Title: Microbial communities from subglacial water of naled ice bodies in the forefield of Werenskioldbreen, Svalbard

Author: Sławomir Sułowicz, Kinga Bondarczuk, Dariusz Ignatiuk, Jacek A. Jania, Zofia Piotrowska-Seget

Citation style: Sułowicz Sławomir, Bondarczuk Kinga, Ignatiuk Dariusz, Jania Jacek A., Piotrowska-Seget Zofia. (2020). Microbial communities from subglacial water of naled ice bodies in the forefield of Werenskioldbreen, Svalbard. "Science of the Total Environment" Vol. 723 (2020), art. no 138025, doi 10.1016/j.scitotenv.2020.138025

Science of the Total Environment 723 (2020) 138025

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Microbial communities from subglacial water of naled ice bodies in the forefield of Werenskioldbreen, Svalbard

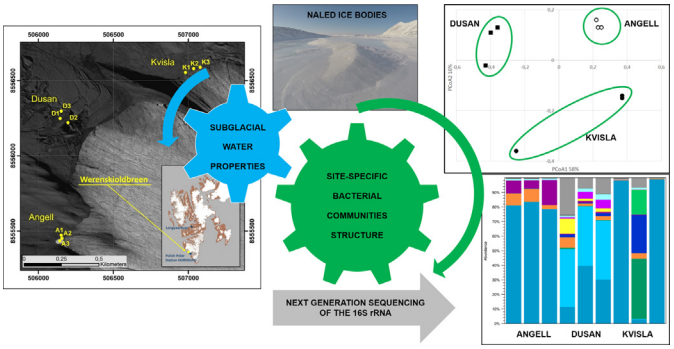
Sławomir Sułowicz [a,*], Kinga Bondarczuk [b], Dariusz Ignatiuk [c,d], Jacek A. Jania [c], Zofia Piotrowska-Seget [a]

[a] University of Silesia in Katowice, Faculty of Natural Sciences, Institute of Biology, Biotechnology and Environmental Protection, Jagiellonska 28, 40-032 Katowice, Poland
[b] Medical University of Bialystok, Centre for Bioinformatics and Data Analysis, Waszyngtona 13a, 15-269 Bialystok, Poland
[c] University of Silesia in Katowice, Faculty of Natural Sciences, Institute of Earth Sciences, Bedzinska 60, 41-205 Sosnowiec, Poland
[d] Svalbard Integrated Arctic Earth Observing System (SIOS), SIOS Knowledge Centre, Svalbard Science Centre, P.O. Box 156, N-9171 Longyearbyen, Svalbard, Norway

HIGHLIGHTS

- Subglacial water from drainage system of Werenskioldbreen glacier was studied.
- Various groups of bacteria were detected in subglacial water from naled ice bodies.
- Microbial community structure was determined by NGS of 16S rRNA.
- Dominant classes were *Beta-, Gamma- and Epsilonproteobacteria.*
- Microbial diversity depended on subglacial water properties.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 18 October 2019
Received in revised form 16 March 2020
Accepted 16 March 2020
Available online xxxx

Editor: Ewa Korzeniewska

Keywords:
Microbial communities
Subglacial microbiome
Naled-ice bodies
NGS of 16S rRNA

ABSTRACT

We assessed the structure of microbial communities in the subglacial drainage system of the Werenskioldbreen glacier, Svalbard, which consists of three independent channels. Dome-shaped naled ice bodies that had been forming and releasing subglacial water in the glacial forefield during accumulations season were used to study glacial microbiome. We tested the hypothesis that the properties of the water transported by these channels are site-dependent and influence bacterial diversity. We therefore established the phylogenetic structure of the subglacial microbial communities using next generation sequencing (NGS) of the 16S rRNA gene and performed bioinformatics analyses. A total of 1409 OTUs (operational taxonomic units) belonged to 40 phyla; mostly *Proteobacteria, Gracilibacteria, Bacteroidetes, Actinobacteria* and *Parcubacteria* were identified. Sites located on the edge of Werenskioldbreen forefield (Angell, Kvisla) were mainly dominated by *Betaproteobacteria.* In the central site (Dusan) domination of *Epsilonproteobacteria* class was observed. *Gracilibacteria* (GN02) and *Gammaproteobacteria* represented the dominant taxa only in the sample Kvisla 2. Principal Coordinate Analysis (PCoA) of beta diversity revealed that phylogenetic profiles grouped in three different clusters according to the sampling site. Moreover, higher similarity of bacterial communities from Angell and Kvisla compared to Dusan was confirmed by cluster analysis and Venn diagrams. The highest alpha index values was measured in Dusan. Richness and phylogenetic diversity indices were significantly ($p < .05$) and positively correlated with pH values of subglacial water and negatively with concentration of Cl^-, Br^-, and NO_3^- anions. These anions negatively impacted the values of richness indices but positively correlated with abundance of some microbial phyla. Our results indicated that subglacial water from naled ice bodies offer the possibility to study the glacial microbiome. In

* Corresponding author.
E-mail address: slawomir.sulowicz@us.edu.pl (S. Sułowicz).

https://doi.org/10.1016/j.scitotenv.2020.138025

the studied subglacial water, the microbial community structure was sampling site specific and dependent on the water properties, which in turn were probably influenced by the local bedrock composition.

1. Introduction

Glaciers and ice sheets contain approximately 75% of Earth's freshwater (Achberger et al., 2017). Land ice masses have been treated as real biomes (Anesio and Laybourn-Parry, 2012) and are increasingly being examined. Together with subglacial meltwater, they are a unique living environment for prokaryotes and eukaryotes (Anesio et al., 2017; Hodson et al., 2008; Lanoil et al., 2009). Microorganisms from these ecosystems are able to survive and proliferate at low temperatures, which implies that they overcame key barriers inherent to permanently cold environments. It was found that subglacial waters are inhabited by various bacteria that play an important part in global microbiological activity and nutrient cycling (Anesio et al., 2017). This water may be a valuable source of the glacial microbiome released from englacial and subglacial environment. However, little information is available on the taxonomic diversity of bacterial communities from these environments.

The study of the subglacial microbial environment is extremely difficult due to the necessity of sterile conditions during sampling. One of the methods used for sampling is a sterile deep drilling method (Hodgson et al., 2016; Tulaczyk et al., 2014). However, this has not yet been applied to the Svalbard glaciers. Another option is surface drilling (Garcia-Lopez et al., 2019; Martinez-Alonso et al., 2019). To study glacier microbial composition, sampling of subglacial water flowing out from underneath the glaciers during accumulation season could be used as well (Cameron et al., 2017). It avoids contamination of samples by superficial meltwater or rain water carrying microbes from sources other than the glacier bedrock interface. The source of uncontaminated subglacial water may be naled ice bodies. These layered dome- or cone-shaped ice bodies are formed by water from the winter seepage underneath glaciers, which refreeze at their forefields (Wadham et al., 2010). The formation of naled ice is connected with the amount of meltwater, air temperatures and glacier forefield topography (Bukowska-Jania and Szafraniec, 2005). The presence of meltwater during winter depends strongly on subglacial drainage systems, channelized during the melting period (Decaux et al., 2019).

Here, direct sampling of water from naled ice bodies were used as a microbial representation of the glacier bed. To the best of our knowledge, this strategy was implemented for the first time. We used Werenskioldbreen as a well-studied area of naled ice glacier located in South Spitsbergen (Svalbard) (Baranowski, 1982; Pälli et al., 2003). Glacier Werenskioldbreen is a polythermal valley-type, 9.5 km long glacier that covers an area of 27.1 km^2. It is divided by a medial moraine into Slyngfjelbreen and Skilryggbreen. The glacier has receded and terminated on land, creating a naled zone in the forefield (Ignatiuk et al., 2014). Most naledi at Werenskioldbreen grow from the bottom rather than the top due to ice accretion, by freezing surface water and incorporation of snow (Stachnik et al., 2016b). The spatial distribution of the naled ice reflected the position of subglacial outflows during summer. The presence of separate naled ice areas in the Werenskioldbreen forefield suggests that the subglacial drainage system is divided into a few channels. A study by Grabiec et al. (2011) showed that some channels of the drainage system are not connected to each other and transport subglacial water independently. Therefore naled water chemistry depended on local lithology (Stachnik et al., 2016b). The main outflow occurs at the Kvisla or central sections of the glacier terminus (Dusan), and minor outflows occur at Angell (Stachnik et al., 2016b).

These separated channels provide a unique opportunity to study the microbial community inhabiting the glacier interior and flowing with subglacial meltwater. We tested the hypothesis that properties of water transported by three independent channels of Werenskioldbreen subglacial drainage system are site-dependent and influenced the composition of microbial communities. To the best of our knowledge, phylogenetic composition of microbial communities in subglacial water from naled ice bodies has not been published thus far.

2. Methods

2.1. Study area and subglacial water sampling

The study of subglacial water from naled ice in front of Werenskioldbreen was performed in April 2016, prior to glacier ablation. Samples of subglacial water were collected from three different naled ice systems located in front of summer outflows in the northern (Kvisla), the central (Dusan) and the most southern part (Angell) of the forefield (Figs. 1, 2). The distance between sampling sites was approximately 800–900 m. In each localization, three independent samples (2–4 m distance) with 20–30 L of volumes from each sampling site were collected. Most naled ice bodies at Werenskioldbreen grow from the bottom because of ice accretion (Stachnik et al., 2016b). Naled ice bodies of interest to us were active, have ongoing contact with the bedrock, and is a source of the fresh water flowing out from the glacier body. When it was necessary to acquire fresh subglacial water, drilling was done using a sterile Mark II Coring System (Kovacs Enterprises, NH, USA). Water was captured in a sterile vessel, placed in a sterile plastic bottle and transported to the laboratory of the Polish Polar Station in Hornsund, Svalbard. There, samples were filtered using a Millipore vacuum filtration kit with sterile 0.45 μm Whatman® membrane filters. Microbial biomass was stored in a sterile Falcon tube at −20 °C for further microbial analysis.

