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PRIMARY RESEARCH PAPER

Glacial recession in Andean North-Patagonia (Argentina): microbial communities in benthic biofilms of glacier-fed streams

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Abstract Global climate change produces important shifts in the glacial runoff, modifying the relative contributions of meltwater and glacial clay discharges into headwater streams. Physical and chemical factors of glacier-fed streams are affected, such as total suspended solids (TSS), and nutrient concentrations. Here, we analyze the composition of the biofilm bacterial community by 16S rRNA sequencing along a glacier-fed network (Upper Río Manso) located in North Patagonian Andes (Argentina). We also analyzed changes in environmental factors in relation to the bacteria composition in different seasons (spring, summer, and autumn). Our results showed that the dominant phyla were Proteobacteria, Cyanobacteria, Bacteroidota, Actinobacteriota, and Acidobacteriota. Bacterial community composition changes longitudinally and seasonally in relation to glacial influence (TSS and phosphorus concentrations). We identified phylotypes of Proteobacteria (*Polaromonas*, *Rhodoferax*, and *Methylotenera*) that were only present in headwaters of the fluvial systems. In addition, Cyanobacteria also presented substantial changes along the main course of Manso River and among seasons. The increase of Cyanobacteria abundance was favored by the glacial influence both longitudinally and seasonally. Overall our results contribute to the understanding of the patterns of biodiversity and bacterial composition under a constant glacial retreat.

Keywords Microbial diversity · Network · Glacial influence · Seasons · Cyanobacteria

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Introduction

Glacier-fed streams are dynamic ecosystems that have an important ecological role because they link glacial ecosystems with downstream habitats (Battin et al., 2003; Wilhelm et al., 2013). These environments are characterized by high conductivity, low temperature, and high turbidity (Ren et al., 2017; Martyniuk et al., 2022). Seasonal fluctuations in glacier retreat produce changes in the runoff, shifting the relative

contribution of melting and glacial clay that are discharged into the headwaters (Brown et al., 2007; Milner et al., 2009). Changes in glacial clay inputs can produce sudden changes in turbidity in glacier-fed streams affecting primary producers, due to transient light protection for attached algae (Martyniuk et al., 2014) or, to an increase in the availability of P (Hodson et al., 2004; Simpson et al., 2021). As a result of climate change, many mountain basins show an accelerated retreat of their glaciers (Huss et al., 2017; Beniston et al., 2018), which, in turn, causes changes in physicochemical, and temperature dynamics of glacial streams due to variation in the rate, timing and volume of ice melt contributions (Milner & Petts, 1994; Milner et al., 2009; Huss & Hock, 2018).

The microbiome of glacial ecosystems is poorly known (Bourquin et al., 2022) and is expected to harbor a particular community composition, that would influence downstream communities (Ren et al., 2019). Microbial life in glacier-fed streams is remarkably diverse, including bacteria, archaea, fungi, protozoa, and viruses (Battin et al., 2001; Besemer, 2015), and is represented mainly by biofilms that colonize the sediment and rock surfaces (Battin et al., 2016). Local environmental conditions (abiotic environment, biotic interactions) are key mechanisms that would affect biofilm biodiversity and, in particular, headwaters are important for maintaining the microbial biodiversity in fluvial networks (Besemer et al., 2012). In addition, the longitudinal nature of stream ecosystems (i.e. differences in landscape) will also provide changes in resources (differences in nutrients, light exposure) that would affect community structure (Wilhelm et al., 2014) and microbial functional traits (Martyniuk et al., 2022).

Biofilms are hotspots for microbial activity, contributing substantially to the metabolism and the biogeochemical cycles of glacial-stream ecosystems with potential implications for downstream biodiversity (Wilhelm et al., 2013; Ren et al., 2017). As moving away from the glacier, the contribution of melted ice and snow decreases (Brown et al., 2007; Gao et al., 2017). Thus, changes in water temperature, channel stability, conductivity, and dissolved nutrients will occur, affecting biofilm communities (Milner et al., 2001; Hannah et al., 2007; Kuhn et al., 2011). Indeed, Wilhelm et al. (2013) found that biofilm diversity in glacier-fed streams increases downstream. In addition, Martyniuk et al. (2022) showed that changes in hydrological factors in glacial-fed streams of Patagonia resulted in shifts in enzymatic activities along the river. However, Malazarte et al. (2022) indicated that biofilm bacterial richness was unrelated to network position in Finland's glacier-fed streams.

In the North Patagonian Andes, the glaciers of Mount Tronador (3481 m above sea level, m a.s.l.) have exhibited a constant retreat over the last four decades (Ruiz et al., 2017; Masiokas et al., 2020). Meltwater from these glaciers is transported by streams that constitute the headwaters for the Upper Manso River that drains to Lake Mascardi. These headwaters have more than 50% of their area covered by glaciers (Martyniuk et al., 2019). Along 24 km, Upper Manso River receives different tributaries that differ in turbidity and canopy and a recent study has shown that biofilm elemental limitation switches from C-limitation in headwaters to P-limitation downstream (Martyniuk et al., 2022). Based on these evidences, we hypothesized that glacial influence will also drive biofilm bacterial community composition due to changes in turbidity and nutrients. Thus, the objectives of the present study were: 1- to analyze the bacterial community composition in biofilms of this glacier-fed network based on 16S ribosomal RNA sequencing. 2- to compare the bacterial community composition on three different occasions, spring, summer, and autumn, corresponding to the early, middle, and end of the ablation period, respectively.

Material and methods

Study site

Mount Tronador (3481 m a.s.l.) is located at 41° 10′ S, 71° 52′ W in Chile and Argentina and constitutes the largest ice-cap of Andean-North Patagonia. This important glacial system includes 13 glaciers that occupy a total area of about ~57 km^2 (Ruiz et al., 2017). The meltwater from these glaciers is transported by several streams that drain the Mount Tronador (Fig. 1), among which are Negro and Blanco streams. Negro stream originates from the drainage of the recently formed proglacial Lake Ventisquero Negro, which remains in contact with the Ventisquero Negro glacier (Modenutti et al., 2018). On the other hand, Blanco stream is formed by the south end of Castaño Overo glacier (2100 m a.s.l.). Both rivers

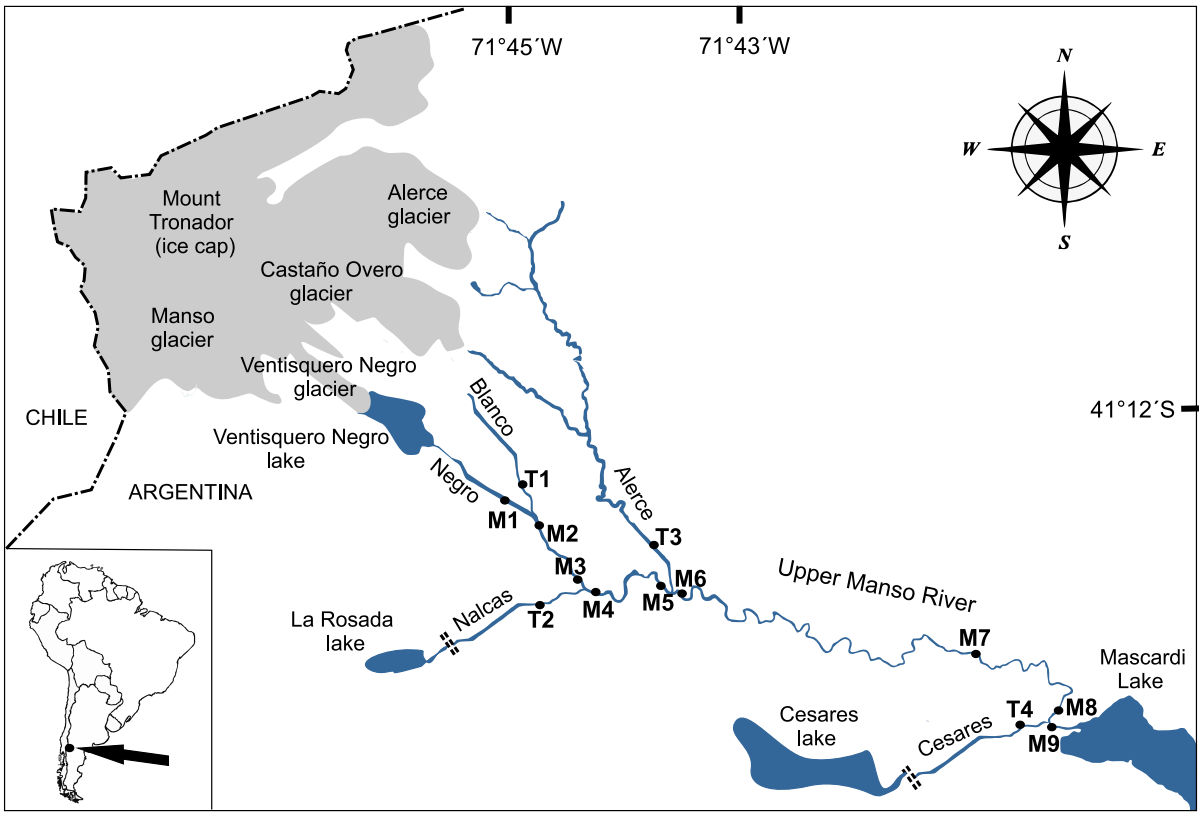

Fig. 1 Map of Upper Río Manso Basin. M1 to M9: sampling sites in the main course of Upper Río Manso. T1–T4: sampling sites in the tributary streams (Blanco, Nalcas, Alerce and Césares)

converge in the Upper Manso River that transports meltwater to Lake Mascardi. Along its catchment, Upper Manso River receives several tributaries (glacial or forested). Alerce stream is supplied by meltwater coming from Alerce and Castaño Overo glaciers. Nalcas and Césares streams are forested tributary streams of Upper Manso River. Nalcas Stream is fed by seasonal snowmelt and a shallow lake (Lake La Rosada), and runs through a *Nothofagus dombeyii* (Mirb.) Oerst. forest (Bianchi & Ariztegui, 2012). Finally, Césares stream receives water from Lake Los Césares located at 1200 m.a.s.l. (Fig. 1). This stream is surrounded by a mixed forest of *N. dombeyi* and *N. antarctica* (G. Forst.) Oerst. (Bianchi & Ariztegui, 2012).

Field sampling procedures

The analysis of the entire network was carried out in 13 sampling points (Fig. 1). On the main course of Upper Manso River, we sampled nine sites (M1 to M9) and we also sampled four tributaries (T1 to T4): Blanco, Alerce, Nalcas, and Césares streams (Fig. 1). This sampling was conducted on the same day in spring (November 28th, 2018).

To analyze seasonal differences we performed two additional samplings in summer and autumn (January and April 2019, respectively). These samplings were carried out in three sites (M1, M3, and M8) of the main course of Manso River (Fig. 1). Based on these data we determined the differences in environmental conditions and bacterial composition between Spring, Summer, and Autumn.

On each sampling occasion, we measured temperature, conductivity, and dissolved oxygen concentration with a YSI 85 (OH, USA). Also, we randomly collected three stones from each site. Stones were stored individually in sterile plastic bags and transported to the laboratory in darkness and thermally insulated conditions (at 4 °C) for biofilm analysis.

Also, in each site, we collected three water samples using sterile 2-l Nalgene ® bottles for nutrient concentration determination and other laboratory procedures. All the water samples were stored in dark conditions in a cooler and were transported to the laboratory to be processed within three hours of sampling.

Laboratory procedures

We quantified the total suspended solids (TSS) by filtering 250 mL of stream water through pre-weighed and pre-combusted GF/F filters, then dried for at least 48 h at 60 °C and then reweighed. Filtered water was utilized to determine total dissolved phosphorus (TDP), total dissolved nitrogen (TDN), and dissolved organic carbon (DOC) concentrations. We digested the TDP samples with potassium persulphate at 125 °C and 1.5 atm for 1 h and then determined their concentrations according to the ascorbate-reduced molybdenum method (APHA, 2005). DOC was measured in 40 mL samples with a high-temperature combustion analyzer (Shimadzu TOC-VCSH), and the TDN was determined using the TNM-1 unit on the Shimadzu TOC-VCSH. The biofilm from each stone was removed using a new and sterilized nylon brush (changed for each sample). The removed biofilm was led to a volume of 150 mL extract with sterile MilliQ water.

DNA extraction, and amplicon sequencing

Bacterial 16S rRNA genes were analyzed to assess biofilm community structure and diversity. For genomic DNA extraction from biofilm, 2 mL of the biofilm extract was centrifuged to concentrate cells (10,000 g Eppendorf refrigerated centrifuge). Then, the excess water was carefully removed, and the pellet was stored at − 80 °C until DNA extraction. Extraction was performed with DNeasy PowerSoil Kit (Qiagen®, Hilden, Germany), following the manufacturer's instructions. The quantity and purity of DNA samples were analyzed spectrophotometrically using a Synergy™ HTX Multi-Mode Microplate Reader, equipped with a Take3 Micro-Volume Plate (BioTek® Instruments, Inc., Winooski, VT, USA). The extracted DNA was stored at − 80 °C until further processing. Extracted DNA was sent to Novogene Bioinformatics Technology Co. (Beijing, China) to be processed using Illumina NovaSeq 6000 platform (250 bp paired-end).

Amplicons of the V3–V4 hypervariable region of the bacterial 16S rRNA gene were used to determine the bacterial community diversity and structure in each of the samples. PCR amplifications were obtained using the barcoded primer pair 341f (CCTAYGGGRBGCASCAG) and 806r (GGACTACNNGGGTATCTAAT) (Muyzer et al., 1993; Caporaso et al., 2011). The PCR conditions informed by Novogene Bioinformatics Technology Co. were: a- an initial denaturation at 98 °C for 1 min, b- 30 cycles of denaturation at 98 °C for 10 s, annealing at 50 °C for 30 s and extension at 72 °C for 30 s, and c- a final extension at 72 °C for 5 min. The PCR products were purified with GeneJET Gel Extraction Kit (Thermo Scientific). Sequencing libraries were generated using NEB Next® Ultra™ DNA Library Prep Kit for Illumina (NEB, USA) following manufacturer's recommendations and index codes were added.

Processing of Illumina sequencing data

Amplicon sequences provided by Novogene Bioinformatics Technology Co. were processed using DADA2 v1.22.0. The entire sequencing project was analyzed with the same filtering parameters following Callahan & collaborators (Callahan et al., 2016). We used the *filterAndTrim* function from DADA2 with the following quality values: maxEE = c(2,2) and truncLen = c(220,200). The chimera sequences were excluded using the function *removeBimeraDenovo*. Sequences were identified as unique amplicon sequence variants (ASV) based on single nucleotide differences. The taxonomic classification was performed using the SILVA database (version 138.1) as a reference. Finally, chloroplasts, mitochondria, eukaryotes, and singletons were removed. The sequencing data have been deposited in NCBI BioProject PRJNA901989 with the Biosample accessions SAMN31743636-SAMN31743660.

Data analysis and calculations

To estimate the glacier influence at each sampling site we calculated de Glacial Index (GI) following Ilg & Castella (2006). The variables used were temperature, electrical conductivity, TSS, and Pfankuch index (PFAN) as an estimation of streambed stability

(Pfankuch, 1975; Snook & Milner, 2001). We used the reciprocal of TSS (1/TSS), and PFAN (1/PFAN) so that all parameters decreased with increasing glacier influence (Ilg & Castella (2006). All parameters were rescaled between 0 and 1 and processed using a non-centered principal component analysis (nPCA, Supplementary Information, Figure S1). The GI values were the ordination scores along the first axis; lower values of GI indicate higher glacial influence.

Differences in DOC, TDN, and TDP concentration were tested with one-way ANOVA with the site as a factor for the network analysis, and two-way ANOVA with season and site as factors for seasonality analysis. In those cases, where normality or homogeneity of variance were not fulfilled, a Kruskal Wallis One-way ANOVA on Ranks was performed. In the case of nutrients in the seasonal analysis, where the dataset could not be normalized, we applied a Generalized Linear Model (GLM) with gamma distribution; then we applied ANOVA type III. When the results showed significant differences, we performed post hoc multiple comparisons by the Holm-Sidak method and multiple pairwise comparisons to estimated marginal means (EMMs) for the seasonal dataset. The same tests were used to analyze the alpha-diversity metrics. These statistical analyses were carried out with *vegan* v.2.6.4 (Oksanen et al., 2013) and *emmeans* v.1.8.1.1 packages (Lenth et al., 2018) in R 4.1.2 (R Core Team, 2013). Alpha diversity metrics (Chao1, Shannon Index) were calculated with the *microbiome* v.1.16.0 package in the R (Shetty & Lahti, 2019). The heatmaps of bacterial most abundant genera were drawn using *pheatmap* v.1.0.12 package in the R (Kolde, 2019).

To visualize differences in bacterial composition among sites in the network, we used non-metric multidimensional scaling (NMDS) analysis based on Bray–Curtis distance with the package *ampvis2* v.2.7.17 in the R (Andersen et al., 2018). ANOSIM was used to analyze significant differences among groupings in the network in ordination analysis (Clarke, 1993). To visualize differences in bacterial composition among seasons we use a matrix of Bray Curtis distance of each season and two-way ANOSIM was applied to analyze significant differences in the seasonal analysis using *vegan* package. The correlations between abiotic factors and community dissimilarities based on Bray–Curtis distance were tested by overlaying a canonical correspondence analysis (CCA). This analysis was carried out with the package *vegan* in the R program.

The environmental heterogeneity in the network was calculated as the average of dissimilarity of abiotic variables (DOC, TDN, TDP, TSS, and conductivity). We computed the Euclidean distance matrix and estimated the dissimilarity among them using *vegan* package in R, and calculated the dissimilarity (Ed) (Huber et al., 2020) for sectors or seasons as:

$$\mathrm{Ed} = \frac{Euc}{Euc_{max}}$$

Where *Euc* is the Euclidean distance between two samples and Euc_{max} corresponds to the maximum Euclidean distance considering all the pairwise distances in the overall dataset. Then, we calculated the Ed of each computed dissimilarity matrix and used it as an index of environmental heterogeneity in each sampling site. Values of 0 and 1 indicated minimum and maximum environmental heterogeneity, respectively.

Results

Bacterial composition, physical and chemical variables in the network

After the quality control, we obtained a total of 1636,750 high-quality sequences, with an average of 125,903 sequences per sample for the network dataset including tributaries (spring sampling), corresponding to a total of 3958 ASVs. The rarefaction curves at each sampling point indicated that most bacterial taxa have been covered (Supplementary Information, Figure S2). The non-metric multidimensional scale (NMDS) analysis identified three sectors, and with the ANOSIM analysis we verified significant differences among them (ANOSIM, Global $R = 0.88$ $P = 0.01$; pairwise test, $P \leq 0.018$ for all comparisons) (Fig. 2A). Sector 1 included three sampling sites: the first reach of Manso river (M1), the glacier-fed stream Blanco (T1), and the junction of the two streams (M2). Sector 2 included four sites in the middle reach of the Manso river (M3, M4, M5, M6), and the glacier-fed stream Alerce (T3). Sector 3 included three sites in the downstream reach of the Manso river (M7, M8, M9), and two forested tributaries (T2, T4)

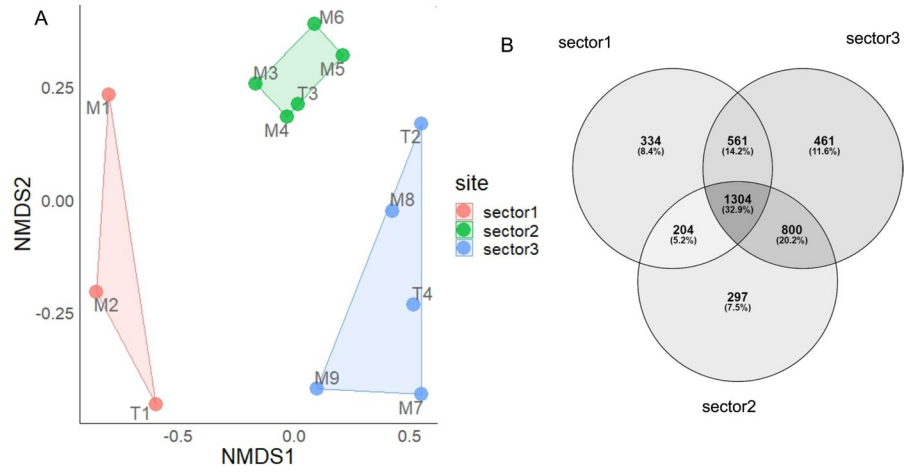

Fig. 2 **A** Non-metric multidimensional analysis based on Bray–Curtis dissimilarity of bacterial community composition with minimum convex polygons encompassing the three groups identified as sectors 1, 2, and 3. **B** Venn diagram showing the number of shared and unique ASVs among sectors

that are the outlet of two mountain shallow lakes. The three sectors shared 1,304 ASVs (32.9%) (Fig. 2B). The percentage of unique ASVs in the sector 1 was 8.4%, while in sectors 2 and 3 were 7.5% and 11.6%, respectively.

The environmental dissimilarity (Ed) between sampling sites in the three sectors indicated a clear trend to decrease internal heterogeneity from sector 1 to 3 (Fig. 3). However, the environmental heterogeneity increased among sectors from sector 1 to 3 (Fig. 3) coinciding with a decrease in glacial influence along the main course of Manso river (Supplementary Information, Table S1). Sector 1 showed high internal heterogeneity (Fig. 3), probably due to low values of TSS and TDP in the tributary Blanco stream. Sector 3 exhibited a low internal heterogeneity while sector 2 has an intermediate situation (Fig. 3). The TSS concentration showed a significant decrease from sector 1 to sector 3 (Kruskal Wallis One-way ANOVA on Ranks: $P=0.014$, d.f.$=2$, $H=8606$) (Supplementary Information, Table S2). The same pattern was observed for conductivity values (Kruskal Wallis One-way ANOVA on Ranks: $P=0.032$, d.f.$=2$, $H=6{,}856$) (Supplementary Information, Table S2). Consequently, we observed a decrease in the glacial influence (GI) along the main course of Manso river (Table S2). Nutrient concentration also varied along the Manso river course. A decreasing pattern was observed for phosphorus concentration, while DOC concentration increased significantly from sector 1 to sector 3 (Supplementary Information, Table S3). Finally, the TDN concentration varied among sectors, with the highest values in sectors 1 and 3 (Supplementary Information, Table S3). Glacial and forested tributaries also differed in TDP and DOC concentrations (Supplementary Information, Table S1). Phosphorus concentration was higher in glacial tributaries while DOC concentration increased in forest ones. In particular, Nalcas stream (T2) was included in sector 3 according to its high DOC and low TSS concentrations.

Proteobacteria was the dominant phylum in the network with 60.98% of the relative abundance, followed by Cyanobacteria, Bacteroidota, Actinobacteriota, and Acidobacteriota which exhibited differences in the different sectors (Fig. 4A). Sector 1 showed the highest proportion of Bacteroidota and Actinobacteriota, and the lowest proportion of Cyanobacteria. On the contrary, sectors 2 and 3 showed an increase in Cyanobacteria relative abundance, and a decrease in Bacteroidota and Actinobacteriota (Fig. 4A). We observed that ASVs relative abundances clustered in three groups (sectors 1, 2 and 3) supporting the previous NMDS analysis (Fig. 4B). Considering the first 21 ASVs in the heatmap (Fig. 4B) we observed a clear contribution to the three sectors. From *Methylotenera* (ASV2581) to *Polaromonas* (ASV711) are ASVs shared by sector 1 and sector 2, and from *Methyloglobulus* (ASV2454) to *Calothrix* PCC-6303 (ASV 3591) are ASVs shared by sectors 2 and 3. These distributions suggest that sectors 1 and 3 have particular bacterial communities while sector 2 is a transition sector since phylotypes are shared by sectors 1 and 3. The alpha-diversity, estimated by both Chao1 and Shannon Index, showed an

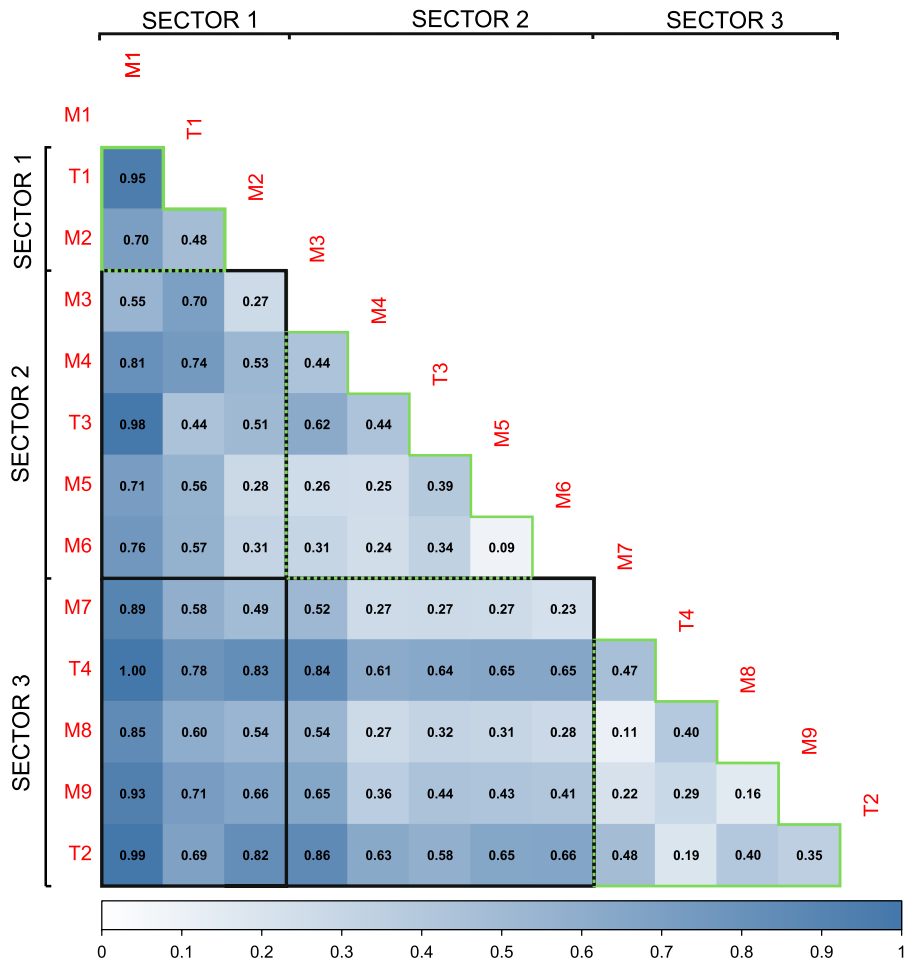

Fig. 3 Environmental heterogeneity (Ed) in the network. The environmental heterogeneity is expressed as the relative environmental dissimilarity between sampling sites of the environmental sectors. For more explanation on the Ed estimation, see Methods. Green and black lines show comparison within and among sectors, respectively

increasing trend towards sector 3 although these differences were not significant differences among sectors (One-way ANOVA, Chao1, $P = 0.205$, df = 2, $F = 1.83$; Shannon, $P = 0.402$, df = 2, $F = 0.99$) (Fig. 4C).

Finally, we observed that the more abundant ASVs (Fig. 4B) correlated with environmental variables. *Polaromonas* (ASV710 and 711), CL500-29 marine group (ASV 2192), *Pseudarcicella* (ASV 5024), *Methylotenera* (ASV 2581) are negatively correlated with DOC concentration and the distance to Ventisquero Negro, and positively correlated with TDP. On the contrary, *Scytonema* UTEX 2349 (ASV 3588), correlated positively with DOC concentration and the distance to Ventisquero Negro, and negatively with TDP (Supplementary Information, Table S4).

Seasonal study in the main course of Manso River

For the seasonal study, we only analyzed the sites M1, M3, and M8, corresponding to sectors 1 (S1), 2 (S2), and 3 (S3), respectively, located in the main course of Manso river. Although we observed changes in absolute values, a similar decreasing pattern in TSS, conductivity, and TDP and the increase in DOC prevailed in the three studied seasons (Fig. 5). Conductivity, TDP, and DOC concentrations showed the highest values in spring, while TSS in summer. We did not observe any significant change in TDN concentration (Supplementary Information, Table S5).

We observed important differences in bacterial community composition in the three sampling seasons (Fig. 6A). In particular, in spring more phyla were detected. The relative abundance of

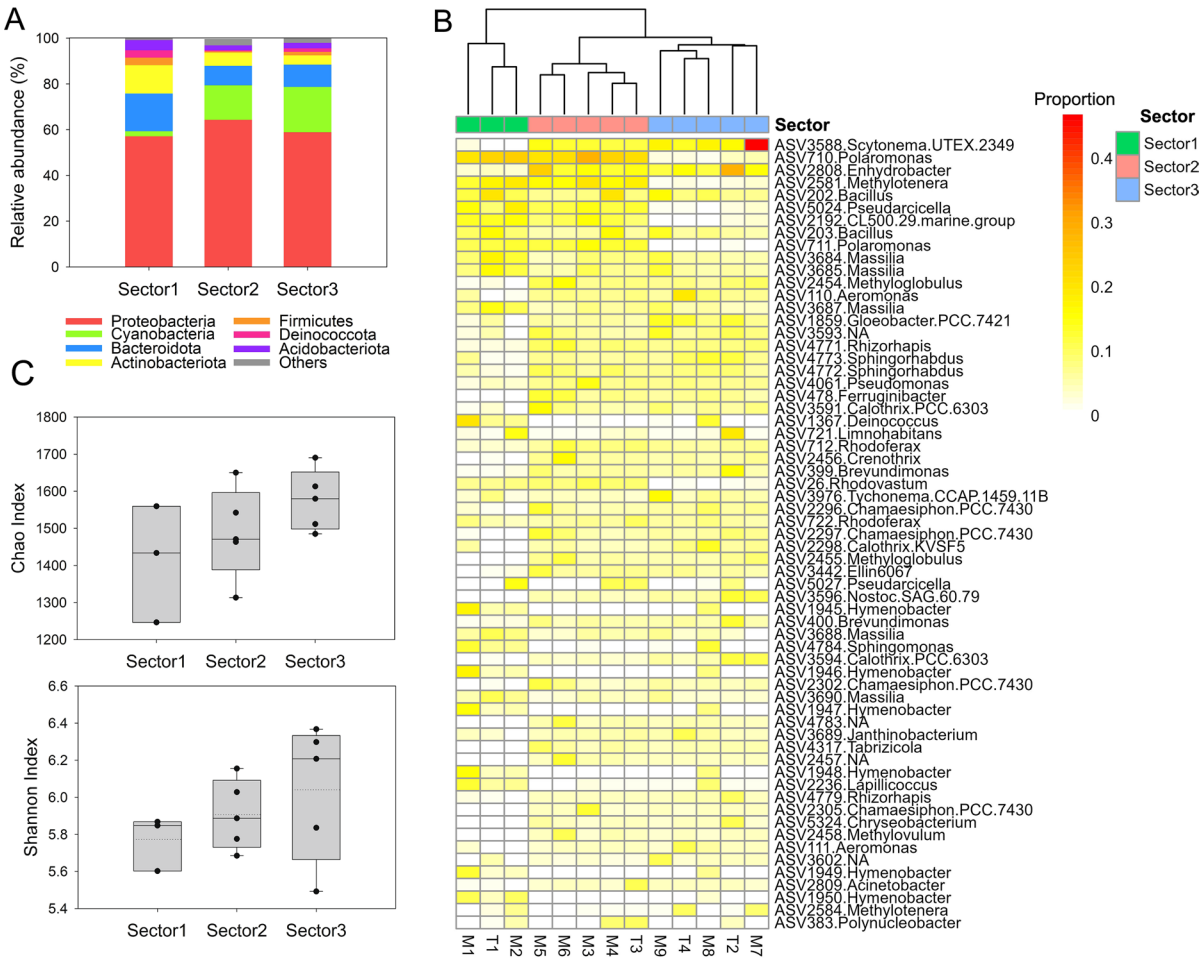

Fig. 4 **A** Relative abundance of phyla in the three sectors. **B** Hierarchically clustered heatmap showing the relative abundance (proportion) of dominant genera in sampling sites. Clustering of sites is represented by a dendrogram based on Bray–Curtis dissimilarity. **C** Box-plot of Chao and Shannon diversity indexes in the three sectors. References: M1 to M9: sampling sites in the main course of Upper Río Manso. T1–T4: sampling sites in the tributary streams (Blanco, Nalcas, Alerce and Césares)

Cyanobacteria and Proteobacteria increased in summer and autumn, particularly in sector 1. These differences were observed also for specific ASVs such as *Polaromonas*, *Calotrix,* and *Aphanizomenon* that change seasonally in sector 1 (Fig. 6B). Considering the beta-diversity (Bray Curtis dissimilarity) we observed differences in the three sectors with maximum dissimilarities between sector 1 and 3. In addition, maximum dissimilarities were observed in summer and an increase in dissimilarities between sectors 2 and 3 in autumn (Fig. 7). The ANOSIM analysis indicates significant differences for both sectors and seasons (Season $R = 0.96$ $P = 0.0003$; Sectors $R = 1$ $P = 0.0003$).

We conducted a canonical correspondence analysis (CCA) to identify possible relationships between microbial community composition and environmental variables in the three studied seasons (Fig. 8). The first two ordination axes explained 41.10% of the observed variance. The first axis was related to DOC and TDP and separates spring from autumn-summer samples. The second ordination axis was related to TSS.

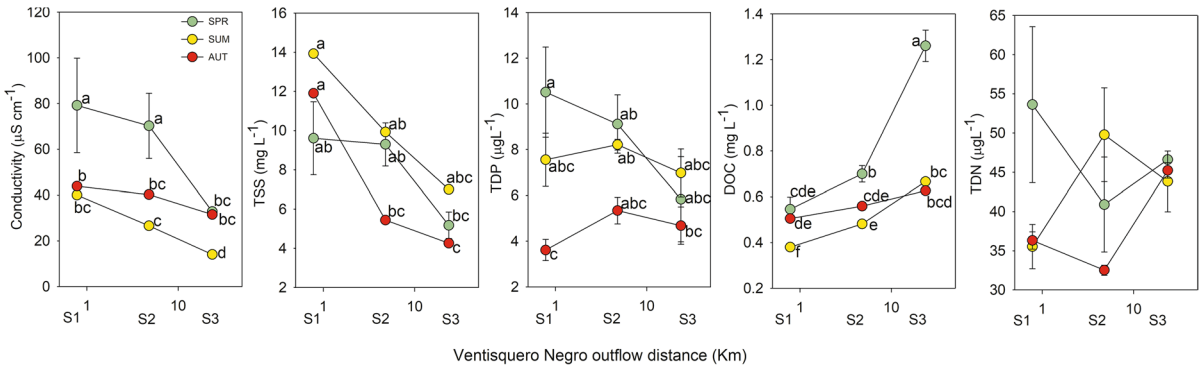

Fig. 5 Physical and chemical parameters of the sampled sites and seasons. References: *TSS* total suspended solids, *DOC* dissolved organic carbon, *TDP* total dissolved phosphorus, and *TDN* total dissolved nitrogen. The different letters above each point indicate significant differences (ANOVA type III, post hoc Tukey, alfa = 0.05)

Discussion

Mountain glaciers are rapidly retreating as a result of climate change, affecting the runoff of glacier-fed streams (Huss & Hock, 2018). The melting of glaciers increases the flow, discharge, and transport of sediments (Milner et al., 2017), altering the dynamics of streams fed by glaciers and, consequently, their biofilms. In the last decades, glaciers in Patagonia are increasingly retreating (Masiokas et al., 2020). In particular, Mount Tronador which is the largest ice cap in North Patagonia Andes is exhibiting a negative overall mass budget of glaciers (Ruiz et al., 2017). Under this scenario, we investigated the biofilm bacterial composition in streams of a glacier-fed network (River Manso) from Monte Tronador. