

## ORIGINAL ARTICLE

# The dynamic bacterial communities of a melting High Arctic glacier snowpack

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**Snow environments can occupy over a third of land surface area, but little is known about the dynamics of snowpack bacteria. The effect of snow melt on bacterial community structure and diversity of surface environments of a Svalbard glacier was examined using analyses of 16S rRNA genes via T-RFLP, qPCR and 454 pyrosequencing. Distinct community structures were found in different habitat types, with changes over 1 week apparent, in particular for the dominant bacterial class present, *Betaproteobacteria*. The differences observed were consistent with influences from depositional mode (snowfall vs aeolian dusts), contrasting snow with dust-rich snow layers and near-surface ice. Contrary to that, slush as the decompositional product of snow harboured distinct lineages of bacteria, further implying post-depositional changes in community structure. Taxa affiliated to the betaproteobacterial genus *Polaromonas* were particularly dynamic, and evidence for the presence of betaproteobacterial ammonia-oxidizing bacteria was uncovered, inviting the prospect that the dynamic bacterial communities associated with snowpacks may be active in supraglacial nitrogen cycling and capable of rapid responses to changes induced by snowmelt. Furthermore the potential of supraglacial snowpack ecosystems to respond to transient yet spatially extensive melting episodes such as that observed across most of Greenland's ice sheet in 2012 merits further investigation.**

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## Introduction

Most of our biosphere is permanently cold (Morita, 1975; Russell, 1990). Among these cold environments, snow permanently or seasonally covers up to 35% of the Earth's terrestrial surface area and drives powerful feedbacks within the climate system (Miteva, 2008).

Microbes have important roles in snow ecosystems, as detailed in reviews of snow ecology (Jones, 1999; Hoham and Duval, 2001). Snow algal blooms are well known as prominent loci of primary production in nival ecosystems (Hoham and Duval, 2001; Takeuchi, 2001). However, although metabolically active bacteria are present in snowpacks the world over, even in remote locations such as the South Pole (Carpenter *et al.*, 2000), these are

less studied. In Alpine snowpacks, bacterial abundances range between  $10^3$ – $10^5$  cells ml<sup>-1</sup> (Alfreider *et al.*, 1996; Sattler *et al.*, 2001). Bacteria in snowpacks may be inoculated by precipitation and dry deposition (Xiang *et al.*, 2009b), however the relative importance of depositional and post-depositional processes in structuring bacterial communities in different environments is less clear. Seasonal shifts in snowpack bacterial communities have been reported in mountainous regions of Japan (Segawa *et al.*, 2005), as increased snow temperature and meltwater content during seasonal melt promote rapid microbial growth, but also induce washout of cells by elution in meltwater, leading to seasonal shifts in snowpack bacterial communities.

In Svalbard, snow bacterial abundances of about  $2 \times 10^4$  cells ml<sup>-1</sup> and the cultivation of *Alphaproteobacteria*, *Betaproteobacteria*, *Gammaproteobacteria*, *Firmicutes* and *Actinobacteria* has been described (Amato *et al.*, 2007). Previous clone library studies of High Arctic snow have identified the predominance of *Betaproteobacteria* in snowpacks containing 19 different classes of bacteria

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(Larose *et al.*, 2010) and six different phyla (Harding *et al.*, 2011). The sequences retrieved were typically related to other sequences from cold environments, although the potential for spatial and temporal variation in snowpack microbial diversity at fine scales of resolution were less well explored.

Nevertheless, interactions between Arctic snowpack communities and environmental changes are currently attracting increasing attention (Larose *et al.*, 2013). As well as impacts on seasonal melting from climate shifts, other impacts include the potential transformation of mercury (Møller *et al.*, 2011) and other pollutants. Atmospheric deposition of nitrogen (Gjessing, 1977; Simoes and Zagorodnov, 2001; Erisman *et al.*, 2008) is important in otherwise pristine Arctic environments. Glacial ecosystems can be influenced by episodic nitrogen deposition events, which may become more frequent in a changing Arctic (Hodson *et al.*, 2010; Roberts *et al.*, 2010; Kühnel *et al.*, 2011). Furthermore, it is thought that microbially mediated nitrification may occur within the snowpack itself (Amoroso *et al.*, 2010) and ammonia-oxidizing *Betaproteobacteria* are active nitrifiers in glacial ice microcosms (Miteva *et al.*, 2007), and *nifH* genes indicate the potential for nitrogen fixation in supraglacial snow (Boyd *et al.*, 2011). Finally, significant increases in anthropogenic nitrogen inputs to Svalbard and other glacierized regions (Döscher *et al.*, 1996; Fischer *et al.*, 1998; Kekonen *et al.*, 2005) modulated via supraglacial snowpacks affect the nitrogen cycle (Telling *et al.*, 2011) of supraglacial hotspots of biodiversity and activity such as cryoconite holes (Edwards *et al.*, 2011).

In the light of our limited understanding of Arctic snowpack bacterial communities that may prove significant in terms of spatial extent, biogeochemical cycling and sensitivity to a changing climate, greater insight into the distribution and dynamics of snowpack communities is needed. A particular lacuna in our knowledge is how seasonal melting, the extent, rate and duration of which is affected by a changing climate, affects these microbial communities.

In this study, samples collected from a High Arctic glacier's snowpack during seasonal melting were subjected to T-RFLP, pyrosequencing and qPCR to examine the bacterial community and betaproteobacterial ammonia-oxidizers. The primary objective was to evaluate whether habitats associated with the supraglacial snowpack of a High Arctic glacier harboured bacterial communities that could rapidly respond to changes induced by advancing melting.

## Materials and methods

### *Study site and sampling*

To test for changes in the snowpack bacterial community at advancing stages of melting, samples were taken on the 6th and 11th of July 2010

(hereafter, Day 1 and Day 2) on Larsbreen, which is a High Arctic polythermal valley glacier near Longyearbyen (Svalbard, 78° 13'N, 15° 38'E; Figure 1). The glacier is 2800 m long and 700 m wide, with an area of 2.96 km<sup>2</sup> (Etzelmüller *et al.*, 2000).

Three sample stations 1 km apart (1, 2 and 3) along an altitudinal transect on the glacier were chosen (Figures 1 and 2) to sample across a gradient of melting season duration and intensity. On each side, three pits (A, B and C), 10 m away from each other, were dug to measure snow- and slush depth.

Samples from the snowpack, slush and the underlying near-surface ice (Figure 1) were collected. Due to high amounts of aeolian depositions observed on the second day of sampling, surface snow (1 cm depth) was additionally sampled. Further details of the sampling protocol, chemical and microbial activity analysis are provided as Supplementary Methods.

Within 24 h of sampling, 1.5–2 l sample volume was filtered through sterile 0.2-µm pore-size polycarbonate filters (Millipore Ltd., Watford, UK) at the laboratory in Longyearbyen. Filters were transported frozen on dry ice to Austria and stored at –20 °C until DNA was extracted.

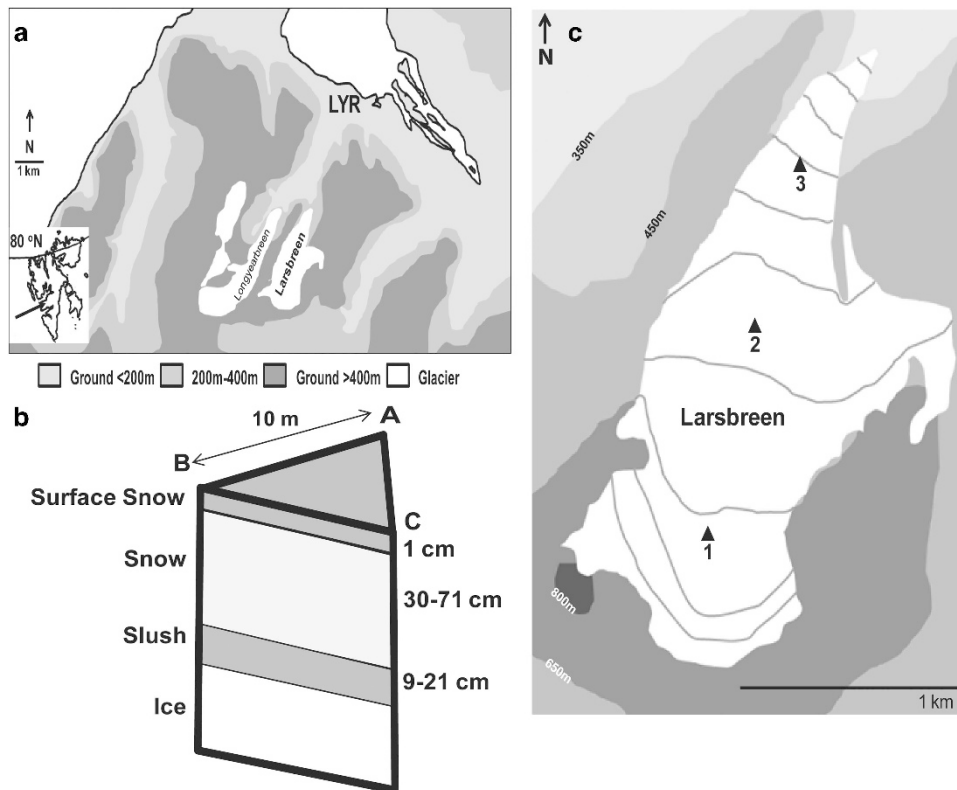
In addition to sampling, snow depths were taken on five spots in the close vicinity of each pit, together with the measurement of slush depths. Conductivity and pH were measured on day 1.

### *DNA extraction and PCR amplification*

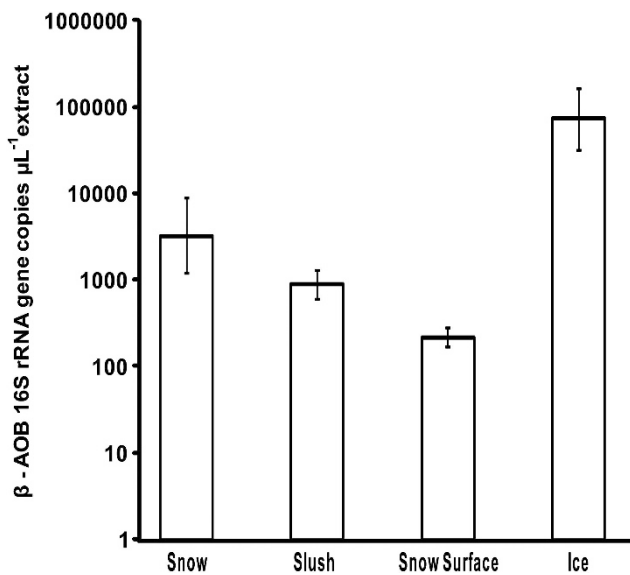
DNA was extracted from membrane filters using the MoBio, Inc., PowerSoil DNA extraction kit as directed by the manufacturer (MoBio, Inc., Solana, CA, USA), with the exceptions that following bead-beating, three freeze–thaw cycles were conducted (30 min at –80 °C followed by 5 min at 65 °C each time) and DNA was eluted in 10 µl of buffer C6. All pre-PCR manipulations were conducted in sterile conditions on bleach-disinfected work surfaces in laminar flow hoods in rooms not used for handling amplified sequences, using filter tips and certified DNA- and DNase-free plasticware. PCR mixtures used for amplifications contained 2.5 µl of 10 × *Taq* buffer with KCl, 200 µM each of deoxynucleoside triphosphate, 0.24 µM of each primer, 1.5 mM MgCl<sub>2</sub>, 0.2% bovine serum albumin, 0.625U *Taq* DNA Polymerase (Fermentas, York, UK), 2 µl of extracted DNA and nuclease-free water to a volume of 25 µl. Details of all PCR primers used in the study are specified as Supplementary Table 1.

### *Terminal restriction fragment length polymorphism (T-RFLP)*

T-RFLP was conducted generally as described in Edwards *et al.* (2011), with the reaction components described above for primers Cy5-27F and 1389R for the bacterial 16S ribosomal RNA gene.



**Figure 1** Study location and design. (a) Overview of the glacier's location. Larsbreen is a High Arctic valley glacier near Longyearbyen, Svalbard (inset). (b) At each sampling station, three snowpits were at the corners of a triangle 10 m apart; snowpack profiles and the range of surface snow, snow and slush habitat thicknesses illustrated. (c) shows the locality of sampling stations 1, 2 and 3, where, triplicate snowpits were excavated as described.



**Figure 2** qPCR estimation of mean  $\beta$ -AOB 16S rRNA gene abundance in different habitats; error bars represent  $\pm 1$  s.e.m.

To target betaproteobacterial ammonia-oxidizing bacteria (hereafter  $\beta$ -AOB), a T-RFLP assay based upon a 465-bp  $\beta$ -AOB 16S rRNA gene PCR product was amplified using an equimolar mixture of the two forward primers CTO189f A + B and CTO189f C,

5'-tagged with fluorophore Cy5 (5-carboxyfluorescein), and a reverse primer CTO654r based upon frequently used  $\beta$ -AOB-specific denaturing gradient gel electrophoresis (Kowalchuk *et al.*, 1997) modified so as to exclude the GC-clamp. In the instance of  $\beta$ -AOB T-RFLP, PCRs were conducted for 40 cycles on 27F/1389R templates to assure ample product for T-RFLP.

For both T-RFLP analyses, PCR products were cleaned, digested with 5U *Hae*III (Promega, Inc., Madison, WI, USA) for 4.5 h, spin column-purified and the fragments were analyzed using a Beckmann-Coulter CEQ8000 (Beckmann-Coulter, High Wycombe, UK) exactly as described by Edwards *et al.* (2011). Analysis of terminal restriction fragment relative abundance profiles was conducted with PERMANOVA (Anderson, 2001) using PRIMER6 and PERMANOVA+ (version 1.0.2 and version 6.1.12; Primer-E, Ivybridge, UK) and CCA (ter Braak, 1986) using MVSP 3.1 (Kovach Computing Services, Sir Fôn, UK), respectively, and was carried out as previously described (Edwards *et al.*, 2011).

#### Real-time quantitative PCR (qPCR) of $\beta$ -AOB partial 16S rRNA genes

To explore variation in the abundance of  $\beta$ -AOB genes in the sample types collected, 2  $\mu\text{L}$  of each

DNA extract was subjected to real-time PCR in 20- $\mu$ l reactions using a SYBR Green I ABSolute qPCR chemistry (ABGene, Epsom, UK) in 40 cycles as described above using a Bio-Rad iQ Cyclor (Bio-Rad, Ltd., Hemel Hempstead, UK) and the  $\beta$ -AOB 16S rRNA gene primers described in Supplementary Table 1. A plasmid standard bearing the 465-bp  $\beta$ -AOB fragment derived from PCR of DNA from cryoconite sampled in the European Alps (A Edwards and B Sattler, unpublished data) cloned as described below was extracted using a QIAQuick plasmid spin minprep kit (Qiagen, Ltd, Crawley, UK) and prepared as a 1:10 dilution series to represent 10–100 000 copies of the  $\beta$ -AOB fragment. A PCR efficiency of 93.7%, and gene copy number per extract was estimated by the iQ cyclor's software. All DNA extracts were used in this study were tested by qPCR. As our objective was the estimation of  $\beta$ -AOB 16S rRNA copy number, and the volume of each DNA extract available was very limited in availability, normalisation to total 16S rRNA gene abundances was not possible. Details of cloning are provided in Supplementary Methods.

#### 454 amplicon pyrosequencing

To complement profiles of community structure by deeply sequencing the bacterial 16S rRNA gene, 454 amplicon pyrosequencing was performed on samples from each habitat type. As barcoded amplicons could not be generated directly from DNA extracts (data not shown), a hemi-nested PCR approach using 15 cycles of PCR with primers 27F and 1389R, as described above, was required prior to amplification using barcoded primers. In the second round of amplification, the primers consisted of 27F and 357R (5'-tagged with the Roche A and MID barcode tags). One microlitre of PCR product was used in 25- $\mu$ l reactions containing 10 $\times$  reaction buffer with 1.8 mM MgCl<sub>2</sub>, 0.2 mM dNTPs, 0.2  $\mu$ M of each primer, 1.25 U FastStart High Fidelity Enzyme Blend (Roche Biosystems, Burgess Hill, UK), and subjected to 30 cycles of 30 s at 95 °C, 30 s at

55 °C and 2 min at 72 °C and terminated by a single final elongation of 7 min at 72 °C.

Amplicons were cleaned using Agencourt AMPure XP beads (Beckman-Coulter Genomics) prior to pyrosequencing using Titanium chemistry and protocols on the Aberystwyth University Roche GS-FLX 454 sequencer (Roche Diagnostics Ltd). Sequences were de-multiplexed, quality-filtered and analyzed using QIIME (Caporaso *et al.*, 2010). Operational taxonomic units (OTUs) were assigned using UCLUST at a threshold of 97% pairwise identity, and representative sequences from each OTU were selected for taxonomy assignment. These sequences were aligned using PyNAST and classified using the Ribosomal Database Project classifier against the RDP 16S rDNA core set using a 0.80 confidence threshold. A matrix of each OTU's relative abundance in each sample was imported into PRIMER6 and PERMANOVA+ for PERMANOVA and CAP (Anderson and Willis, 2003) and calculation of richness and Shannon–Wiener diversity index. Univariate statistics were conducted in Minitab 12.23 (Minitab, Ltd., Coventry, UK).

## Results

Samples of snow ( $n=15$ ), surface layer snow ( $n=16$ ), slush ( $n=15$ ) and ice ( $n=14$ ) were collected for molecular and chemical analysis on both Day 1 and Day 2; summary details of snowpack properties and chemistry are provided in Table 1 and Supplementary Figure 1. As can be determined from Table 1, the decrease in snowpack depth and increase in the slush fraction illustrate the advancing melting of the snowpack in the interim between Day 1 and Day 2.

#### Bacterial community structure of the Larsbreen snowpack

DNA was successfully extracted from all 60 samples of snow, slush and ice, permitting T-RFLP analyses of bacterial and  $\beta$ -AOB 16S rRNA genes. Profiles

**Table 1** Properties of the snowpack at each sampling station

Sampling station	1			2			3		
	A	B	C	A	B	C	A	B	C
<i>Snow pit</i>									
Altitude (m asl)		660			580			460	
pH	8.5	8.5	8.3	8.3	8.7	7.4	8.6	7.3	8.2
Conductivity ( $\mu$ S cm <sup>-1</sup> )	10.1	8.2	8.2	4.1	4.4	4.2	3.6	3.9	3.6
<i>Mean snow depth (cm)</i>									
06 July 2010 (Day 1)	95	95	88	64	65	69	66	68	69
11 July 2010 (Day 2)	87	88	87	59	58	58	54	55	49
<i>Mean slush depth (cm)</i>									
06 July 2010 (Day 1)	14	14	4	8	7	6	7	3	4
11 July 2010 (Day 2)	23	17	6	9	9	13	9	7	1



meeting quality filtering criteria were generated from all samples, with the exception of four samples subjected to bacterial 16S rRNA gene T-RFLP (Slush 2B and 2C from Day 1, Ice 1B III + IV as well as Ice 2B III + IV from Day 2).

To test the hypotheses that (i) different habitat types (seasonal snow-pack, slush and near-surface ice) harbour distinct communities and (ii) these communities are subject to change as the melting season progresses, Bray–Curtis distances of fourth-root transforms of T-RFLP profiles (thus indicative of differences in community structure) were analysed by PERMANOVA to test for significant differences. A three-factor design to test for differences between sampling station, time and habitat type was conducted for both bacterial and  $\beta$ -AOB-specific profiles.

For both bacterial and  $\beta$ -AOB T-RFLP data sets, no significant difference was observed between sampling stations (Pseudo- $F$  = 1.08,  $p(\text{perm})$  = 0.366 and Pseudo- $F$  = 0.911,  $p(\text{perm})$  = 0.522, respectively). However, significant differences were returned for both communities due to time, consistent with a change in the structure of both bacterial and  $\beta$ -AOB communities (Day 1 vs Day 2; Pseudo- $F$  = 5.08,  $p(\text{perm})$  = 0.0016 and Pseudo- $F$  = 2.97,  $p(\text{perm})$  = 0.0149, respectively). Equally, for both communities, highly significant differences according to habitat type were apparent ( $F$  = 3.56,  $p(\text{perm})$  = 0.0001 and Pseudo- $F$  = 4.13,  $p(\text{perm})$  = 0.0001, respectively) for bacteria, in general, and  $\beta$ -AOB. Pairwise PERMANOVA tests between habitats revealed significant differences between habitats for both bacterial and  $\beta$ -AOB 16S rRNA gene T-RFLPs, with the exception of snow surface and ice samples for bacteria and snow and slush for  $\beta$ -AOB at the  $P$  = 0.05 level. Details are provided in Table 2. No interactions between factors (time, habitat type or sampling station) were apparent ( $P$  > 0.1 in all instances). In summary, our T-RFLP results are consistent with the presence of temporally dynamic bacterial and betaproteobacterial ammonia-oxidizer communities partitioned between distinct habitats throughout the melting snowpack of Larsbreen.

**Table 2** Pairwise PERMANOVA comparisons of habitat T-RFLP profiles for both bacteria in general and  $\beta$ -AOB

Pairwise PERMANOVA test	$p(\text{perm})$ value	
	Bacteria	$\beta$ -AOB
Snow $\times$ slush	<b>0.0401</b>	0.2248
Snow $\times$ snow surface	<b>0.0291</b>	<b>0.0034</b>
Snow $\times$ ice	<b>0.0379</b>	<b>0.0006</b>
Slush $\times$ snow surface	<b>0.0003</b>	<b>0.0031</b>
Slush $\times$ ice	<b>0.0019</b>	<b>0.0014</b>
Snow surface $\times$ ice	0.0841	<b>0.0042</b>

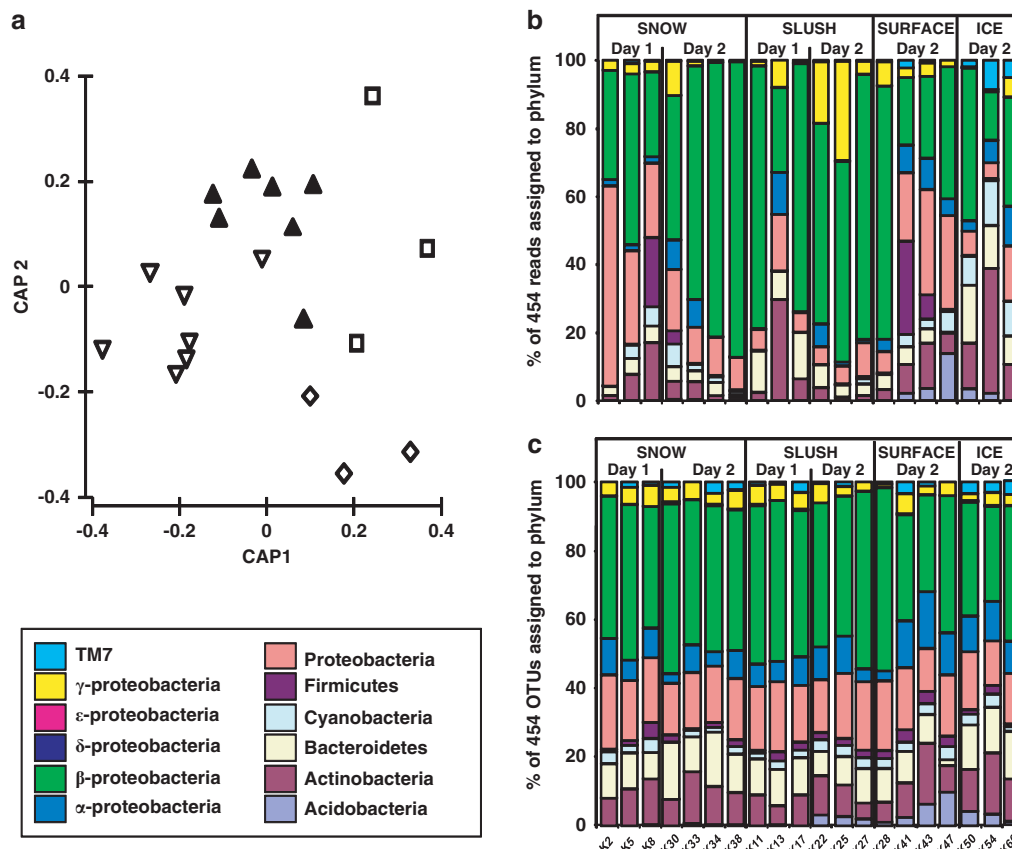
Bold text indicates significance at  $P$  < 0.05.

Although the primers used to amplify  $\beta$ -AOB 16S rRNA genes have been frequently used to specifically profile  $\beta$ -AOB communities using DGGE (Kowalchuk *et al.*, 1997), yielding high levels of  $\beta$ -AOB diversity (Mahmood *et al.*, 2006), and T-RFLP results were broadly congruent with DGGE profiles (for both bacteria, in general, and  $\beta$ -AOB 16S rRNA genes; Supplementary Figures 2–5), we sought to verify the specificity of the  $\beta$ -AOB T-RFLP method given that we believe this is the primers' first application for T-RFLP. Therefore, qPCR products from all samples were pooled and cloned into pGEM T-Easy, and clones yielding PCR products consistent with the presence of an insert were sequenced. Of the clones successfully aligned to the RDP taxonomic hierarchy (28/32 clones; Genbank HF934045–HF934072), these all lay within three clades of *Betaproteobacteria* in neighbour-joining trees, and the best matches using BLASTN were for uncultivated  $\beta$ -AOB, typically from cold environments (Supplementary Figure 6 and Supplementary Table 2). Thus, variation in T-RFLP profiles and qPCR results between habitats is likely to reflect variation in  $\beta$ -AOB community structure and abundance. This is amply illustrated by qPCR results, with mean  $\beta$ -AOB 16S rRNA gene copies per microlitre of DNA extract varying between 216 copies per microlitre of extract in the snow surface and 72 000 copies per microlitre of extract in near-surface ice (Figure 3).

#### Bacterial diversity of the Larsbreen snowpack

Twenty DNA samples representing at least three randomly selected samples each from snow and slush on both Day 1 and Day 2, as well as four surface snow and three ice core samples were subjected to V1–V3 16S rRNA gene amplicon pyrosequencing. Attempts to amplify 16S rRNA gene fragments directly from community DNA using 27F–357R were unsuccessful, potentially due to challenges from amplifying from low-biomass samples with tagged primers. A hemi-nested approach using 27F–1389R as template for the barcoded primer PCR reaction robustly amplified specific products with no contamination apparent in negative controls. This approach has the advantage of minimizing bias associated with direct amplification with barcoded primers (Berry *et al.*, 2011). From this analysis, 254 262 reads successfully passed the QIIME pipeline at an abundance of  $\geq 5$  reads within the data set. The full 454 amplicon dataset is available at EBI-SRA (ERP002433).

These reads constituted 818 operational taxonomic units at the 97% sequence identity level. No statistical difference was apparent according to sample type in terms of richness (mean of 333 OTUs per sample  $\pm$  84.1, 1 s.d.; one-way ANOVA;  $F$  = 2.17,  $P$  = 0.132). However, significant differences were apparent in terms of Shannon–Wiener diversity indices (one-way ANOVA,  $F$  = 5.30,  $P$  = 0.01),



**Figure 3** Barcoded amplicon 454 pyrosequencing of bacterial 16S rRNA genes. **(a)** Canonical Analysis of Principal Components of UCLUST 97%-clustered OTUs modelled by habitat type (total misclassification error = 20%; slush: hollow triangles, snow: filled triangles, surface snow: squares, ice: diamonds.) **(b)** distribution of OTUs aligned and assigned to known bacterial phyla as the cumulative relative abundance of OTUs associated with each phylum. **(c)** Percentage of OTUs affiliated to known bacterial phyla.

accounted for by significantly higher diversity in ice samples compared with snow and slush according to *post-hoc* Tukey testing; no difference was apparent between surface snow samples and other habitat types. Likewise, significant differences were apparent between habitat types using a single-factor design PERMANOVA (pseudo- $F=3.58$ ,  $p(\text{perm})=0.0001$ ) of Bray–Curtis distances of fourth-root transforms of OTU relative abundances.

Pairwise PERMANOVA comparisons reveal differences between most habitat types with the exception of comparisons of snow surface and snow habitats, which achieves borderline significance ( $P=0.07$ ), and between snow surface and ice ( $P=0.102$ ); these results are summarized in Table 3. Figure 3a illustrates the ordination by CAP of the 454 profiles according to the habitat-type model. This correctly classifies 16/20 samples to the correct habitat type, with the greatest source of error associated with snow surface samples, with one sample each distributed to snow, snow surface and ice habitats. Overall, the robustness of the model specifying habitat-specific communities is supported.

Of the 454 OTUs assigned to phyla by alignment to the RDP core set at a confidence of  $\geq 0.8$ , at least seven phyla are present (Figure 3). *Proteobacteria*

overwhelmingly dominate the communities of all habitat types sequenced. Within the phylum *Proteobacteria*, the class *Betaproteobacteria* are highly abundant in snow and slush in terms of relative abundance of reads, but a broadly even distribution of proteobacterial richness is evident across samples. Alignment of 454 OTUs to the Greengenes taxonomy at a confidence level of 0.5 resulted in the alignment of more OTUs to taxonomy, but these OTUs were predominantly mapped to plastid sequences, and in particular, chloroplast 16S rRNA genes affiliated with *Chlamydomonadaceae*, which include the snow algal taxa. Exclusion of plastid-derived OTUs yielded similar results using the Greengenes taxonomy to stringent alignment to the RDP core set, with *Betaproteobacteria* proving abundant, particularly within snow and slush. (Greengenes-assigned *Betaproteobacteria* OTUs: slush 56%; snow, 36%; snow surface, 24%; ice 24% mean relative abundance, one-way ANOVA,  $F=6.84$ ,  $P=0.004$ ). These results are shown as Supplementary Figure 7. Consequently, we limited further analysis to RDP-aligned taxa.

Further inspection of RDP-based OTU assignments reveal that 28 OTUs affiliated to a single genus, *Polaromonas*, are significant contributors to this abundance. This is most apparent for a single

**Table 3** Pairwise PERMANOVA comparisons of phylum (and proteobacterial class) diversity for the main effects of habitat and time, and their interaction

Taxon	PERMANOVA test $P(\text{perm})$ values								
	Main test			Habitat pairwise tests					
	Habitat	Time	Habitat $\times$ time	Snow $\times$ slush	Snow $\times$ surface	Snow $\times$ ice	Slush $\times$ surface	Slush $\times$ ice	Ice $\times$ Surface
Unclassified	<b>0.0001</b>	0.2038	0.2269	<b>0.0028</b>	0.1121	<b>0.0080</b>	<b>0.0113</b>	<b>0.0004</b>	0.1009
Acidobacteria <sup>a</sup>	<b>0.0431</b>	0.1288	0.1973	nt	nt	nt	nt	nt	nt
Actinobacteria	<b>0.0162</b>	0.3832	0.2455	0.1799	0.3214	<b>0.0501</b>	0.2670	<b>0.0077</b>	0.1991
Bacteroidetes	<b>0.0003</b>	0.1497	0.8362	<b>0.0382</b>	0.0725	<b>0.0061</b>	<b>0.0017</b>	<b>0.0079</b>	0.1024
Cyanobacteria	<b>0.0004</b>	0.2255	0.1768	<b>0.0437</b>	<b>0.0382</b>	<b>0.0118</b>	0.0632	0.0725	0.0987
Firmicutes	0.3166	0.3652	0.9091	nt	nt	nt	nt	nt	nt
Proteobacteria	<b>0.0001</b>	0.0711	0.1972	<b>0.0069</b>	<b>0.0187</b>	<b>0.0342</b>	<b>0.0080</b>	<b>0.0134</b>	0.2011
Alphaproteobacteria	<b>0.0226</b>	0.3279	0.1203	0.2854	0.1287	0.0650	0.1907	<b>0.0040</b>	0.3999
Betaproteobacteria	<b>0.0002</b>	<b>0.0077</b>	0.0855	<b>0.0065</b>	<b>0.0079</b>	0.2813	<b>0.0055</b>	<b>0.0453</b>	0.1059
Gammaproteobacteria	<b>0.0200</b>	0.1102	0.6095	0.0903	0.1834	0.6285	<b>0.0005</b>	<b>0.0255</b>	0.4974
TM7 <sup>a</sup>	0.0655	0.7249	0.5898	nt	nt	nt	nt	nt	nt
All OTUs	<b>0.0001</b>	<b>0.0307</b>	0.1941	<b>0.0032</b>	<b>0.0238</b>	<b>0.0093</b>	<b>0.0071</b>	<b>0.0030</b>	0.0993
All OTUs— <i>Polaromonas</i> OTUs	<b>0.0001</b>	<b>0.0355</b>	0.1678	<b>0.0053</b>	<b>0.0246</b>	<b>0.0070</b>	<b>0.0085</b>	<b>0.0022</b>	0.1003
Betaproteobacteria— <i>Polaromonas</i> OTUs	<b>0.0003</b>	<b>0.0073</b>	0.0589	<b>0.0031</b>	<b>0.0061</b>	0.2393	<b>0.0074</b>	<b>0.0487</b>	0.1034
<i>Polaromonas</i> affiliated OTUs	<b>0.0013</b>	<b>0.0157</b>	0.3965	<b>0.0199</b>	<b>0.0231</b>	0.4639	<b>0.0014</b>	<b>0.0354</b>	0.0970

Abbreviations: OTU, operational taxonomic unit; nt = no test (main effect not significant).

Pairwise comparison of habitat categories is also provided. Bold text indicates significance at  $P < 0.05$ .<sup>a</sup>Samples without affiliated OTUs excluded from dataset.

*Polaromonas* OTU, OTU#1649, which accounts for 1.9–45.8% of all reads per sample in the data set (habitat means: 11.8% for snow, 27.8% for slush, 3.9% for surface snow and 8.4% for ice). OTU#1649 is present at significantly higher abundance in slush habitats compared with all other habitat types (one-way ANOVA with *post-hoc* Tukey  $F=7.10$ ,  $P=0.003$ ) and remains stable within the slush habitat over time with no interactions between habitat and time (general linear model ANOVA: slush vs snow, Day 1 vs Day 2; habitat  $\times$  time. Habitat:  $F=10.29$ ,  $P=0.009$ ; Time:  $F=1.21$ ,  $P=0.297$ ; habitat  $\times$  time  $F=1.26$ ,  $P=0.288$ ; Supplementary Figure 8). A similar trend is apparent for the relative abundance of all *Polaromonas* affiliated OTUs (general linear model ANOVA: slush vs snow, Day 1 vs Day 2; habitat  $\times$  time. Habitat:  $F=10.91$ ,  $P=0.008$ ; time:  $F=3.37$ ,  $P=0.096$ ; habitat  $\times$  time  $F=0.76$ ,  $P=0.404$ ) However, excluding the influence of OTU#1649 on *Polaromonas*-affiliated OTU relative abundances diminishes this trend (general linear model ANOVA: slush vs snow, Day 1 vs Day 2; habitat  $\times$  time. Habitat:  $F=4.76$ ,  $P=0.054$ ; Time:  $F=8.88$ ,  $P=0.014$ ; Habitat  $\times$  time  $F=0.02$ ,  $P=0.890$ ). This would lend support to the hypothesis that this particular OTU is capable of responding to the emergence of a seasonal slush layer, and persist at considerable abundance within this particular habitat.

To assess the responses of *Polaromonas*-affiliated OTUs as an assemblage of taxa, PERMANOVA analysis of Bray–Curtis distances of fourth-root transforms of OTU relative abundances was conducted. This returned highly significant differences between *Polaromonas* community structure according to habitat (pseudo- $F=4.86$ ,  $P=0.0013$ ), and a significant difference due to time (pseudo- $F=3.68$ ,  $P=0.0157$ ), without interaction between these main effects (pseudo- $F=1.01$ ,  $P=0.396$ ). The highly significant difference between habitat types is predominantly due to differences between slush and other habitat types (pairwise PERMANOVA  $P$ -values in the range 0.001–0.03 for all comparisons with slush), with the only other difference at the  $P < 0.05$  level being between the snow and the snow surface ( $P=0.02$ ).

Due to the apparent abundance of *Polaromonas*-affiliated OTUs, to verify whether the peculiar distribution of this genus was predominantly responsible for the patterns of taxon distribution observed, PERMANOVA analyses of all OTUs and all beta-proteobacterial affiliated OTUs were repeated, with the *Polaromonas*-affiliated OTUs excluded from the analysis. These analyses confirmed the robustness of overall patterns in OTU and beta-proteobacterial OTUs from *Polaromonas* influences. Using the above model, PERMANOVA returned similarly significant differences for the full data set and both the full data set and beta-proteobacterial data sets exclusive of *Polaromonas*-affiliated OTUs; these are summarized in Table 3.

In conclusion, although *Polaromonas*-affiliated OTUs demonstrate a distinctive distribution within the habitats sampled, other taxa are involved in structuring the bacterial communities of the glacier's melting snowpack.

Indeed, as highlighted in Table 3, such variation by habitat is apparent for a range of higher-order taxa. Of the seven phyla (including five classes of *Proteobacteria*), all phyla and three of the proteobacterial classes (with the exception of *Deltaproteobacteria* and *Epsilonproteobacteria* with two OTUs each) were present to a sufficient degree of OTU richness to permit multivariate analyses of their distribution. These results are reported in Table 3. It is noticeable that with the exception of the *Firmicutes* and TM7 phyla, all others groups of taxa demonstrate significant differences between habitats at the  $P < 0.05$  level. However, only the *Betaproteobacteria* demonstrate significant temporal variation (pseudo- $F = 4.33$ ,  $p(\text{perm}) = 0.0077$ ) between Day 1 and Day 2. Therefore, while the influence of habitat type are apparent on the snowpack bacterial communities, as evidenced by significant differences in the partitioning of higher-order taxon diversity, the *Betaproteobacteria* prove to be the most dynamic members of the snowpack bacterial community of a High Arctic glacier.

#### *Habitat and microbial activity interactions with community structure*

In the light of the shifts in community structure revealed by molecular analyses, attempts to relate the effect of snowpack chemistry and bacterial carbon production (as determined by [ $^3\text{H}$ ]leucine incorporation, to be fully reported elsewhere by J Zarsky, K Hell, R Psenner and B Sattler) on community dynamics were made. Canonical correspondence analysis of these parameters' influence on the relative abundance of OTUs assigned to the Ribosomal Database Project (RDP) hierarchy discriminated slush habitats from snow and snow surface layers clearly, (Figure 4) with a stepwise-optimized model that explained 63.6% of total variance within the first three axes. The effect of calcium, chloride, nitrate and sulphate concentrations on slush communities is apparent. Examination of intraset correlations (Supplementary Table 4) reveals the influence of total nitrogen and bacterial carbon production as the strongest influences on the first environmental axis, followed by ammonium concentrations. These patterns are broadly congruent with CCA conducted similarly using both T-RFLP data sets (Supplementary Figure 9), where the influence of ammonium, total nitrogen, bacterial carbon production and dissolved organic carbon concentrations are apparent in intraset correlations (Supplementary Table 3). Changes in the properties of the slush habitat were investigated further by ANOVA to provide insight into the dynamic habitat occupied by slush bacteria. No significant spatial

effects on environmental parameters concentrations were identified, but significant decreases were observed in the abundance of several parameters. Concentrations of chloride ( $F = 5.73$ ,  $P = 0.032$ ), nitrate ( $F = 6.07$ ,  $P = 0.028$ ), sulphate ( $F = 6.02$ ,  $P = 0.029$ ), magnesium ( $F = 6.72$ ,  $P = 0.022$ ) and calcium ( $F = 7.90$ ,  $P = 0.015$ ) decreased significantly in slush between Day 1 and Day 2, with a borderline significant decrease in sodium concentration ( $F = 4.81$ ,  $P = 0.047$ ) also apparent. No significant changes in ammonium, potassium, dissolved organic carbon, total nitrogen or bacterial carbon production were detected.

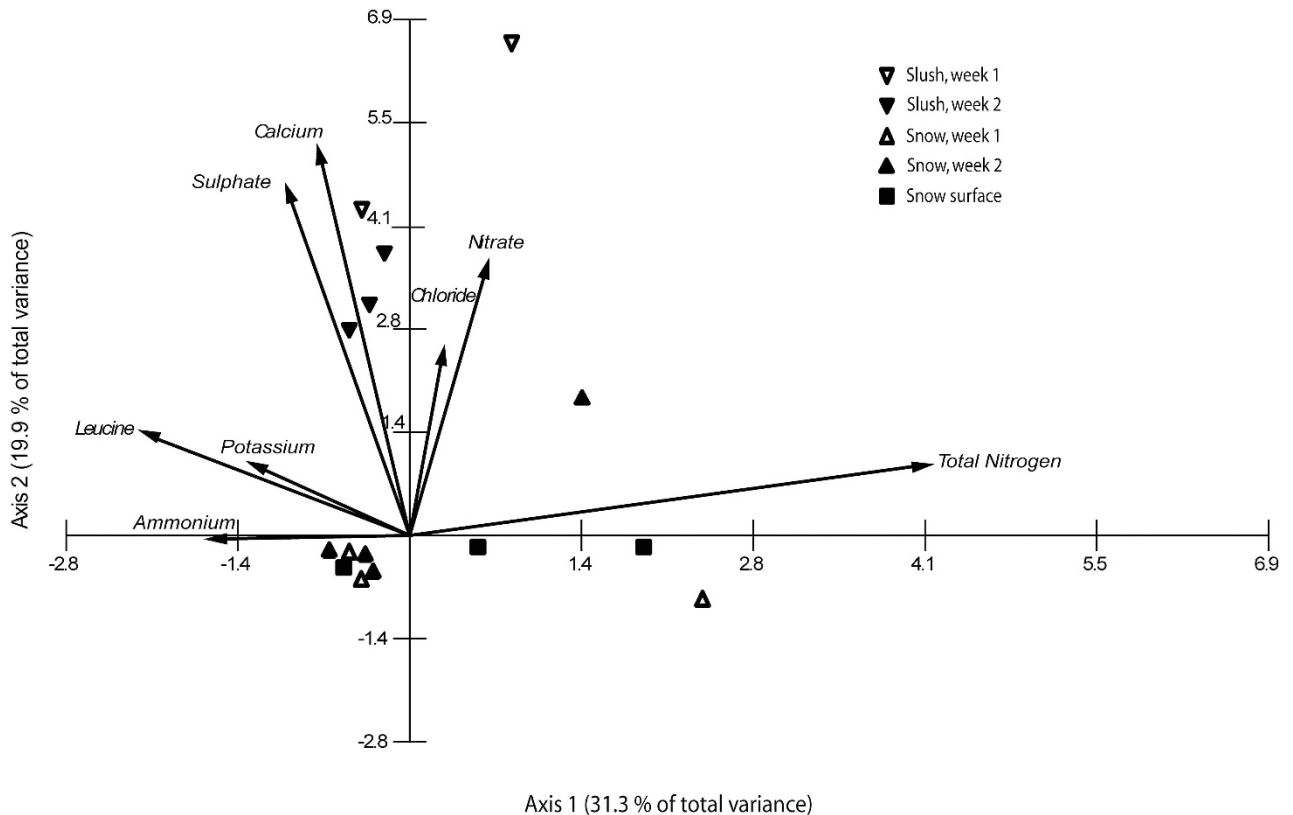
## Discussion

The presence of a diverse microbial community associated with snow environments found all over the world, including the High Arctic, is increasingly recognized (Carpenter *et al.*, 2000; Amato *et al.*, 2007; Xiang *et al.*, 2009a; Larose *et al.*, 2010; Chuvochina *et al.*, 2011; Harding *et al.*, 2011). Although the potential for depositional and post-depositional processes to influence the structure of these microbial communities is proposed (Xiang *et al.*, 2009b), our understanding of the scale of spatial and temporal variation of snow community composition is limited, in spite of the extensive global areal extent of snow cover. This study therefore offers the first quantitative evidence from 16S rRNA gene-based community structure profiling and amplicon pyrosequencing that different snowpack-associated habitats from a High Arctic glacier harbour distinct and diverse bacterial communities, and that these can be temporally dynamic within the melting season.

Little evidence was found of spatial variation in the bacterial communities between sampling stations covering gradients of elevation (200 m) and 2 km horizontal distance. However, at each sampling station, clear effects of habitat type are apparent in influencing the bacterial communities of surface snow, slush and near-surface ice. Most phyla present (*Acidobacteria*, *Actinobacteria*, *Bacteroidetes*, *Cyanobacteria* and *Proteobacteria*) show significantly different habitat distributions (whereas, *Firmicutes* and the TM7 candidate phylum do not) as revealed by PERMANOVA (Table 3). Differences between snow habitats, slush and ice account for variations in the distribution of OTUs affiliated to these phyla.

Within these comparisons, it is interesting to note the resemblance between surface snow samples and ice cores consistently revealed by both T-RFLP analyses and 454 pyrosequencing. In one respect, these results are unexpected, considering the contrasting nature of these habitats, respectively being the most recently formed and the oldest habitats, with surface snow evidently at the atmosphere–





**Figure 4** Canonical correspondence analysis model of environmental variable influence on the relative abundance of RDP-aligned OTUs. The variables displayed were retained following stepwise optimization of multicollinearity. Percentage of total variance explained per environmental axis is indicated for both axes. Supplementary Table 4 provides further details.

snowpack interface, and the glacier ice at the interface with the slush layer.

However, these differences may be accounted for by the mode of cell deposition. Surface snow samples consisted of the top centimetre of the snowpack sampled on Day 2, serendipitously exploiting a dust deposition event in the interim as a means of evaluating the dry deposition of cells. It is therefore likely that the 'surface snow' bacterial community is strongly influenced by organisms introduced by aeolian (or 'dry') deposition. In contrast, snow (and by extension, its decomposition product, slush) constitutes predominantly 'wet', or precipitation-related deposition (Xiang *et al.*, 2009b); all analyses differentiate snow and slush with surface snow in terms of community composition. Glacial ice is likely to integrate microbes introduced by both deposition mechanisms, and thus may conserve dry-deposited microbes. Support for this contention is provided by both the association of biomass with dust-rich layers in ice cores (for example, Xiang *et al.*, 2009a). Flow cytometric analyses of cell and particle budgets of High Arctic glaciers (Irvine-Fynn *et al.*, 2012) demonstrate net accumulation of cells and particulates in surface ice. From the amplicon pyrosequencing data presented here, the significantly increased Shannon–Wiener diversity of ice relative to snow and slush, and the lack of a significant difference between ice and

surface snow in terms of Shannon–Wiener diversity indices would be consistent with this interpretation.

Both bacterial 16S rRNA gene T-RFLP and 454 amplicon pyrosequencing reveal differences in bacterial community structure and composition between snow and slush layers. As slush constitutes the decomposition product of a melting snowpack, these observations are consistent with a population dynamic occurring in the snowpack community during its melting. This is further supported by the significant effect of time on T-RFLP profiles and 454 amplicon compositions in PERMANOVA analyses. In summary, in the five days between Day 1 and Day 2 sampling points, a considerable shift occurred in the structure of bacterial communities within Larsbreen's snowpack. Although seasonal influences on the composition of 16S rRNA gene clone libraries from snow have been reported previously, including on High Arctic glaciers (Larose *et al.*, 2010), the results presented herein illustrate a considerable capacity for even rapid change in the bacterial communities associated with snowpacks, thus underlining a role for snow as a dynamic habitat for active microbial communities.

Nevertheless, it is apparent that rapid changes in community structure are restricted mainly within the dominant phylum, *Proteobacteria*, and particularly the *Betaproteobacteria*. OTUs affiliated with *Betaproteobacteria* appear uniquely responsive to

the effect of time (Table 3,  $p(\text{perm}) = 0.0077$ ), and that this trait appears coherent at the class level, by inference of its retention even when the influence of *Polaromonas*-affiliated OTUs is accounted for (Table 3,  $p(\text{perm}) = 0.0073$ ). The dominance and dynamic nature of betaproteobacterial taxa in the snowpack therefore merits further consideration, especially since its prevalence is consistent with other (clone library) based studies of High Arctic snowpacks (Larose *et al.*, 2010; Harding *et al.*, 2011). In this respect, snowpacks seem comparable to other freshwater systems (Newton *et al.*, 2011).

Although the study of coherence between microbial taxonomy and functionality in the cryosphere is in its infancy, in aquatic and terrestrial systems, *Betaproteobacteria* are characterized as *r*-strategists, capable of rapidly responding to environmental fluctuations (Fierer *et al.*, 2007) and the utilization of diverse nutritional sources. In this regard, they resemble 'opportuni-troph' organisms (Polz *et al.*, 2006), exceedingly capable of exploiting spatially and temporally variable resources. Therefore, the finding of significant shifts in *Betaproteobacteria* diversity over a short period of time is likely to be consistent with exploitation of favourable conditions within a melting snowpack. The ionic environment of a melting snowpack is particularly dynamic (Johannessen and Henriksen, 1978). Impurities are differentially eluted from the snowpack in a nonlinear manner (Brimblecombe *et al.*, 1985), as ions are rejected and relocated as snow metamorphoses (Cragin *et al.*, 1996). Such changes are broadly reflected by the chemistry data (Supplementary Figure 1). Variations in the ionic composition of slush, in particular, calcium, chloride, nitrate and sulphate due to elution clearly ordinate slush from snow habitats in the CCA model of environmental influences on OTU abundances (Figure 4). This lends support to the argument that *Betaproteobacteria* that exploit slush as a habitat must prove exceptionally versatile and tolerant of a dynamic physicochemical environment.

This tendency is illustrated by the distribution of the bacterial genus that appears dominant within the snowpack-associated communities, *Polaromonas*. OTUs affiliated to this genus constitute a considerable proportion of all 454 reads throughout the data set, with one *Polaromonas* OTU alone, OTU#1648, accounting for up to 45% of reads in one sample in the most extreme instance. This OTU is present at a significantly higher relative abundance within the slush layer, and persists within the slush layer between Day 1 and Day 2, in contrast to the summed relative abundance of all remaining *Polaromonas*-affiliated OTUs. It is therefore likely that this taxon is well adapted to life in the seasonal slush layer of the glacier, and thus proliferates rapidly within it, while other members of *Polaromonas* occupy a broader distribution within the snowpack. The appearance of a high ratio of local to global diversity in *Polaromonas*, as recently

reported (Darcy *et al.*, 2011), would provide opportunities for co-dispersed *Polaromonas* species to exploit distinct niches in space and time, utilizing their considerable metabolic versatility (Mattes *et al.*, 2008; Yagi *et al.*, 2009) to do so.

Because of the abundance of *Betaproteobacteria* and isotopic evidence of snowpack nitrification (Amoroso *et al.*, 2010), we sought to evaluate the distribution and dynamics of  $\beta$ -AOB. DGGE analysis (Supplementary Figures 4 and 5), as well as T-RFLP (Table 2) confirmed by qPCR (Figure 2), and a small clone library (Supplementary Figure 6 and Supplementary Table 2) served to demonstrate the presence of  $\beta$ -AOB in all habitats, with different community structures apparent between the snowpack and the ice surface. Coupled with the greater abundance of  $\beta$ -AOB in the ice, these data suggest differing supraglacial distributions of  $\beta$ -AOB. Activity of  $\beta$ -AOB in microcosms representing glacial ice cores suggests that populations accumulated in near-surface ice may be active (Miteva *et al.*, 2007). Amplification of bacterial or archaeal *AmoA* genes was unsuccessful (data not shown), thus limiting the potential for further insights. PCR detection of *AmoA* was recently reported in cryoconite habitats on a glacier <20 km from Larsbreen (Cameron *et al.*, 2012). Many other cryoconite holes yielded multi-meric PCR products (Cameron *et al.*, 2012), while diverse forms of  $\beta$ -AOB *AmoA* were reported from subglacial sediments (Boyd *et al.*, 2011). These highlight challenges in detecting functional genes in (low-biomass) glacial environments, possibly accounting for incongruence. Whether the snowpack is an active locus of nitrification remains to be confirmed, but it is certainly possible that  $\beta$ -AOB accumulated in snowpack-associated habitats, if exported (Irvine-Fynn *et al.*, 2012), serves to inoculate other ice surface (Cameron *et al.*, 2012), subglacial (Boyd *et al.*, 2011) or forefield (Brankatschk *et al.*, 2011) habitats, dependent on the route of meltwater (Hodson *et al.*, 2008).

The decoupling of nutrient dynamics from the elution of other snowpack solutes during melt may infer the importance of the snowpack biota in nitrogen budgets (Hodson, 2006), but amendment experiments in alpine environments (Williams *et al.*, 1996) fail to support this contention. The particular influence of total nitrogen and ammonium concentrations collinear with bacterial carbon production rates is apparent in all CCA models of snowpack bacterial communities reported here. This lends further support to the role of snowpack communities in sequestering nitrogen resources, as argued by workers conducting annual budgets of glacierized catchment biogeochemistry (Hodson *et al.*, 2005; Hodson, 2006).

Although this study was performed within a single glacierized catchment on Svalbard, considering the commonality in the groups of taxa observed with other snowpack diversity studies (Amato *et al.*, 2007; Larose *et al.*, 2010; Harding *et al.*, 2011), and

that specific groups of interest (that is, *Polaromonas*; (Darcy *et al.*, 2011)) are thought to be very efficiently dispersed in the cryosphere, it is tempting to generalize that snowpack communities are able to respond rapidly to snowmelt, even on timescales as brief as several days to a week, in particular, as anthropogenic nitrogen deposition also affects other glacial systems (Fischer *et al.*, 1998). As the recent spatially extensive yet transient melting of the Greenland ice sheet snowpack observed on July 2012 (Nghiem *et al.*, 2012) led to extensive saturation of firn on the Greenland ice sheet interior (A Hubbard, personal communication), the potential for melt-induced pulses to initiate snowpack microbial processes during such episodes merits further investigation. Furthermore, as supraglacial runoff incorporates bacteria (Irvine-Fynn *et al.*, 2012) that may influence ice-marginal habitats (Mindl *et al.*, 2007), the impacts of snowpack bacterial community shifts may extend beyond the glacier itself. *Betaproteobacteria* and, in particular, *Polaromonas* strains were recently shown to significantly accelerate mineral weathering in an Alpine glacier forefield (Frey *et al.*, 2010), so the prominence of these organisms in slush may influence paraglacial dynamics downstream.

In conclusion, the presence of a diverse and dynamic bacterial community is revealed within the melting snowpack of a High Arctic glacier by multiple molecular methods. Clear differences in community structure according to habitat type are evident, implying the role for both depositional and post-depositional influences in structuring the bacterial community of snowpacks. The dominance and dynamic nature of *Betaproteobacteria*, even at the brief timescales examined would indicate their potential to proliferate in transient niches, and hence, exploit the dynamic environment of a melting snowpack with consequent impacts upon glacierized catchment biogeochemistry.

## Conflict of Interest

The authors declare no conflict of interest.

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