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Following the flow—Microbial ecology in surface- and groundwaters in the glacial forefield of a rapidly retreating glacier in Iceland

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Abstract

The retreat of glaciers in response to climate change has major impacts on the hydrology and ecosystems of glacier forefield catchments. Microbes are key players in ecosystem functionality, supporting the supply of ecosystem services that glacier systems provide. The interaction between surface and groundwaters in glacier forefields has only recently gained much attention, and how these interactions influence the microbiology is still unclear. Here, we identify the microbial communities in groundwater from shallow (<15 m deep) boreholes in a glacial forefield floodplain ('sandur') aquifer at different distances from the rapidly retreating Virkisjökull glacier, Iceland, and with varying hydraulic connectivity with the glacial meltwater river that flows over the sandur. Groundwater communities are shown to differ from those in nearby glacial and non-glacial surface water communities. Groundwater–meltwater interactions and groundwater flow dynamics affect the microbial community structure, leading to different microbial communities at different sampling points in the glacier forefield. Groundwater communities differ from those in nearby glacial and non-glacial surface waters. Functional potential for microbial nitrogen and methane cycling was detected, although the functional gene copy numbers of specific groups were low.

INTRODUCTION

Climate change has devastating impacts on glaciers around the globe (Haeberli et al., 1998; Oerlemans, 2005). Over one billion people worldwide inhabit catchments where glacier melt forms a component of the river flow (Kundzewicz et al., 2008; Mackay et al., 2020) and these rivers provide ecosystem services that may be disrupted when glacier dynamics change as a result of glacier retreat in response to climate change. Glacial sediments can form significant aquifers in glacier forefield catchments, containing groundwater stores that play an important role in buffering changes in river discharge induced by melting glaciers (Jiménez Cisneros et al., 2014; Jones et al., 2019; Mackay et al., 2020; Ó Dochartaigh et al., 2019). Glacial meltwaters can contain significant quantities of organic carbon and other nutrients (Stibal et al., 2012), providing a suitable environment for microbes. Microbes are the most diverse and dominant functional drivers in many ecosystems (Gibbons & Gilbert, 2015) and sensitive environmental disruption indicators (Glasl et al., 2017; Karimi et al., 2017).

Climate change alters hydrological cycles and increases seasonal hydrological variability, causing larger fluctuations in nutrient availability and soil moisture (Blaud et al., 2015). Variations in water sources, pathways and fluxes can have significant impacts on water microbial composition in the glacial environment. Groundwater discharging via perennial springs

provides a more continuous year-round moisture source to glacier forefield ecosystems than surface meltwater, as well as having lower turbidity, higher nutrient concentrations and a more stable temperature (Ó Dochartaigh et al., 2019; Vincent et al., 2019). Groundwater discharge via springs also has a positive effect on the growth of microbial mats and vegetation in glacier forefields, and can enhance weathering and soil development (Miller & Lane, 2019). Recharge from glacial meltwater infiltrating into forefield aquifers brings nutrients into groundwater ecosystems. Future alterations in flows will affect the microbiology of the glacier forefield soil and water systems (Wadham et al., 2019; Wilhelm et al., 2013).

Microbial communities in glacial environments have been studied widely (Bradley et al., 2014; Brighenti et al., 2019; Brown & Jumpponen, 2014; Gutiérrez et al., 2015; Hotaling et al., 2017). Microbial community compositions in glacial forefields resemble those detected from supra- and subglacial environments, indicating that the seed community of soils originates from the glacier (Hotaling et al., 2017; Rime et al., 2016). Glacial soil microbial communities are dominated by Proteobacteria, Actinobacteria, Acidobacteria, Cyanobacteria and Bacteroidetes (Brown & Jumpponen, 2014; Rime et al., 2016). In aquatic glacial environments, such as glacier streams, lakes and fjords, typical bacterial phyla include Proteobacteria, Cyanobacteria, Clostridia, Bacteroidetes, Flavobacteria and Acidobacteria (Gutiérrez et al., 2015; Marteinsson et al., 2013; Peter & Sommaruga, 2016; Wilhelm et al., 2013). Heterotrophs are present in newly exposed soils and typically precede the autotrophic community establishment, which is usually initiated by Cyanobacteria (Bardgett et al., 2007; Tscherko et al., 2003). Euryarchaea, Crenarchaea and Thaumarchaea are common archaeal phyla (Pessi et al., 2015; Sheik et al., 2015; Zumsteg et al., 2012). Studies disagree on whether microbial diversity and richness will increase, decrease or remain static in retreating glacier forefields (Besemer et al., 2013; Brown & Jumpponen, 2014; Fernández-Martínez et al., 2016; Schütte et al., 2010; Sun et al., 2016; Wu et al., 2012). Changes in microbial communities along the chronosequence of emerging soils are induced by increases in carbon and other nutrients and changes in pH and conductivity (Freimann et al., 2013; Ohtonen et al., 1999; Rime et al., 2015).

Glacier-fed systems are dominated by specialist microorganisms that can use multiple carbon sources, including amino acids, carbohydrates, carboxylic acids, polymers and phenolic substrates (Freimann et al., 2013; Pessi et al., 2015). In addition to organotrophs, lithotrophs using iron, sulfur and hydrogen oxidation as energy are found in glacial environments (Sheik et al., 2015). There are indications that some microbial functions depend on the age of soil in glacier forefields. Nitrogen fixation is mainly associated with early succession of the glacier forefield soils, while denitrification and nitrification are more prominent in developed soils with plant cover (Brankatschk et al., 2011; Fernández-Martínez et al., 2016). As a result of future climatic warming, nitrogen in arctic soil systems may increase in response to increased rates of nitrogenase activity, which in turn will reduce nitrogen limitation of these ecosystems (Altshuler et al., 2019). Methanogenic metabolism in microbial communities appears to be a distinctive feature in newly emerged glacier forefield soils, but gradually changes to net methanotrophic metabolisms with ageing soils (Bárcena et al., 2010; Fernández-Martínez et al., 2016). Additionally, the diversity of methanotrophic communities changes with the soil age, with older soils showing the highest diversity (Bárcena et al., 2010). Whether the methane-consuming microbiota can counteract the methane release by methanogens in glacier forefield environments, including groundwater, is still unknown.

Little is known of microbial phylotypes in the groundwater systems of glacier forefields, nor how different microbes are distributed in relation to groundwater flow in aquifers and to groundwater–surface water (including meltwater) interaction. Knowing more about the diversity and richness of these communities is important in order to assess the multifunctionality and broader ecosystem functions these communities can provide (Delgado-Baquerizo et al., 2017). In addition, diverse microbial communities are resilient toward environmental disturbances (Allison & Martiny, 2008). Assessing resilience requires describing the biodiversity and functional roles of microbiota in different water bodies originating from retreating glaciers. This study investigates groundwater–meltwater interaction in this proglacial environment and specifically addresses these questions: (i) which bacterial and archaeal taxa are characteristic in shallow (<15 m deep) groundwater in a proglacial floodplain aquifer; (ii) how do these compare with microbial communities in nearby glacial surface waters; (iii) whether these taxa can contribute to nitrogen or methane cycling; and (iv) if and how do the microbial communities vary with the environmental characteristics of their habitats, and if so, how?

EXPERIMENTAL PROCEDURES

Study site: aquifer characteristics and catchment hydrology and hydrogeology

The study site is a well-characterized unconsolidated sandur (a sand and gravel outwash- or flood-plain formed by the depositional action of glacial meltwater) aquifer in front of a rapidly retreating glacier, Virkisjökull

