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Microbial diversity and activity through a permafrost/ground ice core profile from the Canadian high Arctic

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Summary

Culture-dependent and culture-independent methods were used in an investigation of the microbial diversity in a permafrost/massive ground ice core from the Canadian high Arctic. Denaturing gradient gel electrophoresis as well as *Bacteria* and *Archaea* 16S rRNA gene clone libraries showed differences in the composition of the microbial communities in the distinct core horizons. Microbial diversity was similar in the active layer (surface) soil, permafrost table and permafrost horizons while the ground ice microbial community showed low diversity. *Bacteria* and *Archaea* sequences related to the *Actinobacteria* (54%) and *Crenarchaeota* (100%) respectively were predominant in the active layer while the majority of sequences in the permafrost were related to the *Proteobacteria* (57%) and *Euryarchaeota* (76%). The most abundant phyla in the ground ice clone libraries were the *Firmicutes* (59%) and *Crenarchaeota* (82%). Isolates from the permafrost were both less abundant and diverse than in the active layer soil, while no culturable cells were recovered from the ground ice. Mineralization of [1-^{14}C] acetic acid and [2-^{14}C] glucose was used to detect microbial activity in the different horizons in the core. Mineralization was detected at near ambient permafrost temperatures (–15°C), indicating that permafrost may harbour an active microbial population, while the low microbial diversity, abundance and activity in ground ice suggests a less hospitable microbial habitat.

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Introduction

Permafrost, defined as ground that remains frozen for at least two consecutive years (van Everdingen, 2005), underlies approximately one fourth of the northern hemisphere land surface (Zang *et al*., 1999). Permafrost is also present on the shallow continental shelves of the polar seas and at high altitudes in many mountainous regions (Davis, 2001). The extent of permafrost on continental Antarctica remains unknown and observations are mainly restricted to areas around research stations (Turner *et al*., 2007). Ground ice refers to all types of ice that exist in frozen ground (van Everdingen, 2005), while perennial ground ice can only exist in bodies of permafrost (Pollard and French, 1980). Many types of ground ice are recognized, such as pore ice, segregated ice and wedge ice. The term massive ground ice refers to horizontally extensive bodies (> 2 m thick) of subsurface ice.

Permafrost and ground ice are analogues of environments that may exist on cryogenic bodies beyond Earth, such as Mars, where there is evidence of bodies of subsurface ice (Boynton *et al*., 2002) and which appears to have been confirmed by the current National Aeronautics and Space Administration (NASA) Phoenix mission to Mars (http://phoenix.lpl.arizona.edu/index.php). In this regard, permafrost and ground ice are primary targets for future astrobiology studies.

The 20th century has seen a decrease in the extent of permafrost distribution, and current models predict accelerating near-surface permafrost thawing during the first half of the 21st century (Lawrence and Slater, 2005). Areas of permafrost thawing are associated with increased fluxes of CH_4 and CO_2 (Zimov *et al*., 2007) and there is a concern that large-scale emissions of methane and/or carbon dioxide (as a result of increased microbial activity) will occur with this thawing, creating a positive feedback loop on global warming (Goulden *et al*., 1998; Christensen *et al*., 2004). However, it remains unclear how the indigenous permafrost microbial communities will respond and possibly contribute to future permafrost melting.

The majority of studies describing permafrost microbiology originate from Siberian permafrost (e.g. Gilichinsky *et al*., 1995; 2005; Shi *et al*., 1997). Total microscopic

counts of Siberian permafrost microorganisms indicate microbial numbers of 10^7–10^9 cells g^{-1} while viable counts only account for 0.1–10% of these values (reviewed in Steven *et al*., 2006), suggesting that culture-based studies have only described a small proportion of the microbial diversity that exists in Siberian permafrost. Recent culture-independent studies have expanded the diversity of microbial groups associated with permafrost systems. 16S rRNA gene clone libraries from Siberian (Vishnivetskaya *et al*., 2006), Spitsbergen Island (Hansen *et al*., 2007) and Antarctic (Gilichinsky *et al*., 2007) permafrost have revealed diverse microbial communities in permafrost. Compared with permafrost, little is known about the microbial communities that inhabit ground ice. Researchers working in Siberia reported an inability to recover viable cells from Siberian ice wedges (Gilichinsky *et al*., 1995), and there is a single report on the bacterial community inhabiting an Alaskan ice wedge (Katayama *et al*., 2007). The phylogenetic diversity of the ice wedge community was relatively low and all of the recovered isolates and 16S rRNA gene sequences were affiliated with three bacterial phyla, *Actinobacteria*, *Proteobacteria* and *Firmicutes*.

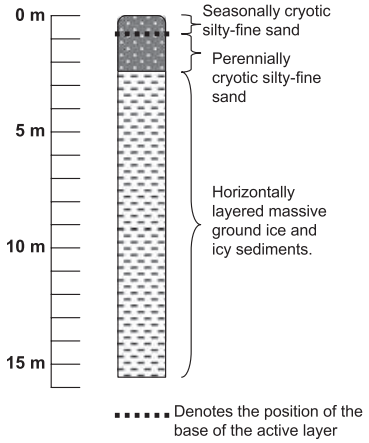
Detection of 16S rRNA gene sequences in the permafrost does not indicate that these organisms are viable or metabolically active *in situ*. The constant subzero temperatures and stability of the permafrost environment make it an ideal system for the preservation of DNA (Willerslev *et al*., 2004a,b). In addition, the majority of isolates from permafrost are psychrotolerant rather than psychrophilic (Gilichinsky *et al*., 2007; Steven *et al*., 2007a), which has led to the hypothesis that permafrost microorganisms are predominantly dormant *in situ* and are revived after extended dormancy in the permafrost (Friedmann, 1994). However, mounting evidences, such as incorporation of ^{14}C into lipids at –10°C (Rivkina *et al*., 2000), CH_4 evolution in permafrost microcosms at –16.5°C (Rivkina *et al*., 2002), growth of permafrost isolates at –10°C (Bakermans *et al*., 2003) and CO_2 respiration by permafrost organisms reported at –35°C (Panikov and Sizova, 2007), all indicate that permafrost communities may be capable of metabolic activity at the ambient subzero temperatures that characterize the permafrost environment. Linking the diversity and activity of microorganisms in permafrost will aid in identifying genetic and physiological adaptations that allow for microbial life at subzero temperatures, as well as better defining the low-temperature limits for microbial metabolism.

Previously, we characterized the microbial diversity in a single permafrost sample from the Eur1 core (Fig. S1) from a depth of 9 m using a combination of culture-dependent and culture-independent methods (Steven *et al*., 2007a). The objectives of this research were to describe the microbial diversity in a permafrost core (Eur3) from the Canadian high Arctic and to determine how this diversity varies in distinct horizons in the core. The Eur3 core is unique as it progresses from surface active layer soil through a zone of permafrost soil and into a layer of massive ground ice. This study represents the first characterization of the microbial abundance, diversity and activity in a vertical permafrost core profile as well as reporting the first characterization of the microbial communities in massive ground ice. Ground ice is considered to be an analogue of subsurface ice deposits that may exist on Mars (Boynton *et al*., 2002; Jakosky *et al*., 2003; Smith and McKay, 2005). In addition, determining if permafrost/ground ice harbours viable microorganisms capable of metabolic activity at ambient temperatures will be important in ascertaining if permafrost retains an active microbial ecosystem or is populated by frozen dormant microbial survivors.

