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ORIGINAL PAPER

Prokaryotic diversity in sediments beneath two polar glaciers with contrasting organic carbon substrates

Marek Stibal · Fariha Hasan · Jemma L. Wadham · Martin J. Sharp · Alexandre M. Anesio

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Abstract Microbial ecosystems beneath glaciers and ice sheets are thought to play an active role in regional and global carbon cycling. Subglacial sediments are assumed to be largely anoxic, and thus various pathways of organic carbon metabolism may occur here. We examine the abundance and diversity of prokaryotes in sediment beneath two glaciers (Lower Wright Glacier in Antarctica and Russell Glacier in Greenland) with different glaciation histories and thus with different organic carbon substrates. The total microbial abundance in the Lower Wright Glacier sediment, originating from young lacustrine sediment, was an order of magnitude higher ($\sim 8 \times 10^6$ cells per gram of wet sediment) than in Russell Glacier sediment ($\sim 9 \times 10^5$ cells g^{-1}) that is of Holocene-aged soil origin. 4% of the microbes from the Russell Glacier sediment and 0.04–0.35% from Lower Wright Glacier were culturable at 10°C. The Lower Wright Glacier subglacial community was dominated by Proteobacteria, followed by Firmicutes. The Russell Glacier library was much less diverse and also dominated by Proteobacteria. Low numbers and diversity of both Euryarchaeota and Crenarchaeota were found in both sediments. The identified clones were related to bacteria with both aerobic and anaerobic metabolisms, indicating the presence of both oxic and anoxic conditions in the sediments.

Keywords Subglacial environment · Bacteria · Archaea · Diversity · Antarctica · Greenland

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M. Stibal · F. Hasan · J. L. Wadham · A. M. Anesio
Bristol Glaciology Centre, School of Geographical Sciences, University of Bristol, Bristol BS8 1SS, UK

Present Address:
M. Stibal (✉)
Department of Geochemistry, Geological Survey of Denmark and Greenland, Øster Voldgade 10, 1350 Copenhagen K, Denmark
e-mail: msti@geus.dk

Present Address:
F. Hasan
Department of Microbiology, Quaid-i-Azam University, Islamabad 45320, Pakistan

M. J. Sharp
Department of Earth and Atmospheric Sciences, University of Alberta, Edmonton, AB T6G 2E3, Canada

Introduction

Microbial ecosystems beneath glaciers and ice sheets have recently attracted significant attention due to their metabolic potential and possible role in regional and global carbon cycling and climate change (Sharp et al. 1999; Wadham et al. 2008). Distinct microbial communities have been found beneath numerous glaciers (e.g. Foght et al. 2004; Skidmore et al. 2005; Lanoil et al. 2009; Yde et al. 2010), feeding on organic carbon overridden by glaciers during period of ice advance or supplied from the glacier surface in melt water (Bhatia et al. 2006; Stibal et al. 2008; Wadham et al. 2008). Betaproteobacteria are the dominant bacteria in most of these communities, but species of other Proteobacteria classes, Bacteroidetes, Acidobacteria, Actinobacteria and other bacterial phyla have also been detected in subglacial sediments (Foght et al. 2004; Skidmore et al. 2005; Lanoil et al. 2009; Yde et al. 2010). Recent work also provides evidence of the presence of archaea in subglacial environments (Boyd et al. 2010; Stibal et al. 2012).

Subglacial sediments are assumed to be largely anoxic due to the removal of dissolved oxygen during the microbial oxidation of organic carbon and sulphide minerals (Bottrell and Tranter 2002; Wadham et al. 2004), and thus various anaerobic pathways of organic carbon metabolism may occur. Production of methane by methanogenic archaea has been suggested to be the last step of carbon metabolism in subglacial ecosystems, with potentially significant effects on global climate due to the release of methane during deglaciation (Wadham et al. 2008; Boyd et al. 2010; Wadham et al. 2012). In addition, the quality of the organic carbon substrate has been shown to affect the abundance and activity of methanogenic archaea in subglacial environments (Stibal et al. 2012).

There is very little information available on microbial diversity and metabolic potential beneath remote ice sheets and glaciers. Here we examine the diversity of all prokaryotic microbes in subglacial sediments from Lower Wright Glacier, Antarctica, and Russell Glacier, part of the Greenland ice sheet, two polar glaciers with contrasting carbon sources recently shown to harbour populations of active methanogenic archaea (Stibal et al. 2012; Wadham et al. 2012). The origin of the Lower Wright Glacier subglacial material is reworked lake sediment and it contains relatively labile organic matter, while the Russell Glacier sediment is overridden soil of Holocene age, with a higher proportion of recalcitrant organic carbon (Stibal et al. 2012). We used both universal bacterial and universal archaeal primers in order to reveal the prokaryotic diversity in these two distinct subglacial environments. We discuss the effect of the different organic carbon sources beneath these glaciers on the genetic and potential metabolic diversity of the present microbes.

Materials and methods

Field sites and sampling

Subglacial sediment was collected from the margins of Lower Wright Glacier in Antarctica and Russell Glacier in Greenland via a combination of ice-cored thrust moraines and pressure ridges. Both sites were described in detail previously (Stibal et al. 2012). Briefly, the origin of the Lower Wright Glacier subglacial sediment is relatively young (~200–300 years) reworked lake sediment. The total OC content in the sediment is low (<0.1% dw), but with a relatively high proportion of modern microbial OC, as indicated by biomarker analysis (Stibal et al. 2012). The bed of Lower Wright Glacier thus represents a very distinct 'end-member' type of subglacial environment, with a relatively high proportion of labile OC. The Russell Glacier sediment mostly originates from overridden soil of Holocene age, and its total OC content is ~0.4% dw. However, it contains a relatively higher proportion of less bioavailable recalcitrant organic carbon, mostly derived from old microbial and plant material (Stibal et al. 2012). The bed of Russell Glacier thus represents another 'end-member' subglacial environment, with older soil-derived OC as the principal substrate for microbial metabolism.

The sampling at Lower Wright Glacier was conducted from large blocks of frozen sediments intercalated with layers of pure glacier ice and basal ice exposed at the crest of the moraine along the interface between the glacier and the perennially ice-covered Lake Brownworth. At Russell Glacier, samples containing subglacial sediment, sediment-laden basal ice and clean glacial ice were collected from glacier termini and pressure ridges containing subglacial sediment bands thrust to the glacier surface in the terminal zone. All the collected samples were transported frozen to the Low Temperature Experimental Facility (LOWTEX) at Bristol and stored at −30°C. Prior to analysis, the samples were placed in a laminar flow cabinet to prevent contamination and the outer layer (10–30 mm) of each sample was removed by washing with sterile deionised water. The ice samples were then placed in a pre-furnaced glass bowl and allowed to thaw. The liberated subglacial sediment was used for further analysis.

Microbial abundance determination

For total microbial counts, 100 mg of freshly melted wet sediment and 1 ml of pre-sterilised deionised water were placed in a sterile Eppendorf tube and mixed by vortexing for 30 s. 0.2 μm filter-sterilised 37% formaldehyde (final concentration 1%) was added to the tube to fix the sample. The suspension was filtered onto a sterile 0.2 μm Anodisc filter (Whatman, Maidstone, UK). Dried filters were placed onto 100 μl drops of 2× SYBR Gold (Invitrogen, Eugene, OR, USA) for 15 min, dried and mounted on microscopic slides with the anti-fade agent Citifluor AF2 (Citifluor, Leicester, UK). More than 300 SYBR Gold stained cells were enumerated on each slide with an Olympus BX41 epifluorescence microscope (Olympus Optical, Tokyo, Japan) using the filter block U-N31001 (excitation 480 nm, emission 535 nm; Chroma Technology, Rockingham, VT, USA). Two slides per sample were counted. For viable counts, 0.1 ml of tenfold serial dilutions of a sediment slurry (1:10 w/v initial dilution) was plated onto R2A media and incubated at 10°C under aerobic and anaerobic conditions for 31 and 47 days, respectively. Viable counts were determined as the mean

of all plates showing between 5 and 200 colonies and normalised to a gram of sediment.

DNA extraction, amplification and purification

Extraction of DNA from the subglacial sediment samples was performed using the PowerSoil DNA Isolation Kit (MO BIO Laboratories, Carlsbad, CA, USA) according to the manufacturer's instructions. Approximately 300 mg (wet weight) of subglacial sediment was used for each extraction, and a blank containing no sediment was extracted in parallel. Bacterial 16S rRNA genes were amplified using the primers 27F (AGA GTT TGA TCC TGG CTC AG) and 1492R (GGT TAC CTT GTT ACG ACT T) (Tavormina et al. 2008). Archaeal 16S rRNA genes were amplified using the universal archaeal primers A109F (ACK GCT CAG TAA CAC GT) and A934R (GTG CTC CCC CGC CAA TTC CT) (Großkopf et al. 1998). 2 µl of the template was used for each PCR reaction. The reaction mixture of 50 µl contained 3 µl of $MgCl_2$ (final concentration 1.5 mM), 1 µl of each primer (200 nM), 1 µl of dNTPs (200 µM), 5 µl of PCR buffer (1×) and 0.25 µl of Taq polymerase (1.25 U). The following protocol was used for DNA amplification: initial melting step at 94°C for 3 min, 32 cycles of 94°C for 45 s, 52°C for 1 min and 72°C for 1 min, and a final extension step at 72°C for 7 min. Negative controls with no template were run in parallel. PCR products were analysed by electrophoresis in a 2% agarose gel stained with SYBR Gold. Amplified DNA was purified using the QiaQuick purification kit (Qiagen, Hilden, Germany).

Clone library construction

Clone libraries were constructed from the DNA extracts. Purified PCR products were ligated into pGEM Easy vector plasmids at 4°C overnight, transformed into *Escherichia coli* JM109 ($>10^8$ cfu μg^{-1}) competent cells (Promega, Madison, WI, USA) and grown overnight at 37°C on LB/ampicillin/IPTG plates spread with 20 µl 2% w/v X-Gal. Plasmid DNA was extracted from up to 60 white colonies using the Wizard Plus SV miniprep kit (Promega, Madison, WI, USA), and sequenced using the M13R primer by Geneservice at the University of Oxford, UK.

Phylogenetic analysis

The obtained bacterial and archaeal 16S rRNA gene sequences from each site were checked for chimeras using Bellerophon (Huber et al. 2004) and grouped into operational taxonomic units (OTUs) according to their similarity. The OTUs were then used for diversity and phylogenetic analysis. 16S rRNA gene sequences of closest relatives were searched for using BLAST at the National Center for Biotechnology Information, and additional sequences potentially related to the obtained ones were also retrieved. All sequences of ~700 bp were aligned using the CLUSTAL W package (Thompson et al. 1994) and edited manually in JalView (Waterhouse et al. 2009). Phylogenetic analyses were restricted to nucleotide positions that were unambiguously aligned in all sequences. The phylogenetic tree was constructed using the maximum likelihood method using the PHYLIP software package, version 3.68 (Felsenstein 2005). The order in which the sequences were added was jumbled in order to avoid potential bias introduced by the order of sequence addition. Bootstrap analysis (1000 replications) was used to provide confidence estimates for phylogenetic tree topologies. The coverage of the clone libraries in % was calculated as $(1 - (n/N)) \times 100$, where n is the number of unique clones detected in the library of size and N the total number of clones. The Berger–Parker index was used to determine the dominance, and the Shannon–Wiener index was calculated to show the diversity.