in SW Iceland (Figure 1). The retreat of this glacier has been extensively monitored and mechanisms of retreat documented (Bradwell et al., 2013; Phillips et al., 2013, 2014). The glacier began retreating in 1990, but in 2005 the rate of ice front retreat increased from an average of 14 m/year (1990–2004) to an average of 33 m/year: between 2007 and 2011, Virkisjökull saw the greatest amount of horizontal retreat (187 m) of any 5-year period since measurements at the glacier began in 1932 (Bradwell et al., 2013). There is little vegetation cover on the sandur—soil development is minimal and only found in areas with less active migration of the river channels (Ó Dochartaigh et al., 2019). Virkisjökull sandur hydrology and hydrogeology have been described previously, showing a clear interaction between meltwater river and groundwater that varies consistently across the aquifer (MacDonald et al., 2016; Ó Dochartaigh et al., 2019). The Virkisá river is sourced from a meltwater lake that lies immediately in front of the glacier ice margin. The river initially flows over bedrock in an area flanked by proglacial moraines; and then onto the Virkisjökull sandur (Figure 1), where it runs for 4 km before joining the Svinafellsá river (MacDonald et al., 2016). Before it flows onto the sandur, the river drains glacial meltwater and precipitation falling on the glacier and proglacial moraines, including some inflows from springs draining small moraine aquifers, recharged from local precipitation. The sandur aquifer is highly permeable and unconfined, with water table depths generally 1–4 m below ground (Ó Dochartaigh et al., 2019). Groundwater in the aquifer is recharged from two sources: local precipitation and infiltration of glacial meltwater through the Virkisá riverbed. Groundwater–river water interactions are controlled by relative differences in water levels between the river and the sandur, and vary spatially down the sandur and seasonally. Consistent hydrogeological variations are observed across the sandur, based on which we define upper (closest to the glacier), middle and lower (farthest from the glacier) sandur aquifer zones (Figure 1). The upper sandur begins at its northeasternmost limit, which was c. 1 km from the 2014 glacier snout (Figure 1). The divisions between sandur zones are not absolute, but the upper to middle sandur boundary is c.1.75 km, and the middle to lower sandur boundary is c.2.5 km, from the 2014 glacier snout (Figure 1). There is a hydraulic gradient from the upper toward the lower sandur aquifer throughout the year, driving groundwater flow in this direction. In the upper and much of the middle sandur—from the point where the Virkisá river flows onto the sandur to c.2 km downstream—there is an almost constant hydraulic gradient from the Virkisá river to the aquifer, driving losses (recharge) of meltwater from the river to groundwater throughout most of the year (Ó Dochartaigh et al., 2019). Below this in the lower sandur there is an opposite hydraulic gradient, from the aquifer to the river, driving year-round flows of groundwater to the river through springs that then flow to the river or directly by baseflow seeping through the river bed. In the middle sandur, the river loses water to groundwater during the summer melt season, and gains baseflow from groundwater during the winter when river flows (and levels) are lowest (MacDonald et al., 2016; Ó Dochartaigh et al., 2019). Stable isotope data support the piezometric evidence for varying river (meltwater) influence on groundwater across the aquifer. A previous study applied a binary mixing model using the stable isotope δ^2H to indicate the relative proportion of precipitation and glacier meltwater in groundwater using glacial meltwater and local precipitation as endmembers (Ó Dochartaigh et al., 2019). This demonstrated a clear relationship with distance from the river: within a zone extending up to 50 m from the river in the upper sandur, 130 m in the middle sandur and 500 m in the lower sandur, borehole groundwater typically comprises >50% meltwater. Outside this zone (further from the river), groundwater consistently comprises <25% meltwater. Consistent variations in hydrochemical tracers (including bicarbonate and specific electrical conductance) and water temperature also distinguished these zones (Ó Dochartaigh et al., 2019).

Methods

Water sampling

Samples of groundwater and both glacial and non-glacial surface water samples were collected during a sampling campaign in May 2014 for microbiological, geochemical and stable isotope analysis (Table 1). Samples of sandur groundwater were collected from eight monitoring boreholes (9–15 m deep), along two transects: downstream from the glacier and at increasing distance from the river (Figure 1) (Ó Dochartaigh et al., 2019). Samples of glacial meltwater were collected from the Virkisá river at two locations: its source at the glacier lake outlet, and 2 km downstream. Surface water from a non-glacial stream within the glacier catchment, which drains hillslopes adjacent to the glacier, was also sampled. In addition, two springs were sampled: one on the upper sandur, which discharges shallow sandur groundwater to the ground surface; and one draining a small moraine aquifer that is hydrologically separate from both the sandur aquifer and glacial meltwater (Table 1; Figure 1).

Groundwater samples were collected from boreholes in the upper, middle and lower sandur aquifer (Figure 1), using a sampling pump after purging boreholes by low-flow pumping until stable readings were obtained for field-measured parameters. Surface water and spring samples were constantly flowing and were collected directly from the sample point, measuring field parameters at the time of sampling.

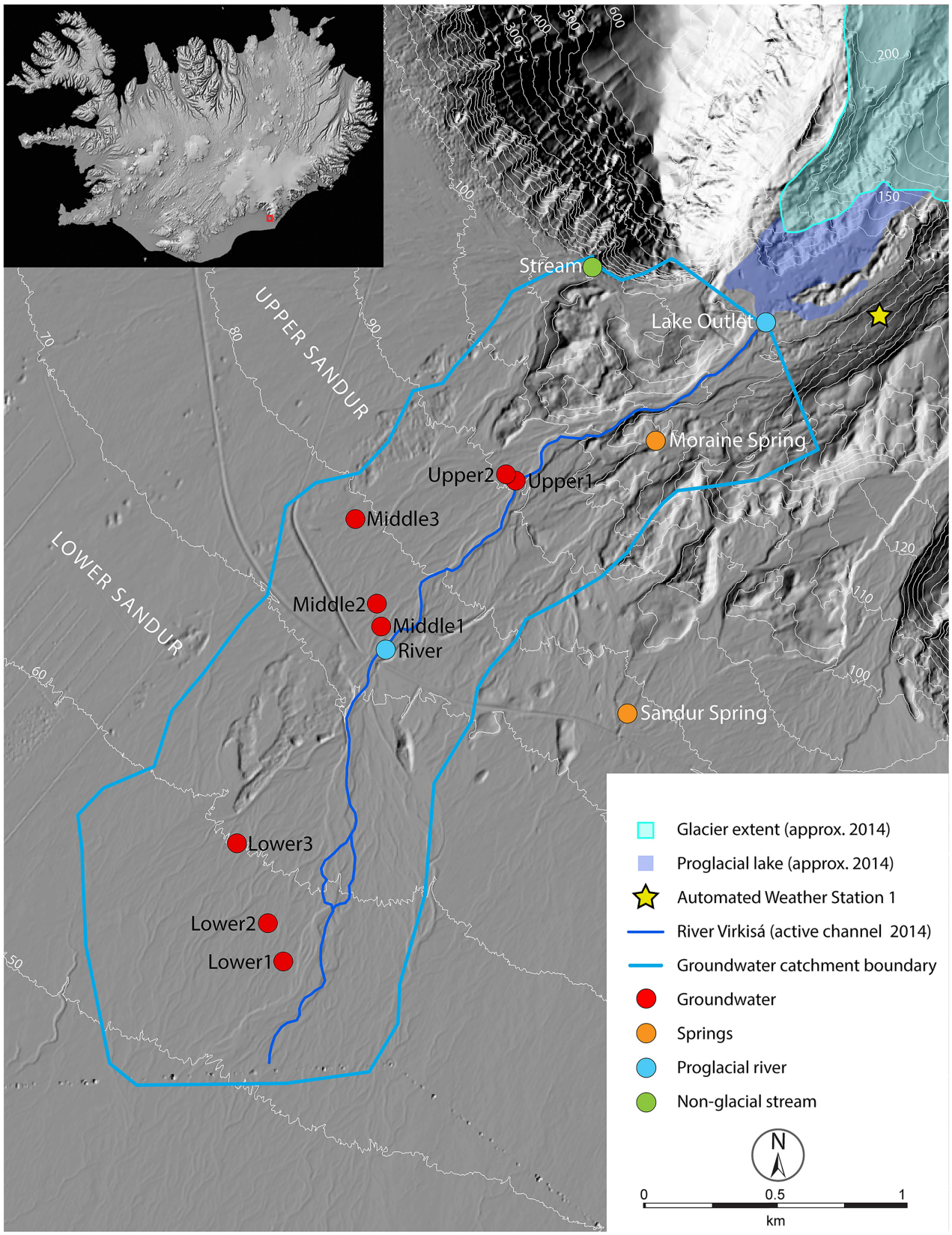

FIGURE 1 Study area in SE Iceland showing sample locations and key hydrological features. Sampling site IDs correspond to those described in detail in Table 1

TABLE 1 Acquired samples (for sample locations see Figure 1)

Sample ID	Description	Coordinates (in decimal degrees)		Geochemistry		Microbial sample size	Parallel samples	Successfully sequenced	
		Longitude	Latitude	Field	Laboratory	(ml)	Pooled	Bacteria	Archaea
Lake outlet	Outlet of proglacial meltwater lake	−16,817871	63,963736	Y	N	70	Y	Y	N
River	River Virkisá (glacial meltwater)	−16,847639	63,953167	Y	Y	70	Y	Y	N
Upper 1	Upper sandur borehole 1 groundwater	−16,83663889	63,9590556	Y	Y	100	Y	Y	N
Upper 2	Upper sandur borehole 2 groundwater	−16,83783333	63,9594167	Y	Y	200	N	Y	Y
Middle 1	Middle sandur borehole 1 groundwater	−16,84830556	63,9541944	Y	Y	500	Y	Y	N
Middle 2	Middle sandur borehole 2 groundwater	−16,84858333	63,955	Y	Y	500	N	Y	Y
Middle 3	Middle sandur borehole 3 groundwater	−16,85008333	63,9580278	Y	Y	500	Y	Y	Y
Lower 1	Lower sandur borehole 1 groundwater	−16,85708333	63,9424722	Y	Y	200	Y	N	Y
Lower 2	Lower sandur borehole 2 groundwater	−16,85822222	63,9438333	Y	Y	100	Y	N	Y
Lower 3	Lower sandur borehole 3 groundwater	−16,8605	63,9466944	Y	Y	500	N	Y	Y
Moraine spring	Car park spring	−16,82266667	63,9603333	Y	Y	500	Y	Y	Y
Sandur spring	VSP1 sandur piezometer	−16,84097222	63,9521389	Y	N	500	Y	Y	Y
Stream	Non-glacial stream in glacier catchment	−16,82969444	63,96675	Y	N	500	Y	Y	Y

Coordinates are given in decimal degrees. Geochemistry sample availability, whether DNA extracts were pooled or not prior to sequencing, and success in sequencing of bacterial and archaeal community of each sample are marked with Y (yes) or N (no).

Microbial biomass was collected by filtering water (70–500 ml, see Table 1) through Sterivex (Merck Millipore, Darmstadt, Germany) filters. Three replicate groundwater samples were filtered from each borehole, and surface water samples from glacier lake outlet, river, two springs and the non-glacial stream. Filters were stored in dry ice and transported to laboratory, where they were frozen to −80°C. Samples for geochemical and stable isotopes analysis were collected at the same time from all sample sites. Repeat geochemical and stable isotope samples of groundwater, spring water and river water were also collected during a series of water sampling campaigns between 2011 and 2018 (MacDonald et al., 2019). Physicochemical variables were measured in the field during sampling: specific electrical conductivity (SEC), temperature, dissolved oxygen and redox potential (Eh), using Mettler Toledo individual parameter portable meters, and bicarbonate alkalinity by pH titration. Samples for major and trace element analysis were filtered through 0.45 μm filters and collected in factory-new polyethylene bottles rinsed with sample water before collection.

One filtered aliquot was acidified to 1% vol./vol. with Aristar HNO_3, for analysis of major cations, total sulfur and Si by ICP-MS. A second filtered aliquot was left unacidified for analysis of anions by ion chromatography (NO_3-N, Cl, Br, F). Samples were collected in chromic-acid-washed glass bottles for dissolved organic carbon (NPOC) analysis, after filtration using the same 0.45 μm filters as for the samples for ionic analysis. Samples for stable isotopes $\delta^{18}O$ and $\delta^{2}H$ were collected and analysed as described in Ó Dochartaigh et al. (2019). Geochemical and stable isotope analyses were conducted at British Geological Survey laboratories. During the 2014 sampling, only SEC, temperature, and $\delta^{18}O$ and $\delta^{2}H$ isotopes were measured on samples from the glacier lake outlet, sandur spring and non-glacial stream, but full geochemistry for lake outlet and sandur spring samples was analysed during other sampling rounds.

DNA extraction and sequencing

Biomass-containing filters were treated as previously described (Purkamo et al., 2017). Briefly, filters were thawed, aseptically cut into small slices under laminar hood flow and placed to the extraction tube of the DNA extraction kit. Microbial community DNA was extracted with Macherey Nagel Nucleospin for soil kit (Macherey-Nagel, Düren, Germany) according to the manufacturer's instructions. An unused filter was treated identically to the sample filters for a DNA extraction control sample. Amplicon sequencing was conducted by an external service provider (MrDNA, Shallowater, TX, USA). The V1–V3 region of bacterial and partial V3–V4 region of archaeal 16S rRNA gene was amplified and sequencing barcodes attached with primers 27Fmod (5′-AGRGTTTGATCMTGGCTCAG-3′) and 519Rmodbio (5′-GWATTACCGCGGCKGCTG-3′) (bacteria) and 349F (5′-GYGCASCAGKCGMGAAW-3′) and 806R (5′-GGACTACVSGGGTATCTAAT-3′) (archaea) and HotStarTaq Plus Master Mix Kit (Qiagen, Hilden, Germany) using following thermal cycling program: 94°C for 3 min, followed by 35 cycles of 94°C for 30 s, 53°C for 40 s and 72°C for 1 min, and final elongation step at 72°C for 5 min. PCR products were checked on agarose gel, pooled and purified using Ampure XP beads (Beckmann Coulter Life Sciences, Indianapolis, IN, USA) before preparing Illumina MiSeq DNA libraries according to manufacturers' instructions.

Raw sequence data were indexed using MrDNA's in-house tool FastQProcessor (v. 1.1.5 http://www.mrdnalab.com/mrdnafreesoftware/fastq-processor.html). Sequences were processed according to MOTHUR's (v. 1.46.1) modified MiSeq SOP (Kozich et al., 2013). Quality of the combined forward and reverse reads was checked with FastQC (Andrews, 2010). Screening of bacterial sequences was done with screen.seqs command using following parameters: maxambig = 0, maxlength = 500, and archaeal sequences maxambig = 0, maxlength = 400. *Escherichia coli* J01859.1 (for bacteria) or *Methanosarcina mazei* AF028691.1 (for archaea) 16S rRNA gene sequence was used to reveal the start and end position of our sequences in the alignment. Reference alignment was reduced to the region of interest using pcr.seqs command with start and end parameters from the previous step. Preclustering was done with parameter diffs = 4 for bacteria with average sequence length of 450 bp and diffs = 3 for archaea with the average of 360 bp. Chimeric sequences were removed, and Mitochondria, Chloroplast, Eukarya, unknown and Archaea or Bacteria (from bacterial or archaeal sequencing data, respectively) were removed from the dataset with remove. lineage. Sequences were classified using silva v.138 taxonomy as a reference. The sequences found in the DNA extraction control were also removed from the dataset. Sequences were assigned to OTUs using phylotype method as described in MOTHUR's MiSeq SOP. Data were rarefied according to the smallest library size, and diversity, coverage and richness estimates were calculated.

Quantification of total amount of microbes and numbers of functional marker genes

The 16S rRNA gene copy number was used as a proxy for total numbers of bacteria and archaea. Potential nitrate-reducing, ammonia-oxidizing, methanotrophic and methanogenic communities were quantified using respective functional genes. Copy numbers were determined with quantitative PCR using BioLine SensiFAST SYBR® No-ROX 5X mastermix (Meridian Life Science, Inc., Memphis, TN, USA) in LightCycler® 480 Instrument (Roche Diagnostics Corp., Indianapolis, IN, USA). The primers used in each assay, fragment sizes, annealing temperatures and information on standard curves are shown in Supplementary data, Table 3. The thermal cycling program was as follows: 95°C initial melting for 10 min and 40 cycles of amplification with three steps: 10 s at 95°C, 35 s at 57°C–59°C (depending on the assay) and 30 s at 72°C. Melting curve analysis was performed, consisting of 10 s at 95°C, 1 min at 65°C, ramping to 95°C with 0.11°C/s and five acquisitions per°C, ending with cooling to 40°C. Each sample was analysed as a triplicate, including DNA extraction control. In addition, no-template control was analysed in each run. The detection limit of each assay is reported in Supplementary data, Table 3.

Statistical analyses

We estimated the observed richness (S_{obs}), Shannon diversity (H'), abundance-based species richness

TABLE 2 Measured geochemical variables from Virkisjökull

	Field																				
Sample ID	SEC $\mu S\ cm^{-1}$	T °C	pH	DO $mg\ L^{-1}$	Eh mv COR	Ca $mg\ L^{-1}$	Mg $mg\ L^{-1}$	Na $mg\ L^{-1}$	K $mg\ L^{-1}$	HCO_3^- $mg\ L^{-1}$	Cl^- $mg\ L^{-1}$	SO_4^{2-} $mg\ L^{-1}$	HCO_3^{2-} Balance %	HPO_4^{2-} $mg\ L^{-1}$	F^- $mg\ L^{-1}$	NPOC $mg\ L^{-1}$	Total P $mg\ L^{-1}$	Total S $mg\ L^{-1}$	$d^{18}O$ ‰	d^2 ‰	Vegetated around sample site[a]	% water derived from glacier meltwater[b]
Lake outlet	39.5	0.7	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	−11.17	−78.2	No	97
River	39.8	2.2	7.57	4.14	401	3.2	1.26	6.6	0.89	22.3	2.97	1.64	18.18	0.08	0.11	<0.5	0.15	0	−11.16	−78.3	No	95
Upper 1	48	2.7	8.24	3.29	368	4.6	1.67	7.0	1.05	28.7	3.25	2.31	13.58	0.16	0.16	0.512	0.17	0	−10.46	−75.7	No	79
Upper 2	81	5.5	8.03	2.76	375	6.9	2.06	10.0	1.26	44.6	4.67	3.94	0.46	0.41	0.37	0.807	0.16	1	−8.08	−58.8	Minimal	6
Middle 1	54.3	4.3	7.99	10.04	406	4.2	1.30	7.5	1.09	30.5	3.31	2.34	1.94	0.31	0.20	<0.5	0.13	0	−9.70	−69.8	Minimal	50
Middle 2	58.5	4.7	8.06	3.02	388	4.4	1.36	8.1	1.04	34.0	3.45	2.21	−0.42	0.34	0.21	<0.5	0.14	0	−9.09	−67.3	Minimal	28
Middle 3	57	4.1	7.39	3.23	404	5.5	1.69	6.6	1.19	34.1	3.77	1.36	2.02	0.13	0.14	0.832	0.07	0	−9.04	−66.4	Yes	11
Lower 1	53	3.1	8.31	2.78	395	4.3	1.49	7.8	1.00	24.4	3.38	2.68	14.22	0.54	0.24	<0.5	0.25	0	−10.64	−75.0	No	86
Lower 2	48.1	1.9	8.39	3.39	411	4.6	1.66	6.9	1.05	25.6	3.51	2.36	16.61	0.15	0.11	<0.5	0.16	0	−10.88	−77.9	Minimal	90
Lower 3	82.9	4.9	7.57	5.68	392	8.4	2.65	8.7	1.58	49.5	5.21	2.15	1.86	0.33	0.22	0.740	0.14	0	−8.81	−63.3	Yes	29
Moraine spring	53.8	6.3	7.76	4.09	377	5.1	1.61	5.7	0.94	31.8	3.57	1.58	−0.23	0.08	0.12	1.62	0.06	0	−8.94	−66.3	Yes	4
Sandur spring	53.9	5.9	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	−9.49	−69.3	Yes	22
Stream	56.1	7.1	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	−8.25	−57.8	Yes	0

MacDonald, A.M., Ó Dochartaigh, B.É., & Fallas, H.C. (2019) Water chemistry and stable isotope data, Virkisjokull Glacier Observatory, 2011–2018, British Geological Survey, Dataset, https://doi.org/10.5285/14da9c02-c5ec-4019-8e5c06c744d8be9d.
Abbreviation: NA, not available.
[a]No = a few isolated bits of moss; Minimal = more extensive moss cover and a few tufts of grass but no other plants; Yes = typical local vegetation, e.g. thick grasses, flowers, dwarf willow, bilberry, and so on.
[b]Based on binary mixing model for δ^2H as described in Ó Dochartaigh et al. (2019); original data provided here. [Note: Values based on averages of all samples collected from each site from 2011 to 2018. δ^2H data are available in MacDonald et al. (2019)].

TABLE 3 Diversity and richness indices of (A) bacteria and (B) archaea

Sample ID	Parallel	Nseqs	Sobs	Shannon H'	Chao1	ACE
A						
Lake outlet		20,004	251	3.98	462	478
River		32,025	275	3.85	449	472
Upper 1		25,321	269	2.25	463	473
Upper 2	a	19,171	317	3.85	509	553
	b	15,474	293	3.73	498	552
	c	10,090	339	3.65	707	610
Middle 1		14,546	215	1.96	475	423
Middle 2	a	9790	366	4.24	556	569
	b	ND	ND			
	c	16,593	312	4.19	503	561
Middle 3		7481	384	4.23	629	675
Lower 1		ND	ND	ND	ND	ND
Lower 2		ND	ND	ND	ND	ND
Lower3	a	26,091	336	4.26	481	489
	b	33,626	327	4.24	620	526
	c	15,584	329	4.23	535	582
Moraine spring		93,751	228	2.18	428	415
Sandur spring		45,342	395	4.19	655	603
Stream		90,170	282	3.41	489	492
B						
Lake outlet		ND	ND	ND	ND	ND
River		ND	ND	ND	ND	ND
Upper 1		ND	ND	ND	ND	ND
Upper 2	a	25,995	14	1.32	17	23
	b	20,588	16	2.06	17	18
	c	ND	ND	ND	ND	ND
Middle 1		ND	ND	ND	ND	ND
Middle 2	a	89,712	17	1.97	17	17
	b	22,225	16	1.43	21	22
	c	77,943	19	2.22	19	19
Middle 3		64,721	17	2.00	17	18
Lower 1		31,899	14	2.07	14	13
Lower 2		8366	12	1.22	13	13
Lower 3	a	64,560	19	2.08	20	21
	b	50,351	20	1.87	20	22
	c	41,936	19	2.08	21	23
Moraine spring		63,109	20	1.92	21	22
Sandur spring		32,620	24	1.87	26	28
Stream		50,915	22	1.87	28	27

ND = not determined as no data available. Triplicate samples marked with a, b, c. Nseqs—number of sequences, Sobs—observed 'species'.

(Chao) and coverage estimate (ACE) based on evenly rarefied OTU matrices (bacteria: 7481 sequences; archaea: 8366 sequences) with phyloseq in RStudio v. 1.4.1106 (McMurdie & Holmes, 2013). PERMANOVA was performed to the rarefied dataset to test if there are differences between the community composition in the samples from different sandur environments using adonis function of vegan package in RStudio (Oksanen et al., 2017). Samples were grouped according to aquatic habitat [upper, middle and lower sandur groundwater, glacial surface water (Virkisá river and lake outlet); springs; and non-glacial stream].

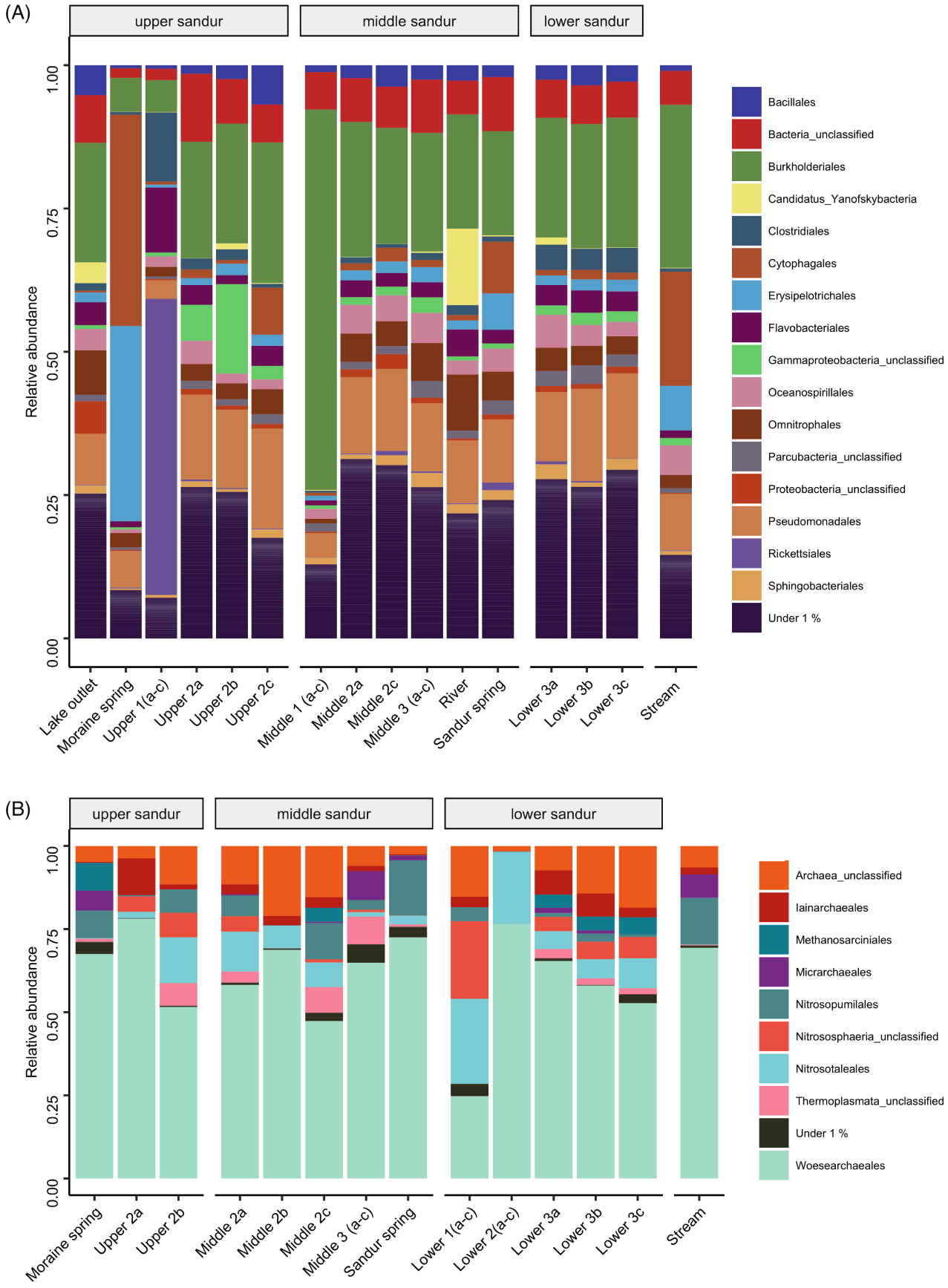

FIGURE 2 Bacterial (A) and archaeal (B) community structure in order level. Orders with <1% relative abundance are grouped together. Suffix a, b or c in the end of the sample name denotes the parallel of the same sample. If no suffix is added, the parallels have been pooled

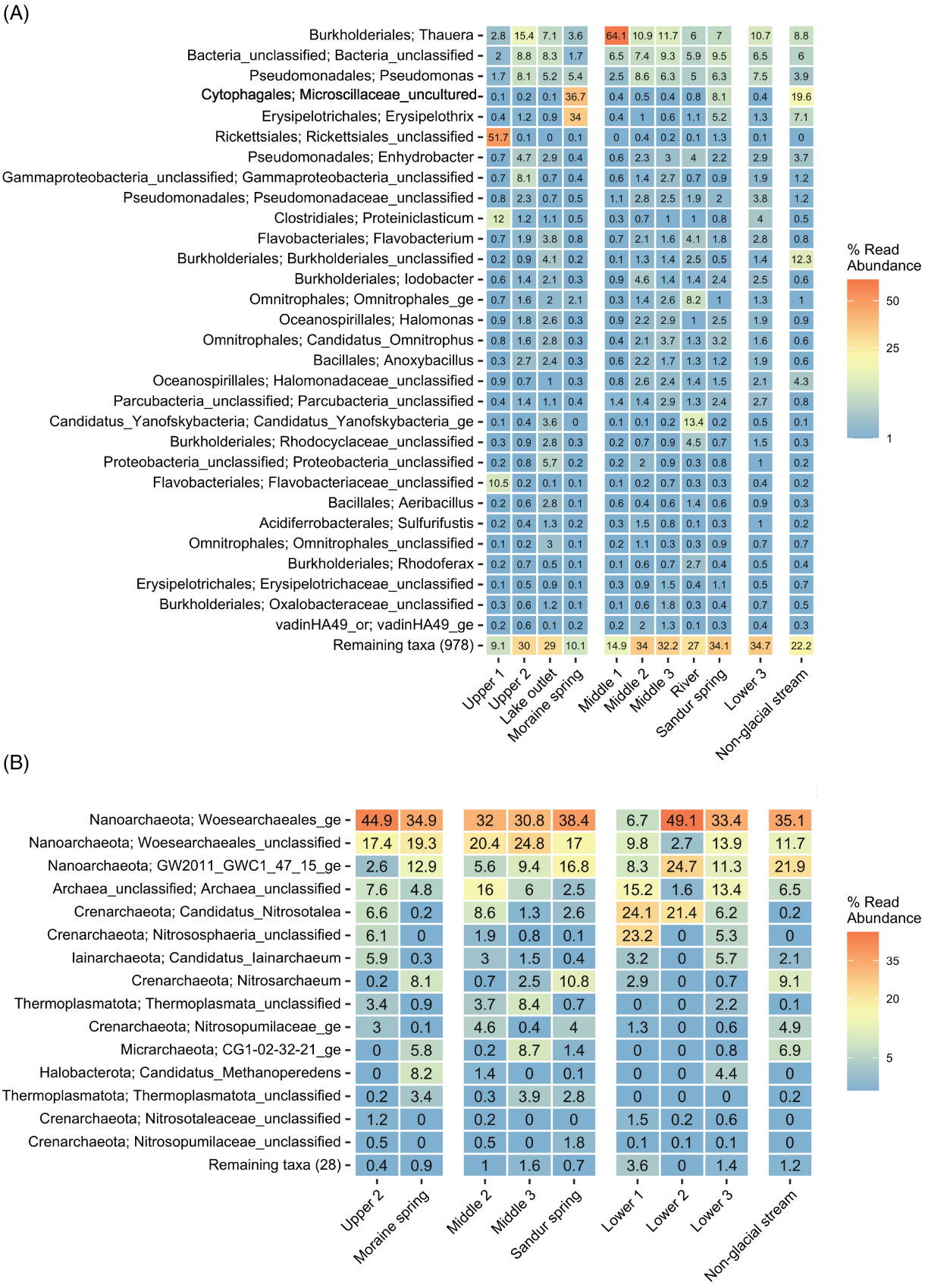

FIGURE 3 Heatmaps of bacterial (A) and archaeal (B) phylotypes detected from each sampling site in genus level. With parallel samples an average abundance is shown

Relative abundance data were further analysed using the PAST4 program (v. 4.08, 2019) (Hammer et al., 2001). These analyses were undertaken on all bacterial and archaeal phylotypes except singletons and data were Hellinger-transformed. Principal coordinates analysis was carried out using the relative abundance data of bacterial and archaeal phylotypes using the Bray–Curtis similarity index with transformation factor $c = 4$ in PAST. SIMPER was used to determine which phyla contributed most to the dissimilarity between sites.

RESULTS

Hydro-physico-chemistry and stable isotope composition of the sample sites

Results are described for a number of aquatic habitats within the Virkisjökull catchment (Figure 1; Table 1): groundwater from a sandur (proglacial sand and gravel floodplain) aquifer from boreholes at 9–15 m depth; glacial meltwater from a proglacial lake; surface water from the Virkisá river, which drains this lake and is dominated by glacial meltwater; two springs where groundwater is discharging to the surface environment (one discharging from sandur groundwater and one on adjacent moraines that is fed only by local precipitation); and a non-glacial stream draining precipitation from a hillside in the glacier catchment, but not connected to the glacier (Figure 1; Table 1). The samples are referred in the text from here on according to the sample ID as in Table 1.

The sampled waters had different temperature, pH, conductivity, ionic chemistry, stable isotopes and particulate organic carbon (Table 2). The lake outlet and Virkisá river were coldest with lowest conductivity. Sampled springs and the non-glacial stream were the warmest aquatic habitats studied. The moraine spring contained the highest amount of organic carbon (1.62 mg/L). There were also distinct differences between groundwaters from the different sample sites. The highest Ca, Mg and Cl^- concentrations were in lower sandur 3; the highest HPO_4^{2+} and total phosphorus in lower sandur 1; and highest Na, SO_4^{2+} and F^- concentrations in upper sandur 2. The major ion chemistry of the groundwater samples fell into two distinct groups: most of them (the upper sandur 1, all three middle sandur and the lower sandur 1 and 2 samples) were similar to each other; but the lower sandur 3 and upper sandur 2 samples which are spatially distant stood out by being more similar to each other than to closer groundwater samples (Table 2). Groundwater stable isotope compositions fell into three groups, with upper sandur 1 and lower sandur 1 and 2 at one end (stable isotopes most depleted) and upper sandur 2 and middle sandur 3 at the other (least depleted); while middle sandur 1 and 2 and lower sandur 3 lay in the middle of these extremes (Table 2). These patterns are consistent with other hydrochemical and stable isotope evidence from the Virkisjökull study site, and are explained by varying degrees of interaction between sandur groundwater and the Virkisá river, and in particular the relative proportion of groundwater recharge from precipitation and from glacier meltwater (Ó Dochartaigh et al., 2019).

