryptophyta* and *Bacillariophyta*, however most of the chloroplast sequences could not be matched to any particular group currently in GenBank.

Cyanobacteria were detected in cultures of all the different snow samples tested. Among the cultures we isolated viable cyanobacteria belonging to the orders *Oscillatoriales*, *Nostocales* and *Chroococcales*. The most common taxa in the cultures were filamentous oscillatorians. We also constructed a clone library from one of the Ward Hunt Lake snow samples using the same primers that have been used to determine the cyanobacterial community composition in the benthic microbial mats of Ward Hunt Lake and in meltpools on the adjacent ice shelves (29). We detected a total of 17 cyanobacterial OTUs (Suppl. Table 3), and eight of these had >97% identity with those from microbial mats in Ward Hunt Lake, Markham and Ward Hunt ice shelves, the inflow of Lake A, and Antoniades Pond, a small water body in the catchment of Lake

A. Five snow sequences representing five different OTUs had their closest match in GenBank to the Jungblut et al. (29) mat sequences sampled in 2007, with up to 100% identity. The clone library for the large fraction also indicated the presence of *Gloeobacterales* in the snow. Many sequences in the clone libraries did not match the 16S rRNA gene sequences in GenBank at the >97% level, most of them having only a 92% similarity to their closest cultivated neighbors.

Eukaryotes in the snow. Eukaryotic clone libraries were constructed for snow from five of the sites, and we obtained 333 clones. The 18S rRNA gene sequences indicated the presence of ciliates, *Chrysophyceae*, *Pelagophyceae*, *Bacillariophyta*, *Dinophyceae*, *Cercozoa*, *Basidiomycota*, *Cryptophyta*, *Streptophyta* and *Chlorophyceae* (Suppl. Table 4). Twelve of the 23 OTUs (52%) were previously recorded in samples from cold environments, such as cold marine waters, sea ice, mountain stream sediment and snow. Eight OTUs (35%) had no matches with >98% similarity to other sequences in GenBank. The eukaryotic clones mostly belonged to phototrophic taxa, notably *Ochromonas*, *Pelagomonas*, *Ancylonema*, *Chloromonas* and *Chlamydomonas*. Species known to form resting stages were also present, such as *Polarella glacialis*, *Chloromonas* sp., *Chlamydomonas* sp. and *Ochromonas* sp.

The Shannon diversity index indicated the same high variability as for the bacterial communities, ranging from extremely low diversity in the Lake A and Char Lake snow samples to higher values in the fjord vicinity (Table 1). The low diversity communities of Lake A and Char Lake snow were dominated by the same eukaryotic OTU, which was 99.8% similar to *Ochromonas* sp. CCMP1899 isolated from sea ice in Antarctica; this taxon accounted for 96% and 64% of the clones respectively. This dominance is consistent with the abundance of the *Ochromonas* cell type observed by fluorescence microscopy one year later (see Microbial DNA and cells).

Similar to the bacterial results, the site nearest to the sea on Disraeli Fjord (DFC), had a large proportion of its community represented by marine taxa (7/16 OTUs or 38/88 clones, Fig. 7). Conversely, the communities of the innermost sites (DFA and DG) had sequences with closest matches to a number of terrestrial representatives (notably the genera *Leucosporidium*, *Ancylonema* and *Chloromonas*), although the marine signal was still detectable in the Disraeli Fjord A sample.

DFAe and DFCe were the only clone libraries for which the rarefaction curves did not reach an asymptote (Suppl. Fig. 1). The Chao1 values were 83% for DFAe and 70% for DFCe, but 100% for all of the other samples, indicating good coverage.