Nucleotide sequence accession numbers

Sequences have been deposited in GenBank under accession numbers JF273561–JF273599 and HQ214465–HQ214473.

Results and discussion

Microbial abundance

Table 1 shows the total and viable microbial counts in the subglacial sediment samples. The total microbial abundance in the Lower Wright Glacier sediment was $\sim 8 \times 10^6$ cells per gram of wet sediment, which is similar to that in nearby deglaciated soil (Ayton et al. 2010) and Antarctic lake sediments (Mancuso et al. 1990), and an order of magnitude higher than in Russell Glacier sediment ($\sim 9 \times 10^5$ cells g^{-1}). The abundance of microbes in Russell Glacier material is more typical of other subglacial sediments from Antarctica (Lanoil et al. 2009), Svalbard (Kaštovská et al. 2007) and New Zealand (Foght et al. 2004). It is likely that the origin of the sediment and the organic carbon present is a major factor affecting the abundance of microbes in subglacial environments, with younger sediments containing more labile organic substrate and thus being more conducive to microbial survival and growth.

Approximately 4% of the microbes from the Russell Glacier sediment and 0.04–0.35% from Lower Wright were culturable at 10°C on R2A media (Table 1). The fraction of culturable bacteria from Lower Wright is similar to that

Table 1 Total and viable microbial counts in the two subglacial sediment samples

	Total cells ($\times 10^6$ cells g^{-1})	Aerobic viable counts		Anaerobic viable counts	
		$\times 10^3$ cfu g^{-1}	% culturable	$\times 10^3$ cfu g^{-1}	% culturable
Lower Wright Glacier, Antarctica	7.9 ± 2.7	28 ± 15	0.35	2.9 ± 0.14	0.037
Russell Glacier, Greenland	0.87 ± 0.39	34 ± 12	3.9	33 ± 9.3	3.8

Table 2 Characteristics of clone libraries of the universal bacterial- and archaeal primer-amplified 16S rRNA genes from the two subglacial sediment samples

	Clones	Unique OTUs	Coverage (%)	Dominance (Berger–Parker index)	Diversity (Shannon–Wiener index)
Bacteria					
Lower Wright Glacier, Antarctica	58	32	45	0.22	3.26
Russell Glacier, Greenland	20	7	65	0.71	1.86
Archaea					
Lower Wright Glacier, Antarctica	39	6	85	0.21	1.69
Russell Glacier, Greenland	18	3	83	0.72	0.73

from subglacial sediment beneath West Antarctica (0.1–0.2% at 4°C; Lanoil et al. 2009), while the culturable fraction from Russell Glacier is at the lower end of the range reported from two New Zealand subglacial sediments (3–12 and 18–82%, respectively; Foght et al. 2004). Similar numbers of colonies ($\sim 3 \times 10^4$ cfu g^{-1}) were obtained when incubating the Russell Glacier sediment under aerobic and anaerobic conditions and the Lower Wright Glacier sediment aerobically, whereas anaerobic cultivation ($\sim 3 \times 10^3$ cfu g^{-1}) of the Lower Wright Glacier material yielded significantly lower numbers of colonies. This contrasts with previous work in West Antarctic subglacial sediment, where no anaerobic microbes were isolated (Lanoil et al. 2009). This result is consistent with the presence of both oxic and anoxic environments beneath these glaciers, containing viable aerobic and anaerobic microbes.

Bacterial diversity

Table 2 and Fig. 1 show the characteristics of the bacterial clone libraries of 16S rRNA genes from the two subglacial sediments. The bacterial library from the Lower Wright Glacier sediment, containing more labile organic carbon, showed a higher diversity of bacteria and a lower dominance (expressed as Berger–Parker index) than the Russell Glacier library (Table 2; Fig. 1). It was dominated by Proteobacteria (22 OTUs, 66% of clones), followed by Firmicutes (5 OTUs, 24% of clones). Acidobacteria, Cyanobacteria, Bacteroidetes, Planctomycetes and the candidate division OP10 were represented by one OTU each. The Russell Glacier library was much less diverse, with three

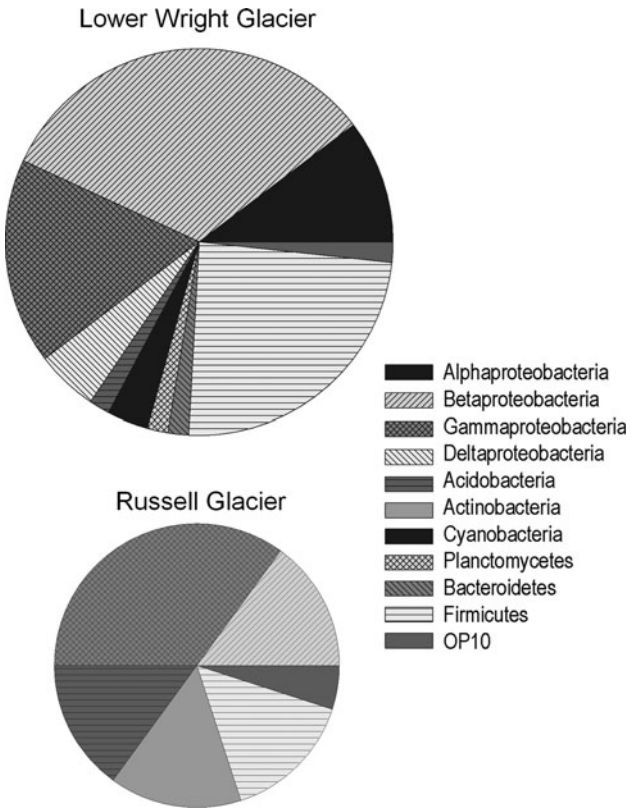


Fig. 1 Distribution of 16S rRNA gene clones between major bacterial groups in the Lower Wright Glacier and Russell Glacier subglacial sediment clone libraries