2.2. Physicochemical analysis

Physicochemical analyses of water were performed in the laboratory of the Polish Polar Station, Hornsund. The pH and electrical conductivity were measured using a multimetre ELMETRON CP-401. Concentration of major ions was estimated using an ion chromatograph Metrohm Compact IC 761. Analysis of total organic carbon (TOC) and dissolved organic carbon (DOC) was performed using TOC-L Shimadzu at the laboratory in Poland. Ion concentration and TOC and DOC content were expressed as mg L^{-1} and electrical conductivity σ as μS cm^{-1}. Significant changes between mean values estimated for different sampling sites were tested using the one-way ANOVA and Tuckey's post-hoc test ($\alpha = 0.05$) using the Statistica 13.0 PL Software package. To test the correlation between subglacial water parameters and phylogenetic indices, the Pearson correlation was calculated using Past 3.26 Software.

2.3. DNA isolation and Next Generation Sequencing (NGS)

Phylogenetic structures of microbial communities were established using sequencing technique and bioinformatics analysis. Microbial biomass was collected from filters (500 mL of the water sample was filtered) for DNA isolation. Total microbial DNA was isolated using the PowerWater® DNA Isolation Kit (MoBio, Carlsbad, CA, US) according to the manufacturer's instruction. The concentration of DNA measured using the NanoPhotometer NP80 was in the range of 6–11 ng/μL. The 16S rRNA fragment was amplified using bacterial primers 341F and 785R (Thijs et al., 2017), spanning the V3–V4 hypervariable regions. PCR amplifications were prepared using the Q5 Hot Start High-Fidelity 2× Master Mix (NEBNext - New England BioLabs, US) according to the

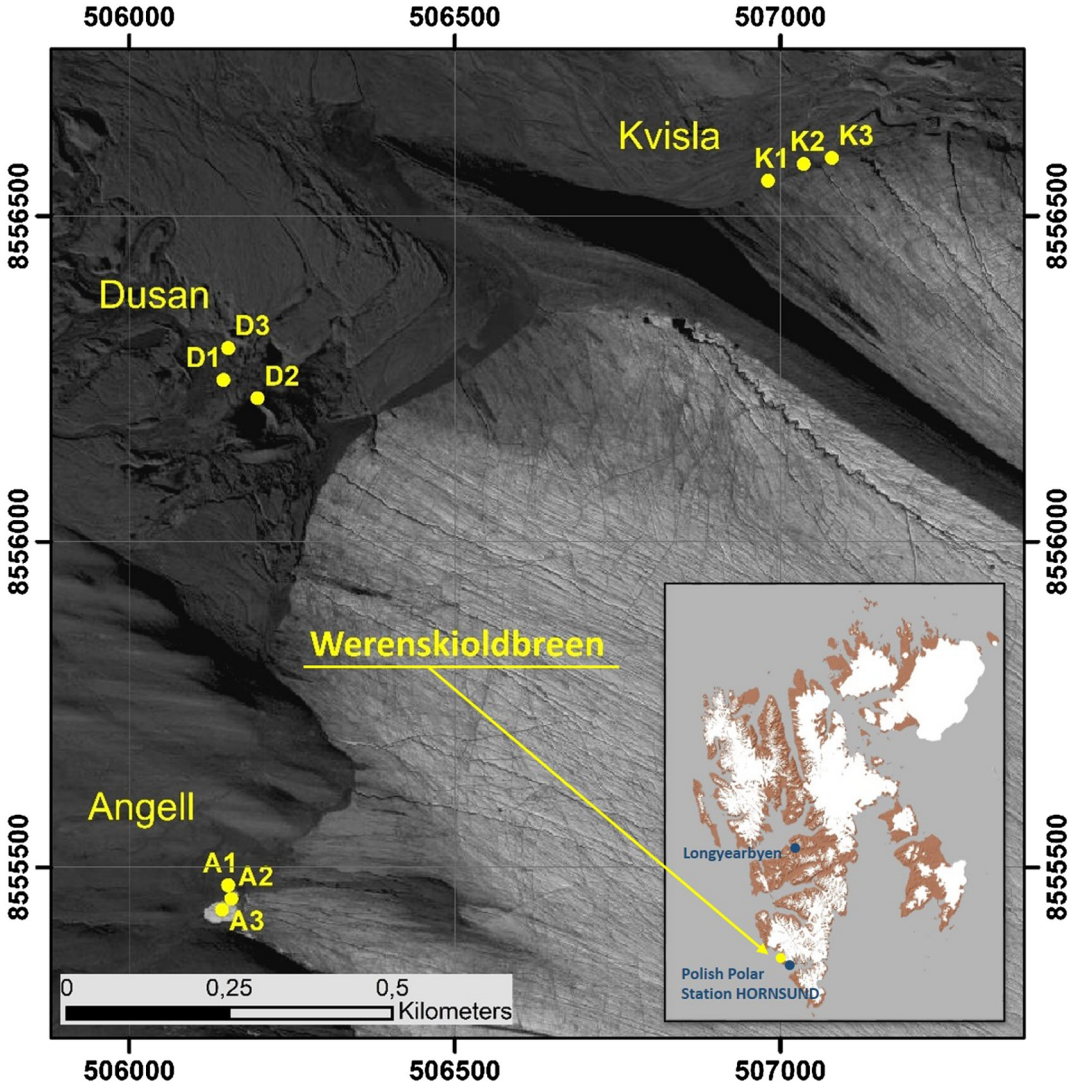


Fig. 1. Location map of Werenskioldbreen in Svalbard. Sampling sites (yellow dots) from naled ice patches located in front of the glacier, presented on a portion of the panchromatic image from Pléaides acquired on 20 August 2017 (Błaszczyk et al., 2019). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

manufacturer's instruction. Sequencing was performed on Illumina MiSeq platform using paired-end reads (2 × 250 bp) MiSeq Reagent Kits v2 (Illumina, Inc., San Diego, CA, USA). Amplicon dataset was deposited at Sequence Read Archive (ID: PRJNA601111).

2.4. Bioinformatics analysis

CLC Genomic Workbench 11.0 and CLC Microbial Genomics Module 3.5 (Qiagen, USA) was used for bioinformatics analysis. Raw sequence

Fig. 2. Dome-shaped and cracked naled ice bodies in the forefield of Werenskioldbreen. Only significant ($p < .05$) p-values are shown. Different letters indicate significant differences between mean values calculated for sampling sites (Tuckey's post-hoc test, $\alpha = 0.05$).

data were filtered, trimmed and merged using *Data QC and OTU Clustering* workflow under default settings (QIAGEN, 2018). Thereafter, the total number of reads ranged from 96,328–195,122. These 641,699 total reads were filtered from chimeric reads and chloroplast sequences. Remaining sequences were assigned to different operational taxonomic units (OTUs) defined by 97% sequence similarity with reference OTU database SILVA 16S v128 16S rRNA (bacteria and archaea). Additionally, the *Allow creation of new OTUs* option was used to allow sequences not represented at the given similarity distance in the SILVA database to form a new cluster. The default (80%) taxonomy similarity percentage was used when annotating new OTUs (Qiagen, 2014). Finally, sequences were assigned to 1409 different OTUs (therein 705 OTUs based on the SILVA database). Coverage was calculated by Good's method as 1 - (N/Individuals), where N is the number of clones that occurred once only (Liu et al., 2011). Coverage assesses efficiency of sampling analysis of the entire sampled population and was calculated in the range of 0.989–0.998.

Relative abundance, as well as alpha and beta diversity, were assessed using *Estimate Alpha and Beta Diversities* workflow (QIAGEN, 2018) based on the filtered OTU Table, where OTUs with low combined abundance (<10) were removed (*Remove OTUs with Low Abundance* tool). Finally, 343 OTUs were used for diversity and phylogenetic analyses. Relative abundance at the class level was presented in the form of a stacked bar chart. The alpha diversity of an individual community isolated from tested sites was assessed based on richness and diversity indices. Richness was estimated by the total number of OTUs, Chao1 index (1) and Chao1 bias-corrected index (2). Diversity indices were calculated based on Simpson's index *SI* (3), Shannon entropy *H′* (4), and phylogenetic diversity *PD* (5) defined as the minimum total length of all phylogenetic branches required to span a given set of taxa on the phylogenetic tree (Faith and Baker, 2006; Qiagen, 2014).

The following formulae were used:

$$Chao1 = D + \frac{f_1^2}{2f_2} \tag{1}$$

$$Chao1{-}bc = D + \frac{f_1\,(f_1{-}1)}{2(f_2+1)} \tag{2}$$

$$SI = 1 - \sum_{i=1}^{n} p_i^2 \tag{3}$$

$$H\prime = \sum_{i=1}^{n} p_i\ log_2 p_i \tag{4}$$

where n is the number of features; D is the number of distinct features observed in the sample; f_1 is the number of features for which only one read has been found in the sample; f_2 is the number of features for which two reads have been found in the sample; and p_i is the fraction of reads that belong to feature i.

$$PD = \sum_{i=1}^{n} b_i I\ (pi{>}0) \tag{5}$$

where n is the number of branches in the phylogenetic tree, b_i is the length of branch i; pi is the proportion of taxa descending from branch i; and the indicator function $I\ (pi > 0)$ and $I\ (pB\ i > 0)$ assumes the value of 1 if any taxa descending from branch i is present in the sample, or 0 otherwise.

Significant changes between mean values of these indices calculated for different sampling sites were tested by one-way ANOVA and Tuckey's post-hoc test ($\alpha = 0.05$) (Statistica 13.0 software).

Beta diversity, a comparison of sample groups based on phylogenetic distance metrics, was estimated using generalised UniFrac distances $d^{(0.5)}$ (Chen et al., 2012). Principal Coordinates Analysis (PCoA) was used for visualization of the beta diversity distance matrix. To describe significant changes in beta diversity of microbial communities dependent on sampling sites (Angell, Dusan, Kvisla), the *PERMANOVA analysis* tool was used with the number of permutations 99,999.

Additionally, to visualise dissimilarity among microbial communities from tested sites, a heat map and Venn diagram were generated (*Create Heat Map for Abundance Table* and *Differential Abundance Analysis* tools, respectively). The heat map was constructed based on the Euclidean distance using the most important (the false discovery rate corrected *p*-value, FDR p-value < 0.0000007) 20 OTUs that differentiated samples. Differential abundance analysis was performed in the form of a Venn diagram based on the number of overlapping OTUs that significantly (FDR-value < 0.05) differentiated across sites.

3. Results

3.1. Physicochemical analysis of subglacial water

Samples of subglacial water collected in the forefield of Werenskioldbreen from naled-ice bodies were differentiated based on chemical properties (Table 1). Concentration of Na^+, Cl^-, Br^-, and NO_3^- was significantly ($p < .05$) lower in water from the central part of Werenskioldbreen forefield (Dusan) and the highest concentration of these ions was generally detected in Angell site. Similarly, dissolved organic carbon content was significantly higher in Angell than in Dusan and Kvisla. The highest mean pH value was measured in water from Dusan, but was not significantly ($p < .05$) different compared to values measured in other sampling sites (Angell, Kvisla). It should be taken into consideration that the highest pH value of individual water sample was measured for Kvisla 2 (pH = 8) and this result significantly influenced the microbial community structure.

3.2. Community structure – taxa distribution

The microbial community structure was estimated according to the abundance of the OTU identified based on the SILVA database. A total of 1409 identified OTUs retrieved from water samples belonged to 40 phyla. Most identified sequences (98.3% of combined abundance – total number of reads belonging to the OTU across all samples) belonged to five taxa – *Proteobacteria* (mainly *Beta-, Gamma- and Epsilonproteobacteria*), *Gracilibacteria, Bacteroidetes, Actinobacteria* and *Parcubacteria* (Table 2). The relative abundance of other 35 phyla was estimated below 1% for each and classified as rare biosphere (Kilias et al., 2014). Additionally, 512 reads classified to 112 OTUs were not identified based on the used database.

Table 1
Physicochemical parameters of subglacial water (ions concentration, dissolved organic carbon (TOC) and dissolved organic carbon (DOC) expressed as mg L^{-1}; σ as μS cm^{-1}).

	p-Value	Angell	Dusan	Kvisla
pH		6.83 ± 0.63	7.62 ± 0.07	6.40 ± 1.42
σ		330.00 ± 133.10	232.00 ± 33.87	303.03 ± 210.42
Na^+	0.029	45.60 ± 14.23^a	11.59 ± 1.54^b	37.46 ± 14.73^a
K^+		4.08 ± 3.38	4.71 ± 0.37	3.25 ± 2.65
Ca^{2+}		11.38 ± 10.71	26.47 ± 4.21	18.18 ± 27.37
Mg^{2+}		8.20 ± 5.54	6.36 ± 0.99	7.66 ± 7.13
HCO_3^-		31.11 ± 36.61	73.22 ± 10.37	67.12 ± 110.44
F^-		0.02 ± 0.01	0.03 ± 0.01	0.11 ± 0.17
Cl^-	0.003	75.31 ± 20.33^a	16.86 ± 2.01^c	45.21 ± 3.93^b
Br^-	<0.001	0.19 ± 0.03^a	0.02 ± 0.01^c	0.11 ± 0.03^b
NO_3^-	0.023	1.91 ± 0.50^a	0.10 ± 0.09^b	1.07 ± 0.85^{ab}
SO_4^{2-}		21.65 ± 13.36	34.30 ± 5.29	35.60 ± 44.18
TOC		1.92 ± 0.57	1.21 ± 0.30	2.14 ± 1.52
DOC	0.040	1.67 ± 0.31^a	0.88 ± 0.34^b	0.95 ± 0.31^b

Only significant ($p < .05$) p-values are shown. Different letters indicate significant differences between mean values calculated for sampling sites (Tuckey's post-hoc test, $\alpha = 0.05$).

Table 2
Bacterial phyla in subglacial water from naled ice bodies on the Werenskioldbreen forefield.

Phylum (aggregated)	Number of detected OTUs	Combined abundance[a]	% abundance
Proteobacteria	424	119,947	73.76
Gracilibacteria	71	13,607	8.37
Bacteroidetes	148	12,078	7.43
Actinobacteria	95	7346	4.52
Parcubacteria	240	6815	4.19
Rare biosphere (<1%)			
Nitrospirae, N/A (not identified), *Chloroflexi, Elusimicrobia, Microgenomates, Firmicutes, Omnitrophica*, WWE3, *Gemmatimonadetes, Fibrobacteres, Saccharibacteria, Acidobacteria, Planctomycetes, Verrucomicrobia, Woesearchaeota* (DHVEG-6), *Peregrinibacteria, Cyanobacteria, Candidatus Berkelbacteria, Chlorobi*, WS2, *Aminicenantes, Ignavibacteriae*, TM6 (*Dependentiae*), GAL15, *Atribacteria*, Miscellaneous Euryarchaeotic Group(MEG), CPR2, *Lentisphaerae, Diapherotrites, Deinococcus-Thermus, Bathyarchaeota, Parvarchaeota, Armatimonadetes*, BRC1, WS6	431	2815	1.73
Total	1409	172,845	100%

[a] Combined abundance – total number of reads belonging to the operational taxonomic units (OTU) across all samples.

Dominant taxa in microbial communities of subglacial water from naled ice belonged to the phylum *Proteobacteria* (Fig. 3). The relative abundance of these bacteria in samples was 61.8–99.3%. Only in sample Kvisla 2, the dominant taxa belonged to the phylum *Gracilibacteria* (GN02) with a 60.1% contribution to the whole microbial community. Contrary to results obtained from other sampling sites, the contribution of *Proteobacteria* in Kvisla 2 was only 31.5% and the majority was classified to *Gammaproteobacteria* with the most abundant species linked to the *Methylobacter* genus (27.2% of the total contribution).

Aside from the Kvisla 2 sample, microbial communities from sites located on the edge of the Werenskioldbreen forefield (Angell, Kvisla) were generally dominated by the *Betaproteobacteria* class (78.7–90.1%), with high contribution of unknown species of the *Paucibacter* genus (11.9–31.0%), member of the family *Oxalobacteraceae* (12.3–33.6%), uncultured species of *Polaromonas* (13.4–20.5%), as well as the *Polynucleobacter* (6.7–19.9%) genus.

In contrast to the Angell and Kvisla sites, the dominant bacterium in subglacial water collected in the central site of Werenskioldbreen forefield (Dusan) was an uncultured bacterium classified to the *Sulfuricurvum* genus (40.2–41.4%) belonging to the *Epsilonproteobacteria* class, whereas, the contribution of the *Betaproteobacteria* class was 11.8–40.1%.

3.3. Alpha diversity indices

Alpha diversity of microbial communities of subglacial water from Werenskioldbreen was quantified based on species richness (total number of OTUs), richness estimators Chao1 and Chao1, bias-corrected, biodiversity indices (Simpson's index and Shannon entropy H′) and phylogenetic diversity (PD). Mean values of alpha diversity estimates are presented in Table 3.

There was a significant ($p < .002$) difference in richness indices across sampling sites. The highest values of the total number of OTUs, Chao1 and Chao1-bc estimators were obtained for samples collected from Dusan located in the central part of Werenskioldbreen forefield. These indices were significantly twice or three time higher (Tuckey, $\alpha = 0.05$) compared to values obtained for Angell and Kvisla.

The sampling site was also a crucial factor that differentiated Shannon entropy ($p < .001$). Microbial diversity estimate based on of this index was significantly ($p < .05$) higher in Dusan than other tested sites. Moreover, ANOVA also indicated that microbial diversity in Angell was significantly ($p < .05$) higher compared to Kvisla. Similarly, analysis based on PD values indicated that the highest phylogenetic diversity was estimated for Dusan and was significantly ($p < .05$) higher than Kvisla and Angell. No significant impact of sampling site was detected for Simpson's index ($p = .109$).

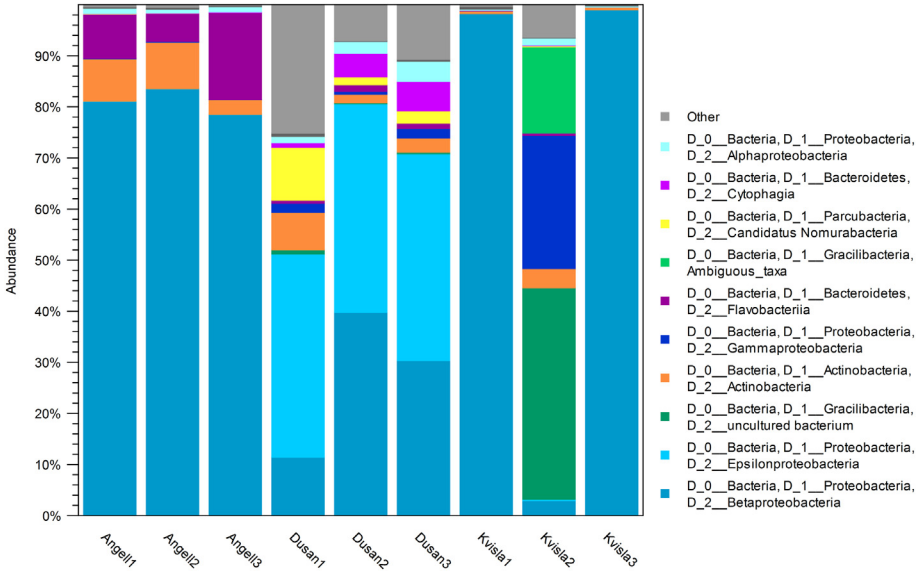


Fig. 3. The microbial community structure – bacterial taxa distribution to class level (10 most abundant features of each sample were shown in all columns).

Table 3
Alpha diversity indices.

Estimators		p-value	Site		
			Angell	Dusan	Kvisla
Richness indices	Total number of OTUs	0.002	121.8 ± 21.1^{b}	271.4 ± 16.3^{a}	83.6 ± 59.2^{b}
	Chao1	0.002	145.0 ± 20.6^{b}	289.0 ± 23.9^{a}	93.6 ± 60.9^{b}
	Chao1-bc	0.002	148.7 ± 19.6^{b}	290.4 ± 23.9^{a}	96.2 ± 59.7^{b}
Diversity indices	Simpson's index (SI)	0.109	0.9 ± 0.1	0.9 ± 0.1	0.8 ± 0.1
	Shannon entropy (H')	0.001	4.0 ± 0.1^{b}	4.7 ± 0.4^{a}	3.1 ± 0.2^{c}
	Phylogenetic diversity (PD)	0.005	5.4 ± 0.7^{b}	12.3 ± 0.2^{a}	4.2 ± 3.3^{b}

Different letters indicate significant differences between mean vales calculated for sampling sites (Tuckey's post-hoc test, $\alpha = 0.05$).