Results

Eur3 core physical and chemical characterization

A schematic diagram of the Eur3 core is presented in Fig. 1. The term active layer refers to the upper part of the soil profile that is exposed to seasonal freezing and thawing. The mean annual temperature at Eureka is –19.7°C with a mean maximum temperature of 8.4°C in July and a mean minimum temperature of –41.4°C in February (Environment Canada, 2004). In Eureka, the average depth of summer thaw is 50–70 cm. The zone of transition from the active layer to permafrost is known as the permafrost table. Below the permafrost table temperatures are continually below 0°C, and permafrost begins. The temperature profile (extrapolated from the Eur1 site) was as follows: 5 m, –18°C with 3–4°C annual variation; 12 m, –17°C with 1.5°C annual variation; and 15 m, –17.5°C with less than 1°C annual variation. The stratigraphic profile observed at Eureka consists of finely laminated silty sand and clay 2.3 m thick overlying > 15 m of horizontally layered intrasedimental ice. The contact between the soil and ground ice is gradual and is marked by a dramatic increase in ice content. Detailed analysis of surface deposits in this area determined a marine origin, which corresponds with higher sea levels during the early Holocene period; the estimated age of emergence at this site is 5000–6000 years BP (Pollard and Bell, 1998; Pollard, 2000a,b). Permafrost aggradation and subsequent ground ice formation are more or less synchronous with emergence, thus the massive ice is also approximately 5000–6000 years old (Pollard and Bell, 1998; Pollard, 2000a,b). The organic carbon throughout the Eur3 core profile was in the range described for Siberian, Canadian and Spitsbergen permafrost (Vorobyova *et al*., 1997; Hansen *et al*., 2007; Steven *et al*., 2007a) but was



Depth	pH	Salinity[1]	Gravimetric Moisture Content	Total Carbon[2]	Total Nitrogen[2]
Surface	7.9	1.5	12.7%	3.0	0.3
1 m	7.8	1.9	11.5%	3.5	0.3
2 m	7.8	3.1	27.0%	1.4	0.1
4 m	8.1	2.0	75.6%	3.9	0.2
7 m	8.3	1.7	72.1%	5.3	0.3
14 m	8.2	2.2	86.3%	6.1	0.5

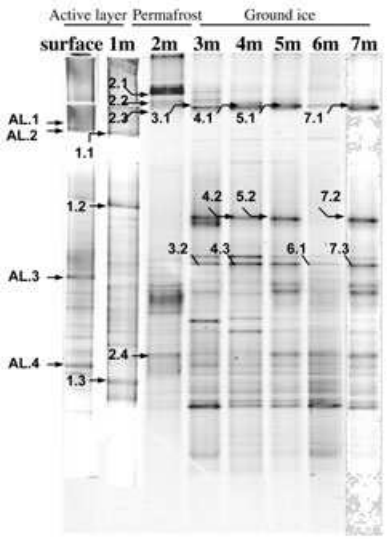
[1]meq/100 g dry soil
[2]% dry weight
Estimated age of permafrost is 5,000- 6,000 years BP

Fig. 1. Schematic diagram of the Eur3 core and the associated physiochemical properties of different-depth samples.

higher than in Antarctic permafrost (Gilichinsky *et al*., 2007).

Denaturing gradient gel electrophoresis analysis of amplified 16S rRNA genes

Denaturing gradient gel electrophoresis (DGGE) fingerprinting was used to identify horizons in the Eur3 core that showed distinct microbial community profiles. The concentration of DNA isolated from 1 m depth intervals, decreased with increasing depth, suggesting lower microbial biomass in deeper samples (data not shown). In ground ice samples deeper than 7 m, PCR amplification of *Bacteria* 16S rRNA genes gave inconsistent results from replicate DNA extractions and positive reactions had low PCR product yields. For this reason, efforts were focused on the samples from the surface to 7 m. The number of bands and banding pattern showed unique profiles for the surface layer, 1 and 2 m samples, while the ground ice samples showed similar DGGE fingerprints (Fig. 2). The relatedness of the community in the ground ice samples was verified by sequencing of DGGE bands. Bands with comparable mobility in the gel had highly similar 16S rRNA gene sequences (≥ 99% sequence identity) indicating that the community structure in the ground ice samples from 3 to 7 m was similar. Based on these results the 7 m sample was selected as a representative ground ice sample.



DGGE Band	Closest BLAST Match	Phylogenetic Group
AL.1	Bacterium G50III (AJ786087)	CFB
AL.2, 1.1	*Flavobacteriaceae* (AY576695)	CFB
AL.3	Beta proteobacterium RG-46 (AY561605)	Proteobacteria
AL.4	Nocardioides sp. NCFB3007 (X76179)	Actinobacteria
1.2	Anoxic soil isolate PB90-2 (AJ229236)	CFB
1.3	Uncultured soil clone (AY988826)	Actinobacteria
2.1	*Flavobacterium gelidilacus* (AJ507151)	CFB
2.2	Flavobacterium sp. MSY2 (DQ196345)	CFB
2.3	*Gillisia* sp. UST050418-085 (DQ202393)	CFB
2.4	*Clostridium* sp. CYP5 (DQ479415)	Firmicutes
3.1, 4.1, 5.1, 7.1	*Clostridium* sp. ArC6 (AF443595)	Firmicutes
3.2, 4.3, 6.1, 7.3	Uncultured soda lake clone (AY245479)	Firmicutes
4.2, 5.2, 7.2	Uncultured vent clone (AJ969457)	CFB

Fig. 2. DGGE analysis of *Bacteria* 16S rRNA genes amplified from total community DNA from different depths (indicated above the lanes) in the Eur3 core. Marked DGGE bands were excised and sequenced. The closest BLAST match and phylogenetic affiliation determined with the RDP classifier software of the sequenced bands are also presented in the table. Bands with similar mobility had the same BLAST match and shared ≥99% sequence similarity and are grouped together in the table.

Table 1. Diversity estimates in the 16S rRNA gene clone libraries.

Sample	Clones[a]	OTUs[b]	Chaol[b]	H′[b]	Coverage (%)
Bacteria clone library					
Active layer (surface)	67	45	115	3.6	47
Permafrost table (1 m)	60	37	192	3.2	51
Permafrost (2 m)	49	41	167	3.6	26
Ground ice (7 m)	22	13	25	2.2	63
Compiled	198	136	568	4.7	44
Archaea clone library					
Active layer	20	13	22	2.4	55
Permafrost table	23	13	31	2.3	61
Permafrost	17	14	47	2.5	29
Ground ice	22	13	22	2.3	59
Compiled	82	43	109	3.3	62

a. Number of clones retained after chimera editing.
b. Calculated at 0.01% difference in DOTUR.

Diversity of Bacteria *16S rRNA gene sequences in the clone libraries*

Bacteria 16S rRNA gene clone libraries were constructed from total community DNA from distinct horizons in the Eur3 core profile. The samples consisted of surface soil representing the active layer, the 1 m sample, which is approximately the depth of the permafrost table, the 2 m permafrost soil sample and a ground ice sample from 7 m (Fig. 1). Clone sequences were assigned to operational taxonomic units (OTUs) at the 1% difference level, approximating a strain level difference between 16S rRNA gene sequences (Stackebrandt and Goebel, 1994; Schloss and Handelsman, 2005). Grouping sequences at the 99% similarity threshold, using the DOTUR software (Schloss and Handelsman, 2005), resulted in several clones being assigned to the same OTU. However, 111 of the 136 OTUs detected in the compiled *Bacteria* clone library were represented by a single sequence (< 99% sequence similarity to any other sequences in the clone library). The number of OTUs in the different horizons and compiled clone libraries are presented in Table 1. Two OTUs were detected in the permafrost table, permafrost and ground ice layers but were not found in the active layer. The RDP classifier software identified both as belonging to the phylum *Actinobacteria* and the closest BLAST matches in the GenBank database were to 16S rRNA genes of uncultured bacteria from Spitsbergen Island permafrost (Hansen *et al*., 2007). The lack of OTU overlap in the clone libraries from different depths suggested that the microbial communities in the different Eur3 core horizons were predominantly different from each other. LIBSHUFF analysis verified that the *Bacteria* clone libraries were significantly different ($P \leq 0.008$ for all combinations). The diversity of the *Bacteria* 16S rRNA gene libraries as indicated by Shannon's index, Chaol estimators (Table 1) and rarefaction curves (Fig. S2) was similar between the active layer, permafrost table and permafrost samples, while diversity was lower in the ground ice library. The non-asymptotic nature of the rarefaction curves combined with the coverage values (Table 1) indicate that only ~44% of the *Bacteria* microbial community was described in the Eur3 core.

Phylogenetic composition of Bacteria *clone libraries*

The active layer *Bacteria* clone library was composed primarily of sequences related to the *Actinobacteria* (54% of clones) and *Proteobacteria* (42%), predominantly related to the *Betaproteobacteria* (25%) (Fig. 3). A small number of clones (4%) were related to the *Cytophaga–Flavobacterium–Bacteroides* (CFB) group. Many (70%) of the active layer clones could be classified to 22 described genera of which the most common genera were *Rhodococcus* (13% of clones) and *Dietzia* (6%).