Microbial communities in groundwaters and surface waters

The dataset after quality control comprised 475,059 bacterial (not including DNA extraction control sample) and 644,940 archaeal sequences (Table 3). The number of bacterial sequences varied between the samples from 7481 (middle sandur 3) to 93,751 (moraine spring). The highest number of archaeal sequences was retrieved from middle sandur 2 (89,712 sequences) and the lowest from lower sandur 2 (8366 sequences). Bacterial community structure varied across the glacier forefield habitats [Figures 2(A) and 3(A), Supplementary data, Figure 1]. Burkholderiales and Pseudomonadales orders were typical and abundant in groundwater habitats in Virkisjökull sandur [Figure 2(A)]. Higher relative abundance of Cytophagales was detected in the moraine spring and non-glacial stream communities compared to other samples. In many of the samples, orders representing <1% of the total relative abundance composed more than 25% of the community, and unclassified bacteria constituted a notable part of the bacterial communities [Figure 2(A)]. The upper sandur 1 sample was dominated by Rickettsiales-affiliating phylotype (51.7% relative abundance) [Figure 3(A)]. Clostridial *Proteiniclasticum* (12% relative abundance) and unclassified Flavobacteriaceae phylotype (10.5% relative abundance) were other common members of the bacterial community in the upper sandur 1 sample. The upper sandur 2 community, analysed from three parallel samples, was dominated by gammaproteobacterial phylotypes affiliating with *Thauera*, *Pseudomonas* and unclassified gammaproteobacteria [Figure 3(A)]. The middle sandur 1 community had the highest relative abundance of *Thauera*-affiliating phylotype (64.1%). Middle sandur 2 and 3 bacterial communities were similar to those of upper sandur 2 and lower sandur 3 communities. However, the middle sandur 2 sample had higher relative abundance of *Iodobacter* (4.6%) compared to other samples, and *Halomonas*/unclassified Halomonadaceae-affiliating phylotypes were more common in middle sandur 2 and 3 communities than in the other groundwater samples [Figure 3(A)]. Firmicute *Proteiniclasticum* was more abundant in the lower sandur 3 sample than the middle 2 and 3, and upper

sandur 2 samples. Glacial surface waters hosted bacterial communities generally similar to those seen in the groundwater samples, but with significantly higher relative abundance of *Candidatus* Yanofskybacteria (Virkisá river 27.5%, lake outlet 3.6%), compared to both groundwater and non-glacial surface water environments. Furthermore, Omnitrophales-affiliating phylotype was more common in the Virkisá river sample (16.9%) compared to the other habitats. The phylotype with the highest absolute number of sequences in the dataset, which was detected in all samples, affiliated with unclassified Microscillaceae, and was most abundant in moraine spring (36.7%), then non-glacial stream (19.7%) and sandur spring (8.2%) communities [Figure 3(A), Supplementary data, Table 1]. The *Erysipelothrix*-affiliating phylotype was also abundant in the spring and non-glacial stream samples, representing 34.0% and 5.2% relative abundance in moraine and sandur springs, respectively, and 7.1% of the non-glacial stream bacterial community. One of the most common phylotypes of the bacterial dataset could not be classified (Supplementary data, Table 1). The representative sequence was compared to NCBI's nucleotide database using blastn. According to blast, highest sequence similarity (88%) for this OTU was with uncultured bacterium clone (FJ612214.1) so no further classification could be deduced.

Archaea were detected from upper sandur 2, middle sandur 2 and 3 and in all lower sandur groundwater samples, in addition to both springs and the non-glacial stream. Archaeal amplicons were not obtained from Virkisá river and lake outlet samples, or from upper sandur 1 and middle sandur 1 (those sites closest to the river). Woesearchaeales was relatively the most abundant archaeal order in the communities [Figure 2(B) and 3(B), Supplementary data, Figure 2]. Woesearchaeales represented >50% of the relative abundance of archaeal communities in the nearly all aquatic habitats studied here [Figures 2(B) and 3(B)]. In lower sandur 1 groundwater sample, however, crenarchaeotal *Candidatus* Nitrosotalea (24.1%) and unclassified *Nitrososphaera* (23.2%) were the most abundant archaeal phylotypes [Figure 3(B)]. *Candidatus* Nitrosotalea was also abundant in lower sandur 2 (21.4.%), but otherwise this community was dominated by Woesearchaeales. A *Nitrosarchaeaum*-affiliating phylotype was abundant in springs (8.1% and 10.8% in the moraine and sandur springs, respectively) and the non-glacial stream (9.1%) habitat. *Candidatus* Methanoperedens was detected in the moraine spring (8.2% relative abundance) and lower sandur 3 groundwater (4.4% of the community). Unclassified archaea were abundant in most samples, as for bacterial communities.

Microbial diversity and statistical comparison between different habitats

The lowest average number of observed bacterial OTUs was detected in the lake outlet, Virkisá river, moraine spring (which is approximately 500 m further away from the ice margin than the lake outlet sample site), and the upper sandur 1 and middle sandur 1 groundwater samples (which are the closest groundwater sample sites to the Virkisá meltwater river) (Table 3A; Figure 1). Groundwater further away from the Virkisá river (middle sandur 2 and 3, lower sandur 3) had higher numbers of observed OTUs, and the sandur spring had the highest average number of OTUs (395).

The highest Shannon (H') diversity estimates were seen in the middle sandur 2 (H' on average 4.23) and 3 (H' 4.23) and lower sandur 3 (H' on average 4.24) groundwater, and the sandur spring sample (H' 4.19)

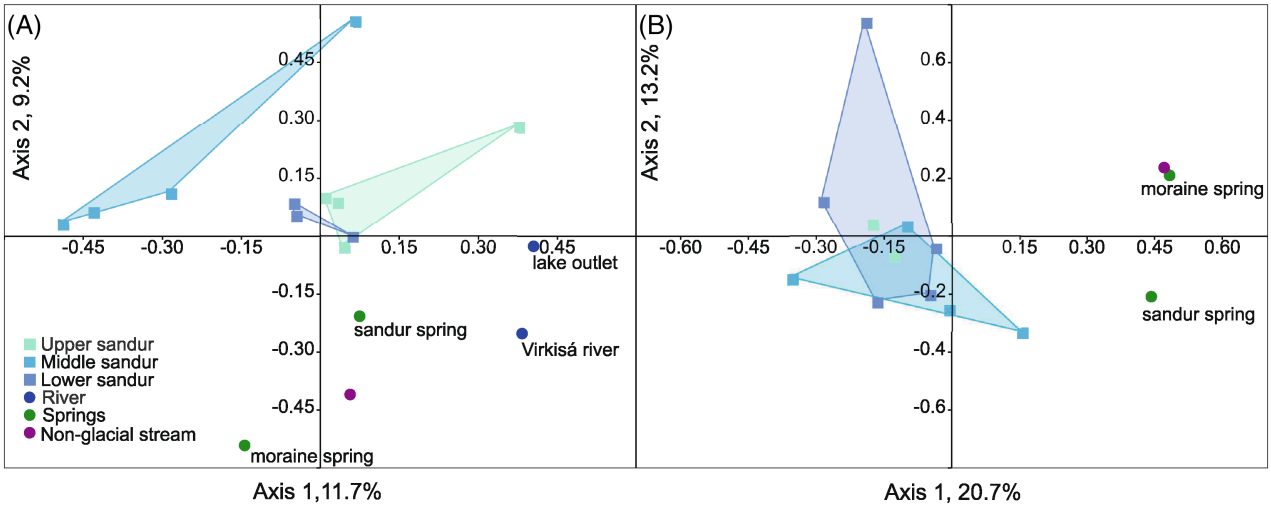

FIGURE 4 Principal coordinates analysis plot of (A) bacterial community and (B) archaeal community. Variation in bacterial community structure was explained by 11.7% by axis 1 and 9.2% by axis 2. For archaeal data, axis 1 explained 20.7% and axis 2 13.2% of the variation

TABLE 4 Gene copy numbers of phylogenetic and functional marker genes.

	Gene copies per ml, ± standard error					
	16S rRNA					
Sample ID	Bacteria	Archaea	*amo*A	*nar*G	*pmo*A	*mcr*A
Lake outlet	25,348 ± 9161	94 ± 41	211 ± 63	6 ± 3	1 ± 0.5	3 ± 1
River	48,508 ± 11,853	143 ± 33	369 ± 75	6 ± 2	2 ± 0.5	33 ± 29
Upper 1	12,562 ± 2430	397 ± 83	71,±10	10 ± 2	0	4 ± 1
Upper 2	15,978 ± 2737	1457 ± 352	0	26 ± 9	0	2
Middle 1	11,047 ± 3176	791 ± 247	163 ± 26	9 ± 2	1 ± 0.1	4
Middle 2	6811 ± 1646	933 ± 42	10 ± 1	4 ± 0,5	0	1 ± 1
Middle 3	5640 ± 1084	682 ± 64	2 ± 1	3 ± 1	0	1
Lower 1	4841 ± 780	500 ± 118	7 ± 3	2	0	1
Lower 2	3556 ± 599	225 ± 69	0	2 ± 1	0	1
Lower 3	6204 ± 1111	626 ± 50	4 ± 2	2	1 ± 0.3	1
Moraine spring	216,356 ± 14,983	2324 ± 38	26 ± 5	31 ± 5	4 ± 0.6	20
Sandur spring	2,169,111 ± 916,014	11,804 ± 2484	120 ± 27	2725 ± 1351	72 ± 3	906 ± 810
Stream	5,363,333 ± 3,663,256	8709 ± 3523	122 ± 44	446 ± 171	534 ± 513	81 ± 19

Abbreviations: 16S rRNA: Ribosomal rRNA, 16S subunit; *amo*A: ammonia monooxygenase; *nar*G: nitrate reductase; *pmo*A: particulate methane monooxygenase; *mcr*A: methyl coenzyme M reductase.

(Table 3A). In the upper and middle sandur, groundwater samples closest to the river had lower diversity (e.g. upper sandur 1 H' 2.25) and diversity increased with increasing distance from the river (e.g. middle sandur 3 H' 4.23). The estimated diversity of the Virkisá river was H' 3.85 and lake outlet H' 3.98. Lowest Chao1 richness in groundwater was in samples obtained closest to the river: upper sandur 1 (463) and middle sandur 1 (475). Highest bacterial richness was observed in the sandur spring. Abundance-based coverage estimate (ACE) showed a similar trend (Table 3A).

Groundwater samples had a lower number of observed archaeal OTUs compared to the springs and stream samples (Table 3B). Average archaeal diversity (Shannon H' diversity index) in groundwater was lower than bacterial diversity. Archaeal diversity in groundwater samples ranged from H' 1.22 to 2.22, with some deviation between the parallel samples. The highest Chao1 estimated richness was detected in non-glacial stream and sandur spring communities, and the lower sandur 2 groundwater sample had the lowest richness. Similarly to Chao1 richness, abundance-based coverage was highest in the sandur spring and non-glacial stream and lowest in groundwaters at the lower sandur 1 and 2 sample sites (Table 3B).

PCoA revealed the dissimilarities in bacterial community composition between the different groundwater and surface water samples [Figure 4(A)]. Groundwater communities grouped loosely together in PCoA plot according to whether they were in the upper, middle or lower sandur. The glacial surface water (Virkisá river and glacial lake outlet) communities are plotted close together but separately from the groundwater communities. The sandur spring, moraine spring and non-glacial stream bacterial communities showed no clear association with each other or with groundwater or glacial surface water communities. PcoA axis 1 explained 11.7% and axis 2 9.2% of the variance.

PCoA indicated that the archaeal community varied according to habitat type [Figure 4(B)]. Upper, middle and lower sandur groundwater communities grouped together, overlapping each other. The moraine spring community plotted close to the non-glacial stream, perhaps reflecting their shared water source in local precipitation. The sandur spring was plotted separately from the other samples. PCoA axis 1 explained 20.7% and axis 2 13.2% variance.

The relative abundance of phylotypes detected in bacterial communities differed somewhat between the habitats (upper, middle or lower sandur groundwater, glacial meltwater samples, springs and non-glacial stream, PERMANOVA, $F = 1.5$, $R^2 = 0.49$, $p = 0.066$). In archaeal communities, PERMANOVA did not show significant differences between habitats ($F = 1.4$, $R^2 = 0.39$, $p = 0.16$). The contribution of each phylotype to the dissimilarity of microbial communities in sandur habitats was assessed with SIMPER (Supplementary data, Table 2). The most likely contributor to the dissimilarity of bacterial communities was *Thauera*, which was dominant in middle sandur groundwater samples. *Microscillaceae* and *Erythropelothrix* also contributed to the observed dissimilarity, especially between groundwater habitats and the non-glacial stream, as well as between groundwater and spring habitats. *Candidatus* Yanofskybacteria was the most likely contributor to the dissimilarity between the glacial surface water and groundwater bacterial communities. Phylotypes affiliating with *Thauera*, unclassified

Rickettsiales, unclassified gammaproteobacteria and *Proteiniclasticum* were the main contributors to the observed dissimilarity between groundwater communities in the upper, middle and lower sandur. *Candidatus* Nitrosotalea, phylotypes affiliating with Woesearchaeales, and unclassified *Nitrososphaera* contributed to the dissimilarity between archaeal communities in groundwater, both springs and the non-glacial stream habitats (Supplementary data, Table 2).

Total number of bacteria and archaea

Bacterial numbers in springs and glacial surface waters (Virkisá river and lake outlet) were distinctly higher than in groundwater, but the highest numbers were in the non-glacial stream. In groundwater, the highest bacterial 16S rRNA gene copy numbers were detected from the upper sandur, and a reducing trend of bacterial copy numbers was detected along the groundwater flow path from upper to lower sandur (Table 4). Archaeal copy numbers were lowest in the glacial surface waters and highest in the sandur spring and non-glacial stream. Archaeal numbers were <1.1% of the total 16S rRNA copy number in the lake outlet, Virkisá river, both springs and the non-glacial stream, while in groundwater, archaeal numbers ranged from 3.1% (upper sandur 1) to 12.0% (middle sandur 2) of the total 16S rRNA gene copies in samples.

Quantification of nitrogen and carbon cycling involved genes

Highest numbers of ammonia oxidation marker gene *amo*A copies were detected in the Virkisá river, followed by the lake outlet (Table 4). Groundwaters hosted low numbers of *amo*A. Nitrate reduction marker gene qPCR assay revealed a diminishing trend in copy numbers of *nar*G in groundwater from upper to lower sandur. Springs and the non-glacial stream hosted a higher number of *nar*G gene copies.

Methane oxidation marker genes (*pmo*A) were not detected in groundwater, and highest numbers were detected from the non-glacial stream. Methanogenesis marker genes (*mcr*A) were above detection limit in the Virkisá river, both springs and the non-glacial stream.

DISCUSSION

Characteristics of microbial communities in shallow groundwater and surface waters in a proglacial sandur

A large number of phylotypes with low abundance (i.e. representing <1% of each community) are contributing to the microbial community diversity in Virkisjökull sandur groundwater. In addition, a large proportion of the communities in the Virkisjökull glacier forefield did not resemble previously described microorganisms. Some of the most abundant bacterial and archaeal OTUs (with up to 36.7% and 20.0% abundances, respectively) could not be taxonomically assigned beyond phylum level. This is not uncommon for glacier environments (Schütte et al., 2010; Yang et al., 2016). The number of unclassifiable OTUs demonstrates the still unknown microbial diversity in groundwater and soils that have undergone recent deglaciation. Nevertheless, the identifiable microbial communities in Virkisjökull sandur groundwater share similarities with other cold climate environments. For example, Schütte et al. (2010) described Proteobacteria, Bacteroidetes, Firmicutes and Verrucomicrobia at a glacier forefield at Spitsbergen, which were also abundant in Virkisjökull, but also Planctomycetes, Acidobacteria, Cyanobacteria and Actinobacteria, which were present but in relatively low abundance in Virkisjökull. Moreover, groundwater microbial communities in a non-glacial hill transect in Germany were dominated by Proteobacteria and *Candidatus* Patescibacteria (Yan et al., 2020). Proteobacteria were also the dominating phylum in all groundwater habitats in Virkisjökull, while patescibacterial phylotypes formed the second-most abundant group in Virkisá river and were common in other habitats as well. Proteobacteria, Patescibacteria and Nitrospirae were identified as core OTUs of groundwater microbial communities in Yan et al. (2020) study. Omnitrophia, present in all samples in this study and relatively abundant in glacial surface water in Virkisjökull, are abundant in many different microbiomes on Earth, but remain elusive as uncultured (Lloyd et al., 2018). Omnitrophia appear to be a stable component of the microbial community in river bank filtrated groundwater throughout seasonal changes nearby the Danube river in Austria (Fiedler et al., 2018). In addition to Omnitrophia, Patescibacteria (including Parcubacteria) are among the most abundant uncultured phyla in different environments (Lloyd et al., 2018). Members of these phyla are also part of the groundwater microbial communities at Virkisjökull, and belong to the uncultivable candidate phyla radiation, which may constitute a major proportion of the global microbial diversity (Castelle & Banfield, 2018), and may be transferred to groundwater from soils during extreme precipitation events (Zhang et al., 2018).

Microbial community structure in relation to catchment hydrology

The microbial communities in different aquatic habitats in the Virkisjökull glacier catchment show distinct variations and similarities, which appear to relate to

hydraulic connectivity between the different water environments (groundwater and both glacial and non-glacial surface waters). Previous work has shown that there are complex hydraulic connections between some of these environments, while others are hydraulically disconnected from each other (Ó Dochartaigh et al., 2019, MacDonald et al., 2016; see summary above: *Study site*: *aquifer characteristics and catchment hydrology and hydrogeology*). We saw similarities in microbial communities between the Virkisá river and sandur groundwater, which are hydraulically connected through recharge from river to groundwater and groundwater discharge to the river. By contrast, bacterial communities in the non-glacial stream and the moraine spring are distinctly different to those in both glacial surface water and sandur groundwater, from which they are hydraulically disconnected. The degree and direction of connectivity between river and groundwater differ from upper, through middle, to lower sandur, and this is reflected in the groundwater microbial communities which show distinct differences in diversity and composition in different zones of the sandur.

A companion study showed variations in sandur groundwater ionic chemistry, stable isotopes and temperature near the river are dominated by the influence of recharge from the river (and therefore by glacial meltwater), but further from the river, groundwater chemistry, stable isotopes and temperature are influenced by recharge from local precipitation (Ó Dochartaigh et al., 2019). Bacterial community diversity, according to the Shannon index, also varied with distance from the river, being lower in groundwater samples near the river than in those further away. By comparison, Yan et al. (2020) observed a higher bacterial diversity in groundwater in direct proximity to a recharge area in a non-glacial hill transect in Germany, probably due to high non-glacial surface water inputs (Yan et al., 2020). We detected less diverse bacterial communities in groundwater samples near the river compared to those further out in the sandur. The middle sandur 2 and 3 and lower sandur 3 are very similar in community composition, and all these are least affected by river recharge (Ó Dochartaigh et al., 2019). The groundwater communities that differed most from others were detected from upper and middle sandur 1. These sites have been previously shown to be strongly hydraulically influenced by the river (Ó Dochartaigh et al., 2019). However, the microbial community in these was not similar with the river community. The upper and middle sandur 1 hosted the least diverse groundwater bacterial communities of the dataset, both with a single dominating phylotype. In the upper sandur 1, this was unclassified Rickettsiales, and in the middle sandur 1 it was *Thauera*. While rickettsia are typically parasites or endosymbionts of eukaryotic organisms, *Thauera* is facultative anaerobe capable of denitrification and common in soil and water environments (Heider & Fuchs, 2015). *Thauera* have previously been a member of basal ice microbial community in Svinafellsjökull, the neighbouring glacier to Virkisjökull in Iceland, where they co-occurred with methanogens (Toubes-Rodrigo et al., 2021).

Detection of microbes important in soil formation

Microorganisms are the primary producers in newly emerged soils, providing an organic carbon and nitrogen source for plants to further colonize these environments (Brown & Jumpponen, 2014; Zhelezova et al., 2019). Bacteroidota have been proposed as the pioneers of newly formed soils after glacier retreat (Rime et al., 2015; Sun et al., 2016). Bacteroidota were also detected in groundwater communities in Virkisjökull (e.g. *Flavobacterium* and *Microscillaceae*). Bacteroidota was especially abundant in both the moraine and sandur springs, which are the aquatic habitats most closely associated with the newly emerged soil surfaces, draining the moraines and sandur, so we can assume that these also play a role in initial colonization of emerging soils at Virkisjökull area. Acidobacteria are another common bacterial phyla associated with soil formation, especially in the later stages, as their relative abundance increases along the chronosequence in glacier forefields (Kim et al., 2017; Rime et al., 2015; Sun et al., 2016). Acidobacteria have been shown to become more abundant with decreasing pH, for example along the chronosequence of retreating glaciers (Jones et al., 2009; Kim et al., 2017). Kim et al. (2017) also observed an opposite pattern with Bacteroidota. Groundwater pH in Virkisjökull is mildly alkaline, so as expected, Bacteroidota were more abundant in Virkisjökull groundwater than Acidobacteria.

Bacteria commonly regarded as promoters of plant growth were detected from Virkisjökull, including *Pseudomonas*, Rhizobiales, Burkholderiales and Bacillales (Backer et al., 2018 and references within). These microbes promote plant growth directly by assisting the plants accession to nutrients, regulating plant stress responses by secretion of extracellular hormones and other bioactive compounds, or by acting as biological control for pathogens (Backer et al., 2018; Glick, 2012). Their presence in groundwaters and springs in the Virkisjökull catchment indicates that these microbes are likely to be playing an important role in plant colonization processes in recently emerged proglacial soils.

Potential functionality of the microbial taxa in the proglacial environment

Nitrogen fixation and nitrification can aid plant growth in nutrient-limited environments such as the Virkisjökull

sandur (Castle et al., 2017; Nemergut et al., 2007). This is especially important in newly emerged soils where primary plant colonizers can suffer from poor nutrient availability (Brankatschk et al., 2011; Glausen & Tanner, 2019; Tanner et al., 2013; Vilmundardóttir et al., 2015). In our study, the numbers of bacteria potentially capable of ammonia oxidation, the initial step for nitrification—were higher in the Virkisa river, springs and non-glacial stream than in groundwaters. Higher numbers of nitrogen cycling microbes in springs indicate that there might be an active microbial nitrogen cycle in emerging soils in the catchment.

Additionally, ammonia-oxidizing archaea, such as *Nitrososphaeria*, *Nitrosopumilus* and *Nitrosotalea*, were also detected in Virkisjökull sandur habitats. Ammonia-oxidizing archaea are usually abundant in nutrient-depleted, oligotrophic environments (Erguder et al., 2009; Sims et al., 2012), and their presence in Virkisjökull sandur groundwater means they are likely to be participating in the nitrification process in groundwater. Unfortunately, the *amo*A qPCR assay used in this study does not target the archaeal ammonia oxidizers (Könneke et al., 2005), so no more detail on the quantity of these in different aquatic habitats of the Virkisjökull sandur environment is available from this study. The distinctive relative abundances of different ammonia-oxidizing microorganisms in different environments at Virkisjökull suggest a niche-specific distribution, although with a unified functional capacity of the microbial community.

Denitrification contributes to greenhouse gas emissions (Thomson et al., 2012). Several gaseous compounds are formed in the denitrification process, among these, nitrous oxide N_2O, which is up to 300 times more effective as a greenhouse gas than CO_2 (US EPA, n.d.). Denitrification and potential nitrogen loss from newly emerged surfaces may hinder soil formation and initial plant growth in glacier forefield areas, as nitrogen is a key nutrient for both microbes and plants (Duc et al., 2009). Here, using *nar*G as a functional marker gene, we detected potential for denitrification from both groundwater and glacial river samples. Catchment water flows could transport such denitrifiers to newly formed sandur soils, and therefore increase nitrogen losses from the soil, especially if they become waterlogged and anoxic (Hamonts et al., 2013). However, as the numbers of the denitrification marker gene were low, we assume that current conditions at Virkisjökull do not favour denitrification, which is probably because oxygen was present in all studied environments.

We did not quantify nitrogen-fixing organisms, but some of the microbial community members detected in the Virkisjökull catchment are known diazotrophs: alphaproteobacterial Rhizobiales, Rhodobacteraceae and Sphingomonadaceae (Franche et al., 2009; Valdespino-Castillo et al., 2018).

It is critical to understand the microbial processes related to methane, another significant greenhouse gas in deglaciated soils (Bárcena et al., 2010). Microbial methanogenesis produces methane in anoxic conditions, for example waterlogged soils, whereas methanotrophic microbes can counteract methane production, either in oxic or anoxic conditions in glacier forefield soils (Chiri et al., 2017; Wadham et al., 2007). The low numbers of methanogens present in all the sampled Virkisjökull sandur environments indicate the potential of the community to adapt to environmental changes, but prevailing oxic conditions in both surface and groundwaters are likely to suppress methanogens. Nevertheless, if environmental conditions change, the currently small population could gain ecological advantage and contribute more to methane emissions (Cavicchioli et al., 2019; Knoblauch et al., 2018; Sogin et al., 2006). Methanotrophs, which could counteract methanogenesis, were detected from oxic surface waters at Virkisjökull, but not in groundwater.

In conclusion, microbial community structures in the Virkisjökull sandur aquatic environment are strongly influenced by the type and source of water (glacial meltwater or local precipitation), and by hydrological flow paths and interactions. We detected many unclassified taxa, with identifiable taxa similar to other deglaciating environments or groundwater. Nitrogen fixation may be carried out by diazotrophs, and both archaea and bacteria are involved in nitrification processes. Methane cycling groups are present in low abundances in response to the oxic conditions, but their presence indicates the potential of the community to adapt, should the local environmental change to favour a more active methane cycle.

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CONFLICT OF INTEREST

The authors declare no competing interests.

DATA AVAILABILITY STATEMENT

Sequences are deposited in European Nucleotide Archive under study PRJEB41187 (bacterial sequence IDs ERS5330886-ERS5330902, archaeal sequence IDs ERS5335200-ERS5335213).

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