OTUs of Proteobacteria (50% of clones) and one of Firmicutes, Acidobacteria, Actinobacteria and OP10 (Fig. 1). The dominance of Proteobacteria in both investigated

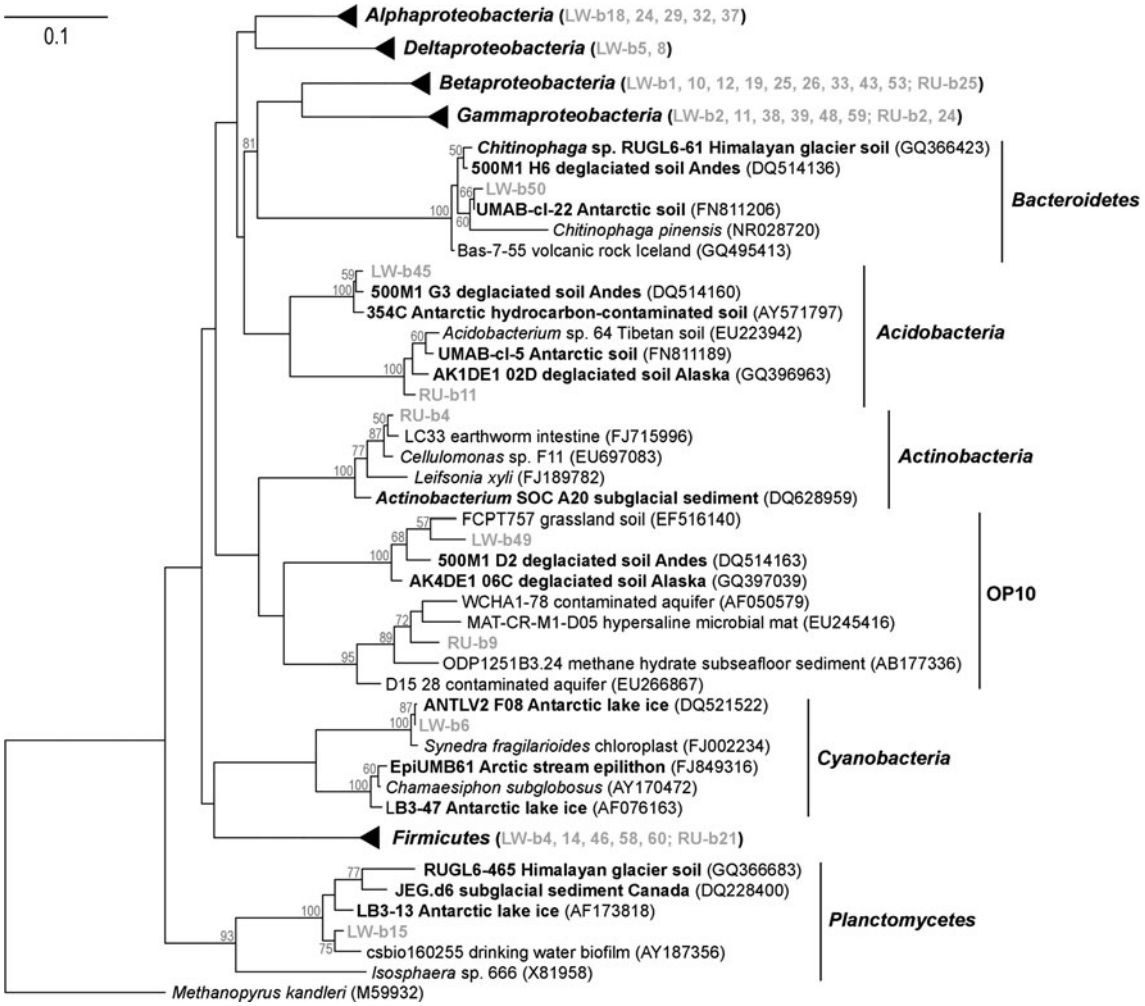


Fig. 2 Phylogenetic analysis of all bacterial clones from Lower Wright Glacier (*LW*) and Russell Glacier (*RU*) subglacial sediments (*bold grey*). The maximum likelihood tree was constructed based on 659-base-pair-long 16S rRNA gene sequences, and is rooted with the archaeon *Methanopyrus kandleri*. Two sequences from each of the most represented group (Alpha-, Beta-, Gamma- and Deltaproteobacteria, and Firmicutes) were used to indicate their position. Bootstrap values (1000 replications) >50 are shown. *Scale bar* represents 10% sequence divergence. *Sequences marked in bold* originate from cold environments

sediment samples is consistent with the results of diversity studies of other subglacial environments (Foght et al. 2004; Skidmore et al. 2005; Lanoil et al. 2009; Yde et al. 2010) and Antarctic lake sediments (Sjöling and Cowan 2003), while Actinobacteria, Acidobacteria, Bacteroidetes and Cyanobacteria were shown to play a significant role in Antarctic soil communities (Aislabie et al. 2006; Smith et al. 2006).

Figure 2 shows the distribution of the bacterial 16S rRNA gene clones detected across the major bacterial groups, and the most represented groups are shown in detail in Fig. 3a–e. Most clones isolated from Lower Wright sediment, and some from Russell Glacier, were related to clones from low-temperature environments, such as soils, subglacial sediments and lake ice from both polar and alpine settings.