Sequences related to the *Actinobacteria* (45%) dominated the permafrost table clone library followed by the CFB (23%). *Proteobacteria*-related sequences (20%) were related to the *Alphaproteobacteria* (10%), *Deltaproteobacteria* (3%) and *Gammaproteobacteria* (2%). Two of the sequences could not be assigned to any described *Bacteria* phyla. One was closely related (97% sequence similarity) to a 16S rRNA gene (AY921704) identified in a metagenomic analysis of farm soil (Tringe *et al*., 2005) and the other was 97% similar to a 16S rRNA gene sequence from urban lake sediments (GenBank Record, DQ165087). Relatively few (27%) of the permafrost table clones could be classified to described genera. While only cautious extrapolations about the physiology of an organism can be made from 16S rRNA sequence data, the detection of a small number of 16S rRNA sequences (5) related to strictly anaerobic genera such as *Geobacter* (Lovley *et al*., 1993) and *Desulphuromonas* (Pfennig and Biebl, 1976) may indicate the presence of anoxic microzones within the 1 m horizon.

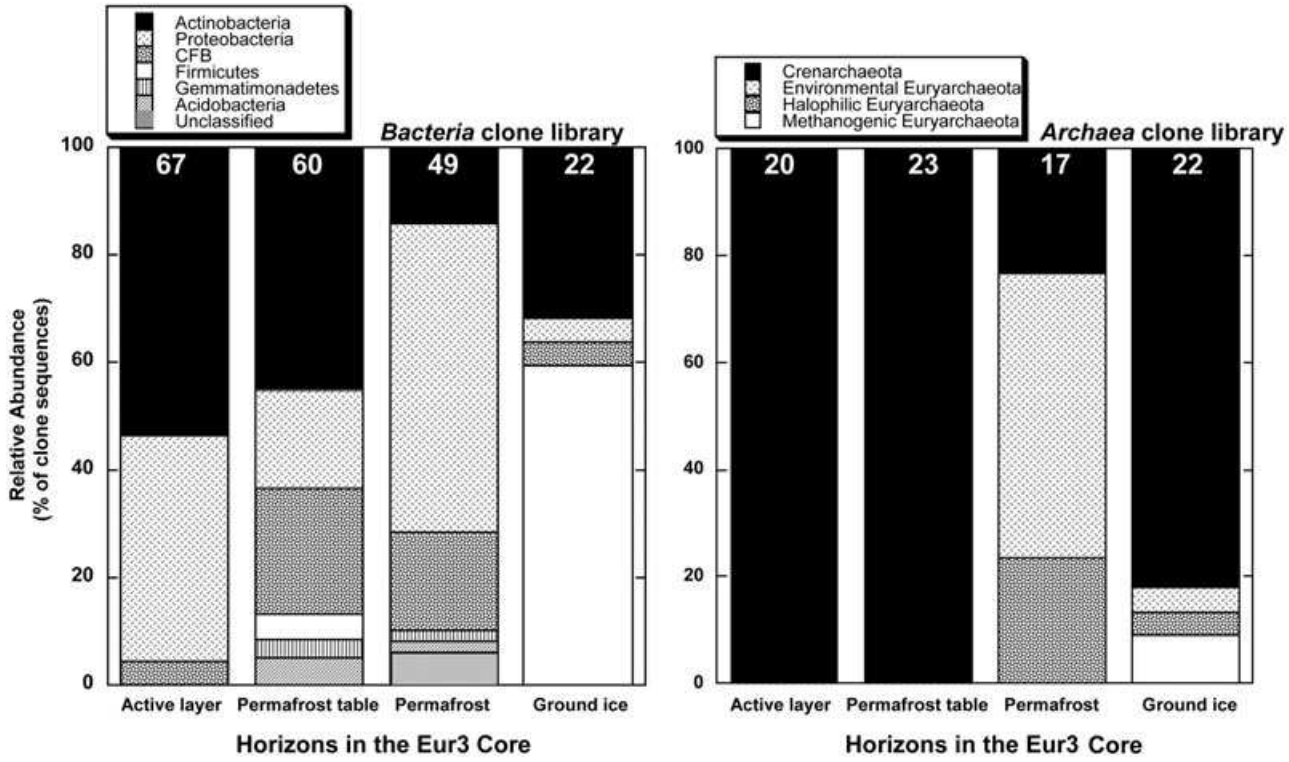


Fig. 3. Phylum level phylogenetic diversity and abundance of *Bacteria* and *Archaea* 16S rRNA gene sequences in the clone libraries. Sequences were assigned to their respective phyla using the RDP classifier software. Numbers above the columns represent the number of clones in each respective library.

Proteobacteria-related sequences (57%) dominated in the permafrost clone library with the *Betaproteobacteria* (41%) being the most abundant group. The phylogenetic groups CFB, *Actinobacteria*, *Acidobacteria* and *Gemmatimonadetes* were also represented. Four sequences in the permafrost clone library could not be assigned to a bacterial phylum using the RDP classifier software and were only distantly related (87–96% sequence similarity) to any 16S rRNA gene sequences in the GenBank database. The most common genus identified in the permafrost clone library was *Rhodoferax* (16%), which is associated with anaerobic Fe(III) reduction (Finneran *et al*., 2003).

The ground ice clone library was dominated by sequences related to the *Firmicutes* (59%). Eleven of the 13 *Firmicutes*-related sequences were classified to the genus *Clostridium*, which differed from the other horizons in the Eur3 core where the majority of the *Firmicutes*-related 16S rRNA genes belonged to spore-forming genera within the family *Bacillaceae*. *Actinobacteria*, *Proteobacteria* and CFB affiliated clones were also present in the ground ice clone library.

Diversity of Archaea *clone libraries*

We constructed *Archaea* 16S rRNA gene clone libraries to determine if the diversity of the *Archaea* microbial community varied in the different horizons in the Eur3 core profile. The Chao1 estimator H′ and rarefaction curves demonstrated a similar level of *Archaeal* diversity in the different horizons (Table 1). The number of OTUs estimated by Chao1 and the H′ diversity index were lower for the *Archaea* clone libraries compared with the *Bacteria* clone libraries in the active layer, permafrost table and permafrost whereas the diversity of the *Bacteria* and *Archaea* communities were similar in the ground ice sample (Table 1), suggesting that the *Archaea* communities in the upper horizons of the Eur3 core were less diverse than the *Bacteria* communities. Six of the OTUs in the compiled *Archaea* clone library were identified in multiple depth samples. One OTU, identified in all of the Eur3 core horizons, was related with 100% sequence identity, to an uncultured 16S rRNA gene from a Canadian high Arctic perennial spring (DQ521207; Pereault *et al*., 2007).

Phylogenetic composition of Archaea *clone libraries*

All of the *Archaea* clones in the active layer and permafrost table clone libraries were identified as belonging to the phylum *Crenarchaeota* (Fig. 3). In contrast, the permafrost clone library was dominated by *Euryarchaeota* related sequences (76%); four of these permafrost clones (23%)

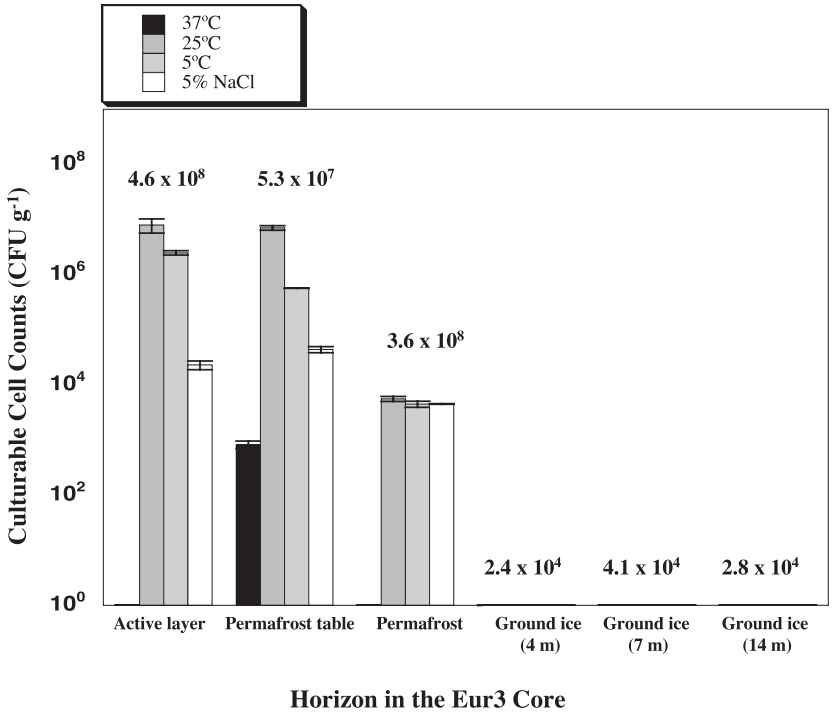


Fig. 4. Total microscopic counts and enumeration of viable aerobic heterotrophic cells in the Eur3 core. Culturing was performed aerobically on R2A media. Culturable cell counts are indicated by the bars and represent the mean of triplicate assays (error bars; 1 standard error of the mean). Five per cent NaCl plates were incubated at 5°C. Microscopic cell counts determined by epifluorescent microscopy are indicated above the bars and represent the mean of 10 random fields counted during microscopy.

were identified as belonging to the genus *Halorubrum*. *Crenarchaeota* sequences dominated the ground ice clone library while *Euryarchaeota*-related sequences were also detected. Two of the *Euryarchaeota*-related clones were classified as methanogens belonging to the genus *Methanobrevibacter* and a single clone in the ground ice clone library was also classified to the genus *Halorubrum*.

