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Publisher's version / Version de l'éditeur:

<https://doi.org/10.1139/w11-004>

Canadian Journal of Microbiology, 57, 4, pp. 303-315, 2011-04-08

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Microbial diversity of active layer and permafrost in an acidic wetland from the Canadian High Arctic

Roland C. Wilhelm, Thomas D. Niederberger, Charles Greer, and Lyle G. Whyte

Abstract: The abundance and structure of archaeal and bacterial communities from the active layer and the associated permafrost of a moderately acidic (pH < 5.0) High Arctic wetland (Axel Heiberg Island, Nunavut, Canada) were investigated using culture- and molecular-based methods. Aerobic viable cell counts from the active layer were ~100-fold greater than those from the permafrost (2.5×10^5 CFU·(g soil dry mass)⁻¹); however, a greater diversity of isolates were cultured from permafrost, as determined by 16S rRNA gene sequencing. Isolates from both layers demonstrated growth characteristics of a psychrotolerant, halotolerant, and acidotolerant community. Archaea constituted 0.1% of the total 16S rRNA gene copy number and, in the 16S rRNA gene clone library, predominantly (71% and 95%) consisted of *Crenarchaeota* related to Group I. 1b. In contrast, bacterial communities were diverse (Shannon's diversity index, $H = \sim 4$), with *Acidobacteria* constituting the largest division of active layer clones (30%) and *Actinobacteria* most abundant in permafrost (28%). Direct comparisons of 16S rRNA gene sequence data highlighted significant differences between the bacterial communities of each layer, with the greatest differences occurring within *Actinobacteria*. Comparisons of 16S rRNA gene sequences with those from other Arctic permafrost and cold-temperature wetlands revealed commonly occurring taxa within the phyla *Chloroflexi*, *Acidobacteria*, and *Actinobacteria* (families *Intrasporangiaceae* and *Rubrobacteraceae*).

Key words: active layer, permafrost, wetland, Arctic, microbial diversity, 16S rRNA gene library.

Résumé : L'abondance et la structure des communautés d'archées et de bactéries de la couche active et du pergélisol d'un milieu humide modérément acide (pH < 5,0) du Grand Nord (Île de Axel Heiberg, Nunavut, Canada) ont été étudiées à l'aide de méthodes en culture et moléculaires. La numération des cellules aérobies viables de la couche active était ~100 fois supérieure à celle du pergélisol ($2,5 \times 10^5$ UFC·(g ps de sol)⁻¹); cependant, une diversité plus élevée d'isolats a été cultivée à partir du pergélisol, tel que déterminé par le séquençage génique de l'ARNr 16S. Les isolats des deux couches montraient les caractéristiques de croissance d'une communauté psychrotrophe, halotolérante et acidotolérante. Les archées constituaient 0.1% du nombre de copies de gènes d'ARNr 16S total et, dans la banque de clones de gènes d'ARNr 16S, comprenaient de façon prédominante (71 % et 95 %) le groupe I. 1b relié aux *Crenarchaeota*. Par contre, les communautés bactériennes étaient diversifiées (indice de diversité de Shannon, $H = \sim 4$), les *Acidobacteria* constituant la division la plus large des clones de la couche active (30 %), et les *Actinobacteria* étant les plus abondantes dans le pergélisol (28 %). Des comparaisons directes des données du séquençage génique de l'ARNr 16S ont mis en évidence des différences significatives entre les communautés bactériennes de chaque couche, les différences les plus importantes apparaissant au sein des *Actinobacteria*. La comparaison des séquences géniques d'ARNr 16S trouvées ici avec celles d'isolats d'autres pergélisols et d'autres milieux humides arctiques de basse température a révélé que les phylums des *Chloroflexi*, *Acidobacteria* et *Actinobacteria* (familles *Intrasporangiaceae* et *Rubrobacteraceae*) constituaient les taxons les plus communs.

Mots-clés : couche active, pergélisol, milieu humide, arctique, diversité microbienne, banque génique d'ARNr 16S.

[Traduit par la Rédaction]

Introduction

Soil in polar and alpine regions generally comprises an active layer, which undergoes seasonal thaw, and a permafrost layer, which remains below 0 °C for more than two consecutive years (Permafrost Subcommittee 1988). Together, the thermally distinct soil layers are referred to as cryosol (Can-

ada, Europe), Gelisol (United States), or Cryozem (Russia), depending on the soil classification system (European Commission – Joint Research Centre 2010). These layers are not only stratified by annual temperature regime (i.e., depth of warming) but also by impermeable ice at the permafrost boundary, which impedes gas and nutrient exchange (for greater detail on cryosol see Kimble 2004). The differences

Received 12 October 2010. Revision received 17 December 2010. Accepted 7 January 2011. Published at www.nrcresearchpress.com/cjm on 8 April 2011.

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in the microbial communities surviving in these extreme ecological niches are new areas of exploration for science since the discovery of diverse and functional cold-adapted communities in each (Steven et al. 2009; Wagner and Liebner 2009). Primary research on the microbial ecology of these inhospitable environments aims to better understand the low-temperature thresholds of life, assisting in the search for life beyond Earth's atmosphere, and is of importance for monitoring the ecological shifts anticipated with the gradual thawing of the upper permafrost table (Intergovernmental Panel on Climate Change 2007).

Despite the harsh conditions of cryosol, such as prolonged subzero temperatures, low water activity, sparse nutrient availability, and long-term background radiation exposure (Steven et al. 2006), molecular-based estimates of diversity for microbial communities in both layers of cryosol are high. Active layer soils in the High Arctic tundra (latitude 82°N) show greater bacterial diversity than more temperate soils based on extensive ribotagged pyrosequencing (Neufeld and Mohn 2005) and are reported to share similar biodiversity with soils of all latitudes (Chu et al. 2010). The first comparative metagenomic study of an active layer and 2-m-deep permafrost sample reported a comparably high level of diversity in both layers, with 711 taxa common to both and 268 unique to either layer (Yergeau et al. 2010). Enumerations of the viable heterotrophic community demonstrate the presence of a moderately sized culturable community ($\sim 10^5$ cells·(gram of soil dry mass)⁻¹ in various permafrost environments, e.g., Spitsbergen permafrost (Hansen et al. 2007), Ellesmere Island permafrost (Steven et al. 2008), and Alpine permafrost (Bai et al. 2006), while counts as high as 10^8 cells·(g dm)⁻¹ have been reported from Siberian permafrost (Gilichinsky 2002). The viable cell counts in the active layer are generally 100–1000 times greater than in the permafrost due to harsher conditions (Steven et al. 2006; Gilichinsky et al. 2008). In situ experiments on activity in permafrost have detected the mineralization of radiolabeled substrates (acetate and glucose) at temperatures as low as -15 °C (Steven et al. 2008). Furthermore, the presence of functional and phylogenetic gene markers in environmental metagenomes suggests a complex functional community where methanogens, methanotrophs, nitrogen-cycling Bacteria, and a diverse and abundant population of carbon-cycling organisms are present in both layers (Yergeau et al. 2010). Yet, the majority of knowledge from which comparisons of active layer and permafrost are made originates from independent study sites, and the differences within a single, associated cryosol have yet to be thoroughly explored.