Bacteria in the atmosphere. For the Ward Hunt air sampling, field blanks and PCR negative controls all showed undetectable amounts of DNA, implying an absence of contamination. The air sample extract contained 16 ng DNA/mL corresponding to a total of 0.64 ng of DNA in ~49 m^3 of sampled air (13 pg/m^3). We retrieved 71 16S rRNA gene clones from the air sample library. These clustered into 14 OTUs (Suppl. Table 1). The taxa belonged to the major phyla *Bacteriodetes*, *Acidobacteria*, *Firmicutes* and *Proteobacteria* with representatives from *Alpha-*, *Beta-* and *Gammaproteobacteria* Classes. Eleven of the OTUs were previously reported from cold environments, including two OTUs previously only isolated from the sea. These included *Roseobacter* and an uncultured *Cytophagales*. Two other OTUs belonged to the genera *Lactobacillus* and *Staphylococcus,* and the final OTU had no match >97% similar in GenBank but showed 95.4% similarity to an *Acidobacteria* isolate. Four OTUs detected in snow the preceding year were also found in this air sample. They were related to *Aquaspirillum arcticum*,

Janthinobacterium, *Rhodoferax* and *Pseudomonas syringae*. One *Polaromonas* sequence was also found in a chimera revealing its presence in the air but its sequence was excluded from the analysis. As in the snow bacterial clone libraries, chloroplast 16S rRNA gene sequences were detected with eight sequences that clustered into the algal class *Prasinophyceae* and an unknown phylum (Suppl. Table 2).

DISCUSSION

Microbial cells and DNA were detected in the snow at all locations, and microbial DNA was also collected in the 24 h air-sampling on Ward Hunt Island. These observations imply that microbiota are widely dispersed via wind and precipitation across the High Arctic, and are a common feature of the snowpack environment. The clone library analysis revealed a relatively diverse ensemble of taxa with a total of 25 bacterial OTUs in the snow and air and 23 eukaryotic OTUs in the snow. No previous molecular studies have been conducted on the snow and aerial microbiology at these remote, far northern sites, and there is little data of this type from the polar regions in general. The annual snow cover melts at these sites each summer, and the microbial content of the snow is therefore the accumulation of aerial transport by wind and precipitation, and possibly growth, over the preceding months.

Spatial distribution of cells. Extracted DNA and prokaryotic cells concentrations were at or below the lower end of the range of published values for snow, which vary from 200 cells/mL in South Pole snow (14) to 7.2 x 10^5 cells/mL in the snow cover of Zadong Glacier on the Tibetan Plateau (32). The low microbial concentrations in High Arctic snow may reflect the remote geographic location of the sampling sites relative to temperate snow packs that receive much greater microbial loading from surrounding ecosystems, and the persistent low temperature

regime of the high latitude environment that inhibits *in situ* growth rates. Post-depositional processes can have a strong influence on microbial abundance in snow (67). DNA quantification and the DAPI cell counts showed that the spatial distribution of microbes was non-uniform across the snow cover. This high degree of patchiness is consistent with the distribution of sediments on Ward Hunt Ice Shelf near our Ellesmere Island sampling sites (39). In 2001, sediment cover varied from 12% of the western surface of the ice shelf, to 14% in the middle and 2% on the eastern sector. This trend was also reported for other Ellesmere Island ice shelves, and is reflected in the patchy spatial distribution of microbial mats that grow in these environments. On Markham Ice Shelf, the sediment content of the 20-mm thick mats was substantial and constituted 75-91% of the mat dry weight (63). Eolian processes are likely to contribute to this patchy distribution of sediments and microbes. Although our snow samples contained fine sediment, which was visible after melting the snow, because of logistic constraints this was not quantified. Wind transport has also been shown as an important vector of microbes in temperate locations (22). There is also likely to be a positive feedback, with nutrients released from the sediments enhancing the local growth of associated, wind-blown microbes, and heat absorption by the sediments causing meltwater production for microbial growth (see below). These microbial hot-spots of colonization and growth may also act as local inoculum for broader dissemination across the snowpack.

Spatial distribution of taxa. Samples grouped into subclusters according to the potential source environments for their communities (Fig. 6). In the vicinity of the fjord, most sequences matched to obligate marine taxa. In the northernmost site (DFC), both bacterial and eukaryotic communities were rich in marine species. Disraeli Fjord site B community was also made up of marine bacteria such as *Glaciecola* spp. and *Loktanella* sp. The clustering of Disraeli Fjord site A

and the Disraeli Glacier site, located southward, is likely due to the presence of characteristic terrestrial species (*Leucosporidium* sp. and *Chloromonas* sp.) *Polarella* sp., a sea ice dinoflagellate (38) was the only taxon found both in the snow of site A and site C, with a relative abundance of at least 20% in each library. These observations suggest that the fjord valley may channel the wind through to these sites. The wavelike ridges observed at the surface of the glacier oriented in the direction of the valley also support this idea.

Interestingly, the communities in Lake A and Char Lake snow were highly similar. The two lakes have a similar topology but greatly vary in area (Char Lake 0,53 km^2; 53, and Lake A 5 km^2; 59), and lie 1000 km apart.

The low level of similarity between the bacterial communities of the air and the snow samples could reflect the different time spans of sampling since most of the snow microbiota would have accumulated over weeks to months prior to being sampled, while the air sampling provided only a snapshot of the dispersed microbiota. However, some taxa (*Aquaspirillum arcticum*, *Rhodoferax* sp. and *Polaromonas* sp.) were detected in both the air and snow, suggesting that these bacteria are especially abundant and commonly dispersed.

Local sources. Despite harsh environmental conditions in the Canadian High Arctic, this region supports several ecosystem types in the vicinity of our sampling sites (64 and references therein), and these may provide local sources of inocula for the snow microbial assemblages. In particular, microbial mats form biomass-rich communities on ice shelves, wetlands and at the bottom of lakes and ponds. These mats consist of diverse microbial assemblages consolidated by intertwined trichomes of cyanobacteria, and they include bacteria, archaebacteria, eukaryotic

469 microbes, viruses and metazoans such as rotifers, tardigrades, nematodes and the free living
470 tubellarian platyhelminths, all contained within a cohesive matrix of exopolymeric substances
471 (63). Metagenomic analysis has shown that these mats are functionally as well as taxonomically
472 diverse, and that they are genetically dominated by *Proteobacteria* and *Cyanobacteria* (60).
473 We detected cyanobacteria in the snow by molecular analysis and by cultivation, and many of
474 these OTUs had a close match to sequences previously determined for microbial mats in this
475 High Arctic region. The bacterial communities of Ward Hunt and Markham ice shelf microbial
476 mats were examined by clone library analysis of their 16S rRNA genes in 2005 and 2006 (8).
477 Again, some of those taxa were highly similar to our High Arctic sequences: four snow OTUs
478 were 97.2% to 100% similar to the mat sequences. In particular, the genera *Polaromonas,*
479 *Rhodoferax* and *Aquaspirillum* were similar in both snow and ice shelf mats. One sequence of
480 *Brevundimonas* detected in the air was 98.9% similar to a mat isolate. These results suggest that
481 Arctic mat microbes are dispersed in the atmosphere and across the snow cover. This dispersion
482 aids new mat development, when the bacteria or cyanobacteria colonize snow or ice regions with
483 favourable conditions. As the mats grow this would result in locally reduced albedo and more
484 rapid meltwater generation, in turn favouring mat growth and the attrition of snow and ice. The
485 role of cyanobacterial mats as accelerators of ice melt was noted by Nordenskiöld on the
486 Greenland Ice Cap (31) and by Mueller and Vincent (41) for the Ellesmere ice shelves. The
487 present results extend these observations by showing that these microbial mat-forming agents are
488 widely dispersed in Arctic snow.
489
490 Three of the bacterial OTUs collected by the air sampler on WHI were affiliated with taxa often
491 associated with humans: *Stenotrophomonas maltophilia*, *Lactobacillus delbrueckii* and
492 *Staphylococcus hominis* (Suppl. Table 1). However, although *S. maltophilia* is commonly