Enumeration and characterization of microbial isolates

Aerobic bacteria were isolated and enumerated on R2A media, as performed in other permafrost systems (Bakermans *et al*., 2003; Gilichinsky *et al*., 2005; 2007). The culturable cell counts were similar in the active layer and permafrost table, while no cells were recovered from three different depths of ground ice (Fig. 4). Total microbial counts, as determined by epifluorescent microscopy, were markedly lower in the ground ice samples. The culturable microbial population, as a percentage of the total counts, were ~1.7%, 13.3% and 0.01% in the active layer, permafrost table and permafrost samples respectively.

The taxonomic affiliation of representative isolates was determined using 16S rRNA gene sequencing and is presented in Table 2. Overall the 42 unique isolates fell into four phylogenetic groups: *Actinobacteria*, *Proteobacteria*, *Firmicutes* and CFB. The number and diversity of the bacterial isolates were highest in the active layer soil. *Firmicutes*-related isolates were predominant in the permafrost table horizon while all isolates recovered from the permafrost were affiliated with the *Firmicutes* (Table 2). Phenotypic characterization of the isolates indicated a microbial population dominated by cold-tolerant organisms, with six of the isolates capable of growth at –5°C (Table 2). For monitoring growth at –5°C, 7% w/v sucrose was incorporated into the media to keep the plates from freezing. All of the permafrost isolates were able to grow in the presence of 7% sucrose at 5°C (data not shown) but several isolates were not capable of growth at –5°C (Table 2), suggesting that growth at subzero temperatures and growth on 7% sucrose are not directly linked. In the active layer and permafrost table horizons, we identified several strains unable to grow with the added sucrose (data not shown) and it is possible that these organisms may be capable of growth at –5°C but were not detected with this experimental design. A high proportion of salt-tolerant isolates in permafrost was indicated by similar cell counts on R2A and R2A supplemented with 5% NaCl. In comparison, cell recovery on R2A media supplemented with 5% NaCl was lower than on unmodified R2A in the active layer and permafrost table horizons (Fig. 4). The difference in the salt tolerance of the culturable microbial communities reflects the salinity data within the Eur3 core (Fig. 1) that indicated higher salinity in the permafrost relative to the active layer and permafrost table horizons.

Microbial activity at low temperatures

Mineralization of acetate and glucose was detected in the

Table 2. Phenotypic and phylogenetic characterization of Eur3 isolates.

Isolate	Closest BLAST Match	Environment of BLAST Match	% Similarity	Growth range (°C)[a]	Phylogeny[b]
Surface					
Eur3 AL.1	*Arthrobacter* sp. PSA A20 (DQ628958)	High Arctic glacier	100	0–25	Act
Eur3 AL.2	*Flavobacterium columnare* (AY747592)	NA[c]	98	0–25	CFB
Eur3 AL.4	*Paenibacillus* sp. H09-08 (AM162297)	NA	99	0–25	Fir
Eur3 AL.5	*Stenotrophomonas* sp. Enf29 (DQ339605)	Alpine plants	99	0–25	Pro
Eur3 AL.6	uncultured clone (DQ447834)	Life support module	99	21–25	Pro
Eur3 AL.7	Glacial ice bacterium	Antarctic sea water	99	0–25	Act
Eur3 AL.8	*Bacteroidetes* bacterium (DQ234533)	Lapland, Finland	98	0–25	CFB
Eur3 AL.10	uncultured clone (EF034288)	Spitsbergen permafrost	100	0–25	Act
Eur3 AL.14	***Planococcus maritimus* (DQ333301)**	**Lake sediment**	**98**	**–5–25**	**Fir**
Eur3 AL.16	*Pseudomonas* sp. An23 (AJ551161)	Antarctic sea	100	0–25	Pro
Eur3 AL.19	uncultured clone (DQ791436)	Geothermal soil	99	0–25	Pro
Eur3 AL.22	*Streptomyces* sp. LK1331.4	Prairie soil	99	0–25	Act
Eur3 AL.24	*Arthrobacter* sp. TSBY-20 (DQ172990)	Alpine permafrost	100	0–25	Act
Eur3 AL.25	*Arthrobacter* sp. TSBY-88 (DQ173034)	Alpine permafrost	100	0–25	Act
Eur3 AL.26	*Rhodoglobus* sp. GICR18 (AY439269)	Glacier ice core	99	21–25	Act
Eur3 AL.28	uncultured clone (AJ863181)	Poplar tree soil	99	0–25	Fir
Eur3 AL.30	***Arthrobacter* sp. PSA A20 (DQ628958)**	**High Arctic glacier**	**99**	**–5–25**	**Act**
Eur3 AL.31	*Sphingobacterium* sp. PDD (DQ512792)	Troposphere cloud	98	0–5	CFB
Eur3 AL.32	*Rhodoglobus* sp. GICR18 (AY439269)	Glacier ice core	99	ND[d]	Act
Eur3 AL.34	***Arthrobacter* sp. TSBY-69 (DQ173023)**	**Alpine permafrost**	**100**	**–5–25**	**Act**
Eur3 AL.37	*Hymenobacter* sp. X2–1 g (DQ888329)	NA	96	0–5	CFB
Eur3 AL.39	*Arthrobacter agilis* (AF440440)	Temperate water	99	0–25	Act
1 m (permafrost table)					
Eur3 1.3	*Flavobacterium* sp. WB (AM177392)	Creek water	98	0–25	CFB
Eur3 1.4	*Staphylococcus aureus* (DQ997837)	NA	100	25–37	Fir
Eur3 1.5	*Arthrobacter* sp. Z63zhy (AM412214)	Deep-sea sediment	100	0–25	Act
Eur3 1.7	*Bacillus psychrosaccharolyticus* (AB021195)	Marsh soil	99	21–25	Fir
Eur3 1.8	*Paenibacillus* sp. Eur1 9.9 (DQ444978)	Canadian permafrost	100	0–25	Fir
Eur3 1.12	*Paenibacillus* sp. Eint3 (AM062705)	Lichen associated	96	0–25	Fir
Eur3 1.15	Uncultured clone (AJ863181)	Poplar tree soil	100	ND	Fir
Eur3 1.20	Uncultured clone (AB240274)	Rhizosphere isolate	99	ND	CFB
Eur3 1.21	**Uncultured clone (DQ628934)**	**High Arctic glacier**	**99**	**–5–25**	**Pro**
Eur3 1.22	*Staphylococcus* sp. R-20811 (AJ786779)	Nitrifying inoculum	99	25–37	Fir
Eur3 1.31	Uncultured clone (DQ444136)	River sediment	97	ND	CFB
2 m (permafrost)					
Eur3 2.1	***Sporosarcina* sp. N-05 (EF154512)**	**Antarctic soil**	**99**	**–5–5**	**Fir**
Eur3 2.2	***Sporosarcina* sp. N-05 (EF154512)**	**Antarctic soil**	**92**	**–5–5**	**Fir**
Eur3 2.3	*Paenibacillus* sp. C7 (AY920751)	Cold active isolate	98	0–25	Fir
Eur3 2.5	*Paenibacillus* sp. YO4-16 (AM162349)	NA	99	0–25	Fir
Eur3 2.6	*Bacillus* sp. cryopeg_9 (AY660701)	Siberian permafrost	99	0–5	Fir
Eur3 2.8	*Bacillus* sp. C2M6 (DQ089754)	Fumigant treated soil	98	5–25	Fir
Eur3 2.10	*Bacillus psychrodurans* (EF101552)	Korean brown alga	99	5–25	Fir
Eur3 2.12	*Staphylococcus* sp. R-20811 (AJ786779)	Nitrifying inoculum	100	5–25	Fir
Eur3 2.14	*Paenibacillus donghaensis*	East Sea Korea	98	5–25	Fir

a. As determined by growth at –5°C, 0°C, 5°C, 21°C, 25°C and 37°C. Strains capable of growth at –5°C are shown in bold.
b. Act, *Actinobacteria*; Fir, *Firmicutes*; Pro, *Proteobacteria*.
c. Not available.
d. Not determined due to an inability to maintain the isolate in serial culture.