To date, two studies have investigated the differences in active layer and permafrost microbial communities along a single soil transect (Steven et al. 2008; Yergeau et al. 2010). Both found significant differences based on direct comparisons of total community DNA, although they also reported general, overarching similarities in taxonomic structure. The metagenomic analysis performed by Yergeau et al. (2010) found that the majority of taxa (73%) were detectable in both active and permafrost layers. Notably, both of these studies originated from the same study site in Eureka, Nunavut, providing a narrow representation of the diversity of cryosol environments. The present investigation of an acidic wetland cryosol from Axel Heiberg Island in the Canadian High Arctic

will further develop our understanding of the similarities and differences between active layer and associated permafrost soil while providing preliminary data on an ecosystem undergoing expansion as a result of Earth's warming climate.

The average temperature increase in the Arctic is estimated at nearly twice the global rate of climate warming (Intergovernmental Panel on Climate Change 2007) and is predicted to lead to significant expansion of wetland areas as low-density ice structures within soils subside creating depressions, termed thermokarst features, where melt water collects (Schoor et al. 2008). A similar transformation occurred from the rise of global temperatures in the early Holocene period, transforming approximately 30% of modern day Siberia into thermokarst-affected terrain (Zimov et al. 1997). Accompanying this broad ecosystem shift was the translocation of an estimated ~ 500 Gt carbon, largely in the form of methane, from soil reservoirs due to microbial activity (Zimov et al. 1997). Knowledge on Arctic wetland microbial communities is therefore of increasing importance.

Current knowledge of the microbial ecology of Arctic wetland systems is limited to specific subpopulations involved in methane cycling in active layer soils (Wagner and Liebner 2009), while that of permafrost has yet to be studied. The broader microbial community of an Arctic wetland, for which "intermediary ecosystem metabolism" is of known importance in methanogenesis (Drake et al. 2009), has yet to be thoroughly characterized. The current lack of understanding of wetland soil trophic interactions and their impact on geochemical cycling, together with the anticipated expansion of Arctic wetland areas, exemplifies the need to study Arctic wetland microbial communities.

In this study, the bacterial and archaeal communities of active and permafrost soils from an acidic High Arctic wetland were examined using a combination of culture-independent (denaturing gel gradient electrophoresis (DGGE), 16S rRNA gene clone libraries, enumeration by quantitative real-time PCR (qPCR), and fluorescent microscopy) and culture-dependent (viable heterotrophic plate counts, strain identification via 16S rRNA gene sequencing, and tolerance testing) analyses, to determine their abundance, diversity, and composition. The goals were to provide a detailed account of an Arctic wetland microbial community, for which we have information only on the active layer communities (Costello and Schmidt 2006; Dedysh et al. 2006; Høj et al. 2008; Liebner et al. 2008), and to contribute to the body of information regarding the differences between the active layer and permafrost microbial communities. To enhance the comparison between the layers and to generalize the findings to other geographically distinct studies of cryosol, the 16S rRNA gene sequence data from this study was compared with sequences from other Arctic permafrost and cold-temperature wetlands.

Methods

Site description

Samples were taken from the edge of an acidic wetland located in Expedition Fjord (79°24.918'N, 90°45.424'W), Axel Heiberg Island, in the Canadian High Arctic in early spring (mid-April 2008). The region is characterized as a polar desert with a mean annual air temperature of -15.2 °C (Doran

1993). Air temperature extremes have been measured as low as -50°C (Anderson 2009) and as high as 25°C (field season, July 2009). The average depth of the active layer throughout the wetland by midsummer (July 2009) was approximately 0.6 m. The soil was previously described as a black Gleysolic cryosol (Buttle and Fraser 1992). The temperature of the active layer soil, at a depth of 40 cm, was -16°C starting early spring (5 May) and increased to 4°C by midsummer (15 July). The soil temperature was measured by placing an iButton data logger (Maxim Integrated Products, California) at the bottom of an insulated drill hole.

Soil core processing and physical and chemical analysis

Subsurface soil was extracted using a "TE-ATC Combhammer" drill (Hilti, Canada) with a 3 inch (1 inch = 25.4 mm) diameter corer sterilized with 70% ethanol prior to each extraction. Core subsections were taken along the soil column from 6 to 107 cm deep in roughly 5–10 cm lengths. Six samples representative of different depths were collected: active layer — MRSC-2 (6–10 cm), MRSC-4 (14–19 cm), and MRSC-5 (30–35 cm); permafrost table — MRSC-8 (55–60 cm); permafrost — MRSC-12 (74–79 cm) and MRSC-18 (97–100 cm). Samples were transported frozen and stored at -20°C . Sterility of samples was maintained by using soil from the interior of the core in a process outlined by Juck et al. (2005). Physicochemical analyses of soil pH, conductivity, dissolved organic carbon, total carbon content, nitrate concentration, and total nitrogen content were performed as previously described in Steven et al. (2007). Gravimetric water content was based on differences in mass of 15 g of soil per sample following drying at 100°C for approximately 24–32 h.

Community DNA extraction

For active layer and the permafrost table samples, community DNA was extracted from 2 g of soil using the UltraClean Soil DNA Isolation kit (Mo Bio Laboratories Inc., Carlsbad, California, USA), as described in the alternative protocol for maximum yields. Each extraction was performed in duplicate and pooled. The permafrost samples MRSC-12 and MRSC-18 required a larger mass of soil (10 g) to yield enough DNA for successful polymerase chain reaction (PCR) amplification. This was achieved using the UltraClean Mega Prep Soil DNA kit (Mo Bio), also performed in duplicate and pooled. All DNA extracts were purified using polyvinylpyrrolidone (PVPP) as described by Berthelet et al. (1996). DGGE comparisons between PVPP-purified and unpurified sample DNA revealed no difference in band patterning and intensity (results not shown), ensuring that PVPP purification did not bias community structure. Negative controls (H_2O in place of DNA or soil) underwent identical handling during the extraction procedure and PVPP purification steps to ensure zero contamination in downstream analyses.

Amplification of bacterial and archaeal 16S rRNA gene

16S rRNA gene PCR was performed on community DNA extracts and cultured isolates using bacterial primers 27F (5'-AGA GTT TGA TCC TGG CTC AG-3') and 1492R (5'-GGT TAC CTT GTT ACG ACT T-3') and archaeal primers 109F (5'-ACK GCT CAG TAA CAC GT-3') and 934R (5'-GTG CTC CCC CGC CAA TTC CT-3') (Steven et al.