3.4. Beta diversity of microbial communities and properties of subglacial water

Beta diversity, the difference in microbial community diversity associated with localisation of tested sites, was estimated based on phylogenetic distance metrics using generalised UniFrac distances $d^{(0.5)}$. Results were visualised by the PCoA. The first two principle coordinate axes accounted for 74% of variability in observed phylogenetic diversity (Fig. 4). Phylogenetic profiles grouped in three clusters depended on the sampling site. To measure the significance on beta diversity changes according to sampling site location, the PERMANOVA was used. This analysis indicated that the separation was significant (pseudo-F = 4.84; p-value = .004), but with only two to three replicates for each group, because clustering was not significant on pair-wise comparisons of the site (Qiagen, 2014). This effect might be caused by the impact of Kvisla 2 data on performed statistical analysis, as its water properties were most similar to samples collected in the central part of Werenskioldbreen forefield (Dusan).

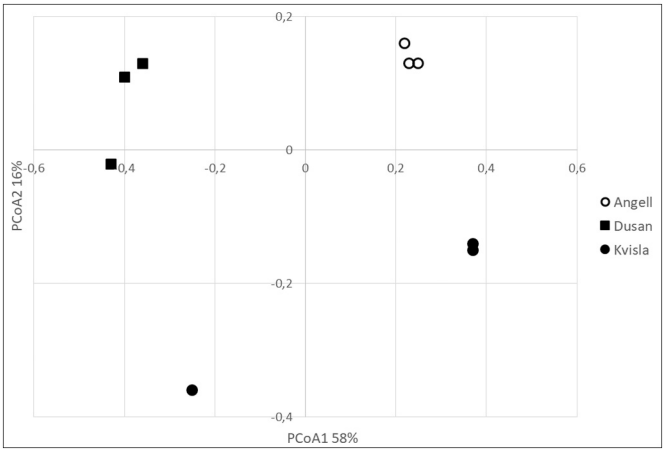


Fig. 4. Principal coordinates analysis (PCoA) plot based on the generalised UniFrac distance matrix.

Nevertheless, comparison of microbial composition of sampling sites were performed using the 20 most important OTUs that significantly differentiated across all samples (FDR p-value < 0.000007), clearly distinguished three clusters connected to the area of outflow channels in Werenskioldbreen forefield. Results of this analysis were presented as a heat map in Fig. 5. Cluster analysis confirmed similarity of microbial communities from sites located on the edge of Werenskioldbreen forefield (Angell, Kvisla) contrary to Dusan, located in the centre of the forefield. Moreover, the most differentiated OTUs isolated from Dusan belonged to *Parcubacteria* and *Proteobacteria* phyla (Supplementary Table 1). In contrast, phylogenetic profiles obtained from Angell differentiated based on OTUs that belonged to the *Bacteroidetes* phylum. Here, cluster analysis indicated that the most different OTU from Kvisla 2 was a *Gracilibacteria* member. However, this profile still groups together with other Kvisla profiles distinguished based on the two *Proteobacteria* OTUs (Supplementary Table 1).

To visualise dissimilarity among microbial communities from tested sites, a Venn diagram was generated (Fig. 6). It shows the number of overlapping OTUs significantly (FDR p-value <.05) differentiated across sites. The highest number of differentiated OTUs sequences was detected for the Dusan vs Angell comparison, two sites with the richest microbial communities. >55% of shared OTUs sequences were significantly ($p < .05$) different between these sites. The lowest number of differentiated OTU sequences was calculated between Kvisla and Angell sites and represented 13% of shared OTUs.

Additionally, to describe the relationship between physicochemical properties of subglacial water and measured microbial diversity,

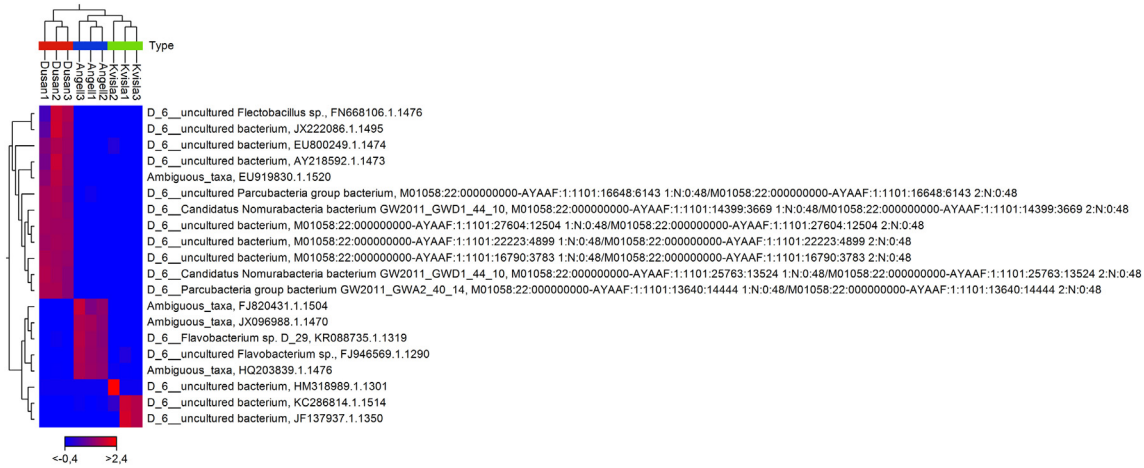


Fig. 5. Normalised distribution of the most important 20 operational taxonomic units (OTUs) that significantly differentiated samples on the Werenskioldbreen forefield.

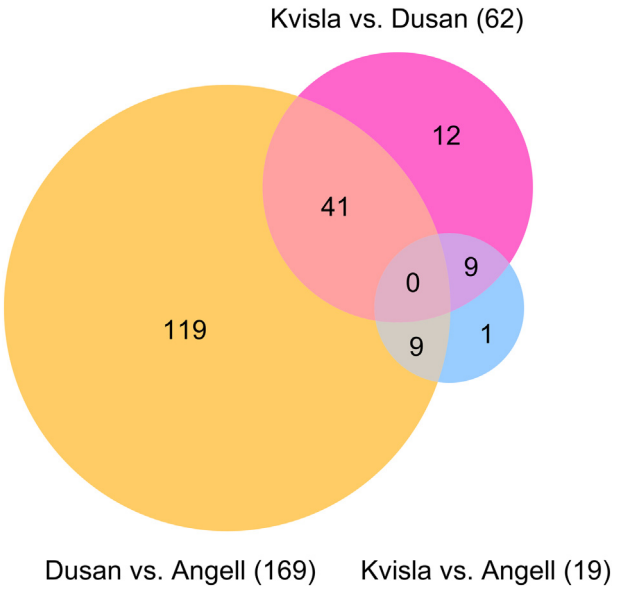


Fig. 6. Venn diagram showing unique and shared operational taxonomic units (OTUs) among the three sampling sites.

correlation analysis was performed. This revealed that richness and phylogenetic diversity indices significantly ($p < .05$) and positively correlated with pH value and negatively correlated with the concentration of Cl^-, Br^-, and NO_3^- anions (Fig. 7). Moreover, concentration of NO_3^- negatively impacted Chao1 and Chao1-bc indices and positively correlated with the abundance of the *Aminicenantes* phylum (Fig. 7) and *Alphaproteobacteria* class (data not shown). Positive correlation was also observed for Br^- concentration and some bacterial phyla (*Aminicenantes, Bacteroidetes, Gemmatimonadetes, Omnitrophica, Peregrinibacteria*) and classes (*Alpha-, Delta- and Epsilonprotebacteria*) (data not shown).

4. Discussion

4.1. Community structure – taxa distribution

The study of microbial communities from the subglacial drainage system of Werenskioldbreen was achieved using next generation sequencing (NGS). Most bacterial sequences isolated from the subglacial water of Werenskioldbreen drainage system belonged to *Proteobacteria, Gracilibacteria Bacteroidetes, Actinobacteria* and *Parcubacteria* phyla. Similarly, *Proteobacteria, Bacteroidetes, Actinobacteria* were also found in ice samples from various Svalbard glaciers (Garcia-Lopez et al., 2019; Perini et al., 2019).

Similar to our study conducted during accumulation season, the *Betaproteobacteria* class, the main component of microbial communities in Angell and Kvisla sites, dominated (21%) in snow and meltwater samples during summer (Larose et al., 2010) in Svalbard and in sequence data obtained from a glacier stream in the Himalayas (Liu et al., 2011). In contrast, the dominant class in the unvegetated alpine glacier forefield (Lazzaro et al., 2012) was *Alphaproteobacteria* (Schostag et al., 2015). *Beta-* and *Alphaproteobacteria* classes were also highly abundant in cryoconite communities on the glacier surfaces. On Arctic glaciers, *Alphaproteobacteria* dominated in cryoconite and their presence negatively correlated with *Betaproteobacteria* (Edwards et al., 2014). These seasonal changes may be related to r-selected, early-colonising *Betaproteobacteria* versus typical K-selected, late-colonising

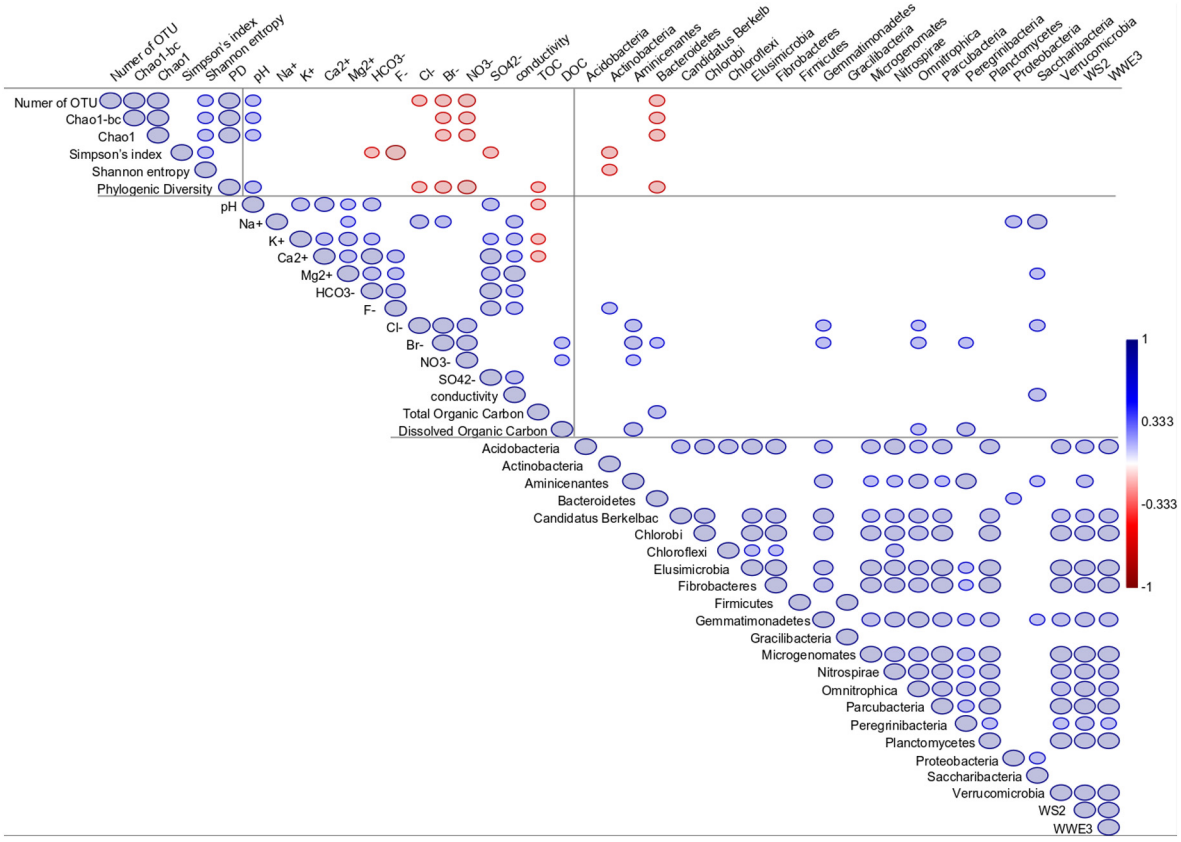


Fig. 7. The Pearson correlation of phylogenetic richness, diversity indices and subglacial water parameters. Only significant ($p < .05$) correlations are marked. The diameter of the sphere is proportional to the value of R.

Alphaproteobacteria (Cook et al., 2015). The lack of *Alphaproteobacteria* in our microbial communities may be related to the sample collecting period. During accumulation season, cryoconites are not formed and the number of *Alphaproteobacteria* members is lower than that of ablation season and their contribution to the glacier microbiome is small (Cook et al., 2015). Moreover, some *Alphaproteobacteria* cells could be lost during the filtration step (Hahn, 2004; Liu et al., 2019), and as a response to starvation, cell size reduction may be observed (Liu et al., 2019).

In Angell and Kvisla, located in the edge of Werenskioldbreen forefield, bacteria typically inhabiting cold environments were present. Bacterial strains classified under the *Paucibacter* genus, were reported present in sediment of meltwater stream of an Arctic glacier, Midtre Lovénbreen, located in Svalbard (Reddy et al., 2009), the subglacial freshwater of the lake Gurudongmar in the Himalayas (Sahay et al., 2013) or glacial river in Iceland (Jóelsson et al., 2013). *Paucibacter* and strains from the *Oxalobacteraceae* family were also isolated from oligotrophic freshwater environments like in sand filters of drinking water treatment plants (Vandermaesen et al., 2017). *Polaromonas* are globally distributed phylotypes in glacial and periglacial environments (Gawor et al., 2016; Willems, 2014), also in Svalbard (Zeng et al., 2013). High contribution of these bacteria in microbial communities was found in a moraine lake and a glacial stream in the Himalayas (Liu et al., 2011). *Polynucleobacter* was found in an oligotrophic Antarctic freshwater lake (Pearce, 2003; Villaescusa et al., 2013), but are considered globally distributed planktonic freshwater microorganisms (Hahn et al., 2015). Similarly, microbial sequences detected in Angell belonging to *Flavobacteriia* and *Actinobacteria* classes (*Bacteroidetes* and *Actinobacteria* phylum, respectively) are typical members of microbial communities of glacial meltwater (Jóelsson et al., 2013; Sinha et al., 2017).

In contrast, microbial communities of subglacial water collected from the central site of Werenskioldbreen forefield (Dusan) were dominated by members of the *Epsilonproteobacteria* class –uncultured bacteria classified under the *Sulfuricurvum* genus. *Epsilonproteobacteria* were detected in Arctic ecosystems, but their contribution in comparison with other *Proteobacteria* classes was relatively low (Edwards et al., 2014; Hell et al., 2013). Moreover, to date, *Sulfuricurvum* is a monotypic genus of the family *Helicobacteraceae* (Mitchell et al., 2014). One known species from this genus, *Sulfuricurvum kujiense,* is a facultative anaerobic, chemolithoautotrophic, sulphur-oxidizing bacterium. Previously, it was isolated from an underground crude-oil storage cavity (Kodama and Watanabe, 2004). These bacteria were also detected in surface sediments from an urban river (He et al., 2017), and in hydrocarbon-contaminated soil (Zhang et al., 2012). Furthermore *S. kujiense* were recognized as pioneers colonising the basaltic lava flow (Kelly et al., 2014) and were detected in subglacial caldera lakes in Iceland (Mikucki et al., 2011). Also, members of *Betaproteobacteria* and *Gammaproteobacteria* were abundant in Dusan and Kvisla 2, respectively, and are known as sulphide and iron-oxidizing bacteria. Sulphide oxidation and organic carbon transformation in subglacial ecosystems are responsible for creating anaerobic conditions (Achberger et al., 2017). It initiates the denitrification process that transforms NO_3^- to N_2, NO, NO_2, NH_4^+ and may explain the lower concentration of NO_3^- and highest pH values detected in Dusan and Kvisla 2 samples (Garcia-Lopez et al., 2019). This may also be supported by the presence of uncultured bacteria only in these sites, from *Aminicenantes*, *Nitrospira* and *Omnitrophica* phyla that are linked to the nitrogen reducing process (Dutta et al., 2018; Kadnikov et al., 2019; Momper et al., 2017). Additionally, high abundance of *Gamma-* and *Alphaproteobacteria* members capable of anaerobic respiration with nitrate (Chen et al., 2018; Vilar-Sanz et al., 2018) was also observed in Dusan and Kvisla 2.

Results also indicated that subglacial water taken from Kvisla 2 might be mixed with forefield sediments as a result of high pressure water flowing in channels. The pH value of water collected from Kvisla 2 (pH = 8) was the highest among collected water samples and the content of cations and anions was four time higher than samples collected from Kvisla 1 and Kvisla 3. The dominant taxa of the microbial community from the Kvisla 2 sample belonged to phylum *Gracilibacteria* (GN02), uncultured bacteria commonly found in permafrost soil (Frey et al., 2016). Additionally, as mentioned above, *Gammaproteobacteria* was the second dominant class with the most abundant species linked to the *Methylobacter* genus. Strains from this genus were isolated from the Arctic wetland (Wartiainen et al., 2006) or peat soil (Tveit et al., 2013) on the Svalbard. High abundance of *Methylobacter* is generally related to water-logged soils (Blaud et al., 2015) and supports the conclusion about mixing subglacial water with forefield sediments in Kvisla 2 site. The presence of these methanotrophic organisms may also indicate utilization of available methane sub glacially, produced by methanogenic archaea (Dieser et al., 2014; Lamarche-Gagnon et al., 2019).

Generally, analysis of relative abundance indicated that the structure of microbial communities was connected with the localisation of sampling sites where subglacial water was collected (central part vs. edge of Werenskioldbreen forefield). This variability is related to the physicochemical properties of water drained from different channels. Kvisla and Angell drainage systems are located between glacier and bedrock, which favours mineralisation of subglacial water. In contrast, the Dusan drainage system is smaller and some parts are located in glacier forefield in deposited sediments and buried ice (Kies et al., 2011; Stachnik et al., 2016a). These site-dependent differences and the result of microbial nitrogen transformation also influenced pH values of water. Value of pH is considered the most important factor impacting microbial diversity, also in Arctic environments (Malard et al., 2019; Malard and Pearce, 2018). Additionally, detected site-specific composition of microbial communities support the thesis of Grabiec et al. (2011), in that these individual channels represent separated subglacial environments. Others found that the spatial differentiation in microbial community structure depended on glacial meltwater properties (Conte et al., 2018; Garcia-Lopez et al., 2019; Piquet et al., 2010; Sinha et al., 2017).

4.2. Alpha diversity

Calculated richness values, markers of alpha diversity of microbial communities, confirmed that subglacial meltwater from Werenskioldbreen was an oligotrophic environment (Anesio et al., 2017). Another study conducted in the Arctic tundra (Canada) indicated higher microbial richness in shallow thaw ponds (Negandhi et al., 2014). Similarly, in our study, a subglacial Arctic deep marine sediment was a richer and more diverse environment (Li et al., 2015) than subglacial meltwater. Nevertheless, low values of these indices were also calculated for other arctic ecosystems. In the study of microbial communities from snow and meltwater samples, higher richness measured as Chao1 index and biodiversity expressed as Shannon index was detected for meltwater samples (Larose et al., 2010). Similar to our results, Chao1 values were estimated over 100. In turn, Shannon index in the range of 3.4–3.6 were generally lower compared to values obtained for meltwater collected from the naled ice on the Werenskioldbreen forefield. Also, in the study of Perini et al., (2019) bacterial diversity H′ in the ice sample from three Svalbard glaciers was generally lower than our samples, and estimated in the range of 2.51–5.03.

Spatial differentiation of microorganisms expressed as alpha diversity indices in our investigation was also observed in another study. Park et al. (2011) indicated that meltwater runoff impacted on microbial communities of coastal marine sediment located in Temelfjorden (Svalbard) and microbial alpha diversity was higher within the glacier–proximal zone than the outer fjord region. This and another study (Garcia-Lopez et al., 2019) confirmed that subglacial meltwater is a source of microorganisms and/or minerals that influence microbial diversity in the impacted environment.

4.3. Beta diversity of microbial communities and properties of subglacial water

The analysis of microbial phylogenetic profiles revealed the difference in microbial communities' diversity associated with differences in tested sites (beta diversity). Visualising by PCoA phylogenetic profiles (Fig. 4) grouped in three clusters depended on the localisation of sampling sites. Generally, profiles from subglacial meltwater collected from naled ice at the edge of Werenskioldbreen (Angell and Kvisla) showed more similarity to each other than to microbial profiles from Dusan. Similar patterns revealed cluster analysis (Fig. 5). Additionally, the Venn diagram was used to visualise dissimilarity among microbial communities from tested sites, and indicated that the highest number of differentiated OTU sequences was detected for Dusan vs Angell and the lowest for Kvisla vs. Angell. Overall, these results confirmed previous conclusions that the structure and biodiversity of microbial communities depended on outflow localization of different channels from the Werenskioldbreen drainage system.

Phylogenetic diversity indices were positively correlated with water pH and negatively correlated with the concentration of Cl^-, Br^-, and NO_3^- anions. Positive correlation of alpha diversity indices with pH was also observed for microbial communities in Arctic soils, where the lowest values were detected in acidic soils and the highest in acidoneutral soils (Malard et al., 2019). Additionally, the concentration of NO_3^- negatively affected richness indices Chao1 and Chao1-bc but positively correlated with the abundance of the *Aminicenantes* phylum and *Alphaproteobacteria* class. As previously mentioned, members of these bacterial groups may be involved in the denitrification process, which may account for lower nitrate concentration than in other sites (Kadnikov et al., 2019; Vilar-Sanz et al., 2018). The product of denitrification, ammonium ions, may be responsible for increases in pH values (Achberger et al., 2017; Garcia-Lopez et al., 2019).

Mineralisation of outflow water is a result of its contact with till and bedrock underneath a glacier, as well as storage in the subglacial drainage system throughout winter (Stachnik et al., 2016a). Mineral composition of bedrock, proglacial sediments and suspended sediment in the Werenskioldbreen basin were studied (Bukowska-Jania, 2007; Czerny et al., 1992; Kabala and Zapart, 2012) and summarised (Stachnik et al., 2016a), showing spatial differentiation. Briefly, amphibolite, quartzite and chlorite schist are situated beneath the southern part of Werenskioldbreen; phyllites with quartzite and silt intercalations, and calcareous and chlorite schists are situated beneath the eastern part of the glacier. The north-western part of the basin is composed of greenschists, and muscovite–carbonate–quartz or carbonate–chlorite–quartz schists. Moreover, in the proglacial area, mica–carbonate–quartz, grey calcite marbles and pyrite accompanied by pyrrhotite, galena, sphalerite, magnetite and haematite are the most common ore minerals. Carbonate minerals are present as siderite, ankerite or Fe-calcite (Czerny et al., 1992). Suspended sediment consists of magnesium chlorite, feldspars, muscovite, quartz, calcite, and dolomite (Bukowska-Jania, 2007). Despite a low proportion of calcium carbonate in the bedrock, calcium carbonate concentration increase in the proglacial sediment partly due to precipitation of calcite from water in subglacial tills. Additionally, during the accumulation season, minerals precipitate due to freezing of highly concentrated solutions within naled ice. Therefore, calcium carbonate content increases in glaciofluvial sediments close to naledi (Bukowska-Jania, 2007). This fact may also explain higher concentration of positively correlated Cl^- an HCO_3^- ions in our study in subglacial water compared to the result obtained during ablation season (Stachnik et al., 2016a). Positive correlation of Br^- and Cl^- ions was observed by others (Banks et al., 1998; Jambon et al., 1995) and Br/Cl ratio in our study was similar like in arctic groundwater and lower compared to sea-water indicating sediment origin (Banks et al., 1998; Jambon et al., 1995). Additionally, positive correlation between Br^- concentration and some bacterial phyla was observed impacting on unique site-specific microbial community structure (Cota and Sturges, 1997). Moreover, there was an observed increase of ion concentration in subglacial outflows compared to the supraglacial stream (Stachnik et al., 2016a). Similarly, the specific electric conductivity value of our samples in the range of 180–546 μS/cm, was much higher than those measured for the ablation water from snow and ice (Pälli et al., 2003). Therefore, physicochemical differences in properties of water transported through different channels from subglacial drainage system may explained observed spatial diversity of microbial communities. Moreover, mineralisation, which is dependent on the bedrock composition is also responsible for unique site-specific microbial community structure. We speculate that during accumulation season, microbial communities from subglacial water are specific for each glacier and are a part of the whole glacier microbiome.

5. Conclusions

Naled ice bodies that form in the forefield of glaciers are sources of subglacial water, which can be used to study glacier microbiomes during the accumulation season. Structures of microbial communities were diverse and site-specific. The main factor responsible for this differentiation was water properties probably influenced by chemical composition of local rock beneath the glacier. This study showed that dominant classes of microorganisms that existed in subglacial water from Werenskioldbreen in Svalbard were *Beta-*, *Gamma-* and *Epsilonproteobacteria*. Nevertheless, further studies conducted over several seasons are necessary to answer if the changes in structure of microbial communities from subglacial meltwater, part of glacier microbiome, may be used to study glacier behaviour and changes in the arctic environment.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2020.138025.

CRediT authorship contribution statement

Sławomir Sułowicz: Methodology, Validation, Formal analysis, Investigation, Writing - original draft, Visualization. **Kinga Bondarczuk:** Methodology, Investigation, Data curation, Writing - review & editing. **Dariusz Ignatiuk:** Methodology, Investigation, Writing - review & editing. **Jacek A. Jania:** Conceptualization, Writing - review & editing, Supervision, Project administration, Funding acquisition. **Zofia Piotrowska-Seget:** Conceptualization, Writing - review & editing, Supervision, Project administration, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The authors would like to thank Krystyna Kozioł from the Polish Polar Station in Hornsund for conducting IC analyses, and Katarzyna Koziorowska from the Institute of Oceanology of the Polish Academy of Sciences (IO PAN)_in Sopot for TOC and DOC analyses. This publication was partially financed by the Leading National Research Centre (KNOW), received by the Centre for Polar Studies of the University of Silesia in Katowice, Poland.

References

Achberger, A.M., Michaud, A.B., Vick-Majors, T.J., Christner, B.C., Skidmore, M.L., Priscu, J.C., Tranter, M., 2017. Microbiology of subglacial environments. In: Margesin, R. (Ed.), Psychrophiles: From Biodiversity to Biotechnology. Springer International Publishing, Cham, pp. 83–110. https://doi.org/10.1007/978-3-319-57057-0_5.

Anesio, A.M., Laybourn-Parry, J., 2012. Glaciers and ice sheets as a biome. Trends Ecol. Evol. 27, 219–225. https://doi.org/10.1016/j.tree.2011.09.012.

Anesio, A.M., Lutz, S., Chrismas, N.A.M., Benning, L.G., 2017. The microbiome of glaciers and ice sheets. npj Biofilms Microbiomes 3, 0–1. https://doi.org/10.1038/s41522-017-0019-0.

Banks, D., Sletten, R.S., Haldorsen, S., Dale, B., Heim, M., Swensen, B., 1998. The thermal springs of Bockfjord, Svalbard: occurrence and major ion hydrochemistry. Geothermics 27, 445–467. https://doi.org/10.1016/S0375-6505(98)00022-4.

Baranowski, S., 1982. Naled ice in front of some Spitsbergen graciers. J. Glaciol. 28, 211–214.

Błaszczyk, M., Ignatiuk, D., Grabiec, M., Kolondra, L., Laska, M., Decaux, L., Jania, J., Berthier, E., Luks, B., Barzycka, B., Czapla, M., 2019. Quality assessment and glaciological applications of digital elevation models derived from space-borne and aerial images over two tidewater glaciers of southern spitsbergen. Remote Sens. 11, 1–21. https://doi.org/10.3390/rs11091121.

Blaud, A., Lerch, T.Z., Phoenix, G.K., Osborn, A.M., 2015. Arctic soil microbial diversity in a changing world. Res. Microbiol. 166, 796–813. https://doi.org/10.1016/j.resmic.2015.07.013.

Bukowska-Jania, E., 2007. The role of glacier system in migration of calcium carbonate on Svalbard. Polish Polar Res 28, 137–155.

Bukowska-Jania, E., Szafraniec, J., 2005. Distribution and morphometric characteristics of icing fields in Svalbard. Polar Res. 24, 41–53. https://doi.org/10.1111/j.1751-8369.2005.tb00139.x.

Cameron, K.A., Stibal, M., Hawkings, J.R., Mikkelsen, A.B., Telling, J., Kohler, T.J., Gözdereliler, E., Zarsky, J.D., Wadham, J.L., Jacobsen, C.S., 2017. Meltwater export of prokaryotic cells from the Greenland ice sheet. Environ. Microbiol. 19, 524–534. https://doi.org/10.1111/1462-2920.13483.

Chen, J., Bittinger, K., Charlson, E.S., Hoffmann, C., Lewis, J., Wu, G.D., Collman, R.G., Bushman, F.D., Li, H., 2012. Associating microbiome composition with environmental covariates using generalized UniFrac distances. Bioinformatics 28, 2106–2113. https://doi.org/10.1093/bioinformatics/bts342.

Chen, D., Wang, H., Yang, K., Ma, F., 2018. Performance and microbial communities in a combined bioelectrochemical and sulfur autotrophic denitri fi cation system at low temperature. Chemosphere 193, 337–342. https://doi.org/10.1016/j.chemosphere.2017.11.017.

Conte, A., Papale, M., Amalfitano, S., Mikkonen, A., Rizzo, C., De Domenico, E., Michaud, L., Lo Giudice, A., 2018. Bacterial community structure along the subtidal sandy sediment belt of a high Arctic fjord (Kongsfjorden, Svalbard Islands). Sci. Total Environ. 619–620, 203–211. https://doi.org/10.1016/j.scitotenv.2017.11.077.

Cook, J., Edwards, A., Takeuchi, N., Irvine-Fynn, T., 2015. Cryoconite: the dark biological secret of the cryosphere. Prog. Phys. Geogr. 40, 66–111. https://doi.org/10.1177/0309133315616574.

Cota, G.F., Sturges, W.T., 1997. Biogenic bromine production in the arctic. Mar. Chem. 56, 181–192. https://doi.org/10.1016/S0304-4203(96)00070-9.