Eur3 core active layer soil and 2 m permafrost microcosms at temperatures as low as –15°C. Mineralization in the ground ice sample (4 m) occurred only at 5°C (Fig. 5). The Eur1 core 9 m permafrost sample showed very low levels of mineralization at subzero temperatures, despite demonstrating high mineralization rates at 5°C. In general, the rates and cumulative mineralization of acetic acid and glucose in the microcosms were similar, indicating that there was not a preference for one substrate over the other. The notable exception was in the 5°C Eur1 9 m permafrost microcosms where the cumulative mineralization of acetic acid was 116% higher than the mineralization of glucose.

Discussion

The aim of the present study was to describe the diversity and abundance of the microbial community in a vertical profile from the Canadian high Arctic that proceeded from the active layer soil into a layer of massive ground

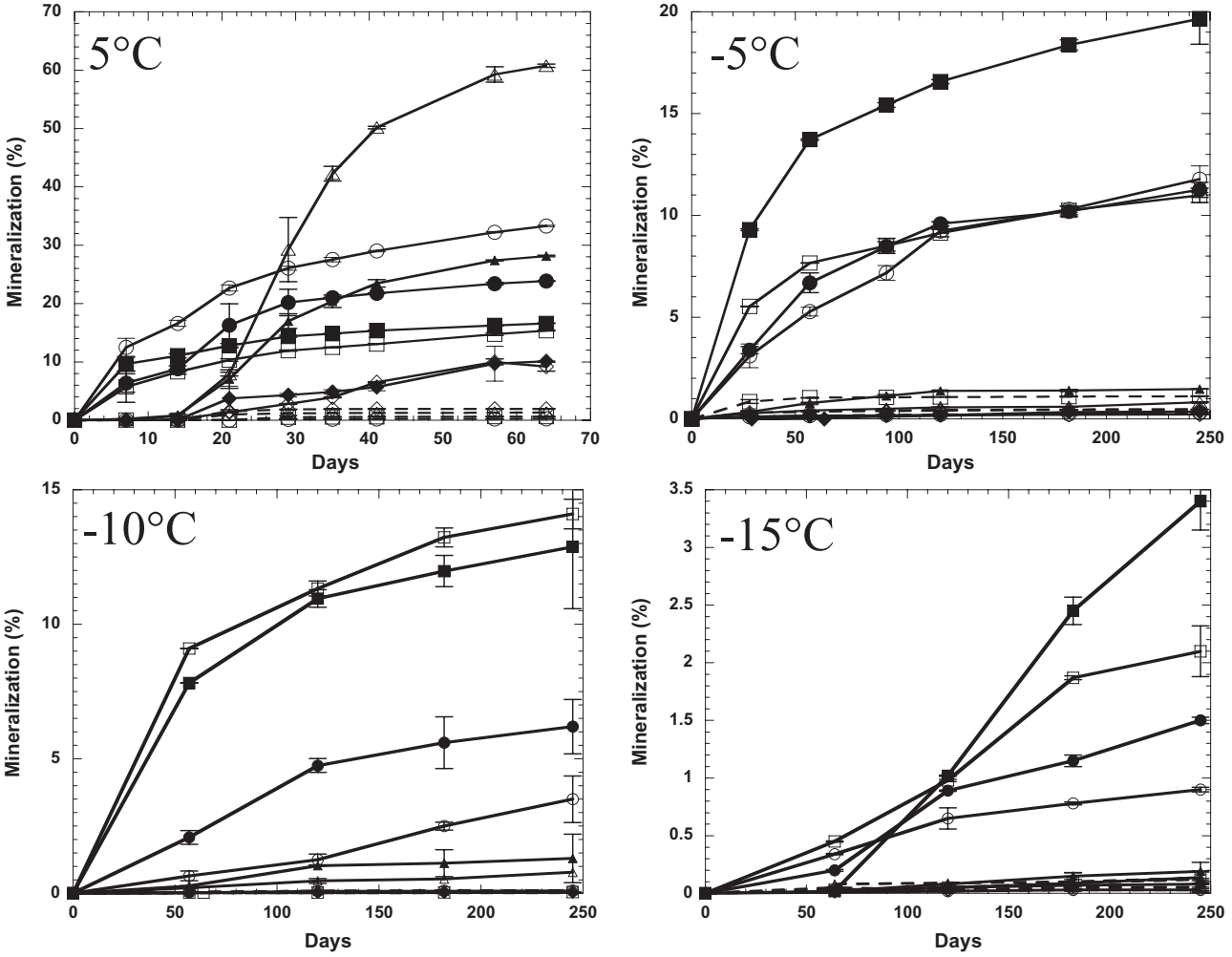


Fig. 5. Mineralization of [1-^{14}C] acetic acid and [2-^{14}C] glucose in microcosms. Each point represents the mean cumulative mineralization (% $^{14}CO_2$ recovered) from triplicate assays including sterile controls (dashed lines). Error bars show the standard error of the mean. Note the different scales on the *x*- and *y*-axes between different-temperature trials. Open markers (□ ○ ◇ △) represent mineralization of acetic acid and closed markers (■ ● ◆ ▲) represent mineralization of glucose. □ active layer soil; ○ Eur3 2 m permafrost; ◇ Eur3 4 m ground ice; △ Eur1 9 m permafrost.

ice. In addition, the mineralization of [1-^{14}C] acetic acid and [2-^{14}C] glucose was assessed to determine if the microbial communities associated with the distinct horizons in the profile were active at near ambient subzero temperatures. The Eur3 permafrost core is notable in that it represents one of the most northern latitudes from which the microbial communities have been described and represents the first microbiological study of a massive ground ice system.

The DGGE profiling was used to investigate changes in the microbial community with increased sample depth and within the distinct Eur3 core horizons. The DGGE analysis showed that the microbial communities varied with depth in the upper soil layers but were similar throughout the ground ice (Fig. 2). The differences in the microbial populations between the active layer, permafrost table, permafrost and ground ice horizons were further tested by constructing clone libraries of partial 16S rRNA gene sequences. Pairwise LIBSHUFF analysis indicated that the differences in the clone libraries were significant, suggesting that the observed differences in phylogenetic composition between the clone libraries is valid and reflect actual differences in the microbial communities.

Similarities in the phylogenetic groups identified in Antarctic active layer soil and permafrost have been proposed as circumstantial evidence that the active layer is the source of permafrost microorganisms (Gilichinsky *et al.*, 2007). This does not appear to be the case in this Canadian high Arctic permafrost system as both culture-dependent and culture-independent methods suggested that the microbial communities in the active layer and permafrost were significantly different. For instance, all of the permafrost isolates were halotolerant, compared with 32% of active layer isolates, possibly due to the higher

salinity in the permafrost (Fig. 1). The permafrost and active layer are very different environments; the permafrost environment is considered extreme because indigenous microorganisms must survive prolonged exposure to subzero temperatures and background radiation for geological time scales in a habitat with low water activity and extremely low rates of nutrient and metabolite transfer (Steven *et al*., 2006). Even if the active layer is the source of permafrost microorganisms, the unique conditions within permafrost have preferentially selected for different phylogenetic groups and phenotypes compared with active layer soils.

Three methods (*Bacteria*-specific DGGE, 16S rRNA gene clone libraries and culturing) were used to describe the microbial diversity in the Eur3 core. Both the culture-independent studies (DGGE and 16S rRNA gene clone libraries) detected many of the same partial 16S rRNA sequences in the same horizons of the Eur3 core. For example, the DGGE bands isolated from the ground ice samples were related to 16S rRNA clones in the ground ice clone library with 98–100% sequence identity. However, there were some notable differences between the DGGE and clone library analysis. For instance, DGGE band 2.4 was related to a *Clostridium* sp. (Fig. 2), while none of the 16S rRNA clones in the 2 m permafrost library were affiliated with the *Firmicutes*. This suggests that these two methods provided complementary but different pictures of the microbial composition in the Eur3 core.