493 isolated from clinical specimens, it is also widely distributed in natural environments, especially
494 in the soil and plant rhizosphere (27) and can utilize simple organic compounds such as acetate,
495 succinate and propionate as its sole carbon source (56). In contrast, *Lactobacillus* and
496 *Staphylococcus* spp. are more likely to come from anthropogenic activities. For example, *S.*
497 *hominis* is a member of the airborne bacterial population within the confined Antarctic Base
498 Concordia (58) and *L. delbrueckii* is used in the manufacturing of dairy products. Our air sampler
499 was located 500 m from the camp on Ward Hunt Island, which has a 50 year history of
500 intermittent summer occupation by military personnel, scientists and explorers, and it is likely
501 that human-associated microbes have been widely dispersed across the site. Pearce et al. (45)
502 reported bacterial populations collected over a two-week period in the atmosphere of the Halley
503 V research station in Antarctica, with 35% of sequences appearing to be related to human-
504 associated bacteria. During summer, Halley Station receives up to 70 people and human-
505 associated microbiota are a likely component of the aerobiology, masking to some extent the
506 natural signal of this otherwise pristine environment.
507
508 Although it is possible that the communities resulted from long range dispersal of cosmopolitan
509 taxa followed by environmental selection for growth of cold-adapted genotypes in the snow, the
510 most parsimonious explanation is that the biota in the snow was predominantly from the local
511 sources that they resembled. The transient nature of the snowpack in which the microbial load is
512 renewed each year leads to only restricted opportunities for growth. The air samples provided
513 more direct evidence of local sources. Growth in the air sampler is unlikely and conditions are
514 very different than in either the sea or terrestrial mats and lakes. Air constantly was pumped
515 through the tube over the whole sampling period likely leading to cell desiccation. Microbes
516 detected in this sample were predominantly cold taxa, many similar to those found in the snow

517 the year before (*Aquaspirillum* sp., *Rhodoferax* sp. and *Polaromonas* sp.), including some
518 obligate marine microbes.
519
520 **Regional sources.** Some of the snow samples contained a relatively high abundance of bacteria
521 characteristic of cold oceanic waters, notably the genera *Glaciecola*, *Colwellia*, *Loktanella* and
522 *Polaribacter.* This unexpected observation, given the low conductivity of the snow, suggests an
523 input of bacteria originating from marine aerosols, and it provides evidence of dispersal of marine
524 microbiota via the atmosphere within the High Arctic. Snow samples also contained eukaryotic
525 microbes that have been exclusively isolated from the marine environment in the past.
526 Furthermore, the air sample contained chloroplast DNA of *Micromonas*, a major component of
527 the Arctic Ocean phytoplankton (36), and sequences of obligate marine bacteria, providing
528 evidence of the active aerial transport of marine microbiota. All of the sampling sites were at or
529 near the coast, and it is likely that the Arctic Ocean and sea ice served as inoculum. Sea ice with
530 extremely high concentrations of cells, especially diatoms, within brine channels is often more
531 productive per m^3 compared to the underlying pelagic zone (12). In the present study, the Disraeli
532 Fjord C clone library contained a high proportion of marine taxa with the eukaryotic community
533 dominated by a marine diatom. Many sequences corresponding to the chloroplast 16S rRNA gene
534 of marine diatoms was also found in the bacterial clone libraries of Disraeli Fjord B and C
535 (Suppl. Table 2). Brinkmeyer et al. (11) found *Octadecabacter* sp. in both Antarctic and Arctic
536 sea ice but it was more abundant in the latter. In our Disraeli Fjord B snow sample, one OTU
537 (represented by DFBb6 in Suppl. Table 1) accounted for 13% of the clones bearing bacterial
538 sequence in the library and was identical (1390/1390 bp) with those reported by Brinkmeyer et al.
539 (11). Although some salty microenvironments in the snow may exist in microfilms around snow
540 and sediment granules and could support marine taxa to some extent, the overall detection of

marine microbes in the Arctic atmosphere strongly suggests a local input from the Arctic Ocean, especially as these taxa were also detected in the air samples and the predominant winds came across the sea from the north (Fig. 3). These results imply that sea ice is a major source for the High Arctic snowpack microflora and that sea ice microbiota are widely dispersed by the wind, not just by ocean currents.

One likely mechanism that could spread microbes from sea ice to the atmosphere is exposure of the ice community to the air by frost flowers. These surface structures are created under extreme cold and dry conditions by salt exclusion of the ice matrix during freezing (46). They have been found to contain 3-6 fold higher concentrations of bacteria than in the underlying ice, and thought to be important for the long-range transport of ice-nucleating particles in the atmosphere (10). In August 2008, we observed frost flowers on Quttinirpaaq Lagoon, a brackish water feature north of Ward Hunt Island, and they are also likely to form on the sea ice along the coast.

The circumpolar flaw lead occurs intermittently along the northern coast of Ellesmere Island throughout the year, and the resultant open water could also provide a microbial inoculum. The sea-surface microlayer is concentrated in microbes compared to the subsurface waters and has been implicated in the enrichment of microbes in marine aerosols produced via wave action and bubble bursting. Marine aerosols can be up to 22-fold enriched in microbes compared to bulk seawater (2).

Cosmopolitanism and long range transport. Most of the microbes detected in the snow and air had best matches to sequences from other cold environments including: Antarctica (some with 100% similarity, e.g. *Octadecabacter,* see Suppl. Table 1), the Tibetan Plateau, alpine regions of

565 Japan, Europe and North America, including the Arctic. No clones were >98% similar to
566 sequences isolated from more typically warm regions, with the exception of WHIb50 that was
567 98.1% similar in its 16S rRNA gene sequence with *Pseudomonas* PsI isolated from a woodland
568 soil of the arid southwest United States. Rodrigues et al. (50) reported a global distribution of the
569 psychrophilic species of *Psychrobacter*, which was abundant in polar environments and in
570 temperate and tropical zones. Similarly, Jungblut et al. (29) reported cyanobacterial ecotypes
571 throughout the cold biosphere, and the absence of any gene sequences from warmer regions that
572 matched High Arctic strains. Our detection of these taxa in the High Arctic snowpack is
573 consistent with the global dispersal of a set of cold-adapted ecotypes throughout the cold
574 biosphere. Other sequences from High Arctic snow had no close matches in GenBank, and might
575 represent novel species, particular to the Arctic. For example, clone DFCb18 in Disraeli Fjord
576 snow showed only 94.7% similarity with its closest match *Deinococcus radiomollis* strain PO-
577 04-20-144; this latter strain was isolated from a Mexican alpine soil at 5065 m and is
578 psychrophilic (13).
579
580 **Viability in the snow.** The snow microbial communities likely contained a mixture of non-living
581 and living cells. The presence of marine bacteria and eukaryotes implied that part of the
582 microflora is from marine microbial aerosols that may have acted as nucleation agents for the
583 subsequent snowfall. Given the obligate requirement of many of these taxa for marine salts, it is
584 unlikely that these cells would grow in the low conductivity waters of snowmelt, although they
585 could briefly survive in microenvironments of boundary layers of concentrated salts surrounding
586 ice and soil grains. The eventual lysis of such cells by osmotic stress during snow melt would
587 contribute nutrients and organic carbon substrates for the growth of the non-halophilic taxa.
588

589 Felip et al. (18) studied the microbial communities in the ice and snow cover of three high
590 altitude lakes in the Tyrolean Alps, and found that algal and protozoan biomass and microbial
591 activity were greater in the snow cover compared to the underlying lakes. Tyrolean Alps genera
592 (*Gymnodinium*, *Ochromonas* and *Chlamydomonas*) were also detected in our High Arctic snow
593 suggesting that these are likely true snow algae. We successfully cultivated cyanobacteria from
594 snow samples, providing evidence that these organisms remain viable in snow. If phototrophs are
595 able to grow in the snow it is likely that some of the heterotrophic bacteria may also be viable and
596 active. Several of the bacteria identified from the snow reportedly have low nutrient
597 requirements and may grow on diverse substrates (e.g. 26, 43, 48, 68). Notably, 11 of the genera
598 we recovered contain psychrophilic representatives, with potential to grow *in situ*. Several
599 heterotrophic bacteria isolated from Ellesmere Island ice shelf mats grow under low nutrient and
600 cold temperature conditions (8), and many of the same OTUs from these mats were found in our
601 snow samples. Environmental surveys based on RNA would provide additional insights into
602 which taxa are metabolically active in the Arctic snowpack. Contrary to our initial expectation,
603 no spore-forming bacteria were detected in the High Arctic snow samples. This absence might be
604 the result of methodological bias, for example, the difficulty of extracting DNA from bacterial
605 spores, and this negative result is inconclusive.
606
607 In summary, our snow and air samples from northern Canada contained a distinct assemblage of
608 heterotrophic and phototrophic microbes, and such communities are likely distributed throughout
609 the High Arctic. A proportion of the microbial community was likely derived from two disparate
610 sources near our sampling sites, specifically freshwater microbial mats and Arctic Ocean sea ice.
611 The heterogeneous distribution of microbes in the snow suggested that microbes were
612 redistributed into patches via wind action and localised melting. The high similarity between

some of the Ellesmere Island SSU rRNA gene sequences to those found in polar and alpine environments elsewhere in the world suggests that High Arctic microbes are globally distributed in cold regions. These results show the value of the Ward Hunt Island region as a set of extreme northern sites for the study of snow and air microbiology. Further studies are needed to test other phylogenetic markers with finer resolution to unravel the relationships between High Arctic microbes and those from elsewhere. Deeper sampling of the rarer taxa could also be achieved with next generation sequencing technologies, such as pyrosequencing, as in Galand et al. (21). However the tradeoff is a loss of fine taxonomic resolution, and a combination of approaches is required. Arctic microbial ecosystems are currently experiencing accelerated climate change (62), and the microbiology of High Arctic snow will require ongoing surveillance. Our detection of close relatives of well-known ice-nucleating bacteria (e.g., *Pseudomonas syringae*) in the air also highlights the need to study the role of microbes in atmospheric chemistry in the rapidly changing north polar environment.

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827 FIG. 1. Location of the sampling sites in High Arctic Canada. On the northern coast of Ellesmere
828 Island, Quttinirpaaq Lagoon (QL, purple dot), Ward Hunt Island (light blue dot) including Ward
829 Hunt Lake (WHL) and the air sampling site (WHI), Ward Hunt Ice Shelf (WHIS, white dot),
830 Lake A (LA, dark blue dot), Disraeli Fjord site C (DFC, orange dot), site B (DFB, yellow dot)
831 and site A (DFA, red dot), Disraeli Glacier (DG, green dot). The WHIS (black area) surrounds
832 Ward Hunt Island. On Cornwallis Island, Char Lake (CL, pink dot) is in the vicinity of Resolute
833 Bay. Modified from Mueller et al. (39) and Van Hove et al. (59).
834
835 FIG. 2. Depth of snow over the ground on the northern shore of Ward Hunt Island between
836 August 2007 and August 2009. Arrows indicate the moment of snow sampling for the molecular
837 analyses (plain arrow) and the microscopic analyses as well as the air sampling (dotted arrow).
838
839 FIG. 3. Direction and speed (m s^{-1}) of winds during the air sampling, from July 7 to 9 2009. Dots
840 indicate the hourly averages of wind speed (values on the axes) and direction (from which winds
841 came from).
842
843 FIG. 4. Extracted DNA concentrations in the small (0.2 to 3 µm) and large (> 3µm) fractions
844 from High Arctic snow samples collected in May-June 2008 (QL = Quttinirpaaq Lagoon, WHL =
845 Ward Hunt Lake, WHIS = Ward Hunt Ice Shelf, LA = Lake A, DF = Disraeli Fjord, DG =
846 Disraeli Glacier, CL = Char Lake, Neg. = field blanks). The sites were sampled in duplicate at a
847 distance of tens of meters apart.
848

849 FIG. 5. Concentration of prokaryotic and *Ochromonas* cells (note the log scale) in the snow
850 cover of Ward Hunt Lake (WHL), Lake A (LA) and Char Lake (CL) in July 2009 determined by
851 DAPI slide counts.
852
853 FIG. 6. Bray-Curtis cluster analysis comparing the bacterial (top) and eukaryotic (bottom)
854 communities detected in the snow in May-June 2008 at Char Lake sites A and B (CLA and
855 CLB), Lake A (LA) Disraeli Fjord sites A, B and C (DFA, DFB and DFC), and Disraeli Glacier
856 (DG), and in the air of Ward Hunt Island (WHI) in July 2009. Comparison takes into account the
857 relative abundance of each OTU in the clone libraries and results from 1000 replicates (values
858 indicated at the nodes).
859
860 FIG. 7. Source environment of eukaryotic clones according to sampling site and compiling the
861 isolation source of all the sequences in GenBank having >98% similarity with a specific clone
862 representing one OTU. Clone abundances were determined from the RFLP pattern repeats. The
863 numbers of clones were 88 in Disraeli Fjord C, 91 in Disraeli Fjord A and 27 in Disraeli Glacier.