2008). PCR reaction mixes (25 μL) contained 1 U of *Taq* polymerase (Invitrogen Canada), $1\times$ PCR buffer (200 $\text{mmol}\cdot\text{L}^{-1}$ Tris-HCl, 500 $\text{mmol}\cdot\text{L}^{-1}$ KCl, at pH 8.4), 3 $\text{mmol}\cdot\text{L}^{-1}$ MgCl_2 (5 $\text{mmol}\cdot\text{L}^{-1}$ for Archaea), 0.2 $\text{mmol}\cdot\text{L}^{-1}$ (each) deoxynucleoside triphosphate (dNTPs), 0.5 $\mu\text{mol}\cdot\text{L}^{-1}$ (each) primer, 0.6 μL of 10 $\text{mg}\cdot\text{mL}^{-1}$ bovine serum albumin, 2 μL of template DNA. Thermocycler conditions for both Archaea and Bacteria involved an initial denaturation of 3 min at 96°C ; followed by 30 cycles at 94°C for 1 min, 55°C for 1 min, and 72°C for 45 s; with a final extension at 72°C for 15 min.

DGGE

Bacterial primers 357F+GC and 518R were used according to O'Sullivan et al. (2008). Nested PCR was required to amplify substantial quantities of archaeal product. The first primer set was A347F (5'-CCA GGC CCT ACG GGG CGC A-3') and A1335R (5'-GTG TGC AAG GAG CAG GGA C-3') followed by A915F (5'-AGG AAT TGG CGG GGG AGC AC-3') and A1335R+GC. PCR reaction mixes (25 μL) were as previously stated with the following exceptions: for the first PCR, 0.2 $\mu\text{mol}\cdot\text{L}^{-1}$ (each) primer and 1.25 μL of dimethyl sulfoxide in place of bovine serum albumin (also for second reaction), and for the second PCR, 1.25 $\text{mmol}\cdot\text{L}^{-1}$ MgCl_2 , 0.2 $\mu\text{mol}\cdot\text{L}^{-1}$ (each) primer, 1.25 μL of DMSO, 0.75 U of *Taq* polymerase, and 1.5 μL of template DNA. Thermocycler conditions for A347F–A1335R were as follows: initial denaturation of 5 min at 94°C ; followed by 30 cycles of 94°C for 30 s, 60°C for 30 s, and 72°C for 2 min; with a final extension of 72°C for 10 min. Thermocycler conditions for A915F–A1335R+GC were as follows: initial denaturation of 5 min at 94°C ; followed by 22 cycles of 94°C for 30 s, 60°C for 1 min, and 72°C for 1 min; followed by 14 cycles of 94°C for 1 min, 60°C for 1 min, and 72°C for 1 min; with a final extension of 72°C for 5 min. DGGE was performed with 500 ng of DNA in a protocol similar to Steven et al. (2008). Comparisons of DGGE banding patterns were made with GelCompar software (Applied Maths NV, Belgium) and dendrograms constructed using the UPGMA method for grouping and the Jaccard coefficient of similarity.

Construction of bacterial and archaeal 16S rRNA gene clone libraries

PCR amplicons from community DNA were purified and pooled from three separate reactions to mitigate individual reaction biases. Ligation and transformation of clones followed by amplified ribosomal DNA restriction analysis were performed as described by Steven et al. (2007). Bacterial and archaeal clones were sequenced using the forward primers 27F and 109F, respectively, by Séquençage Laval (University of Laval, Québec, Québec, Canada) with an ABI Prism 3130XL genetic analyzer.

Bioinformatic analyses

Chimeric sequences were identified using the program Bellerophon (Huber et al. 2004) and were then verified with the PinTail algorithm (Ashelford et al. 2005) before being removed (10 sequences in total). Clone sequences, DGGE bands, and isolates were classified using the nucleotide BLAST search (BLASTn) from NCBI (Altschul et al. 1990)

and the Classifier tool from the Ribosomal Database Project (RDP) release 10 (Cole et al. 2009). Clone libraries were compared using the RDP tool LibCompare and LIBSHUFF (Singleton et al. 2001), as well as the Sørensen similarity index (Sørensen 1948). Phylogenetic trees were created using the MACVECTOR 7.0 software package (Oxford Molecular Ltd.). Tree construction involved a CLUSTALW multiple alignment joined using the neighbour-joining model. Distances were calculated using the Jukes–Cantor distance matrix. Bootstrap analysis was also performed using MACVECTOR 7.0 with 1000 replications. Coverage of clone libraries was estimated using Good's method, where n represented the number of operational taxonomic units (OTUs) at >97% similarity (Good 1953). The determination of OTUs, Shannon's diversity index (H'), Simpson's diversity index ($1/D$), Chao1 richness estimate, and rarefaction curves were performed with DOTUR (Schloss and Handelsman 2005). 16S rRNA gene sequences from other studies were chosen based on agreement between primers and similarity of study site and were acquired using NCBI GenBank accession numbers and compared with sequences from the current study using DOTUR based on OTUs at >95% similarity.

Fluorescent microscopic and viable heterotrophic cell enumerations

Total cell counts were enumerated with 5-(4,6-dichlorotriazinyl) aminofluorescein (DTAF) stain as described by Steven et al. (2007). A dilution series was used to determine viable heterotrophic cell counts on four different media at different pH and temperature. Based on previous permafrost microbial studies (Vishnivetskaya et al. 2000; Hansen et al. 2007; Steven et al. 2007), the media chosen were R2A agar (Becton, Dickson and Co.); 1:1 R2A diluted with water (supplemented with 1.5% agar (m/v)); mineral salts media augmented with yeast, tryptone, and soluble starch (MSM+YTS; 0.25 g each per L); and tryptone soy agar (TSA; Becton, Dickson and Co.). Enumerations were undertaken on media at neutral pH (R2A, pH 7.2; MSM+YTS, pH 7.0; and TSA, pH 7.3) and at acidic pH (pH 4.5 $\pm$ 0.1) mimicking in situ pH. Plates were incubated at 37, 25, and 5 °C until no additional colonies formed (7, 14, and 60 days, respectively). Enumerations were also performed at –5 °C on R2A + 7% (m/v) NaCl and on R2A at pH 4.5 and 7.0 under anaerobic conditions (0 ppm [O_2]) at 5 and 25 °C in an anaerobic chamber (COY Laboratory Products, Inc., Michigan, USA). Plate counts were performed in triplicate and viable cell enumerations were averaged across triplicates.

Identification and characterization of isolates

Isolates were selected from heterotrophic enumeration cultures based on morphology, pigmentation, texture, and colony size. Each colony was streak isolated (3 times) and DNA extracted via boiling lysis (Sambrook and Russell 2001), and the 16S rRNA gene was PCR amplified and sequenced as described for bacterial 16S rRNA gene clones. Eleven isolates were recalcitrant to restreaking (slow growth, or no longer viable) and were sequenced without streak isolation. Isolates were tested for growth at –5, 5, 25, and 37 °C and at increments of 1 pH unit beginning at pH 4 (the lowest

pH at which the agar solidified) to pH 9 on the media on which they originally grew. Salt tolerance was also tested at both pH 5 and 7 in medium supplemented with 0%, 2.5%, 5.0%, 7.5%, and 10% (m/v) NaCl.