The most notable difference between the microbial diversity as determined by the culture-independent methods and culturing was the dominance of endospore-forming *Firmicutes* cultivated from the permafrost (2 m) sample, Previously, we found that 69% of representative isolates from the Eur1 9 m Canadian high Arctic permafrost sample were related to the *Firmicutes* whereas they only accounted for 4% of the 16S rRNA gene clone library sequences (Steven *et al*., 2007a). However, factors such as differential cell lysis during DNA extraction (von Wintzingerode *et al*., 1997) and PCR biases (Suzuki and Giovannoni, 1996; Baker *et al*., 2003) make it difficult to extrapolate the abundance of a PCR-amplified 16S rRNA gene fragment to the actual abundance of the organism in the environment. This may be especially true of organisms present in the environment in recalcitrant forms, such as spores, and may explain the relatively low proportion of spore-forming *Firmicutes* present in the 16S rRNA gene clone libraries. In comparison with Canadian high Arctic permafrost, *Firmicutes*-related bacteria made up 30% of Siberian permafrost isolates (Shi *et al*., 1997), 5% of Spitsbergen permafrost isolates (Hansen *et al*., 2007) and 1% of bacterial isolates from Chinese Alpine permafrost (Bai *et al*., 2006). In Antarctic permafrost, non-spore-forming isolates were also dominant (Gilichinsky *et al*., 2007). The predominance of *Firmicutes* in Canadian high Arctic isolates appears to be unique, and may be a reflection of the distinctive characteristics of this environment.

We have identified many of the same phylogenetic groups in the Eur3 core that have been described from other permafrost systems. Genera such as *Arthrobacter*, *Paenibacillus*, *Rhodococcus* and *Pseudomonas* are commonly identified in culture-dependent and culture-independent surveys of permafrost microbial diversity (Shi *et al*., 1997; Gilichinsky *et al*., 2007; Hansen *et al*., 2007). The high proportion of clone sequences and isolates (Table 2) identified in this study, which were closely related to organisms or 16S rRNA gene sequences from other permafrost systems or similar cryoenvironments, implies that geographically separated permafrost environments may harbour cosmopolitan microorganisms as suggested by Hansen and colleagues (2007). Conversely, there was a relatively high proportion of potentially novel 16S rRNA gene sequences identified in the 2 m permafrost sample, suggesting the possibility of a novel endemic Canadian high Arctic permafrost microbial population.

The descriptions of *Archaea* in permafrost are limited to the detection of methanogenic *Archaea* in Siberian permafrost (Rivkina *et al*., 1998), the detection of *Crenarchaeota*-related sequences in a Russian permafrost sample (Ochsenreiter *et al*., 2003) and the culture-independent description of the Archaeal community in the Eur1 9 m permafrost sample (Steven *et al*., 2007a). In this study, we described the distribution and diversity of *Archaea* in a permafrost/ground ice core profile. The 16S rRNA gene sequences belonging to the *Crenarchaeota* dominated the active layer and permafrost table horizons, whereas *Euryarchaeota* were predominant in the permafrost. Many (27%) of the *Euryarchaeota* clones from the permafrost library were related to halophilic *Archaea*. Previously, we detected sequences related to the halophilic *Archaea* in the Eur1 9 m permafrost sample (Steven *et al*., 2007a). The 16S rRNA gene sequences related to the halophilic *Archaea* in the Eur1 9 m sample were predominantly related to the genus *Halobaculum* whereas *Halorubrum*-related sequences dominated the Eur1 2 m permafrost sample. The detection of 16S rRNA gene sequences from halophilic *Archaea* in the Eur3 core is a notable confirmation of their presence in Canadian high Arctic permafrost. To date, we have been unsuccessful in culturing halophilic *Archaea* from permafrost.

The culture-independent surveys of microbial diversity in the Eur3 core indicated the presence of anaerobic groups such as *Clostridium*, *Desulphuromonas* and *Rhodoferax* that we would not expect to be recovered using the aerobic culturing techniques employed in this

study. Therefore, this is not a comprehensive description of the culturable microbial community in Canadian high Arctic permafrost. However, the difference in the abundance and diversity of the recovered isolates indicates that the different horizons in the Eur3 core differ in their ability to support aerobic heterotrophic microbial populations.

Even the largest 16S rRNA gene clone libraries only describe a fraction of the total microbial diversity predicted to exist in those environments (Leser *et al*., 2002; Eckburg *et al*., 2005; Ley *et al*., 2006). The relatively small size of the clone libraries in this study paired with underestimation of species diversity that may be associated with using non-parametric species estimators such as Chao1 (Hong *et al*., 2006) suggests that the microbial diversity described should only be considered a minimal estimate of the total microbial diversity. Based on the number of phylogenetic groups recovered in the 2 m clone library, richness estimates (Table 1) and rarefaction analysis (Fig. S2), the microbial community in near surface permafrost (2 m) was at least as diverse as in the active layer soil. Using serial analysis of ribosomal sequence tags of 17–55 bp, the diversity of active layer soil from the Canadian high Arctic was found to be remarkably diverse ($H' \sim 5.9$; Neufeld and Mohn, 2005). We detected less diversity in the active layer soil from Eureka ($H' = 3.6$) although the diversity was similar to other high Arctic environments such as cold saline springs ($H' = 2.2–3.2$; Perreault *et al*., 2007) and ice shelf microbial mats ($H' = 3.5–4.4$; Bottos *et al*., 2007).

The 16S rRNA gene diversity in the Eur3 permafrost ($H' = 3.6$, Table 1) was greater than detected in our previous study on the Eur1 9 m permafrost sample ($H' = 1.8$; Steven *et al*., 2007a). In the Eur3 2 m permafrost *Proteobacteria*-related 16S rRNA gene sequences were more predominant (57% of clone sequences) compared with in the Eur1 9 m permafrost sample (39% of clone sequences; Steven *et al*., 2007a). Interestingly, although the diversity and composition of the microbial communities were different, the number of culturable heterotrophic cells recovered was similar (Eur1 9 m = 6.9×10^3; Eur3 2 m = 4.5×10^3) from both permafrost samples. None of the isolates or 16S rRNA sequences from the Eur3 2 m permafrost were identified as having a 16S rRNA gene sequence from the Eur1 9 m sample as a nearest neighbour using BLAST. In fact, only one isolate (Eur3 1.8) from the permafrost table horizon (1 m) had a 16S rRNA sequence from the Eur1 9 m sample identified as the closest BLAST match (Table 2). On the other hand, similar Gram-positive genera (*Arthrobacter*, *Paenibacillus*, *Sporosarcina*) were found in the culturable aerobic heterotrophic populations from both the Eur1 9 m permafrost sample (Steven *et al*., 2007a) and in the Eur3 1 and 2 m samples. None of the sequenced DGGE bands or 16S rRNA gene clones from the Eur3 core samples were identified as having a closest BLAST match from the Eur1 9 m sample. These results indicate that the microbial diversity in the Eur3 core horizons varied considerably from the microbial community in relatively deep permafrost (9 m).

Several recent studies have shown that temperate soils support extremely diverse microbial communities (e.g. Neufeld and Mohn, 2005; Roesch *et al*., 2007). The dominant phyla represented in diverse soil environments were the *Proteobacteria* followed by the CFB, *Acidobacteria*, *Actinobacteria* and *Firmicutes* (Roesch *et al*., 2007), the same phyla that made up the majority of the Eur3 clone libraries. Other less common phyla such as *Nitrospira*, *Verrucomicrobia* and TM7 (Roesch *et al*., 2007) that were detected in temperate soils were not found in the Eur3 core. However, due to the incomplete coverage of the Eur3 clone libraries we cannot conclude that these phyla were not present in Canadian high Arctic soils.