TABLE 1. Bacterial and eukaryotic diversity indices for samples at each site

Sites	Shannon [a, b]		Chao1 [b]	
	Bacteria	*Eukarya*	*Bacteria*	*Eukarya*
Lake A (LA)	0.18 ± 0.19	0.18 ± 0.27	2	2
Disraeli Fjord site C (DFC)	1.44 ± 0.22	1.77 ± 0.36	8	23
Disraeli Fjord site B (DFB)	1,86 ± 0.22	N/A	10.5	N/A
Disraeli Fjord site A (DFA)	N/A	0,89 ± 0.25	N/A	6
Disraeli Glacier (DG)	N/A	0.93 ± 0.25	N/A	4
Char Lake site A (CLA)	0.36 ± 0.25	N/A	4	N/A
Char Lake site B (CLB)	0 ± 0	0 ± 0	1	2

[a] The values after ± specify the lower and upper bound of a 95% confidence interval.

[b] N/A means that no clone library was done for these samples.

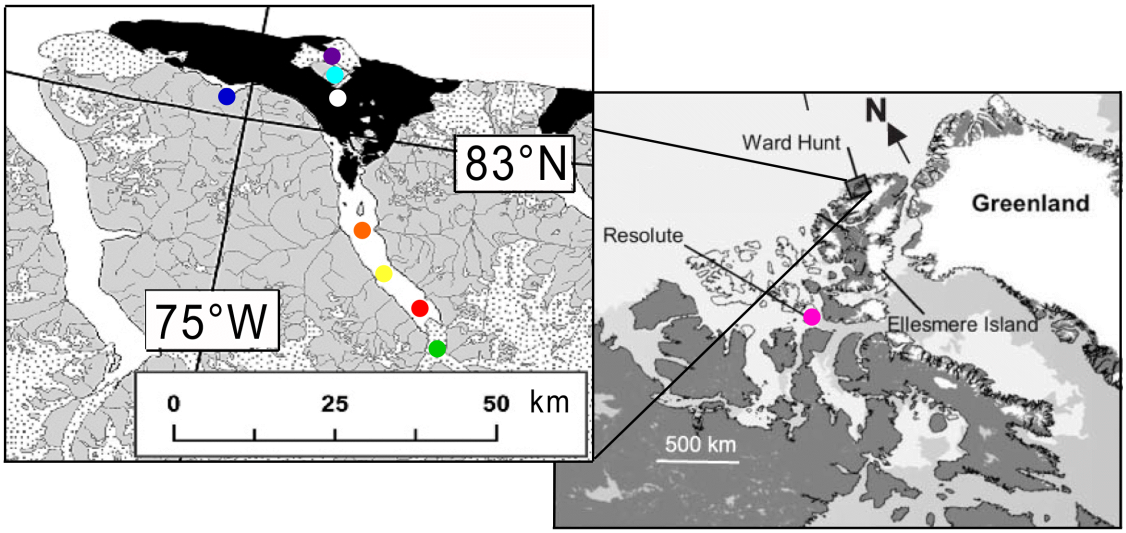

Fig. 1

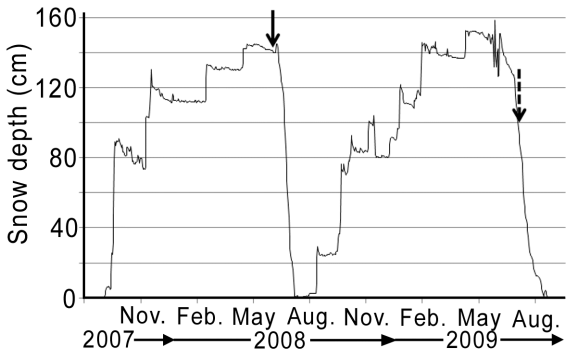

Fig. 2

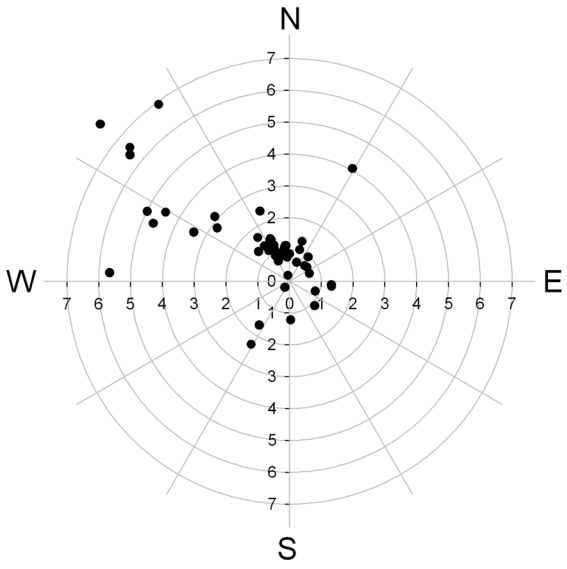

Fig. 3

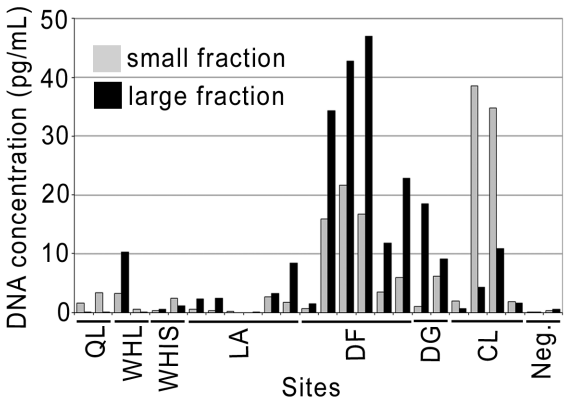

Fig. 4

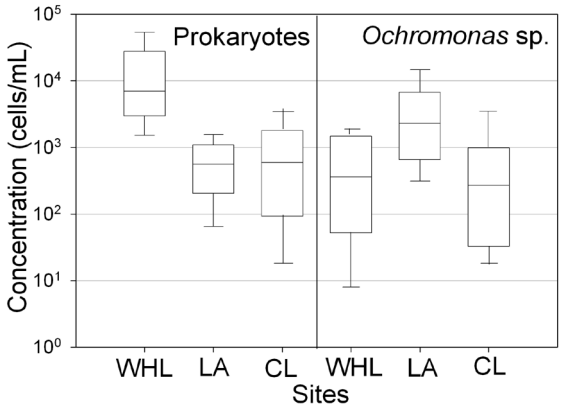

Fig. 5

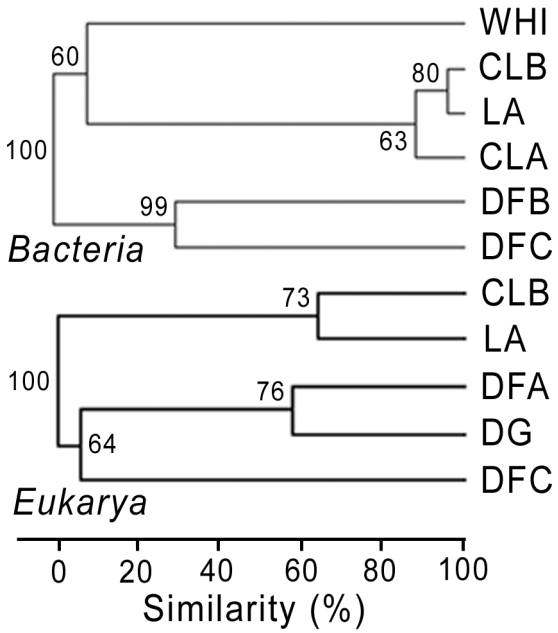

Fig. 6

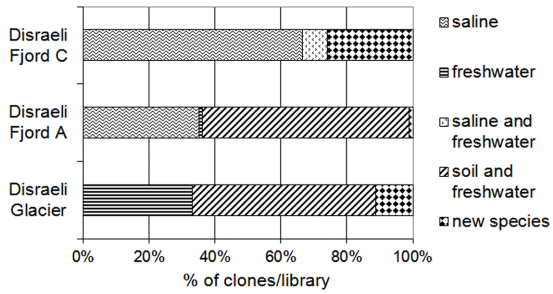

Fig. 7