Microbial Communities in Coastal Glaciers and Tidewater Tongues of Svalbard Archipelago, Norway

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Global warming is having a great impact on the Arctic region, due to the change of air temperature and precipitation. As a consequence, the glacial ice melts and englacial materials are being transported into the ocean. These substances can constitute a source of nutrients in food webs or, on the contrary, a source of contaminants. In this research seven marine Svalbard glaciers and their tidewater tongues were focused. This survey provides a first attempt comparing microbial communities from coastal and tidewater glaciers that reveal a hitherto unknown microbial diversity. A wider diversity was found in glaciers than in seawater samples. Glacier microorganisms mainly corresponded to the phylum Proteobacteria (48.8%), Bacteroidetes (29.1%) and Cyanobacteria (16.3%) (**Figure 3A**). Seawater microorganisms belonged to Bacteroidetes (40.3%), Actinobacteria (31.7%) and Proteobacteria (25.4%). Other phyla found such as Firmicutes, Thermi, Gemmatimonadetes, Verrucomicrobia, Nitrospirae, Chloroflexi, Planctomycetes, and Chlamydiae were less abundant. The distribution of microbial communities was affected in different extent by the concentration of nutrients (nitrogen nutrients, dissolved organic carbon and soluble reactive phosphorus) and by environmental parameters such as salinity. Nevertheless, the environmental variables did not influence in the distribution of the microbial communities as much as the concentration of nutrients did. Our results demonstrate an interchange between glacier and coastal microbial populations as well as the presence of some indicator species (i.e., *Hymenobacter*) as possible sentinels for bacterial transport between glaciers and their downstream seawaters. The consequence of this process could be the alteration of the water composition of the fiords producing serious consequences throughout the marine ecosystem and in the cycling of globally important elements.

Keywords: coastal glaciers, next-generation sequencing, food web, Svalbard archipelago, Arctic
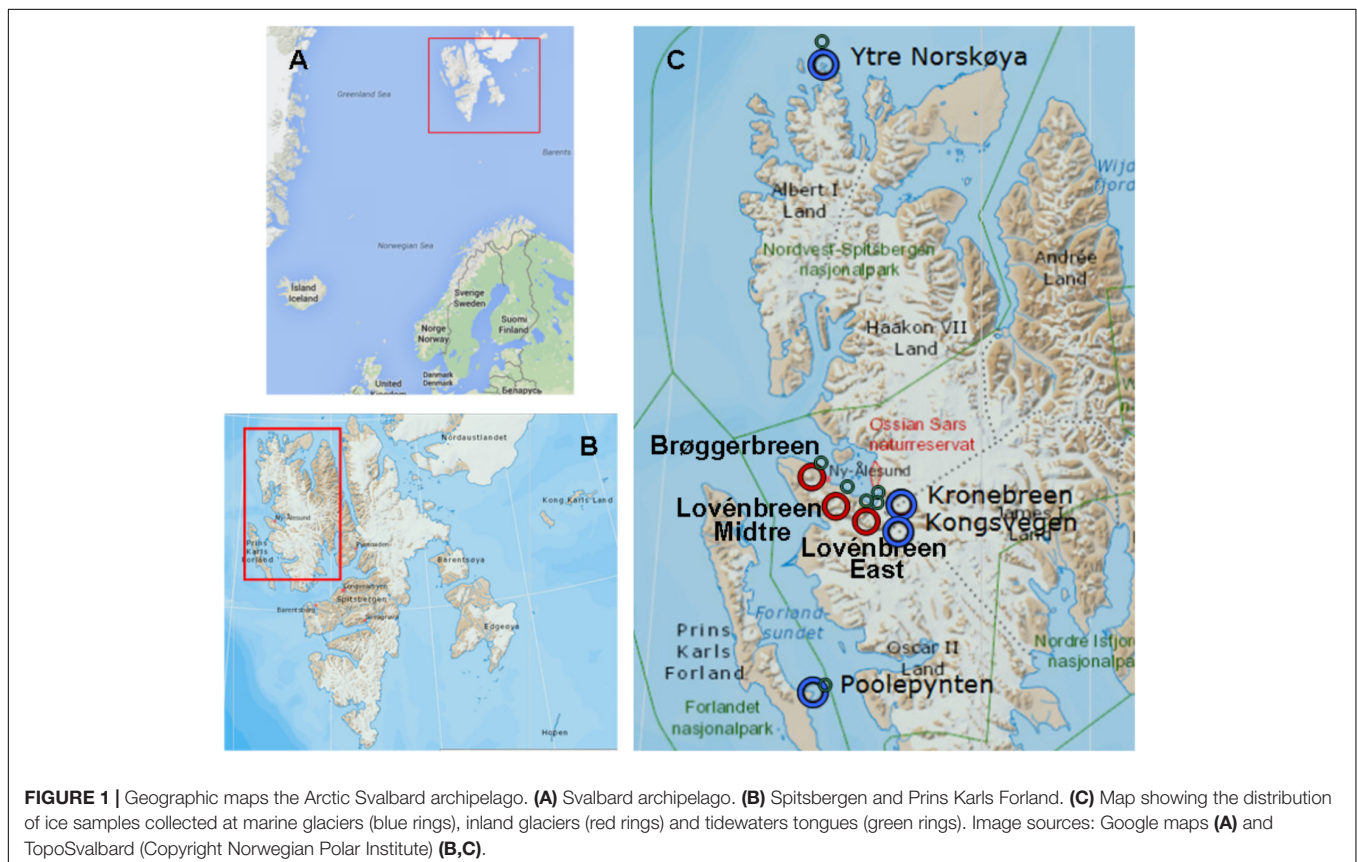
INTRODUCTION

Glaciers have been considered authentic biomes (Anesio and Laybourn-Parry, 2012), in which microbial life goes by enduring a hostile environment. Microbial communities associated with glaciers found all over the world, are being increasingly studied. Specifically, these microorganisms have been reported in Polar Arctic (Amato et al., 2007; Hell et al., 2013; Larose et al., 2013) and Antarctic Regions (Foreman et al., 2007) and in high mountains (Garcia-Descalzo et al., 2013; Hotaling et al., 2017). However, glaciers are very different from each other, which influence the life of their inhabitants. The effect of environmental variables, of chemical composition (Møller et al., 2011) and nutrient concentration on microbial communities have been extensively researched (Mindl et al., 2007).

The Svalbard archipelago (74° to 81° N) (**Figure 1**) comprise an area of 62,248 km^2, with more than 2,000 glaciers; and ice covers 60% of its surface (Lang, 2011). Svalbard glaciers are of various kinds (Hagen et al., 1993; Hagen et al., 2003), although most of them are sub-polar or polythermal, sharing the characteristics of the cold and temperate glaciers (Hagen et al., 1993). Some of them have a coastal margin and terminate in a calving front, what establishes important differences with respect to glaciers with inland margin. Coastal glaciers calve icebergs into the ocean (McNabb et al., 2016). These glaciers greatly affect the physical and chemical characteristics of the sea to which they discharge (Garcia-Lopez et al., 2016). In glaciers ending on land,

there is continuous permafrost at ice front; while calving glaciers present partly or completely temperate tidewater tongues (Hagen et al., 2003).

Global warming is nowadays one of the most worrying problems. It is having a great impact on the Arctic region, because of the change of air temperature and precipitation (Solomon et al., 2007; Førland et al., 2009). As a consequence, the glacial ice melts and disappears, and microbial communities are being seriously affected (Hallbeck, 2009; Zeng et al., 2013). One of the effects of global warming is the change in the basal temperature of the ice, moving from cold to polythermal; this causes the growth of new microorganisms that are not psychrophiles, thus leading to changes in the diversity (Hell et al., 2013; Nowak and Hodson, 2014). It is essential to know how climate change is shaping the distribution and diversity of microbial communities, as microorganisms participate particularly in the ecology of the Arctic ecosystems. They constitute the basis of trophic networks and they are an essential element in carbon and nutrient cycling (Hoham and Duval, 2001; Garcia-Descalzo et al., 2013).

In the last few decades, recently deglaciated areas present in different glacial zones in the world, are available for colonization and primary succession, especially initiated by pioneer microorganisms (Kastovska et al., 2005; Deiglmayr et al., 2006). It has been researched that in glacier forelands, soil microorganisms are essential for plant growth as they play a key role in the nutrient cycling. In this phase, nitrogen, phosphorus, and other nutrients accumulate and facilitate

FIGURE 1 | Geographic maps the Arctic Svalbard archipelago. **(A)** Svalbard archipelago. **(B)** Spitsbergen and Prins Karls Forland. **(C)** Map showing the distribution of ice samples collected at marine glaciers (blue rings), inland glaciers (red rings) and tidewaters tongues (green rings). Image sources: Google maps **(A)** and TopoSvalbard (Copyright Norwegian Polar Institute) **(B,C)**.

succeeding plant growth (Garcia-Lopez and Cid, 2017; Kim M. et al., 2017). Similarly, in seawater, these microorganisms could also contribute to the development and evolution of marine trophic webs.

The role of microorganisms in polar environments is essential (Kirchman et al., 2009). They are responsible for carrying out key functions in the ecosystems. For instance, phosphorus is a major growth-limiting nutrient, which can be made biologically available by a group of heterotrophic microorganisms (Khan et al., 2009). Other important nutrients such as natural polysaccharides from plants and algae (cellulose, and laminarin) are degraded by some bacteria such as Cytophaga and a few *Clostridium* species (Lopez-Ramirez et al., 2015). The denitrification or reduction of NO_3^- to N_2, NO, or N_2O, is the main means by which N_2 and N_2O are biologically formed. Nitrification (the oxidation of NH_3 to NO_3^-) is a main process in oxic environments, and it is carried out by the nitrifying bacteria (Madigan et al., 2012). Sulfate-reducing bacteria are also a highly diverse group. Many reduced sulfur compounds are used as electron donors by sulfur bacteria. The most common sulfur compounds used as electron donors are H_2S, S^0, SO_3^{2-}. Hydrogen sulfide H_2S is oxidized by chemolithotrophic microorganisms to sulfur S^0 and sulfate SO_4^{2-}. SO_4^{2-} can then be reduced to H_2S by activities of the sulfate-reducing bacteria (organisms that consume organic carbon) and this reduction closes the biogeochemical sulfur cycle while regenerating CO_2 (Madigan et al., 2012).

With the aim of studying whether a process of colonization analogous to what happens in soils can be initiated in the coastal glaciers, the microbial populations of seven glaciers and their corresponding discharge seawaters were studied. The studied glaciers were: Lovénbreen Midtre (LVM), Lovénbreen Austre (LBE), Kongsvegen (KGV), Kronebreen (KNB), Brøggerbreen (BGN), Ytre Norskøya (YTRE) (Hamiltonbukta), and Poolepynten (POOL) (Archibald Geikiebreen) (**Figure 2**). Of these seven glaciers, two are more distant (YTRE and POOL) and the other five form a glacial circus. One of them (BGN) is close to a populated area, which can also condition the

FIGURE 2 | Images of the seven Svalbard glaciers. **(A)** Lovénbreen Midtre, Sherdahlfjellet (LVM); **(B)** Lovénbreen Austre, Slatlofjellet (LBE); **(C)** Kongsvegen (KGV); **(D)** Kronebreen (KNB); **(E)** Brøggerbreen (BGN); **(F)** Ytre Norskøya, Hamiltonbukta (YTRE); **(G)** Poolepynten, Archibald Geikiebreen (POOL). GPS Coordinates: Kongsvegen: 78°51′44″N 12°32′29″E; Kronebreen 78°52′23″N 12°39′14″E; Ytre Norskøya: 79°47′11″N 11°44′14″E; Poolepynten: 78°27′29″N 11°32′55″E; Lovénbreen Midtre: 78°53′12″N 12°2′57″E; Lovénbreen Austre: 78°53′51″N 11°49′15″E; Brøggerbreen: 78°54′49″N 11°43′42″ E.

microbial communities. In addition, some are marine and others are terrestrial, and each has its particular physico-chemical characteristics. The study of such microorganisms involved the sequencing of the 16S rRNA genes to identify bacteria. The chemical composition of ice and seawater was also one of the main goals of study. Once the microorganisms in the samples were identified, their distribution and relationship with the chemical composition of glacial ice and seawater were determined.
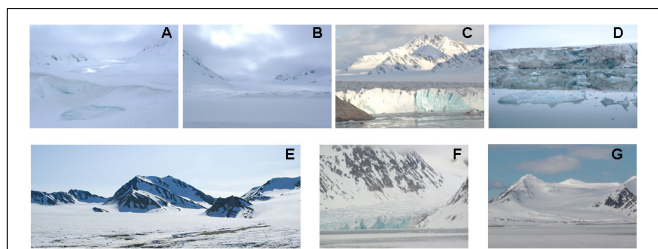
MATERIALS AND METHODS

Sample Collection and Processing

Two types of samples were obtained, during summer 2014, from seven Svalbard glaciers and their corresponding nearest tidewater (**Figures 1**, **2**). The glaciers considered in the study were: Lovénbreen Midtre, Sherdahlfjellet (LVM); Lovénbreen Austre, Slatlofjellet (LBE); Kongsvegen (KGV); Kronebreen (KNB); Brøggerbreen (BGN); Ytre Norskøya, Hamiltonbukta (YTRE); Poolepynten, Archibald Geikiebreen (POOL). Four glaciers, KGV, KNB, YTRE and POOL, present all or part of the glacier front in the sea, and are termed "coastal glaciers"; the rest are termed "inland glaciers." Samples were named as: CG, glacial ice samples from coastal glaciers (KGV, KNB, YTRE, and POOL); IG, glacial ice samples from inland glaciers (LVM, LBE, and BGN); CT, seawater samples from the tidewater area closest to a coastal glacier; and IT, seawater samples from the tidewater area closest to an inland glacier. GPS coordinates of the sampling areas are detailed in **Table 1**.

Glacial ice samples were obtained, by removing 20–30 cm of thick debris and by drilling with a Mark II Kovacs Core System. Each ice core of 9 cm × 3 m was cut into three parts to obtain three sampling replicates. Glacial ice samples (a total of 21) were wrapped in sterile plastic bags, transported in ice and stored at −20°C until analyzing in the laboratory at the Kings Bay Research Station (Ny Ålesund, Svalbard). Then, ice samples were decontaminated following a surface decontamination and melting procedure described in previous studies (Garcia-Descalzo et al., 2013). In a UV-irradiated laminar flow hood, each section of block ice was removed from −20°C and soaked in ice-cold 95% ethanol for 1 min, followed by extensive rinsing with 0.22 μm-filtered MilliQ water, effectively ablating the exterior 2-cm shell of ice samples. The decontaminated interior ice was thawed in a sterile plastic bag at 4°C and used for analyses.

Tidewater samples (3 sampling replicates, a total of 21) were obtained at the seaside, in the nearest point from each glacier. They were collected in Niskin bottles at a depth of 2 m as in Zaikova et al. (2009), transported and immediately filtered in the laboratory.

Both, the meltwater and tidewater samples were individually filtered through filters with pores of 0.22 μm attached to a vacuum pump in a flow hood, previously sterilized with ethanol. Both filters and water were stored at −20°C until use in the laboratory at the Centro de Astrobiología (Madrid, Spain). To control for laboratory contamination, 500 ml of MilliQ water were subjected to identical analytical procedures.

TABLE 1 | Glacier coordinates and geochemical properties.

	Kongsvegen KGV		Kronebreen KNB		Ytre Norskøya YTRE		Poolepynten POOL		Lovénbreen Midtre LVM		Lovénbreen Austre LBE		Brøggerbreen BGN	
	Glacier	Tidewater	Glacier	Tidewater	Glacier	Tidewater	Glacier	Tidewater	Glacier	Tidewater	Glacier	Tidewater	Glacier	Tidewater
GPS Coordinates*	78°51'44''N 12°32'29''E	–2	78°52'23''N 12°39'14''E	–2	79°47'11''N 11°44'14''E	–2	78°27'29''N 11°32'55''E	–2	78°53'12''N 12°2'57''E	–2	78°53'51''N 11°49'15''E	–2	78°54'49''N 11°43'42''E	–2
Altitude(m)	10	–2	25	–2	100	–2	25	–2	59	–2	49	–2	100	–2
Temperature (°C)	0.3	0.8	0.3	0.7	0.1	0.9	0.8	2.2	0.6	2.1	0.6	2.5	0.1	2.1
pH	4.5	4.5	4.5	4.5	4.5	5.1	4.5	4.0	5	5.5	5	4.5	4.5	5.2
Salinity (ppt)	30.11 (2.98)	224.45 (7.32)	30.11 (2.98)	234.65 (3.82)	100.13 (6.23)	31.25 (2.36)	120.01 (4.45)	29.21 (1.32)	0.24 (0.03)	25.33 (2.04)	0.35 (0.06)	29.52 (1.99)	0.22 (0.02)	26.12 (2.14)
Type of samples	CG	CT	CG	CT	CG	CT	CG	CT	IG	IT	IG	IT	IG	IT
Glacier margin	Coastal		Coastal		Coastal		Coastal		Inland		Inland		Inland	

*According to http://toposvalbard.npolar.no/ (Norwegian Polar Institute).

Chemical Analysis of Meltwater

Basic measurements of physical and chemical parameters of meltwater from sampling sites were made with a temperature-calibrated pH, conductivity, and salinity meter (WTW, Weilheim, Germany). Assays for dissolved nitrogen and phosphorus (NH_4^+, NO_2^-, NO_3^-, TDN, SRP, and DOC) were performed by ion chromatographic method using suppressed conductivity detection (Garcia-Descalzo et al., 2013) and by HPLC-Size Exclusion Chromatography with UV and On-Line DOC Detection (Her et al., 2002) in a 861 Advance Compact IC system (Metrohm AG, Herisau, Switzerland). Chromatograms were recorded using the Metrohm IC Net 2.3 SR6 software.

Extraction, Quantification and Sequencing of Genomic DNA

Genomic DNA from each sampling replicate (500 ml) was extracted by using the DNA Isolation PowerWater kit (MO BIO Laboratory, Inc.). Extraction procedures were identical for all samples. DNA concentration was determined using a Nanodrop 2000p.

Then, Illumina sequencing was applied to each sampling replicate. Purified DNA was quantified and 1ng of input DNA was used in a first PCR of 20 cycles with Q5® Hot Start High-Fidelity DNA Polymerase (New England Biolabs) in the presence of 100 nM primers of the regions V3 and V4 of the 16S rRNA gene. Primers sequences were: (V3-V4) 341F, 5′-ACACTGACGACATGGTTCTACACCTACGGGNGGCWGCAG-3′ and (V3-V4) 805R, 5′-TACGGTAGCAGAGACTTGGTCTGACTACHVGGGTATCTAATCC-3′ (Herlemann et al., 2011). After the first PCR, a second PCR of 15 cycles was carried out with Q5® Hot Start High-Fidelity DNA Polymerase (New England Biolabs) in the presence of 400 nM of primers 5′-AATGATACGGCGACCACCGAGATCTACACTGACGACATGGTTCTACA-3′ and 5′-CAAGCAGAAGACGGCATACGAGAT-[10 nucleotides barcode]-TACGGTAGCAGAGACTTGGTCT-3′ of the Access Array Barcode Library for Illumina Sequencers (Fluidigm). Amplicons were validated and quantified by Bioanalyzer and an equimolecular pool was purified using AMPure beads and titrated by quantitative PCR using the Kapa-SYBR FAST qPCR kit for LightCycler 480 and a reference standard for quantification. The pool of amplicons were denatured prior to be seeded on a flowcell at a density of 10 pM, where clusters were formed and sequenced using a MiSeq Reagent Kit v3, in a 2×300 pair-end sequencing run on a MiSeq sequencer to obtain 100000 reads per sample approximately.

Sequencing data were analyzed with the Base Space platform. All DNA sequences obtained have been deposited in NCBI Short Read Archive (SRA).

Statistical Analysis

A rarefaction analysis was performed using Analytic Rarefaction 1.3 software[1] (Tipper, 1979).

Statistical differences on the number of sequences and number of OTUs were studied by ANOVA test. Data of sequences and

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[1] https://strata.uga.edu/software

OTUs are media values of three sampling replicates. Significant differences between two types of samples were identified using Student's t-test. All of the statistical analyses were performed using GraphPad Prism version 7.00 (GraphPad Software, La Jolla, CA, United States)[2].

Effects of the type of sample, nutrient concentrations and environmental variables on the bacterial community composition were investigated by a combination of multivariate statistical analysis developed with CANOCO version 5 software (Microcomputer Power, Ithaca, NY, United States) (Jongman et al., 1995; Ter Braak, 1995). For statistical analysis, Monte Carlo permutation tests with 500 permutations were used.

RESULTS AND DISCUSSION

General Characteristics and Chemical Properties of the Glacial Ice and Seawater Samples

Most of the physico-chemical characteristics of glacier samples were very analogous to each other (**Tables 1**, **2**). These samples differed mainly on their salinity, since the samples from coastal glaciers had a much higher salinity than those from inland glaciers.

Nevertheless, it was observed that seawater samples corresponding to the two types of glaciers were very different in terms of their physico-chemical characteristics. IT samples are combined with the runoff waters dragged from glaciers located inland. These waters transport inorganic and organic matter from the snow or soil they pass through in their way to the sea. However, as coastal glaciers emit icebergs directly into the sea, waters of these tidewater tongues (CT samples) contain less organic matter and less inorganic salts in solution. Their different physico-chemical characteristics can affect the microbial populations these samples contain.

Soluble nitrogen nutrients were analyzed in the forms of NH_4^+, NO_2^-, NO_3^-, and TDN. The occurrence of the different forms of ammonia depends on pH.

In an aqueous medium, ammonia (NH_3) and ammonium (NH_4^+) ions take part in an equilibrium reaction. Ammonia reacts as a base, raising the pH by generating OH^- ions. This equilibrium depends on pH and temperature. At a lower pH, the concentration of NH_3 is lower (Weiner, 2008). At the observed pH of between 4 and 5, about 95% of ammonia is in the cationic form ammonium (NH_4^+). Highly variable contents were obtained for each glacier (**Table 2**). Samples from KNB showed the highest amounts of NH_4^+, NO_2^-, and NO_3^- while the highest TDN values were found in LVM, LBE and KNB. The soluble reactive phosphorous (SRP) ranged between 0.51 μM in Brøggerbreen Vestre and 1.55 μM in LBE. No differences with respect to these parameters could be established between samples from coastal or land margin neither between ice or seawater samples.
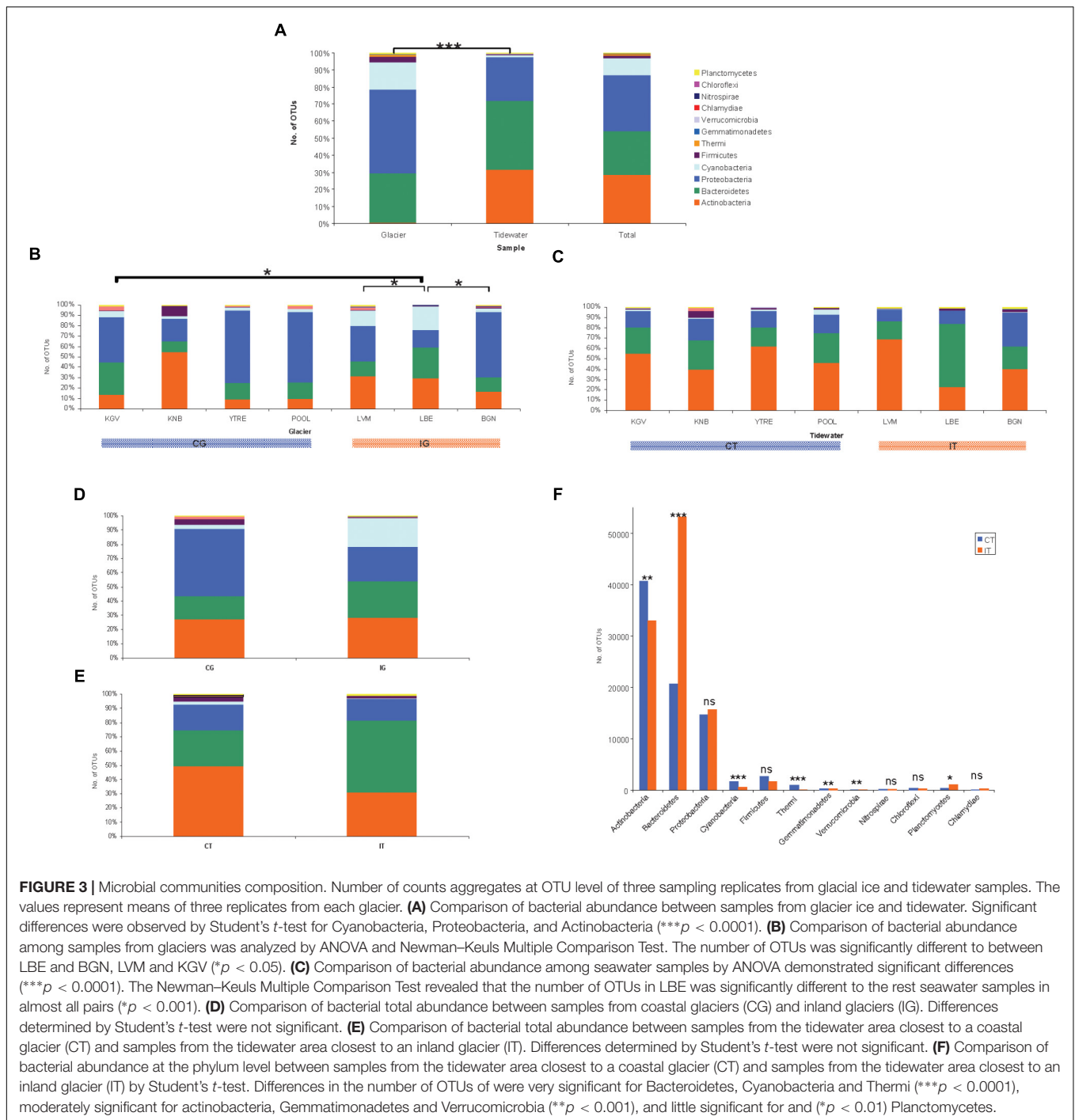
In glaciers, the movement of carbon from the atmosphere to the rocks and moraines begins with snow precipitation.

[2]www.graphpad.com

TABLE 2 | Chemical analysis of soluble nutrients in melt water.

	Kongsvegen KGV		Kronebreen KNB		Ytre Norskøya YTRE		Poolepynten POOL		Lovénbreen Midtre LVM		Lovénbreen Austre LBE		Brøggerbreen BGN	
	Glacier	Tidewater	Glacier	Tidewater	Glacier	Tidewater	Glacier	Tidewater	Glacier	Tidewater	Glacier	Tidewater	Glacier	Tidewater
NH_4^+	2.22 (0.04)	0.81 (0.87)	61.04 (5.36)	0.56 (0.03)	1.81 (0.18)	0.81 (0.04)	2.01 (0.09)	0.59 (0.61)	1.91 (0.26)	0.66 (0.56)	6.32 (0.51)	0.57 (0.02)	2.10 (0.26)	0.68 (0.03)
NO_2^-	4.54 (0.33)	5.32 (0.48)	69.87 (6.66)	1.21 (0.09)	5.36 (0.55)	3.21 (0.21)	6.87 (0.44)	1.02 (0.09)	3.02 (0.30)	3.02 (0.31)	70.21 (5.51)	1.25 (0.11)	4.21 (0.03)	1.08 (0.06)
NO_3^-	6.38 (0.97)	8.52 (0.75)	100.87 (8.33)	2.35 (0.11)	6.33 (0.35)	6.95 (0.74)	7.22 (0.58)	2.48 (0.22)	5.21 (0.44)	4.32 (0.21)	100.01 (7.21)	3.41 (0.33)	5.62 (0.14)	3.97 (0.21)
TDN[a]	90.58 (9.54)	29.98 (3.87)	136.11 (7.55)	21.38 (0.22)	88.78 (5.47)	20.54 (0.19)	99.11 (6.33)	22.14 (0.14)	190.25 (9.33)	25.14 (3.21)	150.33 (6.22)	34.66 (4.21)	96.35 (6.47)	35.01 (0.29)
SRP[b]	0.86 (0.11)	0.79 (0.21)	1.33 (0.01)	1.22 (0.09)	0.51 (0.01)	0.98 (0.09)	BD	BD	0.42 (0.11)	0.45 (0.09)	1.55 (0.12)	1.14 (0.12)	BD[d]	BD
DOC[c]	41.28 (7.23)	39.22 (4.21)	121.22 (5.98)	147.11 (15.27)	54.37 (7.95)	56.88 (6.32)	111.04 (4.54)	125.54 (16.22)	75.21 (9.23)	153.24 (20.11)	81.18 (24.01)	193.54 (11.54)	95.24 (14.17)	167.98 (12.54)

Concentrations are expressed in μM (±SEM) of three replicates. [a]TDN, total dissolved nitrogen. [b]SRP, soluble reactive phosphorus. [c]DOC, dissolved organic carbon. [d]BD, below detection or < 0.3 μM.

FIGURE 3 | Microbial communities composition. Number of counts aggregates at OTU level of three sampling replicates from glacial ice and tidewater samples. The values represent means of three replicates from each glacier. **(A)** Comparison of bacterial abundance between samples from glacier ice and tidewater. Significant differences were observed by Student's *t*-test for Cyanobacteria, Proteobacteria, and Actinobacteria (***$p < 0.0001$). **(B)** Comparison of bacterial abundance among samples from glaciers was analyzed by ANOVA and Newman–Keuls Multiple Comparison Test. The number of OTUs was significantly different to between LBE and BGN, LVM and KGV (*$p < 0.05$). **(C)** Comparison of bacterial abundance among seawater samples by ANOVA demonstrated significant differences (***$p < 0.0001$). The Newman–Keuls Multiple Comparison Test revealed that the number of OTUs in LBE was significantly different to the rest seawater samples in almost all pairs (*$p < 0.001$). **(D)** Comparison of bacterial total abundance between samples from coastal glaciers (CG) and inland glaciers (IG). Differences determined by Student's *t*-test were not significant. **(E)** Comparison of bacterial total abundance between samples from the tidewater area closest to a coastal glacier (CT) and samples from the tidewater area closest to an inland glacier (IT). Differences determined by Student's *t*-test were not significant. **(F)** Comparison of bacterial abundance at the phylum level between samples from the tidewater area closest to a coastal glacier (CT) and samples from the tidewater area closest to an inland glacier (IT) by Student's *t*-test. Differences in the number of OTUs of were very significant for Bacteroidetes, Cyanobacteria and Thermi (***$p < 0.0001$), moderately significant for actinobacteria, Gemmatimonadetes and Verrucomicrobia (**$p < 0.001$), and little significant for and (*$p < 0.01$) Planctomycetes.

Atmospheric carbon combines with water to form carbonic acid that falls to the glacier surface. The acid dissolves rocks and releases several ions such as calcium, magnesium, potassium, or sodium. Calving glaciers transport these ions (Zeebe and Caldeira, 2008), carbon particles and organic matter contained in ice to their downstreaming tidewater (Sipler et al., 2017). Storage and release of organic carbon from glaciers have been extensively studied (Hood et al., 2015); and it has been calculated that climate change contributes to this release approximately 13%

of the annual flux of glacier dissolved organic carbon as a result of glacier mass loss. In coastal glacier samples, DOC concentrations were very similar to DOC values in their downstreaming seawater samples. But DOC concentrations were significantly higher in seawater samples than they were in inland glacier samples. These amounts are comparable to previously described DOC concentrations for glaciers and ice sheets (Hood et al., 2015), and for Arctic seawater (Benner et al., 2005). All results about the chemical composition of ice and seawater will be discussed

in relationship to the structure of microbial communities in glaciers.

Microbial Community Composition

In order to identify the microbial communities that inhabit the glacial and seawater samples, 16S rRNA gene sequencing was performed by Illumina MiSeq. A total of 695307 sequences were obtained (**Supplementary Table S1**). The correspondence of sequences to OTUs was assessed considering a 97% identity threshold. According to **Supplementary Figure S1**, corresponding to the seven glacier samples and seven tidewater samples, graphs tended to an asymptotic behavior. At 3% sequence divergence rarefaction curves reached saturation, indicating that the surveying attempt covered almost the full extent of taxonomic diversity at this genetic distance.

Statistical analyses of microbial abundance were only run on OTUs that were present in two or more sampling point. Glacier microorganisms mainly corresponded to the phylum Proteobacteria (48.8%), Bacteroidetes (29.1%) and Cyanobacteria (16.3%) (**Figure 3A**). Seawater microorganisms belonged to Bacteroidetes (40.3%), Actinobacteria (31.7%), and Proteobacteria (25.4%). Other phyla found such as Firmicutes, Thermi, Gemmatimonadetes, Verrucomicrobia, Nitrospirae, Chloroflexi, Planctomycetes, and Chlamydiae were less abundant. The total number of sequences found in glaciers and seawater was significantly different (***$p < 0.0001$) (**Figure 1A**). When abundances were compared at the phylum level, significant differences were also observed for Cyanobacteria, Proteobacteria, and Actinobacteria (***$p < 0.0001$). It has been published that these bacterial phylum constitute some of the typically most well-represented bacterial groups in glaciers (Lutz et al., 2015) and marine habitats (Michaud et al., 2014) respectively.

Several researches (Michaud et al., 2014) have described the input of marine microorganisms and organic particles which could be transported to glaciers by atmospheric deposition. We observe that, even in the inland glaciers, an important influence from marine environment seems to exist in both directions, from the glaciers to the sea and vice versa. The presence of bacterial genus characteristic of a given glacier could be sentinels for tracking this transport.

The best example of sentinel microorganism in this study would be the OTUs identified as *Hymenobacter*. *Hymenobacter* is the most abundant genus found in this study due to its great presence in the inland glacier LBE. In spite of being the glacier farthest from the coast of all considered in this study, a great influence in its tidewater is observed, because it is also the most abundant OTU in its corresponding tidewater. Currently, about 50 species of the genus *Hymenobacter* are known (Sun et al., 2018). Some of them are psychrotrophs from Arctic and Antarctic environments and high altitudes at Qinghai-Tibet Plateau (Zhang et al., 2008), but they have also been isolated from deep sea water samples (Sun et al., 2018) and from Arctic marine sediments (Kim M.C. et al., 2017).
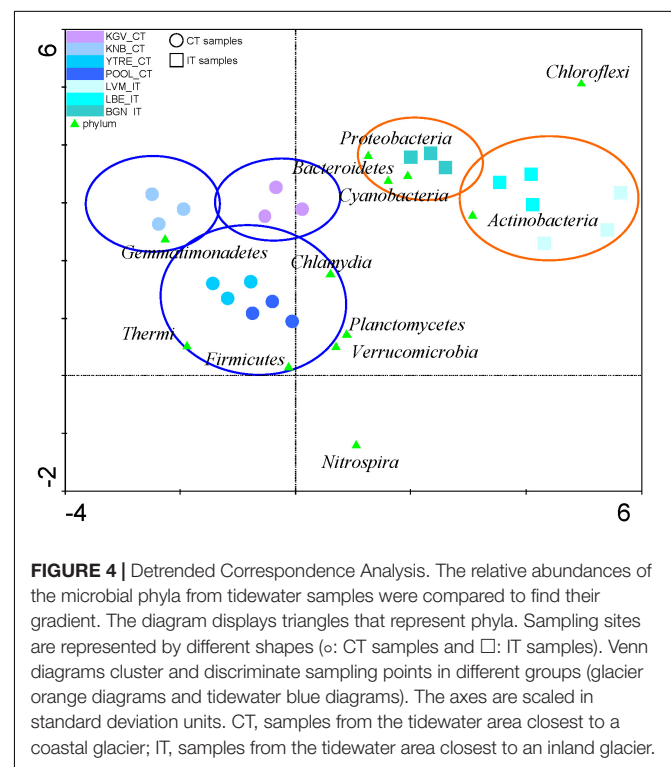
Other sentinel microorganisms from the exchange of materials from the glaciers to the seawaters could be numerous OTUs common to the sampling points. Among them, the most abundant OTUs belong to the genus *Aquaspirillum*,
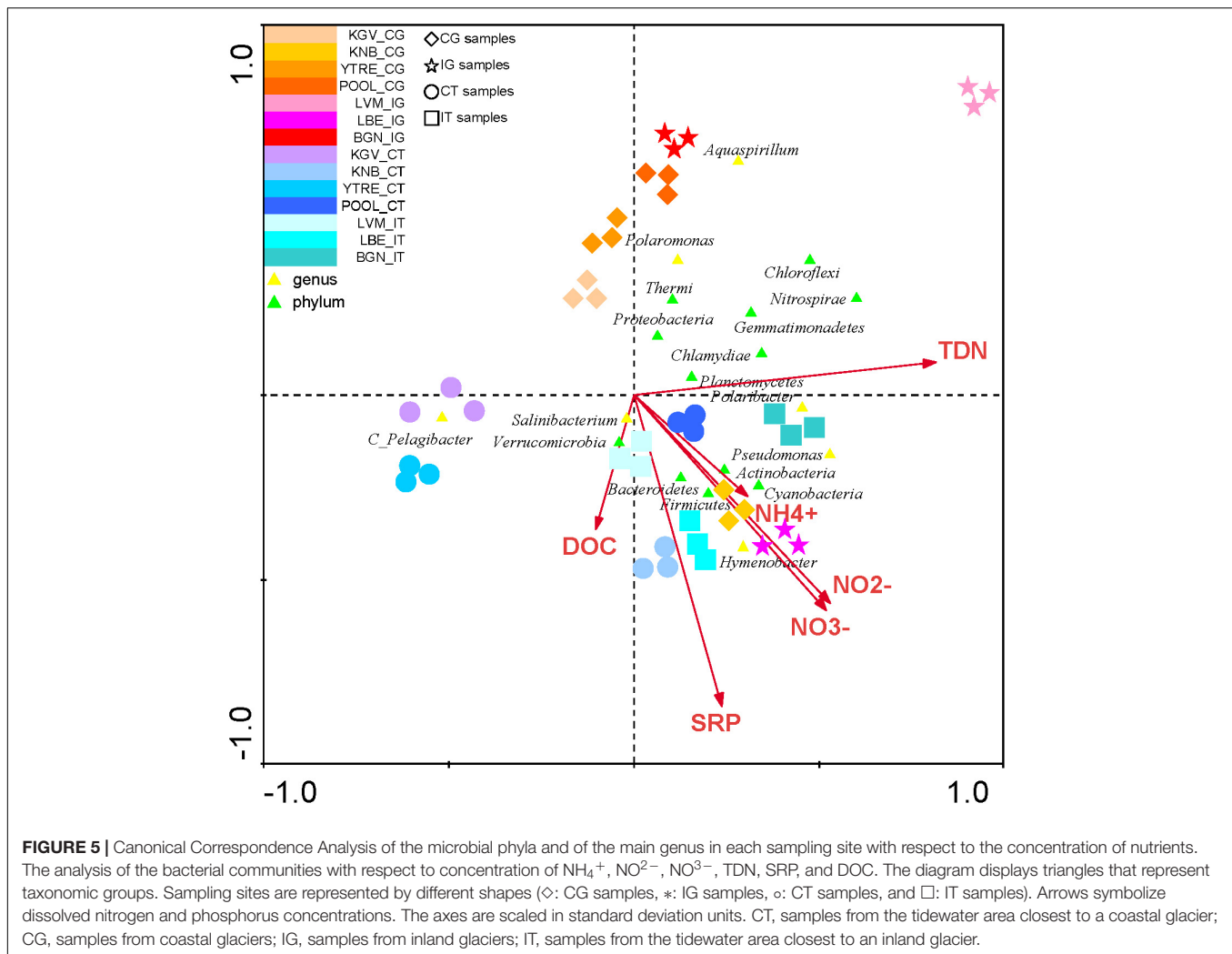
Streptomyces, *Polaromonas*, *Candidatus Pelagibacter*, *Pseudonocardia*, *Polaribacter*, *Salinibacterium*, *Paracoccus,* and *Streptosporangium*.

When of the number of OTUs among all glaciers was analyzed by ANOVA it was little significant. The Newman–Keuls Multiple Comparison Test demonstrated that the most different glacier in terms of the number of OTUs, was LBE, which is significantly different to BGN, LVM, and KGV (*$p < 0.05$) (**Figure 3B**). The number of sequences found in LBE-CG samples was more abundant than the number of sequences in any of the rest glaciers.

In the seawater samples, the number of OTUs was also significantly different by ANOVA (***$p < 0.0001$) (**Figure 3C**). When comparing the number of OTUs using the Newman–Keuls Multiple Comparison Test, LBE samples were significantly different to the rest seawater samples in almost all pairs (*$p < 0.001$), except for Proteobacteria and Firmicutes. The number of sequences found in LBE-CT samples also was more abundant than the number of sequences in any of the other seawater samples.

Regarding the number of sequences corresponding to the most abundant genus (**Supplementary Table S2**), the highest abundance of sequences belonged to *Hymenobacter*, both in glaciers and seawater. The abundance of the genus *Hymenobacter* could be a consequence of its broad presence in the LBE glacier. LBE is an inner glacier but its flux of water can transport this bacterium that is also the most abundant in the seawater samples (LBE-CT). However, in the other glaciers, the highest number of sequences corresponded to *Pseudomonas*, *Polaromonas* and *Aquaspirillum* (**Supplementary Table S2**). In seawater samples, in addition to *Hymenobacter*, the most

FIGURE 4 | Detrended Correspondence Analysis. The relative abundances of the microbial phyla from tidewater samples were compared to find their gradient. The diagram displays triangles that represent phyla. Sampling sites are represented by different shapes (○: CT samples and □: IT samples). Venn diagrams cluster and discriminate sampling points in different groups (glacier orange diagrams and tidewater blue diagrams). The axes are scaled in standard deviation units. CT, samples from the tidewater area closest to a coastal glacier; IT, samples from the tidewater area closest to an inland glacier.

FIGURE 5 | Canonical Correspondence Analysis of the microbial phyla and of the main genus in each sampling site with respect to the concentration of nutrients. The analysis of the bacterial communities with respect to concentration of NH_4^+, NO^{2-}, NO^{3-}, TDN, SRP, and DOC. The diagram displays triangles that represent taxonomic groups. Sampling sites are represented by different shapes (◇: CG samples, ✳: IG samples, ○: CT samples, and □: IT samples). Arrows symbolize dissolved nitrogen and phosphorus concentrations. The axes are scaled in standard deviation units. CT, samples from the tidewater area closest to a coastal glacier; CG, samples from coastal glaciers; IG, samples from inland glaciers; IT, samples from the tidewater area closest to an inland glacier.

abundant OTUs belonged to *Pelagibacter*, *Salinibacterium* and *Polaribacter* (**Supplementary Table S2**).

When comparing by Student's *t*-test the number of OTUs found in the CG samples with the number of OTUs in the IG samples, the differences were not significant (**Figure 3D**). Neither were they when comparing the number of OTUs between the CT and IT samples (**Figure 3E**).

Microbial diversity in glaciers was very similar (**Figure 3B**). The most significant difference was a greater abundance of Actinobacteria in KNB as well as of Cyanobacteria in LVM and LBE.

Microbial diversity among seawater samples (**Figure 3C**) was only higher for Cyanobacteria at POOL, Bacteroidetes at LBE and Firmicutes at KNB. The higher and specific abundance of Firmicutes at KNB glacier and its downstreaming tidewater could be also a consequence of the microbial interchange between both populations.

In addition, OTUs have been found in glaciers that corresponded with typical marine microorganisms such as *Pelagibacter* (Giovannoni, 2017), *Polaribacter* (Staley and Gosink, 1999), and *Roseospira* (Kalyan Chakravarthy et al., 2007)
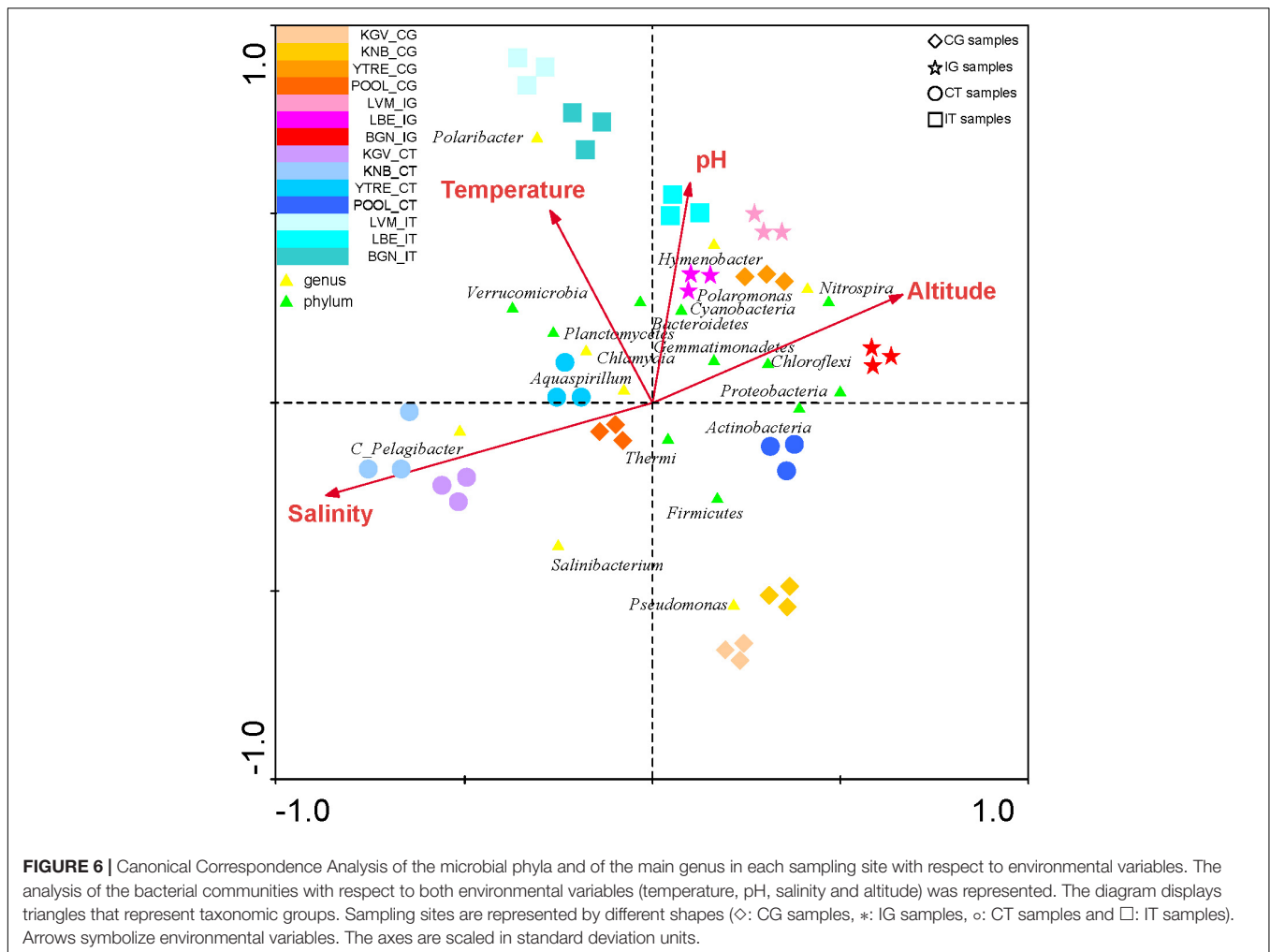
or vice versa, OTUs of terrestrial microorganisms as *Acidisoma* (Belova et al., 2009) or *Propionibacterium* (Koussémon et al., 2001) were found in seawater samples.

A relevant result was the significant difference found between microbial populations inhabiting CT and IT by Student's *t*-test (**Figure 3F**). The difference in the number of OTUs was very significant for Bacteroidetes, Cyanobacteria, and Thermi (***$p < 0.0001$), moderately significant for Actinobacteria, Gemmatimonadetes, and Verrucomicrobia (**$p < 0.001$), and little significant for (*$p < 0.01$) Planctomycetes.

In order to determine the distribution of microbial communities and how they are affected by environmental variables and by the concentration of nutrients in the environment, several multivariate statistical analysis of community data were carried out with.

Detrended Correspondence Analysis with the relative abundances of the microbial phyla from tidewater samples was performed. This analysis clustered tidewater samples into two groups (**Figure 4** and **Supplementary Table S3**): seawater samples corresponding to inland glaciers (samples IT) and seawater samples corresponding to coastal glaciers

FIGURE 6 | Canonical Correspondence Analysis of the microbial phyla and of the main genus in each sampling site with respect to environmental variables. The analysis of the bacterial communities with respect to both environmental variables (temperature, pH, salinity and altitude) was represented. The diagram displays triangles that represent taxonomic groups. Sampling sites are represented by different shapes (◇: CG samples, ∗: IG samples, ○: CT samples and □: IT samples). Arrows symbolize environmental variables. The axes are scaled in standard deviation units.

(CT). These two microbial populations presented an equivalent number of species (**Figure 3C**), but their microbial composition in the tidewater region were different, although they are very close geographically and their seawaters can be mixed easily and quickly by wave effect.

The CCA of all phyla and of the most abundant OTUs in each sampling site demonstrated a significant correlation of the genus *Hymenobacter* with NO_2^-, NO_3^- and NH_4^+ (**Figure 5** and **Supplementary Table S3**). Other abundant genus such as *Pseudomonas* and *Polaribacter* were correlated with levels of all nitrogen species (NO_2^-, NO_3^-, NH_4^+ and TDN). OTUs identified as *Salibacterium* were correlated to DOC, on the contrary other OTUs such as *Polaromonas* presented a negative correlation with these compounds.

The CCA of was also applied to study the influence of environmental variables such as altitude, temperature, pH and salinity in all phyla and in the most abundant OTUs in each sampling site (Analyses 1–4) (**Figure 6** and **Supplementary Table S3**). The presence of some genus such as *Pelagibacter* was correlated to salinity, and the presence of the genus *Polaromonas* and the phylum Thermi were correlated to low temperatures. However, according to the eigenvalues obtained in the analysis 5
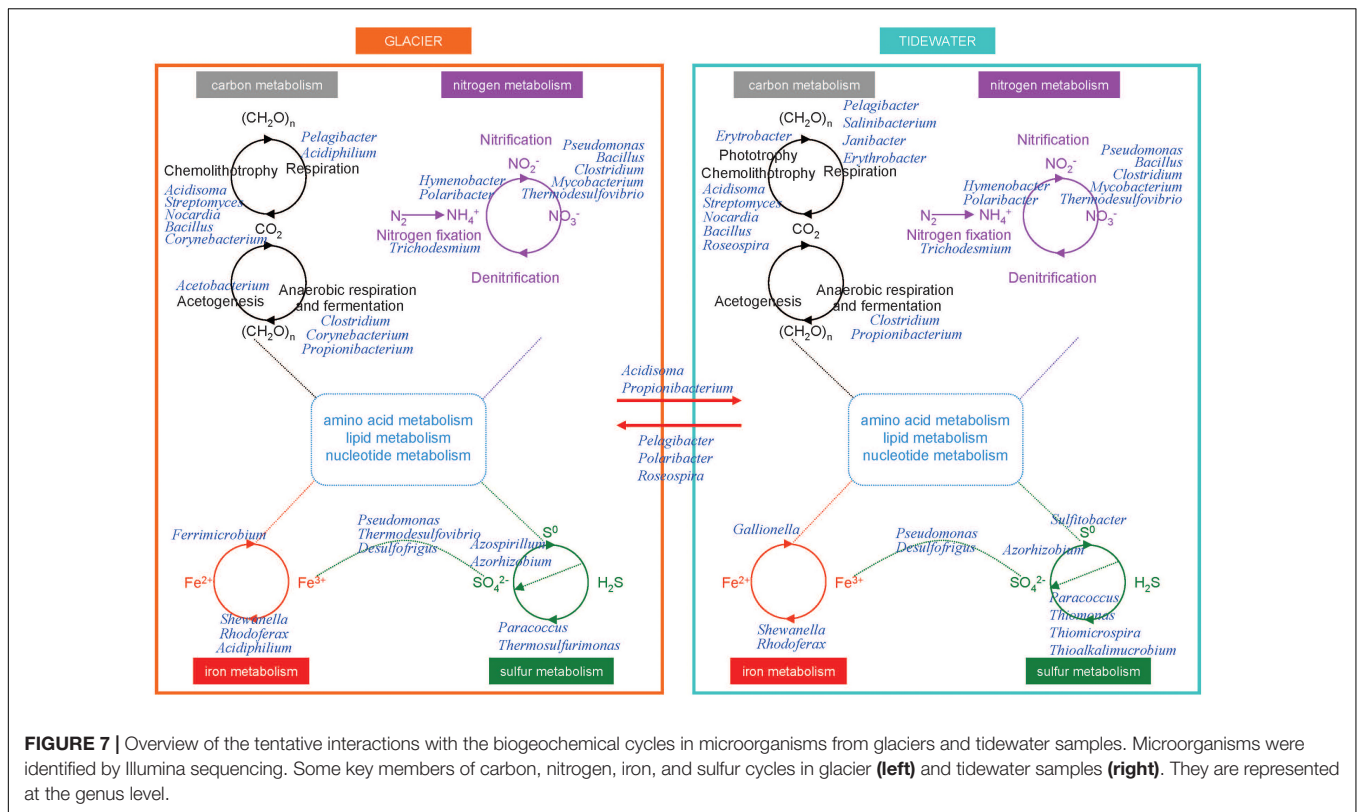
(**Supplementary Table S3**), which are less significant than in the previous analysis, the environmental variables do not influence in the distribution of the microbial communities as much as the nutrients do.

Metabolism and Element Cycling Inferred From Taxonomy
Carbon Cycle

In glaciers, organic compounds CH_2O (**Figure 7**) are scarce. In a dark environment such as the englacial area these compounds must be biologically synthesized by CO_2 fixation by chemolithotrophs. Even though in most environments the contributions of these organisms to the accumulation of organic matter are trivial compared to that of oxygenic phototrophs, in the englacial ecosystem their small inputs are important. They are degraded biologically to CH_4 and CO_2.

Some aerobes and facultative anaerobes oxidize CO to produce CO_2. The aerobic CO oxidizers described in the scientific literature include members of Proteobacteria, Firmicutes, and Actinobacteria (Park et al., 2003). These organisms can grow chemolithotrophically on CO as the sole

FIGURE 7 | Overview of the tentative interactions with the biogeochemical cycles in microorganisms from glaciers and tidewater samples. Microorganisms were identified by Illumina sequencing. Some key members of carbon, nitrogen, iron, and sulfur cycles in glacier **(left)** and tidewater samples **(right)**. They are represented at the genus level.

carbon and energy source under aerobic conditions and play important roles in global CO biogeochemistry; and it has been assessed that they contribute to CO uptake in soils, plant roots, seas and other natural systems (King, 2006), among which glaciers should be included. In our research we have found the Actinobacteria *Streptomyces* as well as other aerobic CO oxidizers like *Bacillus*, *Nocardia,* and *Corynebacterium* (Park et al., 2003).

In tidewater samples, much of the primary productivity comes from photosynthesis by Cyanobacteria. Phototrophs and chemolithotrophs, produce new organic carbon from CO2. Besides oxygenic phototrophs (which are the majority), anoxygenic phototrophs are also present. Aerobic anoxygenic phototrophs include bacteria such as *Erythrobacter.*

Some marine bacteria such as *Pelagibacter* are ecologically important in the so called "secondary production" because they consume high amounts of organic carbon produced from photosynthesis and are responsible for nutrient regeneration. Thus, these marine bacteria return organic matter to the marine food web that would otherwise be lost because of the inability of larger organisms to use such organic nutrients.

Nitrogen Cycle

On glacier surface, phototrophs such as cyanobacteria (i.e. *Trichodesmium*) are important nitrogen-fixing bacteria. In tidewater samples, nitrogen-fixing cyanobacteria were also identified (**Figure 7**).

The nitrogen cycle is driven by both chemolithotrophic and chemoorganotrophic microorganisms. In the biological utilization of N_2, it is reduced to NH_3, and then assimilated into

organic forms, such as amino acids and nucleotides. Nitrification results from the sequential activities of two physiological groups of organisms: the ammonia-oxidizing bacteria (which oxidize NH_3 to nitrite, NO_2^-), and the nitrite-oxidizing bacteria, which oxidize NO_2^- to NO_3^-. Many species of bacteria are nitrifiers. The majority of them belonged to Proteobacteria and Nitrospirae (i.e., *Thermodesulfovibrio*). The first step is performed both in glacier and tidewater samples by very abundant bacteria such as *Hymenobacter* and *Polaribacter*.

Iron Cycle

The biological redox reactions in the iron cycle include both oxidations and reductions. Some bacteria can use Fe^{3+} as an electron acceptor in anaerobic respiration, and Fe^{2+} is oxidized in the chemolithotrophic metabolism. Important Fe^{3+} reducers as *Shewanella*, *Rhodoferax*, and *Acidiphilium* were identified in glacier samples (**Figure 7**). Among Fe^{2+} oxidizers we found *Ferrimicrobium*. The electron donors that are coupled to iron reduction could be inorganic (sulfur or hydrogen), as in the case of chemolithotrophic acidophiles; or it could be organic (glucose, glycerol), as in the case of heterotrophic acidophiles (e.g., *Acidiphilium*). In tidewater samples, *Shewanella*, *Rhodoferax*, were also identified, as well as some Fe^{2+} oxidezers such as *Gallionella*.

Sulfur Cycle

In glaciers, several Epsilonproteobacteria (*Sulfurospirillum* and *Sulfurimonas*) were identified. These bacteria oxidize sulfide and sulfur as electron donors with either O_2 or nitrate as

electron acceptors. Some Deltaproteobacteria are specialized in anaerobic metabolisms using oxidized sulfur compounds as electron acceptors. These include organisms such as *Desulfovibrio* and *Desulfuromusa*, a group of sulfate-reducing bacteria that reduces sulfate to sulfide, with lactate, pyruvate, or H_2 as electron donors. However, in anoxic environments acetate-oxidizing sulfate-reducing bacteria dominate. Acetate-oxidizing sulfate reducers include genera such as *Desulfofrigus* identified in glacier samples.

Other bacteria, *Paracoccus*, found both in glacier and tidewater samples (**Figure 7**), contains a sulfide and thiosulfate oxidation system, named Sox, that is also present in and many other sulfur bacteria both chemolithotrophic and phototrophic. This system oxidizes reduced sulfur compounds.

In tidewater samples, hydrogen sulfide (H_2S) is oxidized by phototrophic and chemolithotrophic microorganisms (*Thiomonas*, *Thiomicrospira,* and *Thioalkalimucrobium*) to sulfur (S_0) and sulfate (SO_4^{2-}), the latter being a key nutrient for algae and marine plants.

However, SO_4^{2-} can be reduced to H_2S by activities of the sulfate-reducing bacteria. These organisms consume organic carbon, and this reduction closes the biogeochemical sulfur cycle while regenerating CO_2.

Phosphorous Cycle

Among the phosphorous solubilizing bacterial communities, strains from *Pseudomonas*, *Bacilli*, *Rhizobium* and *Enterobacter* have been described as phosphate solubilizers (Khan et al., 2009). In glacier samples the presence of microorganisms involved in phosphorus acquisition include strains from bacterial genera *Pseudomonas*, *Mesorhizobium*, *Azorhizobium*, *Bradyrhizobium*, *Bacillus* and *Azospirillum*. In tidewater samples, these same genera except *Azospirillum* were found.

Possible Consequences of Differences in Microbial Composition

Glaciers constitute a key link between coasts and their downstreaming tidewaters and are of increasing importance in land-to-ocean fluxes. In published reports about other coastal glaciers such as the Antarctic Wanda Glacier (Pessi et al., 2015), it has been observed that marine microorganisms could colonize glaciers. In our study we have also observed that some marine bacterial OTUs were present in the glaciers. Additionally, we did also observe that OTUs corresponding to specific seawater bacteria were identified in the glacier samples. That is to say, it is possible that part of the microbial diversity found in the seawater samples comes partially from the upstream glaciers. When the glaciers thaw, the inland runoff waters transport more materials than the coastal glaciers do. This progression would alter the seawater composition, by increasing both

nutrients and contaminants, or by changing the physicochemical characteristics of water. Over time, microbial populations from CT samples would become more similar to populations from IT samples.

CONCLUSION

The glaciers on the Northwest coast of Svalbard are undergoing a rapid change due to climate change. This effect is important in the coastal glaciers, but it is even more so in the interior glaciers. If the glaciers inland continue to melt, they will increasingly drag a greater amount of materials and sediments in their runoff waters. The consequence of this process will be the possible alteration of the water composition of the fiords. If this effect is maintained over time, the microbial populations of the coasts (CT) could be modified and they would be more similar to the current populations of the IT samples. This process can have serious consequences throughout the marine ecosystem and in the cycling of globally important elements.

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All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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SUPPLEMENTARY MATERIAL

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