While there is no universal agreement upon the level of 16S rRNA gene sequence divergence to establish a sequence as novel, $\leq$ 97% similarity is commonly applied to indicate novel species level diversity (Stackebrandt and Goebel, 1994). The majority of sequences (62–69%) in the clone libraries from the different horizons were related with $\geq$ 98% sequence similarity to their nearest neighbour in the GenBank database, indicating a close relationship to previously described species or environmental 16S rRNA gene sequences. However, 20% of the permafrost (2 m) clone library sequences showed $\leq$ 95% sequence similarity to previously described 16S rRNA sequences compared with 8%, 5% and 6% in the active layer, permafrost table and ground ice clone libraries respectively, indicating that a relatively large proportion of the permafrost clones could represent potential novel genera or higher taxonomic levels. Many of the clones that were closely related to 16S rRNA gene sequences in the GenBank database were related to sequences from other Arctic environments. For example, 53% of the permafrost *Archaea* OTUs were most similar (97–100% sequence identity) to sequences recovered from cold perennial springs in the Canadian high Arctic (Perreault *et al*., 2007) and 55% of clones in the permafrost table *Bacteria* clone library were most closely (96–100%) related to 16S rRNA genes identified in a similar-depth Spitsbergen permafrost sample (Hansen *et al*., 2007).

The low diversity of 16S rRNA gene sequences in the ground ice clone library, absence of culturable cells, relatively low direct microscopic cell counts and the inability to recover PCR-amplifiable DNA from depths below 7 m suggest that ground ice is an inhospitable environment compared with permafrost. Similarly, culturable cells were not recovered from Siberian ice wedges (Gilichinsky

et al., 1995). However, an Alaskan ice wedge sample harboured a viable microbial community of ~10^6 cfu g^{-1} with low diversity, predominantly composed of bacteria related to the *Actinobacteria* (Katayama *et al*., 2007). The clone library of partial 16S rRNA sequences from the massive ground ice in the Eur3 core also showed low diversity (Table 1) but was dominated by sequences related to the *Firmicutes*, primarily of the genus *Clostridium* (Fig. 3). The partial 16S rRNA gene sequences of the *Archaea* community in massive ground ice included sequences closely related to methanogens, halophiles and environmental sequences related to both the *Euryarchaeota* and *Crenachaeota* and represents the first description of the *Archaea* community in a ground ice system. Differences in the microbial communities between these two permafrost ice systems is probably due to differences in the origin and deposition of the ice. The similarity of the *Bacteria* community in the different depths of ground ice, as revealed by DGGE (Fig. 2), suggested that the environmental conditions in ground ice selected for specific groups of organisms, predominantly anaerobic spore-forming *Clostridia*.

We cannot rule out the possibility that some of the 16S rRNA gene sequences in this study came from dead microbial cells or naked DNA molecules in the permafrost. Microscopic observations of permafrost microorganisms have shown that up to 74% of Spitsbergen permafrost microorganisms had damaged membranes (Hansen *et al*., 2007) and Siberian permafrost was dominated by microorganisms in a cyst-like state (Soina *et al*., 2004). To determine if uncultured microbial groups detected in permafrost have remained viable will require developing targeted culture methods or using techniques such as stable isotope probing, which can identify active microbes.

Microbial metabolism as measured by mineralization of [1-^{14}C] acetic acid and [2-^{14}C] glucose in cold temperature microcosms indicated the presence of active microbial communities in all of the samples. Mineralization at 5°C in the 4 m ground ice sample (Fig. 5) indicates the presence of viable cells despite our inability to culture any microorganisms from the ground ice on R2A plates (Fig. 4). Mineralization at 5°C was highest in the 9 m permafrost sample, but the onset of mineralization showed an early lag phase compared with the other samples. This delay in mineralization may be due to the time required for germination of spores or recovery of cells from a resting state. At extremely low temperatures the amount of liquid water available in the pore spaces of permafrost is related to temperature and the physical characteristics of the permafrost (Gilichinsky *et al*., 1995; Price, 2007). Therefore, it is not clear whether the differences in metabolic activity between the horizons in the Eur3 core are due to differences in the physical environment or whether it reflects differences in the metabolic capabilities of the indigenous microbial community. In the horizons that did support subzero microbial activity, both the rates and cumulative mineralization increased with increasing temperature, suggesting that even a small elevation in the mean annual temperature in permafrost may result in a rise in CO_2 emissions from permafrost soils.

In conclusion, we have shown that the microbial community in Canadian high Arctic permafrost is diverse and may include microbial populations active at the ambient subzero temperatures. The ice content in permafrost appears to have a large influence on the community structure, as ground ice microbial communities had lower abundance, diversity and activity compared with permafrost soils, indicating that such relatively ice-rich permafrost environments may be poor astrobiology targets for detecting potential microbial life or biosignatures. Identifying the environmental factors that control the diversity and abundance of microbes in permafrost will also be necessary to model changes in permafrost microbial ecosystems predicted to occur with global climate change.

Experimental procedures

Site description and sampling

Permafrost and ground ice samples were collected in May 2003, as part of a collaboration with the NASA Astrobiology Science and Technology Instrument Development Mars Deep Drill Project. The Eur3 core was collected from Eureka (80°0.029 N, 85°50.367 W), Ellesmere Island, Nunavut, Canada, using the coring equipment, fluorescent microspheres and protocols described by Juck and colleagues (2005). Samples were returned frozen to Montreal, Canada and stored at –20°C until sampling.

Soil and ground ice physical and chemical analyses

Physical and chemical analyses of active layer and permafrost soils were performed as previously described (Steven *et al*., 2007a). For ground ice samples, 20 g composite samples were oven-dried (105°C), and the pH and electroconductivity (for determination of salinity) of the dried sediment was determined in a 1:2 sediment/deionized water slurry (Rhoades, 1982). Total carbon and nitrogen were measured in the dried sediments by combustion at 900°C (Lim and Jackson, 1982) with a Carlo Erba Flash EA NC Soils Analyser (Milan, Italy). Bore-hole temperature was monitored at the Eur1 site (Steven *et al*., 2007a; Fig. S1) but temperature measurements during drilling indicated a similar temperature profile at the Eur3 site.

DNA isolation from soil and ice samples

Total community DNA was isolated from successive 1 m depths throughout the Eur3 core profile (15 samples; surface to 14 m). At 1 m depth intervals the core was sectioned with a sterile chisel and each of the subsections was painted with an overnight culture (7.8×10^8 cells ml^{-1}) of *Pseudomonas*

sp. strain Cam1-*gfp2* (Ahn *et al*., 2001). Pristine subcores were collected using the subcoring method described by Juck and colleagues (2005). Interior subcore material was demonstrated to be pristine by culturing and PCR directed towards the *gfp* gene of *Pseudomonas* sp. strain Cam1-*gfp2* using previously described methods (Juck *et al*., 2005). These analyses demonstrated that the subcore samples were pristine and suitable for both culture-dependent and culture-independent microbial analyses.

For active layer and permafrost samples, total community DNA was extracted from 10 g composite samples using the method of Miller and colleagues (1999). Extracted community DNA was then purified using polyvinylpolypyrrolidone spin columns as previously described (Berthelet *et al*., 1996). For the ground ice samples, the amount of ground ice required to recover 10 g of sediment (60–100 g) was calculated from the gravimetric water content. The required amount of ground ice was placed in a sterile beaker and rapidly thawed at 65°C in a water bath (~5 min). The melted sample was transferred to a centrifuge tube and the sediments were collected by centrifugation (10 000 $g \times 10$ min at 4°C). The water was decanted and total community DNA was extracted from the pellet using the same methods as for the active layer and permafrost samples. All DNA extractions were verified by agarose gel electrophoresis using standard protocols (Sambrook and Russell, 2001).

DGGE

Primers 341FGC with a 40-base **GC clamp** (5′-**CGC CCG CCG CGC GCG GCG GGC GGG GCG GGG GCA CGG GGGG** CCT ACG GGA GGC AGC AG-3′) and 758R (5′-CTA CCA GGG TAT CTA ATC C-3′) were used to amplify a 417 bp region of *Bacteria* 16S rRNA genes from total microbial community DNA in each of the 1 m depth horizons throughout the Eur3 core. Unless otherwise stated, the PCR reactions and conditions were as previously described (Steven *et al*., 2007a). PCR products were purified using the QIAaquick PCR purification kit (Qiagen, Mississauga, Canada) and the purified products were quantified using a NanoDrop ND-1000 spectrophotometer (Wilmington, DE). Each lane in a 8% acrylamide gel with a denaturing gradient of 35–65% was loaded with 300 ng of PCR product, and electrophoresis was performed using the D-Code system (Bio-Rad, Hercules, CA) in 1× TAE (20 mM Tris acetate, 10 mM sodium acetate, 0.5 mM EDTA) at a constant temperature of 60°C and 60 V for 16 h. The gel was stained with 1× SYBR green (Molecular Probes, Eugene, OR) for 10 min, destained in sterile water for 10 min and visualized with a ChemiGenius Bioimaging system (Cambridge, UK). Bands of interest were excised and DNA was eluted with an equal volume of TE overnight at 4°C. The resulting solution (2 μl) was used as template DNA for a subsequent PCR amplification. The purity and correct running position of each fragment was confirmed by a second DGGE gel. The resulting bands were excised from the gel, eluted as above and re-amplified for DNA sequencing.