Quantitative real-time PCR for archaeal and bacterial 16S rRNA genes

qPCR was performed using an iCycler iQ Real-Time PCR Detection System (Bio-Rad, California, USA). Plasmid standards were constructed by cloning the PCR-amplified target region into the pGEM-T Easy vector (Promega, Wisconsin). Plasmid DNA was isolated using the QIAprep Spin Miniprep kit (Qiagen, Mississauga, USA) and quantified using a NanoDrop spectrophotometer (Nanodrop Technologies, Delaware, USA). All reactions were done in triplicate. Each 25 μ L reaction contained 12.5 μ L of 2 $\times$ iQ SYBR Green Supermix, 0.5 μ L of DMSO, 1 μ g μ L^{–1} bovine serum albumin, 0.2 μ mol L^{–1} (each) primer, and 5 μ L of template DNA. The primers used for amplifying the bacterial 16S rRNA gene were 338F (5'-ACT CCT ACG GGA GGC AGC AG-3') and 518R (5'-ATT ACC GCG GCT GCT GG-3'). Thermocycler conditions included an initial 5 min denaturation; followed by 40 cycles each of 95 °C for 20 s, 55 °C for 30 s, 72 °C for 30 s, and 80 °C for 10 s. Fluorescence was measured during the 80 °C primer–dimer removal step. The archaeal 16S rRNA gene was quantitatively amplified with primers A571F (5'-GCY TAA AGS RIC CGT AGC-3') and 915R (5'-GTG CTC CCC CGC CAA TTC CT-3'). Thermocycler conditions included an initial 5 min denaturation step, followed by 40 cycles of 94 °C for 1 min with minimum ramping speed, followed by 48 °C for 30 s, 72 °C for 1.5 min, and 80 °C for 10 s.

Nucleotide sequence accession numbers

The accession numbers for the nucleotide sequences deposited in the GenBank database are as follows: bacterial clones, GU047424–GU047696; bacterial isolates, GU047384–GU047423; and archaeal clones, GU047697–GU047854.

Results

Sampling site characteristics

The physicochemical parameters measured at various depths displayed similar characteristics along the soil column (Table 1). The permafrost boundary had slightly greater salinity (as measured by conductivity), dissolved carbon, and total carbon and nitrogen than either the active or permafrost layers. The upper active layer soil was most saturated, while the moisture content at the permafrost boundary was lower than in the permafrost layer, indicating either poor infiltration to that depth or the drawing of moisture down by the freezing of the permafrost layer.

DGGE

Archaeal and bacterial 16S rRNA gene DGGE fingerprint patterns were compared to assess differences in community structure at various depths along the soil column (Fig. S1¹). At all depths, the bacterial community had more complex banding patterns than the archaeal community, suggesting greater diversity in the bacterial domain. Dendrograms based

¹Supplementary data are available with the article through the journal Web site (www.nrcresearchpress.com/cjm).

Table 1. Physicochemical parameters, total cell counts, and 16S rRNA gene copy number at various depths of the soil profile.

	Active layer	Permafrost boundary	Permafrost layer
Soil depth (cm)	14–19	55–60	97–100
pH	4.48	4.99	4.69
Moisture content (%)	29.6	11.0	19.6
Conductivity ($\mu\text{S}\cdot\text{cm}^{-1}$)	52.5	89.3	87.4
Dissolved carbon ($\text{mg C}\cdot(\text{kg soil})^{-1}$)	3.24	5.41	1.15
Total carbon ^a (%)	0.98	1.10	1.01
Nitrate ($\text{mg NO}_3\text{-N}\cdot\text{kg}^{-1}$)	BDL	BDL	0.34
Total nitrogen ^a (%)	0.12	0.17	0.11
DTAF cell counts ($10^7\cdot(\text{g soil dry mass})^{-1}$)	4.36	4.09	1.93
Bacterial 16S gene copy number ($10^7\cdot(\text{g soil dry mass})^{-1}$)	3.81	4.74	7.53
Archaeal 16S gene copy number ($10^4\cdot(\text{g soil dry mass})^{-1}$)	3.68	10.6	2.83

Note: BDL, below detection limit; DTAF, 5-(4,6-dichlorotriazinyl) aminofluorescein stain.

^aPercent dry mass permafrost.

Table 2. Statistical analyses of archaeal and bacterial 16S rRNA gene clone libraries from active layer and permafrost samples.

	Bacterial ^a		Archaeal ^b	
	Active layer	Permafrost	Active layer	Permafrost
Clones analyzed	139	134	59	134
Operational taxonomic units	86	75	12	75
Good's percent coverage	38%	44%	80%	90%
Chao1 richness index	246	205	17	13
Shannon's diversity index (H')	4.14	4.00	1.82	1.54
Simpson's diversity index ($1/D$)	54.5	51.5	2.24	3.49
Sørensen index	0.15 ^c		0.5 ^c	

^a97% similarity of OTUs.

^b98% similarity of OTUs.

^cClone libraries for active layer and permafrost are significantly different based on LIBSHUFF analysis.

on the similarity in banding pattern between bacterial profiles showed clustering between active layer samples (S_{AB} 65%) and between permafrost samples (S_{AB} 62%), with the bottom active layer sample showing similarity to the permafrost boundary. The majority of bands were common to all samples (S_{AB} 53.5%) (Fig. S1¹). To a lesser extent, the archaeal profiles reflected similar profile differences, although less demonstratively, as the overall number of bands was low. Band sequencing from bacterial DGGE was ineffective due to difficulties in removing single bands from dense band clusters. All bands sequenced from archaeal DGGE corresponded to Group I. 1b organisms in the phylum *Crenarchaeota* (Fig. S1¹).