Five OTUs from Lower Wright Glacier clustered within Alphaproteobacteria, specifically within Rhodobacterales (2 OTUs), Sphingomonadales, possibly related to *Kaistobacter* (1 OTU), and Rhizobiales, probably within the genus *Devosia* (2 OTUs) (Fig. 3a), all of which are likely aerobic heterotrophs (Nakagawa et al. 1996; White et al. 1996).

Betaproteobacteria was the most represented class of Proteobacteria in the Lower Wright Glacier library, with 9 unique OTUs. Eight of these clustered within the order Burkholderiales and one within Nitrosomonadales (Fig. 3b). All the putative Burkholderiales were closely related to clones detected in similar environments, including subglacial sediment from Canada (Skidmore et al. 2005; Cheng and Foght 2007), permanent lake ice in Antarctica (Mosier et al. 2007) and recently deglaciated

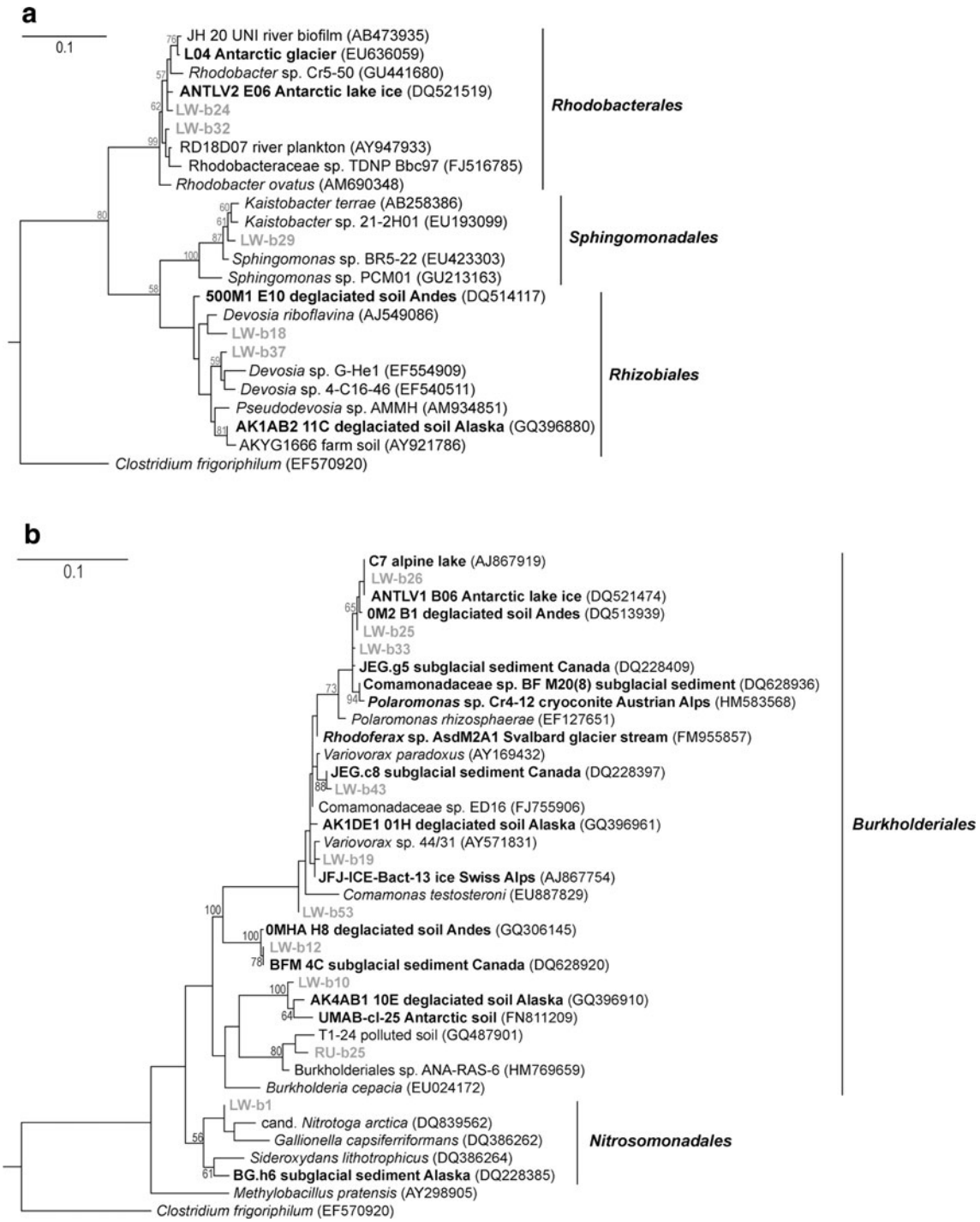
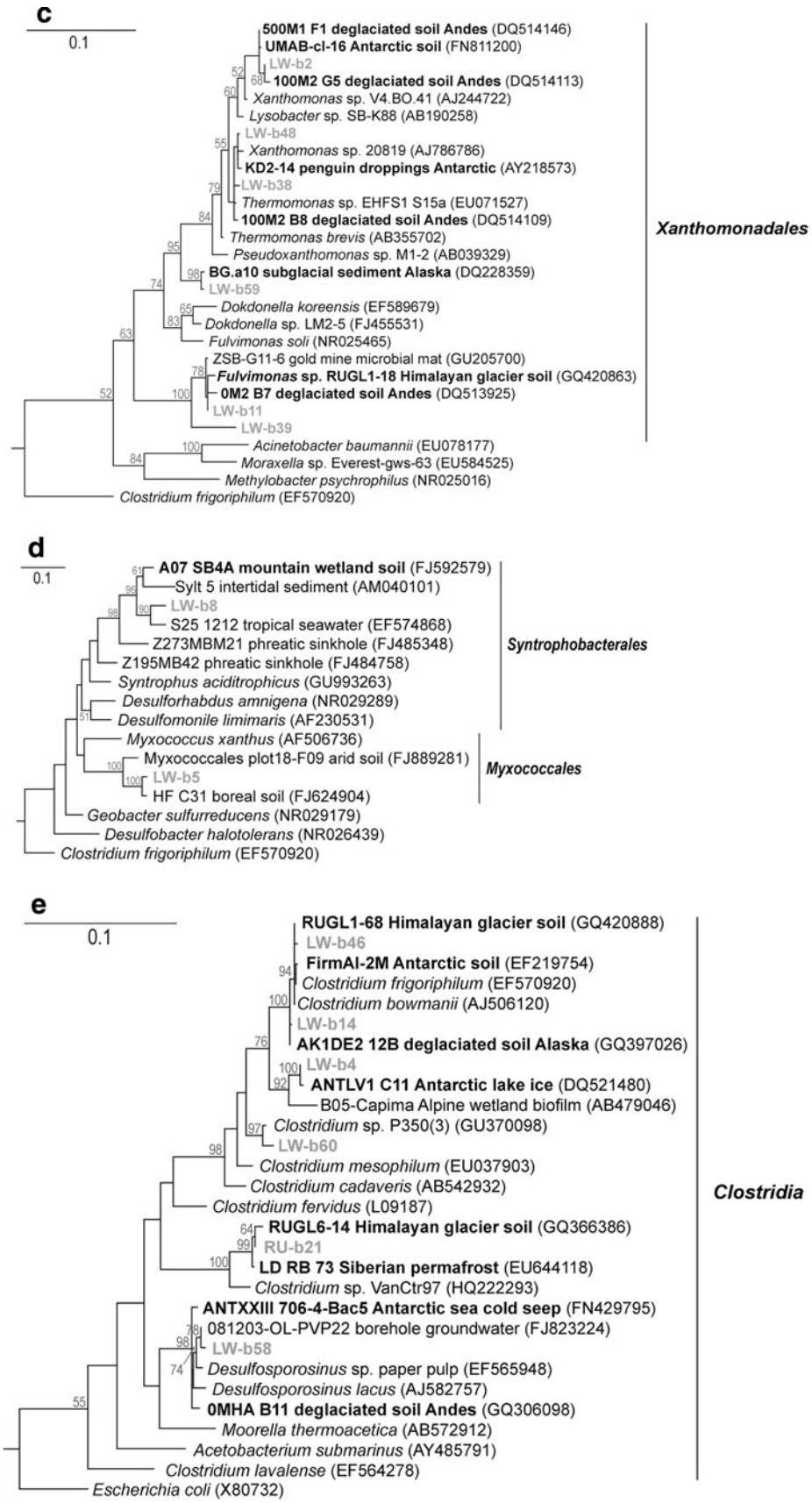