Czerny, J., Lipien, G., Manecki, A., Piestrzynski, A., 1992. Geology and ore-mineralization of the Hecla Hoek Succession (Precambrian) in front of Werenskioldbreen, south Spitsbergen. Stud. Geol. Pol. 98, 67–113.

Decaux, L., Grabiec, M., Ignatiuk, D., Jania, J., 2019. Role of discrete recharge from the supraglacial drainage system for modelling of subglacial conduits pattern of Svalbard polythermal glaciers. Cryosph., 735–752 https://doi.org/10.5194/tc-2017-219.

Dieser, M., Broemsen, E.L.J.E., Cameron, K.A., King, G.M., Achberger, A., Choquette, K., Hagedorn, B., Sletten, R., Junge, K., Christner, B.C., 2014. Molecular and biogeochemical evidence for methane cycling beneath the western margin of the Greenland Ice Sheet. ISME J 8, 2305–2316. https://doi.org/10.1038/ismej.2014.59.

Dutta, A., Gupta, S.D., Gupta, A., Sarkar, J., Roy, S., Mukherjee, A., Sar, P., 2018. Exploration of deep terrestrial subsurface microbiome in late cretaceous Deccan traps and underlying Archean basement. India. Sci. Rep. 8, 1–16. https://doi.org/10.1038/s41598-018-35940-0.

Edwards, A., Mur, L.A.J., Girdwood, S.E., Anesio, A.M., Stibal, M., Rassner, S.M.E., Hell, K., Pachebat, J.A., Post, B., Bussell, J.S., Cameron, S.J.S., Griffith, G.W., Hodson, A.J., Sattler, B., 2014. Coupled cryoconite ecosystem structure-function relationships are revealed by comparing bacterial communities in alpine and Arctic glaciers. FEMS Microbiol. Ecol. 89, 222–237. https://doi.org/10.1111/1574-6941.12283.

Faith, D.P., Baker, A.M., 2006. Phylogenetic Diversity (PD) and biodiversity conservation: some bioinformatics challenges. Evol. Bioinforma. 2, 121–128. https://doi.org/10.1177/117693430600200007.

Frey, B., Rime, T., Phillips, M., Stierli, B., Hajdas, I., Widmer, F., Hartmann, M., 2016. Microbial diversity in European alpine permafrost and active layers. FEMS Microbiol. Ecol. 92, 1–17. https://doi.org/10.1093/femsec/fiw018.

Garcia-Lopez, E., Rodriguez-Lorente, I., Alcazar, P., Cid, C., 2019. Microbial communities in coastal glaciers and tidewater tongues of Svalbard archipelago, Norway. Front. Mar. Sci. 5. https://doi.org/10.3389/fmars.2018.00512.

Gawor, J., Grzesiak, J., Sasin-Kurowska, J., Borsuk, P., Gromadka, R., Górniak, D., Świątecki, A., Aleksandrzak-Piekarczyk, T., Zdanowski, M.K., 2016. Evidence of adaptation, niche separation and microevolution within the genus Polaromonas on Arctic and Antarctic glacial surfaces. Extremophiles 20, 403–413. https://doi.org/10.1007/s00792-016-0831-0.

Grabiec, M., Jania, J., Budzik, T., Puczko, D., Gajek, G., Kolondra, L., 2011. Development of subglacial drainage conduits in relation to geometry changes of Spitsbergen Glaciers, ocean influence on climate and cryosphere in the Arctic. Workshop Abstracts Sopot, Poland-November 28–30, p. 32.

Hahn, M.W., 2004. Broad diversity of viable bacteria in "sterile" (0.2 μm) filtered water. Res. Microbiol. 155, 688–691. https://doi.org/10.1016/j.resmic.2004.05.003.

Hahn, M.W., Koll, U., Jezberová, J., Camacho, A., 2015. Global phylogeography of pelagic Polynucleobacter bacteria: restricted geographic distribution of subgroups, isolation by distance and influence of climate. Environ. Microbiol. 17, 829–840. https://doi.org/10.1111/1462-2920.12532.

He, Z., Long, X., Li, L., Yu, G., Chong, Y., Xing, W., Zhu, Z., 2017. Temperature response of sulfide/ferrous oxidation and microbial community in anoxic sediments treated with calcium nitrate addition. J. Environ. Manag. 191, 209–218. https://doi.org/10.1016/j.jenvman.2017.01.008.

Hell, K., Edwards, A., Zarsky, J., Podmirseg, S.M., Girdwood, S., Pachebat, J.A., Insam, H., Sattler, B., 2013. The dynamic bacterial communities of a melting High Arctic glacier snowpack. ISME J 7, 1814–1826. https://doi.org/10.1038/ismej.2013.51.

Hodgson, D.A., Bentley, M.J., Smith, J.A., Klepacki, J., Makinson, K., Smith, A.M., Saw, K., Scherer, R., Powell, R., Tulaczyk, S., Rose, M., Pearce, D., Mowlem, M., Keen, P., Siegert, M.J., 2016. Technologies for retrieving sediment cores in Antarctic subglacial settings. Philos. Trans. R. Soc. A Math. Phys. Eng. Sci. 374. https://doi.org/10.1098/rsta.2015.0056.

Hodson, A.J., Anesio, A.M., Tranter, M., Fountain, A., Osborn, M., Priscu, J., Laybourn-Parry, J., Sattler, B., 2008. Glacial Ecosystems. Ecol. Monogr. 78, 41–67. https://doi.org/10.1002/ecy.2446.

Ignatiuk, D., Piechota, A., Ciepły, M., Luks, B., 2014. Changes of altitudinal zones of Werenskioldbreen and Hansbreen in period 1990-2008, Svalbard. AIP Conf. Proc. 1618, 275–280. https://doi.org/10.1063/1.4897727.

Jambon, A., Déruelle, B., Dreibus, G., Pineau, F., 1995. Chlorine and bromine abundance in MORB: the contrasting behaviour of the Mid-Atlantic Ridge and East Pacific Rise and implications for chlorine geodynamic cycle. Chem. Geol. 126, 101–117. https://doi.org/10.1016/0009-2541(95)00112-4.

Jóelsson, J.P., Frijónsdóttir, H., Vilhelmsson, O., 2013. Bioprospecting a glacial river in Iceland for bacterial biopolymer degraders. Cold Reg. Sci. Technol. 96, 86–95. https://doi.org/10.1016/j.coldregions.2013.03.001.

Kabala, C., Zapart, J., 2012. Initial soil development and carbon accumulation on moraines of the rapidly retreating Werenskiold Glacier, SW Spitsbergen, Svalbard archipelago. Geoderma 175–176, 9–20. https://doi.org/10.1016/j.geoderma.2012.01.025.

Kadnikov, V.V., Mardanov, A.V., Beletsky, A.V., Karnachuk, O.V., Ravin, N.V., 2019. Genome of the candidate phylum Aminicenantes bacterium from a deep subsurface thermal aquifer revealed its fermentative saccharolytic lifestyle. Extremophiles 23, 189–200. https://doi.org/10.1007/s00792-018-01073-5.

Kelly, L.C., Cockell, C.S., Thorsteinsson, T., Marteinsson, V., Stevenson, J., 2014. Pioneer microbial communities of the Fimmvörðuháls lava flow, Eyjafjallajökull, Iceland. Microb. Ecol. 68, 504–518. https://doi.org/10.1007/s00248-014-0432-3.

Kies, A., Nawrot, A., Tosheva, Z., Jania, J., 2011. Natural radioactive isotopes in glacier meltwater studies. Geochem. J. 45, 423–429. https://doi.org/10.2343/geochemj.1.0141.

Kilias, E.S., Peeken, I., Metfies, K., 2014. Insight into protist diversity in Arctic sea ice and melt-pond aggregate obtained by pyrosequencing. Polar Res. 33, 1271–1287. https://doi.org/10.3402/polar.v33.23466.

Kodama, Y., Watanabe, K., 2004. Sulfuricurvum kujiense gen. nov., sp. nov., a facultatively anaerobic, chemolithoautotrophic, sulfur-oxidizing bacterium isolated from an underground crude-oil storage cavity. Int. J. Syst. Evol. Microbiol. 54, 2297–2300. https://doi.org/10.1099/ijs.0.63243-0.

Lamarche-Gagnon, G., Wadham, J.L., Lollar, B.S., Arndt, S., Fietzek, P., Beaton, A.D., Tedstone, A.J., Telling, J., Bagshaw, E.A., Hawkings, J.R., Kohler, T.J., Zarsky, J.D., Mowlem, M.C., Anesio, A., Stibal, M., 2019. Greenland melt drives continuous export of methane from its bed. Nature 565, 73–77. https://doi.org/10.1038/s41586-018-0800-0.

Lanoil, B., Skidmore, M., Priscu, J.C., Han, S., Foo, W., Vogel, S.W., Tulaczyk, S., Engelhardt, H., 2009. Bacteria beneath the West Antarctic Ice Sheet. Environ. Microbiol. 11, 609–615. https://doi.org/10.1111/j.1462-2920.2008.01831.x.

Larose, C., Berger, S., Ferrari, C., Navarro, E., Dommergue, A., Schneider, D., Vogel, T.M., 2010. Microbial sequences retrieved from environmental samples from seasonal Arctic snow and meltwater from Svalbard, Norway. Extremophiles 14, 205–212. https://doi.org/10.1007/s00792-009-0299-2.

Lazzaro, A., Brankatschk, R., Zeyer, J., 2012. Seasonal dynamics of nutrients and bacterial communities in unvegetated alpine glacier forefields. Appl. Soil Ecol. 53, 10–22. https://doi.org/10.1016/j.apsoil.2011.10.013.

Li, Y., Liu, Q., Li, C., Dong, Y., Zhang, Wenyan, Zhang, Wuchang, Xiao, T., 2015. Bacterial and archaeal community structures in the Arctic deep-sea sediment. Acta Oceanol. Sin. 34, 93–113. https://doi.org/10.1007/s13131-015-0624-9.

Liu, Y., Yao, T., Jiao, N., Tian, L., Hu, A., Yu, W., Li, S., 2011. Microbial diversity in the snow, a moraine lake and a stream in Himalayan glacier. Extremophiles 15, 411–421. https://doi.org/10.1007/s00792-011-0372-5.