Clone library construction

Bacteria 16S rRNA genes were amplified from total community DNA using the primers 27F and 758R under previously described PCR conditions (Steven *et al*., 2007a). Because of the low yields of *Archaea* 16S rRNA amplification products from a direct PCR reaction from total community DNA, a semi-nested PCR reaction was employed (Boon *et al*., 2002; Bomberg *et al*., 2003). In the first PCR reaction, primers 109F (5′-ACK GCT CAG TAA CAC GT-3′) and 934R (5′-GTG CTC CCC CGC CAA TTC CT-3′) were used to amplify *Archaea* 16S rRNA genes from total community DNA using the PCR conditions previously described (Steven *et al*., 2007a). The second round of PCR was performed with primers 344F (5′-ACG GGG TGC AGC AGG CGC GA-3′) and 934R. PCR was performed with the same PCR conditions as in the first amplification, with exception that only 10 cycles were performed after the touchdown. All PCR reactions were evaluated by agarose gel electrophoresis and resulted in PCR products of the expected size (~590 bp). Clone libraries were constructed as described previously (Steven *et al*., 2007a).

Enumeration and characterization of microbial isolates

Culturable heterotrophic bacteria from active layer and permafrost soils were enumerated by standard spread plate counts on R2A media as previously described (Steven *et al*., 2007a). Ground ice samples were processed in a –20°C freezer to maintain the integrity of the core. Several subsample were collected from each horizon using the subcoring procedure described by Juck and colleagues (2005). The resulting subcores (~10 g of ice) were thawed for 1 h at 5°C in pre-chilled glass tubes containing 2.5 g of sterile glass beads and vortexed for 1 min. Serial dilutions, including undiluted melt water, were plated on the same medium. Cold temperatures during plating were maintained by performing all manipulations on ice and using pre-chilled glass tubes, dilution buffers and culturing plates. R2A plates were incubated at 37°C, 25°C and 5°C. The R2A plates supplemented with 5% NaCl, to test for the presence of salt-tolerant microbial communities, were also incubated at 5°C. Plates were incubated until the appearance of new colonies was no longer noted, 9, 28 and 35 days at 37°C, 25°C and 5°C respectively.

Unique colonies from plates with $\geq$ 50 cfu were isolated and characterized based on colony morphology (e.g. size, pigmentation, expolysaccharide production) and growth characteristics (e.g. temperature of growth and time of colony appearance) and representative colonies were selected for 16S rRNA gene sequence analysis. DNA from the isolates was prepared by boiling lysis and 16S rRNA genes were amplified with primers 27F and 758R using the PCR conditions as previously described (Steven *et al*., 2007a). Amplification products were verified by agarose gel electrophoresis and sequenced. Phenotypic characterization was performed by subculturing the isolates onto R2A plates incubated at 37°C (7 days), 25°C and 21°C (20 days), 5°C and 0°C (30 days) or R2A supplemented with 7% w/v sucrose incubated at –5°C (90 days).

Phylogenetic and statistical analyses

The 16S rRNA gene sequences were compared with the GenBank database using the nucleotide BLAST (Altscul *et al*., 1990) and with the RDP using the sequence Match software (Cole *et al*., 2007) to identify closest relatives of the queried

16S rRNA gene sequences. Phylogenetic classifications of 16S rRNA gene sequences were made with the RDP classifier software (Cole *et al*., 2007) using default parameters (80% confidence threshold). Alignments were constructed using the Clustal_W program in the MacVector 7.0 software package (Oxford Molecular, Oxford, UK). Potential chimeric sequences in the clone libraries were identified using the Mallard software package (Ashelford *et al*., 2006) and putative chimeric sequences were verified by Pintail (Ashelford *et al*., 2005) as per the author's directions. All identified chimeras were removed from further analyses. The OTUs, Shannon's diversity index (H′), Chaol estimator and rarefaction curves were determined using the DOTUR software package (Schloss and Handelsman, 2005) with PHYLIP-generated DNADIST files (http://evolution.genetics.washington.edu/phylip.html). The significance of difference between the clone libraries was examined in paired comparisons using LIBSHUFF (Singleton *et al*., 2001). Coverage of the clone libraries was calculated using Good's method (Good, 1953; Kemp and Aller, 2004).

Mineralization of ^{14}C-glucose and ^{14}C-acetate in microcosms

To determine if distinct horizons in the Eur3 core harboured microbial populations active at cold temperatures, microcosms containing 5 g composite samples of soil or ground ice were set up as previously described (Steven *et al*., 2007b). The horizons used for activity detection were active layer soil, permafrost from a depth of 2 m and a ground ice sample (4 m). Also included was a permafrost sample from the Eur1 core from a depth of 9 m, which has been described previously (Steven *et al*., 2007a). Each microcosm, performed in triplicate, including sterile controls (autoclaved twice for 30 min at 120°C and 1.0 atm, with a 24 h period between autoclavings), was spiked with 0.045 μCi μl^{-1} (100 000 disintegrations per min) of either [1-^{14}C] acetic acid or [2-^{14}C] glucose in a volume of cold substrate to give a final concentration of 100 p.p.m. of substrate in the microcosm. The CO_2 trap consisted of 1 M KOH + 20% v/v ethylene glycol for microcosms incubated at 5°C, −5°C and −10°C. For the −15°C microcosms the CO_2 trap was a solution of 1 M KOH + 30% v/v ethylene glycol. Microcosms were incubated in Refrigerated Incubators (Fisher Scientific) with a rated temperature uniformity of ±0.5°C and temperature constancy of ±0.2°C; actual temperatures of the incubators were monitored continuously using digital data logger thermometers (Fisher Scientific) and the incubator internal temperature did not vary more than ±3°C, including during sampling, when the incubators were opened to sample the microcosms. All manipulations and sampling of the microcosms were performed on ice and the microcosms were never outside of the incubator for more than ~2 min per sampling. The CO_2 traps were sampled at timed intervals and radioactive counts were determined by liquid scintillation spectrometry on a Beckman Coulter (CA, USA) LS 6500 Multi-purpose Scintillation Counter.

Nucleotide accession numbers

GenBank accession numbers for the nucleotide sequences deposited in this study are as follows: *Archaea* clone library, EU218553-EU218634; *Bacteria* clone library and DGGE bands, EU218635-EU218837; and *Bacteria* isolates, EU218838-EU218875.

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Supporting information

Additional Supporting Information may be found in the online version of this article:

Fig. S1. Comparison of the Eur1 (79°59.900 N, 85°53.755 W) and Eur3 (80°0.029 N, 85°50.367 W) drilling sites.
A. NAPL Air photo A27038-162 (1972) showing approximate locations of the drill sites relative to the runway and topography. Scale = 1:10 000 (scale bar = 100 m).
B. Schematic diagram of the Eur1 and Eur3 cores. Sample depths used for microbiological examinations are indicated. The main difference between the two sites pertains to the pattern of sediment and ground ice linked to depositional and freezing history. The small amounts of ice in the Eur1 core occurs due to the gradual freezing associated with permafrost aggradation into a marine terrace composed primarily of a thick blanket of rhythmically deposited silty sand with clay sediments. Ice contents are relatively low due to the fine-grained nature of these sediments and the presence of clay in the sequence. So the ice observed in the Eur1 core is basically *in situ* pore water. By comparison, the core from the Eur3 site is associated with a body of massive ice and ice-rich permafrost. The marine sediment veneer (underlying permafrost) at this site is relatively thin (roughly 1–1.2 m) and is only slightly thicker than the active layer. Below the surface veneer of massive silty clay, the sequence is mainly one of ice-rich silt or massive ice and layers of silt. This is a typical sequence where massive ice forms through injection–segregation.
Fig. S2. Rarefaction curves for *Bacteria* (A) and *Archaea* (B) 16S rRNA gene clone libraries. ○ active layer clone libraries; ● permafrost table clone libraries; ▲ permafrost clone libraries; × ground ice clone libraries.

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