Fig. 3 Phylogenetic analyses (maximum likelihood) of bacterial clones of the dominant groups from Lower Wright Glacier (*LW*) and Russell Glacier (*RU*) subglacial sediments (*bold grey*). **a** Alphaproteobacteria, based on 660-base-pair-long 16S rRNA gene sequences. **b** Betaproteobacteria, 660 bp. **c** Gammaproteobacteria, 652 bp. **d** Deltaproteobacteria, 754 bp; all Proteobacteria rooted with *Clostridium frigoriphilum*. **e** Firmicutes, 640 bp; rooted with *Escherichia coli*. Bootstrap values (1000 replications) >50 are shown. *Scale bars* represent 10% sequence divergence. *Sequences marked in bold* originate from cold environments

soil in the Peruvian Andes (Nemergut et al. 2007). One OTU in the Russell Glacier library (RU-b25) also clustered within Burkholderiales, although it was not closely related to any low-temperature environmental clones (Fig. 3b). The dominance of Betaproteobacteria, especially the family Comamonadaceae (Burkholderiales) containing aerobic and facultatively anaerobic chemoorganotrophs and chemolithotrophs (Willems et al. 1991), in subglacial and

Fig. 3 continued



similar cold environments has been documented in previous papers (Foght et al. 2004; Skidmore et al. 2005; Yde et al. 2010), and attributed to some possible adaptations to the physical environment of glacier beds (Skidmore et al. 2005). The ability of some Comamonadaceae to switch between oxygen and nitrate or ferric iron as electron acceptor (Willems et al. 1991) could be advantageous in an environment where both oxic and anoxic conditions occur. This is also supported by the detection of the facultatively anaerobic iron reducer *Rhodoferax* (Comamonadaceae) in basal ice from Russell Glacier (Yde et al. 2010).

Gammaproteobacteria were represented by 6 unique OTUs in the Lower Wright library and two in the Russell Glacier library. The two OTUs from Russell Glacier were not included in the analysis due to their sequences being too short. All the Lower Wright clones clustered within Xanthomonadales (Fig. 3c) and were related to the mostly chemoorganotrophic genera *Xanthomonas*, *Thermomonas*, *Fulvimonas* and *Dokdonella* (Mergaert et al. 2002, 2003). Most of the clones were similar to other cold environment clones, including subglacial sediment from an Alaskan glacier (Skidmore et al. 2005) and deglaciated soil in the Andes (Nemergut et al. 2007) and the Himalayas (Pradhan et al. 2010). Two OTUs of Deltaproteobacteria were also detected in the Lower Wright Glacier sample, one clustering within Myxococcales and one within Syntrophobacterales (Fig. 3d).