Liu, J., Li, B., Wang, Y., Zhang, G., Jiang, X., Li, X., 2019. Passage and community changes of filterable bacteria during microfiltration of a surface water supply. Environ. Int. 131, 104998. https://doi.org/10.1016/j.envint.2019.104998.

Malard, L.A., Pearce, D.A., 2018. Microbial diversity and biogeography in Arctic soils. Environ. Microbiol. Rep. 10, 611–625. https://doi.org/10.1111/1758-2229.12680.

Malard, L.A., Anwar, M.Z., Jacobsen, C.S., Pearce, D.A., 2019. Biogeographical patterns in soil bacterial communities across the Arctic region. FEMS Microbiol. Ecol. 95, fiz128. https://doi.org/10.1101/655431.

Martinez-Alonso, E., Pena-Perez, S., Serrano, S., Garcia-Lopez, E., Alcazar, A., Cid, C., 2019. Taxonomic and functional characterization of a microbial community from a volcanic englacial ecosystem in Deception Island. Antarctica. Sci. Rep. 9, 1–14. https://doi.org/10.1038/s41598-019-47994-9.

Mikucki, J.A., Han, S.K., Lanoil, B.D., 2011. Ecology of psychrophiles: subglacial and permafrost environments. In: Horikoshi, K. (Ed.), Extremophiles Handbook. Springer, Japan, Tokyo, pp. 755–775. https://doi.org/10.1007/978-4-431-53898-1_37.

Mitchell, H.M., Rocha, G.A., Kaakoush, N.O., O'Rourke, J.L., Queiroz, D.M.M., 2014. The family Helicobacteraceae. In: Rosenberg, E., DeLong, E.F., Lory, S., Stackebrandt, E., Thompson, F. (Eds.), The Prokaryotes: Deltaproteobacteria and Epsilonproteobacteria. Springer, Berlin Heidelberg, Berlin, Heidelberg, pp. 337–392. https://doi.org/10.1007/978-3-642-39044-9_275.

Momper, L., Jungbluth, S.P., Lee, M.D., Amend, J.P., 2017. Energy and carbon metabolisms in a deep terrestrial subsurface fluid microbial community. Nat. Publ. Gr. 11, 2319–2333. https://doi.org/10.1038/ismej.2017.94.

Negandhi, K., Laurion, I., Lovejoy, C., 2014. Bacterial communities and greenhouse gas emissions of shallow ponds in the high Arctic. Polar Biol. 37, 1669–1683. https://doi.org/10.1007/s00300-014-1555-1.

Pälli, A., Moore, J.C., Jania, J., Kolondra, L., Piotr, G., 2003. The drainage pattern of Hansbreen and Werenskioldbreen, two polythermal glaciers in Svalbard. Polar Res. 22 (2), 355–371.

Park, S.J., Park, B.J., Jung, M.Y., Kim, S.J., Chae, J.C., Roh, Y., Forwick, M., Yoon, H. Il, Rhee, S.K., 2011. Influence of deglaciation on microbial communities in marine sediments off the coast of Svalbard, Arctic Circle. Microb. Ecol. 62, 537–548. https://doi.org/10.1007/s00248-011-9860-5.

Pearce, D.A., 2003. Bacterioplankton community structure in a maritime Antarctic oligotrophic lake during a period of holomixis, as determined by denaturing gradient gel electrophoresis (DGGE) and fluorescence in situ hybridization (FISH). Microb. Ecol. 46, 92–105. https://doi.org/10.1007/s00248-002-2039-3.

Perini, L., Gostinčar, C., Gunde-Cimerman, N., 2019. Fungal and bacterial diversity of Svalbard subglacial ice. Sci. Rep. 9, 20230. https://doi.org/10.1038/s41598-019-56290-5.

Piquet, A.M.T., Scheepens, J.F., Bolhuis, H., Wiencke, C., Buma, A.G.J., 2010. Variability of protistan and bacterial communities in two Arctic fjords (Spitsbergen). Polar Biol. 33, 1521–1536. https://doi.org/10.1007/s00300-010-0841-9.

Qiagen, 2014. User Manual for CLC Microbial Genomics. pp. 1–69.

QIAGEN, 2018. OTU Clustering Using Workflows.

Reddy, P.V.V., Rao, S.S.S.N., Pratibha, M.S., Sailaja, B., Kavya, B., Manorama, R.R., Singh, S.M., Srinivas, T.N.R., Shivaji, S., 2009. Bacterial diversity and bioprospecting for cold-active enzymes from culturable bacteria associated with sediment from a melt water stream of Midtre Lovenbreen glacier, an Arctic glacier. Res. Microbiol. 160, 538–546. https://doi.org/10.1016/j.resmic.2009.08.008.

Sahay, H., Babu, B.K., Singh, S., Kaushik, R., Saxena, A.K., Arora, D.K., 2013. Cold-active hydrolases producing bacteria from two different sub-glacial Himalayan lakes. J. Basic Microbiol. 53, 703–714. https://doi.org/10.1002/jobm.201200126.

Schostag, M., Stibal, M., Jacobsen, C.S., Bælum, J., Tas, N., Elberling, B., Jansson, J.K., Semenchuk, P., Priemé, A., 2015. Distinct summer and winter bacterial communities in the active layer of Svalbard permafrost revealed by DNA- and RNA-based analyses. Front. Microbiol. 6, 1–13. https://doi.org/10.3389/fmicb.2015.00399.

Sinha, R.K., Krishnan, K.P., Kerkar, S., Thresyamma, D.D., 2017. Influence of glacial melt and Atlantic water on bacterioplankton community of Kongsfjorden, an Arctic fjord. Ecol. Indic. 82, 143–151. https://doi.org/10.1016/j.ecolind.2017.06.051.

Stachnik, Ł., Majchrowska, E., Yde, J.C., Nawrot, A.P., Cichała-Kamrowska, K., Ignatiuk, D., Piechota, A., 2016a. Chemical denudation and the role of sulfide oxidation at Werenskioldbreen, Svalbard. J. Hydrol. 538, 177–193. https://doi.org/10.1016/j.jhydrol.2016.03.059.

Stachnik, Ł., Yde, J.C., Kondracka, M., Ignatiuk, D., Grzesik, M., 2016b. Glacier naled evolution and relation to the subglacial drainage system based on water chemistry and GPR surveys (Werenskioldbreen, SW Svalbard). Ann. Glaciol. 57, 1–12. https://doi.org/10.1017/aog.2016.9.

Thijs, S., De Beeck, M.O., Beckers, B., Truyens, S., Stevens, V., Van Hamme, J.D., Weyens, N., Vangronsveld, J., 2017. Comparative evaluation of four bacteria-specific primer pairs for 16S rRNA gene surveys. Front. Microbiol. 8, 1–15. https://doi.org/10.3389/fmicb.2017.00494.

Tulaczyk, S., Mikucki, J.A., Siegfried, M.R., Priscu, J.C., Barcheck, C.G., Beem, L.H., Behar, A., Burnett, J., Christner, B.C., Fisher, A.T., Fricker, H.A., Mankoff, K.D., Powell, R.D., Rack, F., Sampson, D., Scherer, R.P., Schwartz, S.Y., 2014. WISSARD at Subglacial Lake Whillans, West Antarctica: scientific operations and initial observations. Ann. Glaciol. 55, 51–58. https://doi.org/10.3189/2014AoG65A009.

Tveit, A., Schwacke, R., Svenning, M.M., Urich, T., 2013. Organic carbon transformations in high-Arctic peat soils: key functions and microorganisms. ISME J 7, 299–311. https://doi.org/10.1038/ismej.2012.99.

Vandermaesen, J., Lievens, B., Springael, D., 2017. Isolation and identification of culturable bacteria, capable of heterotrophic growth, from rapid sand filters of drinking water treatment plants. Res. Microbiol. 168, 594–607. https://doi.org/10.1016/j.resmic.2017.03.008.

Vilar-Sanz, A., Pous, N., Puig, S., Balaguer, M.D., Colprim, J., Bañeras, L., 2018. Bioelectrochemistry denitrifying nirK-containing alphaproteobacteria exhibit different electrode driven nitrite reduction capacities. Bioelectrochemistry 121, 74–83. https://doi.org/10.1016/j.bioelechem.2018.01.007.

Villaescusa, J.A., Casamayor, E.O., Rochera, C., Quesada, A., Michaud, L., Camacho, A., 2013. Heterogeneous vertical structure of the bacterioplankton community in a non-stratified Antarctic lake. Antarct. Sci. 25, 229–238. https://doi.org/10.1017/S0954102012000910.

Wadham, J.L., Tranter, M., Hodson, A.J., Hodgkins, R., Bottrell, S., Cooper, R., Raiswell, R., 2010. Hydro-biogeochemical coupling beneath a large polythermal Arctic glacier: implications for subice sheet biogeochemistry. J. Geophys. Res. Earth Surf. 115, 1–16. https://doi.org/10.1029/2009JF001602.

Wartiainen, I., Hestnes, A.G., McDonald, I.R., Svenning, M.M., 2006. Methylobacter tundripaludum sp. nov., a methane-oxidizing bacterium from Arctic wetland soil on the Svalbard islands, Norway (78 N). Int. J. Syst. Evol. Microbiol. 56, 109–113. https://doi.org/10.1099/ijs.0.63728-0.

Willems, A., 2014. The family Comamonadaceae. In: Rosenberg, E., DeLong, E.F., Lory, S., Stackebrandt, E., Thompson, F. (Eds.), The Prokaryotes: Alphaproteobacteria and Betaproteobacteria. Springer Berlin Heidelberg, Berlin, Heidelberg, pp. 777–851. https://doi.org/10.1007/978-3-642-30197-1_238.

Zeng, Y.X., Yan, M., Yu, Y., Li, H.R., He, J.F., Sun, K., Zhang, F., 2013. Diversity of bacteria in surface ice of Austre Lovénbreen glacier, Svalbard. Arch. Microbiol. 195, 313–322. https://doi.org/10.1007/s00203-013-0880-z.

Zhang, D.C., Mörtelmaier, C., Margesin, R., 2012. Characterization of the bacterial archaeal diversity in hydrocarbon-contaminated soil. Sci. Total Environ. 421–422, 184–196. https://doi.org/10.1016/j.scitotenv.2012.01.043.