Structure and diversity of bacterial 16S rRNA gene clone libraries

Based on the differences between the upper two and bottom DGGE banding patterns, a representative active layer (MRSC-2, 14–19 cm deep) and a permafrost (MRSC-18, 97–100 cm deep) sample were chosen for 16S rRNA clone library analyses. The diversity of active layer and permafrost clone libraries was similar based on diversity indices (Table 2). A comparison between libraries using LIBSHUFF ($p = 0.001$, significant at ≤ 0.025) and, more qualitatively, the Sørensen similarity index found a significant difference in community structure (Table 2). The RDP tool LibCompare

identified the phylum *Actinobacteria* ($p = 0.01$) as the only significantly disproportioned taxonomic group. The permafrost library contained a greater abundance of *Actinobacteria*, specifically unclassifiable members of the family *Rubrobacteraceae* (12 clones) and the family *Intrasporangiaceae* (17 clones), which were found in relatively minor proportions in the active layer (2 and 0 clones, respectively). The dominant phyla differed between the active layer (*Acidobacteria* (30%), *Proteobacteria* (19%), *Gemmatimonadetes* (14%)), and the permafrost (*Actinobacteria* (28%), *Acidobacteria* (20%), *Proteobacteria* (15%), *Gemmatimonadetes* (15%)) (Fig. 1). Large proportions of both libraries, 16% of active layer and 15% of permafrost, could not be classified by RDP. These sequences formed branches on the phylogenetic tree associated with *Chloroflexi* and a branch with which no cultured organisms were associated (Fig. S2¹). The single most common genus found in both libraries was *Gemmatimonas*, occupying 15% and 14% of clones from the active layer and permafrost, respectively. Nine of the 11 phyla detected occurred in both libraries, with only *Nitrospira* occurring in the permafrost, while candidate divisions *OP10* and *OP11* occurred only in the active layer. Good's percent coverage of bacterial clone sequences from the active layer (38%) and permafrost (44%) suggests a very diverse community requiring extensive sequencing to identify all taxonomic groups present.

Fig. 1. Distribution of phyla derived from 16S rRNA bacterial clone sequences in the active layer (left) and permafrost (right), as classified by the Ribosomal Database Project. The percentage corresponds to the total number of sequences in the active layer ($n = 139$) and permafrost ($n = 134$).

Structure and diversity of archaeal 16S rRNA gene clone libraries

In contrast to the diversity of bacterial libraries, both archaeal libraries had lower diversity based on indices (Table 2). Both the active layer and permafrost consisted predominantly of *Crenarchaeota*, at 71% and 95%, respectively. The active layer had a greater proportion of *Euryarchaeota* (22%) compared with permafrost (4%) and both contained minor portions of unclassifiable sequences, 7% and 1%, respectively. Similar to the bacterial communities, LIBSHUFF found significant differences between the two libraries ($p = 0.001$, significant at ≤ 0.025), while the Sørensen index indicated ~50% of the OTUs were common to both libraries (Table 2). No phylotype could be assigned beyond the class level by the RDP, with all *Crenarchaeota* reported as unclassified *Thermoprotei*, whose cultured representatives are thermophilic organisms common to acidic environments (Itoh et al. 2003). In phylogenetic tree building, the closest cultured representative to the *Thermoprotei*-related clones was a novel candidate division in crenarchaeotic Group I. 1b, *Candidatus Nitrososphaera* (Fig. 2), a moderately thermophilic ammonia-oxidizing crenarchaeote (Hatzenpichler et al. 2008). *Thermoprotei*-related clones had top BLASTn matches with clones from the rhizosphere, a cave colony, and uranium tailings in the phylogenetic tree. The majority of clones classified as *Euryarchaeota* clustered together with clones from temperate soil, permafrost peat, and an Arctic river, while the remaining clones were distantly related to cultured acidophilic or methanogenic representatives. Archaeal primers yielded DNA related to Bacteria, where 33/92 active layer clones and 23/122 permafrost clones were of bacterial origin. These sequences were discarded for phylogenetic analysis and not included in the bacterial library owing to the lack of sequence overlap required for alignment. The RDP classification of these sequences mirrored the bacterial taxa derived from bacterial primers (results not shown).

Culture-dependent and culture-independent enumerations

The highest numbers of colony-forming units (CFUs) occurred at 25 °C and neutral pH (Fig. S3¹). The highest cell count for the active layer was 2.5×10^5 CFU·(g dm⁻¹), obtained on MSM+YTS, and for permafrost was 6.6×10^2 CFU·(g dm⁻¹) on TSA. Growth was observed at both acidic and neutral pH and 5 and 25 °C; no colonies formed at 37 or -5 °C. A single colony formed on R2A under strict anaerobic conditions at pH 4.5 at 5 °C and was identified as *Streptacidiphilus* sp. Total 16S rRNA gene copy number, as determined by qPCR, was highest for Bacteria in the permafrost, with 7.5×10^7 copies·(g dm⁻¹), while the highest copy number for Archaea occurred in the permafrost boundary, with 1.1×10^5 copies·(g dm⁻¹) (Table 1). The greatest total cell count using DTAF fluorescent staining was in the active layer, with 4.4×10^7 cells·(g dm⁻¹) (Table 1). Total cell estimates based on DTAF and 16S rRNA gene copy number were in general agreement and were 100×–10 000× greater than those observed by heterotrophic plate counts. The 16S rRNA gene copy number observed in permafrost is high compared with microscopic counts, indicative of DNA preservation in the continually subzero permafrost environment.

Isolate classification and temperature, pH, and salt tolerance characterization

Forty isolates were chosen from both acidic and neutral pH and from incubations at 5 and 25 °C. Of the 40 isolates, 16 unique 16S rRNA gene phylotypes were identified at 97% similarity (Table 3). *Arthrobacter* sp. (*Actinobacteria*) dominated the culturable community of the active layer as the sole culturable organism. The diversity of permafrost isolates was greater than active layer isolates, with members of the phyla *Actinobacteria*, *Alphaproteobacteria* and *Betaproteobacteria*, and *Firmicutes* present (Table 3). The most distantly related isolate belonged to the genus *Kaistibacter* sp. with 97% similarity to its top BLASTn match.

The pH, salt, and temperature tolerance testing of 32 of 40 isolates resulted in the identification of 24 unique phenotypes

Fig. 2. Combined active layer and permafrost archaeal 16S rDNA distance-based phylogenetic tree including bootstrap values. Root to *Aquifex pyrophilus* (M83548.2) not shown. Clones from this study are in bold, top BLASTn matches are in plain text, and cultured representatives are italicized. The number of clones represented by a branch is given by the notation #x, and representative branches shared by both libraries are shaded with the following notation on the number of clones from each library (active layer/permafrost). Clones are named based on Ribosomal Database Project classifications.

Table 3. List of isolates recovered from acidic active layer and permafrost soils and their classification by the Ribosomal Database Project and BLASTn.

Source	Isolate acc. No.	Classification	Closest cultured BLASTn match ^a	Similarity to top BLASTn match	Origin of top BLASTn culture
Firmicutes					
Permafrost	GU047423	<i>Paenibacillus</i>	<i>Paenibacillus</i> sp. (EU839659)	99%	Endophyte
Permafrost	GU047414	<i>Staphylococcus</i>	<i>Staphylococcus</i> sp. (GQ503327)	100%	Polluted soil
Permafrost	GU047407	<i>Bacillus</i>	<i>Bacillus</i> sp. (FJ977609)	99%	Sea water
Permafrost	GU047403	<i>Sporosarcina</i>	<i>Paenisporosarcina</i> sp. (FJ999934)	99%	Tibetan mountain
Permafrost	GU047418	<i>Sporosarcina</i>	<i>Sporosarcina</i> sp. (X68415)	99%	Anaerobic waste
Actinobacteria					
Permafrost	GU047413	<i>Microbispora</i>	<i>Microbispora rosea</i> (AB440707)	99%	Cabbage root
Permafrost	GU047421	<i>Corynebacterium</i>	Uncultured organism (GQ065273)	100%	Human skin
Permafrost	GU047420	<i>Streptacidiphilus</i>	<i>Streptacidiphilus</i> sp. (AM422450)	99%	Natural cave
Permafrost	GU047404	<i>Streptomyces</i>	<i>Streptomyces</i> sp. (AB184688)	98%	Temperate soil
Active layer	GU047396	<i>Arthrobacter</i>	<i>Arthrobacter</i> sp. (EU787019)	99%	Temperate soil
Active layer and permafrost	GU047387	<i>Arthrobacter</i>	<i>Arthrobacter</i> sp. (FJ938117)	100%	Temperate soil
Permafrost	GU047417	<i>Frigoribacterium</i>	<i>Frigoribacterium</i> sp. (AB461107)	100%	Plant tissue
Permafrost	GU047388	Unclassified <i>Microbacteriaceae</i>	<i>Cryobacterium</i> sp. (FM955863)	99%	Antarctic soil
Permafrost	GU047422	Unclassified <i>Actinomycetales</i>	<i>Kaistibacter</i> sp. (EU423306)	97%	Temperate soil
Proteobacteria					
Permafrost	GU047405	<i>Burkholderia</i>	<i>Burkholderia</i> sp. (FJ772043)	100%	Forest soil
Permafrost	GU047393	<i>Sphingomonas</i>	<i>Sphingomonas</i> sp. (FJ267571)	100%	Plant tissue

^aIsolates with greater than 97% similarity were aggregated, a complete list of isolates appears in Table S1.¹

(Table S1¹). Thirteen out of 17 isolates classified as *Arthrobacter* sp. demonstrated unique tolerance phenotypes. All isolates, except one, were capable of growth at 5 and 25 °C, and one isolate (unclassified *Microbacteriaceae*) was capable of growth at –5 °C (at pH 7.2), demonstrating overall characteristics of a psychrotrophic community. Two isolates were capable of growth at 37 °C (*Bacillus* sp. and *Burkholderia* sp.). At 25 °C, isolates demonstrated more robust tolerance to acidic pH and higher salt concentrations than at 5 °C. At 25 °C, the majority of isolates had a broad range of pH tolerance, with 65% growing at pH 4, 79% at pH 5, and all isolates were capable of growth at pH 8 or 9. At 5 °C, only 12% of isolates were capable of growth at pH 4, although 85% were capable at pH 5. Regarding halotolerance, at 25 °C, 21 (65%) of the isolates grew at an NaCl concentration of 5% or greater; whereas, four grew at 7.5% and one grew at 10% NaCl. At 5 °C, 18 isolates (56%) grew at 5% NaCl, but none grew at higher concentrations. The broad pH tolerance demonstrated the absence of obligate acidophiles in our cultured representatives. The anaerobic isolate, *Streptacidiphilus* sp., was an obligate anaerobe and grew at 5 °C (this was not tested for subzero growth).

Comparisons to previously published permafrost and Arctic wetland clone libraries

Comparisons between 16S rRNA gene sequences from our Axel Heiberg Island clone libraries with other permafrost and wetland clone libraries were made using DOTUR (Table S2¹). Sequences with ≥95% similarity were identified between Axel Heiberg Island and three out of four permafrost clone libraries. There were no common sequences found in Siberian permafrost (Vishnivetskaya et al. 2000). There were four phylotypes common to both previous studies at Eureka,

Ellesmere Island. The 1 m permafrost sample from Eureka (Steven et al. 2007) shared genetic similarity with taxa *Gp8*, *Intrasporangiaceae*, *Gemmatimonas*, and *Rhizobiales*, while the 9 m sample shared similarity with *Rubrobacteraceae*, *Pseudomonas*, *Gemmatimonas*, and *Rhizobiales* (Steven et al. 2008). From the most acidic permafrost studied to date (pH 5.01), from Spitsbergen Island, Norway (Hansen et al. 2007), six phylotypes shared sequence similarity: *Gp16*, *Intrasporangiaceae*, *Rubrobacteraceae*, *Pseudomonas*, *Rhizobiales*, and an unclassified *Betaproteobacteria*. To date, *Gp8* and *Gp16* are the only *Acidobacteria* groups detected in permafrost; these particular groups were present in Axel Heiberg Island permafrost and absent in the active layer (Hansen et al. 2007; Steven et al. 2008).

A total of five bacterial Axel Heiberg Island clone sequences shared similarity with the active layer of an acidic subarctic (56°N) wetland (Dedysh et al. 2006). One of the related sequences was from a clone that formed a novel lineage in the study (clone ID B125) from the family *Rubrobacteraceae*. A library of 154 clones from an alpine tundra wetland in Colorado matched with 44 Axel Heiberg Island clones from 28 different phylotypes (Costello and Schmidt 2006). Large numbers of the alpine tundra clones were related to *Acidobacteria* (five out of six genera from our active layer) and unclassifiable clones most closely related to the phyla *Chloroflexi* and *Actinobacteria*.

From the two Arctic studies with archaeal 16S rRNA gene clone libraries, Axel Heiberg Island clones were related to one *Euryarchaeota* clone from a Spitsbergen active layer, clustering to a nonmethanogenic group (Høj et al. 2008), and one *Crenarchaeota* clone, of the class *Thermoprotei*, from Eureka, Ellesmere Island (Steven et al. 2008).

Discussion

The analyses of the microbial communities in acidic wetland cryosol provided details on the type, diversity, and abundance of organisms capable of surviving in one of the harshest environments on Earth. This record of the biodiversity of active layer and permafrost microbial communities will be a valuable ecological resource for comparing present-day and future states of the Arctic wetland microbial ecosystem. However, the results provided serve only as a preliminary study, since logistical challenges and the cost of field work in such an extreme, remote environment limit extensive sampling, a factor common to studying permafrost (Steven et al. 2007).

The results demonstrate the presence of moderately sized (10^2 – 10^5 cells·(g dm)⁻¹), psychrotolerant, halotolerant, and acidotolerant communities in both the active layer and permafrost. The taxonomic structures of these communities share broad similarities based on 16S rRNA gene analysis, although at the highest taxonomic resolution, significant structural differences were apparent. Overarching similarities were noticeable in DGGE profiles, where greater than 50% of bacterial and archaeal bands were common in upper and lower soil layers, in the overlap of 9 of the 11 bacterial phyla in 16S rRNA clone libraries, in the shared abundance of *Crenarchaeota* phylotypes from Group I. 1b, and in the comparable abundance of *Acidobacteria* and *Gemmatimonadetes* clones in both layers. The semblance may reflect the similar origin, type, and chemical and (or) physical parameters (i.e., low pH) of the soil layers. Previous comparisons of cryosol, from the same field site in Eureka, Nunavut, also found overarching similarities between the two layers. An abundance of *Actinobacteria* was common in both layers (Steven et al. 2008), and similarly the bulk of taxa (711/979) detected in a metagenomic study overlapped both communities (Yergeau et al. 2010). These findings suggest that a general population of cold-adapted microorganisms cohabit both layers.

Beyond outward similarities, both bacterial and archaeal clone libraries proved significantly different in comparisons of total community DNA composition. One major distinction was the abundance of *Actinobacteria* clones in the permafrost (28%), which occupied only a minority of the active layer library (7%). Both the abundance and type of *Actinobacteria* clones differed, with clones related to *Intrasporangiaceae* and a polyphyletic grouping classified as *Rubrobacterineae* found only in the permafrost layer. Sequences related to these two families have been found in abundance in Ellesmere Island and Spitsbergen permafrost (Hansen et al. 2007; Steven et al. 2008; Yergeau et al. 2010), and have also been found in active layer soil in the dry valleys of Antarctica (Shrivage et al. 2007). Overall, the greater abundance of *Actinobacteria* in permafrost supports the theory proposed by Johnson et al. (2007) that members of the *Actinobacteria*, non-spore formers, have adapted a form of long-term, low-energy anabiosis that enables them to survive for extended periods in cryoenvironments, such as permafrost.

Varying proportions within the divisions of *Proteobacteria* also appeared to differentiate active layer and permafrost communities. Previous findings from the Canadian High Arctic show higher abundances of *Betaproteobacteria* than any other *Proteobacteria* division in the permafrost (Steven et al.

2008; Yergeau et al. 2010). This was true of the Axel Heiberg Island permafrost library, while the active layer library contained greater proportions of *Alphaproteobacteria*. In general, members of phylum *Proteobacteria* are recurrently a major component in active layer (Zhou et al. 1997; Costello and Schmidt 2006; Dedysh et al. 2006) and permafrost communities (Steven et al. 2007, 2008; Gilichinsky et al. 2008) around the world.

Distinctions between archaeal active layer and permafrost phylotypes were not as pronounced as in the bacterial clone libraries. *Crenarchaeota*, belonging to Group I. 1b, were the most abundant phylotype in both layers. Previous Archaea-specific 16S rRNA gene libraries from Ellesmere Island cryosols (1 m permafrost) also contained a majority of clones related to Group I. 1b (Steven et al. 2008), a characteristic also present across a range of locations in soils from the Ross Sea region of Antarctica (>99% of clones) (Ayton et al. 2010). Notably, *Crenarchaeota* were observed to increase in abundance during the winter period in an Arctic active layer peat sample (Høj et al. 2008), which suggests that members of *Crenarchaeota* may be particularly adapted to cold conditions. In phylogenetic analysis, Axel Heiberg Island Group I. 1b clones clustered with *Nitrosphaera gargensis*, a candidate ammonia-oxidizing Archaea, implicating a functional role in ammonia oxidation. However, 16S rRNA gene phylogeny is not a comprehensive predictor of functional relevance, since active transcription and in situ activity do not necessarily correlate. The relatively few euryarchaeotal sequences (22% and 4%) were unclassifiable and did not group closely with cultured representatives, although a small number of active layer clones clustered loosely with known methanogens. In comparison, the archaeal community of a 2-m-deep permafrost sample from Ellesmere Island comprised mainly *Euryarchaeota*, predominantly halophiles (Steven et al. 2008), although functional genes related to methanogenesis were detected in the same Ellesmere Island samples using metagenomic analysis (Yergeau et al. 2010). Overall, Archaea constituted a minor proportion of the total community based on quantitative PCR (0.1%); this is consistent with previous reports where Archaea have constituted between 1% and 12% of total cells in Siberian active layer soils (Kobabe et al. 2004; Høj et al. 2008). The overall low diversity of archaeal 16S rRNA sequences suggests there may be a narrower ecological niche for Archaea, though taxa from *Crenarchaeota* Group I. 1b are of likely significance to cryosol environments.

Compared with previous studies of permafrost soils, the Axel Heiberg acidic wetland permafrost community represents a distinct type of cold-adapted community with an abundance of *Acidobacteria* (20%) and *Gemmatimonadetes* (15%) at previously unrecorded levels. A permafrost library from Siberia contained neither phylum (Vishnivetskaya et al. 2000), while libraries from Ellesmere Island contained approximately 1%–3% of each phyla (Steven et al. 2008; Yergeau et al. 2010), and a moderately acidic sample from Spitsbergen (pH 5.01) contained only 0.5% *Acidobacteria* and did not contain any *Gemmatimonadetes* (Hansen et al. 2007). The abundance of *Acidobacteria* and *Gemmatimonadetes* in Axel Heiberg Island cryosol suggests considerable variability in what is considered common taxa for polar soils. This conforms to expectations, since pH is known to influ-

ence community structure in Arctic bacterial communities (Chu et al. 2010), as well as in a range of other environments (Baker et al. 2009; Rousk et al. 2010). In contrast to other polar environments, the Axel Heiberg permafrost clone library most closely resembles that of active layer soil from the McMurdo Dry Valleys in Antarctica, where *Actinobacteria* (33%), *Acidobacteria* (31%) and *Gemmatimonadetes* (8%) were the most abundant phylotypes (Pointing et al. 2009).

The phylum *Acidobacteria* comprises moderately acidophilic cultured representatives (pH 3.0–6.5) originally detected in 16S rRNA gene libraries from acidic environments (reviewed by Ward et al. 2009). *Acidobacteria* can also be found in a wide range of neutral and alkaline environments (Eichorst et al. 2007). Their abundance in an acidic cryosol may be attributable to their general acid tolerance; their wide range of adaptation to stressors, such as the ability to rapidly regulate DNA and protein synthesis; and their ability to metabolize complex sugars, enabling their survival under low nutrient conditions (Ward et al. 2009). Their capacity for low temperature growth has recently been demonstrated by psychrotolerant isolates (*Gp1* and *Gp3*) from a subarctic wetland with a temperature growth range of 2 to 31 °C (Pankratov et al. 2008).

An understanding of the ecological importance of *Gemmatimonas* in cryosol is limited by a lack of cultured representatives. The sole cultured representative, *Gemmatimonas aurantica*, is an aerobic heterotroph isolated from an anaerobic–aerobic waste treatment reactor and is noted for its capacity to accumulate polyphosphates, a possible advantage for organisms undergoing long-term nutrient deprivation (Zhang et al. 2003). *Gemmatimonas* sp. was previously detected in Canadian High Arctic permafrost (Steven et al. 2008) and in the McMurdo Dry Valleys of Antarctica (Niederberger et al. 2008) and, thus, may be a common inhabitant of polar desert environments.

A common observation in the broader microbial communities of subarctic and alpine wetlands (active layer) is the presence of a phyletic grouping distantly related to *Actinobacteria* (Costello and Schmidt 2006; Dedysh et al. 2006). This feature was observed in the Axel Heiberg Island clone libraries (Fig. S2a¹), where clones demonstrated similar remote branching, some with top BLASTn matches to the novel phylotypes. The large proportion of *Acidobacteria* in the Axel Heiberg Island cryosol may be characteristic of wetland communities, with both the subarctic and alpine studies reporting 28% and 30%, respectively, of clones classified as *Acidobacteria* (Dedysh et al. 2006; Costello and Schmidt 2006). Furthermore, a large number of Axel Heiberg Island bacterial clones and the majority of archaeal clones from both active layer and permafrost were top BLASTn hits with clones from an alpine wetland (Costello and Schmidt 2006), suggesting that the two environments have comparable communities. The greatest number of closely related sequences occurred with phylotypes from a novel branch of *Chloroflexi*, the discovery of which by Costello and Schmidt (2006) constituted a major expansion of the division of *Chloroflexi*.

To date, most Arctic wetland studies have focused on the community of methane-cycling microorganisms (Kobabe et al. 2004; Wagner and Liebner 2009). In the Axel Heiberg libraries, the methanotroph *Methylocystis* sp. was detected in the active layer. No strong indication of the presence of

methanogens was observed in either layer. However, the anaerobic genera *Opitutus* and *Propionibacterium* were detected and are of known importance in the intermediary ecosystem metabolism commonly associated with methane cycling. These Bacteria can act as sources of acetate and propionate for methanogens and their syntrophs. For instance, in an in vitro study, the acetate and propionate generated by *Opitutus* accounted for 48%–83% and 18%–28% of the methane produced, respectively (Chin and Janssen 2002). The presence of these organisms in the active layer is indicative of methanogenic potential, although no methanogens were observed.

Differences in the abundance and type of viable cells recovered further illustrate the distinctions in the microbial composition of each layer. Active layer culturable heterotroph enumerations were approximately 100-fold greater than those of the permafrost. Previous studies corroborate that the permafrost contains approximately 100-fold fewer organisms than the active layer (see review by Gilichinsky et al. 2008). One hypothesis is that permafrost harbours a community of survivors rather than an active community (Steven et al. 2006). The high percentage (47%) of spore-forming isolates recovered from the Axel Heiberg Island permafrost supports this hypothesis. In addition to culturing biases, the discrepancy between viable and total cell counts observed in permafrost indicates a large population of viable but not culturable cells or the preservation of impaired, deteriorating cells resulting in overestimations in DNA or stain-based enumerations. The number of CFUs for both cryosol layers was low compared with previous reports — between 10⁷–10⁹ CFUs in the active layer and 0–10⁸ CFUs in the Siberian permafrost (Gilichinsky et al. 2008). The low estimate of culturable cells likely reflects the bias of conventional culturing techniques. A study of an acidic subarctic wetland utilized a specialized biofilm-mediated culturing approach that enabled the isolation of rare organisms from the phyla *Planctomycetes* and *Acidobacteria* (Dedysh et al. 2006). Both taxa were detected in Axel Heiberg clone libraries, *Acidobacteria* in considerable abundance, yet the taxa were not represented in the inventory of cultured isolates. This discrepancy suggests that more elaborate culturing techniques are required for characterizing acidic environmental samples.

The culturing of viable organisms from acidic permafrost has previously been attempted at neutral pH and pH 5.5 (Hansen et al. 2007). In the present study, the use of low pH media (pH 4.5) improved the diversity of recovered isolates, although culturing at neutral pH produced a greater number of CFUs. All isolates grew at both 5 and 25 °C, with a single isolate (*Microbacteriaceae*) capable of growth at –5 °C (pH 7.2), characteristic of a psychrotrophic, cold-adapted community commonly found in cryosols (Steven et al. 2007; Gilichinsky et al. 2008). In addition to expanding our knowledge of a unique terrestrial environment, the acidity of the Axel Heiberg Island cryosol (pH < 5.0) and the habitual cold temperatures may provide a suitable analog to Martian soil conditions. In the Arctic, the temperature range of active layer soil (–35 to 15 °C) (Mangelsdorf et al. 2009) and permafrost (–27 to –10 °C) (Gilichinsky et al. 2008) approach the daily mean surface temperature on Mars (–47 °C), which ranges from –87 to –5 °C (Harvey 2008). A soil survey done by the Mars Exploration Rover in the Meridiani Planum

discovered jarosite, a mineral indicative of highly saline, acidic brines (pH ~2.3), demonstrating a possible habitat for halotolerant, acidophiles (Fairén et al. 2008). The high percentage of isolates from Axel Heiberg cryosol capable of growth at 5 °C, at ≥5% NaCl (*m/v*), and at acidic pH is evidence for the possibility of microbial life in acidic, low temperature Martian salt brines.

In summary, this research provides the first comprehensive account of the bacterial communities contained within the active layer and permafrost of an acidic Arctic wetland and is only the second of its kind describing archaeal communities. The culture-independent and culture-dependent analyses revealed both novel and corroborating data on the biodiversity, abundance, and community structure of the microbial communities of Arctic cryosol. The results indicate that at the subphylum level, the active layer community differs significantly from the permafrost. The presence of large numbers of *Acidobacteria* and *Gemmatimonadetes* clones, previously identified as minor community members in Arctic soil, permafrost, and (or) sediments, demonstrates the variability in Arctic cryosol environments, even between cryosol of similar soil pH (see Hansen et al. 2007). The overlap between the Axel Heiberg Island clone library and that of an alpine wet meadow (Costello and Schmidt 2006) demonstrates that wet, cold-temperature soil environments from different geographic locations may share similar archaeal and bacterial taxa. The process of cataloguing active layer and permafrost microbial communities is an important task for it provides a point of comparison for assessing the effects of climate change in the vulnerable polar regions.

Acknowledgements

Logistic support was provided by the Canadian Polar Continental Shelf Project (PCSP Nos. 08 and 09) and McGill University's High Arctic Research Station. This work was funded by the Natural Sciences and Engineering Research Council of Canada (NSERC) Discovery Program, the Northern Supplements Program, the Special Research Opportunities IPY (International Polar Year) Program, the Cooperative Research Centres (CRC) program, and the Canadian Space Agency (CSA) Canadian Analogue Research Network program. Additional funding for student research was provided by the Department of Indian and Northern Affairs – Northern Scientific Training Program. We would also like to thank our scientific colleagues: Dr. Joann Whalen and her laboratory for performing soil chemical analyses, Sara Klemm for performing qPCR, Dr. Etienne Yergeau for technical support, and Christine Martineau for temperature data and support during field work.

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