Five OTUs of Firmicutes from Lower Wright Glacier and one from Russell Glacier were detected (Fig. 3e). One of them (LW-b58) clustered within sulphate-reducing species of *Desulfosporosinus*, while the others formed clusters with various *Clostridium* species and environmental clones, including those from polar and alpine soils, permafrost and Antarctic lake ice (Mosier et al. 2007; Liebner et al. 2008; Sattin et al. 2009; Pradhan et al. 2010). The occurrence of anaerobic *Clostridia* indicates anoxic conditions in the sediment, although some of them may survive oxic conditions (Wiegel et al. 2006).

Archaeal diversity

Table 2 shows the characteristics of the clone libraries of the universal archaeal primer-amplified 16S rRNA genes from the two glaciers. The Lower Wright Glacier subglacial sediment library had a higher archaeal diversity and lower dominance of OTUs (Table 2), similar to those from proglacial and coastal soils in the vicinity of Lower Wright Glacier (Ayton et al. 2010). The Russell Glacier library showed a very low diversity of archaea, and a higher dominance, similar to that of bacteria in the same community.

Four unique Euryarchaeotal clones were found in the Lower Wright Glacier library and one in the Russell Glacier library (Fig. 4). Two of them clustered within Rice cluster V and were closely related to a clone from a High Arctic lake (Pouliot et al. 2009) and to a clone from Svalbard permafrost peat (Høj et al. 2008), respectively. Three putative methanogenic clones were also identified in the samples, two in the Lower Wright Glacier sediment sample, clustering with Methanomicrobiales and Methanosarcinales, respectively, and one in the Russell Glacier sample, clustering with Methanomicrobiales (Stibal et al. 2012; Fig. 4).

Two Crenarchaeotal clones were identified in the Lower Wright Glacier sediment and two in the Russell Glacier sediment, clustering into group 1.1b and closest to a saline soil clone from the former lake Texcoco in Mexico (Valenzuela-Encinas et al. 2008), a biological soil crust clone from North American arid soils (Soule et al. 2009) and clones from subglacial sediments from Svalbard and Norway (M. Stibal, F. Hasan, J. L. Wadham, unpublished data) (Fig. 4). Similar clones have also been found in proglacial soil in the Austrian Alps (Nicol et al. 2006).

The lower diversity of archaea in the investigated subglacial sediments when compared to bacteria is consistent with other similar environments including Antarctic soils (Ayton et al. 2010), lake sediments (Sjöling and Cowan 2003) and microbial mats (Brambilla et al. 2001). The difference in diversity and dominance of archaea between Lower Wright Glacier and Russell Glacier was very similar to that of bacteria (Table 2), and it is therefore likely that the different glaciation histories and the consequent differences in the amount and quality of organic carbon substrates exert a control on the whole prokaryotic community.

Implications for subglacial metabolism and carbon cycling

Overridden organic matter of various age and bioavailability is the principal source of organic carbon for heterotrophic metabolism beneath ice sheets and glaciers worldwide (Skidmore et al. 2000; Wadham et al. 2008; Stibal et al. 2012). Therefore, complex microbial communities are assumed to take part in metabolising the overridden organic carbon to end products—methane and carbon dioxide—depending on the prevailing redox conditions.

Some of the bacterial clones detected in the Lower Wright Glacier and Russell Glacier subglacial sediment samples clustered within Bacteroidetes, Acidobacteria and Actinobacteria (Fig. 2) that are often involved in the degradation of large C compounds such as chitin and cellulose (Sangkhobol and Skerman 1981; Goodfellow and Williams 1983; Ward et al. 2009). It is possible that some of these bacteria can break up plant-derived and other more recalcitrant organic material and so provide more labile organic substrate for other microbes, especially in the Russell

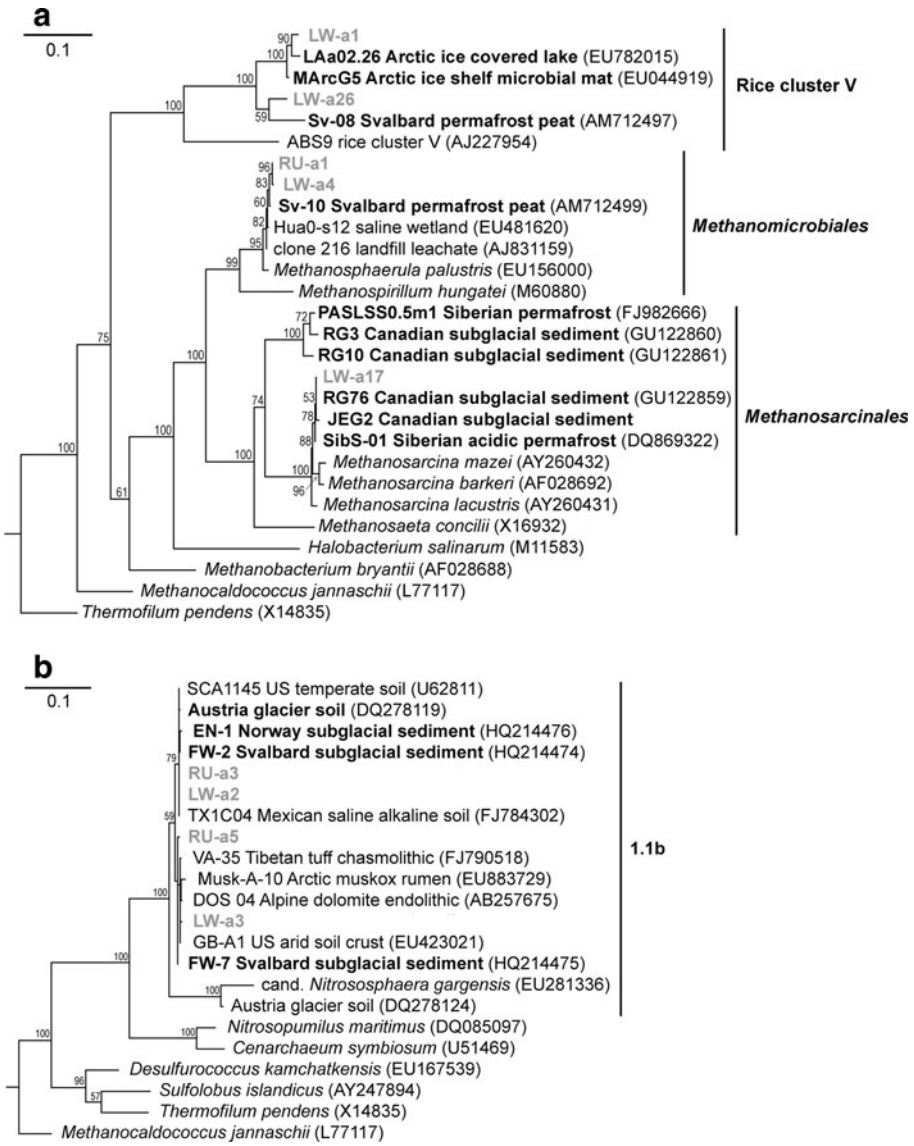


Fig. 4 Phylogenetic analyses (maximum likelihood) of archaeal clones from Lower Wright Glacier (*LW*) and Russell Glacier (*RU*) subglacial sediments (*bold grey*). **a** Euryarchaeota: based on 700-base-pair-long 16S rRNA gene sequences and rooted with the crenarchaeote *Thermofilum pendens*. **b** Crenarchaeota: 761 bp, rooted with the euryarchaeote *Methanocaldococcus jannaschii*. Bootstrap values (1000 replications) >50 are shown. *Scale bar* represents 10% sequence divergence. *Sequences marked in bold* originate from cold environments

Glacier environment containing overridden plant material (Stibal et al. 2012).

The identified clones in Russell Glacier and Lower Wright Glacier subglacial sediment were related to bacteria with both aerobic and anaerobic metabolisms. This may indicate the presence of both oxic and anoxic conditions in the sediment, possibly as oxic microenvironments within mostly anoxic sediment, as suggested in previous geochemical and microbiological studies (Bottrell and Tranter 2002; Wadham et al. 2004; Raiswell et al. 2009; Yde et al. 2010). Various types of heterotrophic metabolism are thus likely to occur, including aerobic respiration, anaerobic respiration with nitrate or ferric iron as electron acceptors and fermentation.

The assumed prevalence of anoxia (Bottrell and Tranter 2002; Wadham et al. 2004) is supported by the presence and activity of strictly anaerobic methanogenic archaea beneath glaciers (Skidmore et al. 2000; Boyd et al. 2010; Stibal et al. 2012). Some *Clostridia* are also capable of acetogenic fermentation (Wiegel et al. 2006), which might provide the link between more complex organic substrates and acetoclastic methanogenesis, detected in the Lower Wright Glacier sediment (Stibal et al. 2012; Wadham et al. 2012). However, most of the detected clones of *Clostridia* were related to uncultured organisms (Fig. 3e) with unclear metabolisms, and it is therefore extremely difficult to infer their metabolic potential.

Subglacial archaea may play significant roles in carbon and nitrogen cycling in Arctic and Antarctic terrestrial ecosystems. The potential role of methanogenic Euryarchaeota beneath glaciers and ice sheets as an important component of the carbon cycle on Earth has been suggested in recent papers (Boyd et al. 2010; Stibal et al. 2012; Wadham et al. 2012). Crenarchaeota from group 1.1b,

detected in both investigated subglacial sediments, may also be metabolically important in these environments, possibly as nitrifiers, since they are usually more abundant in soils and sediments than ammonia-oxidising bacteria (Leininger et al. 2006; Francis et al. 2007). They may oxidise ammonia that was overridden during glacier advance or deposited on glacier surfaces and washed to subglacial environments by melt water. This is supported by field observations from Svalbard where the NH_4^+/NO_3^- ratio in subglacial runoff was much lower than that in the original atmospheric deposition, suggesting significant subglacial nitrification (Hodson et al. 2010).

Conclusions

Diversity and abundance of prokaryotes was investigated in subglacial sediment from two glaciers from Greenland and Antarctica with different glaciation histories and organic carbon substrates. The Lower Wright Glacier subglacial sediment originates from relatively young (~200–300 years) reworked lake sediment and it contains relatively labile, and thus readily available, organic matter. The Russell Glacier sediment mostly originates from overridden soil of Holocene age, with a relatively higher proportion of less bioavailable recalcitrant organic carbon. The total microbial abundance in the Lower Wright Glacier sediment was $\sim 8 \times 10^6$ cells per gram of wet sediment, an order of magnitude higher than in Russell Glacier sediment ($\sim 9 \times 10^5$ cells g^{-1}). 4% of the microbes from the Russell Glacier sediment and 0.04–0.35% from Lower Wright were culturable at 10°C. The Lower Wright Glacier subglacial bacterial community was dominated by Proteobacteria, mostly from the Betaproteobacteria class, followed by Firmicutes. Acidobacteria, Cyanobacteria, Bacteroidetes, Planctomycetes and the candidate division OP10 were also represented. The Russell Glacier library was much less diverse and also dominated by Proteobacteria. The identified clones were related to bacteria with both aerobic and anaerobic metabolisms, indicating the presence of both oxic and anoxic conditions in the sediment. The majority of the detected clones were also related to clones from similar low-temperature environments. It is likely that the amount and quality of organic carbon beneath glaciers and ice sheets exert a control on the present community of bacteria and archaea. Younger sediments with more labile organic carbon available are likely to support a higher diversity of microbes and, potentially, of metabolic pathways and carbon cycling-related processes.

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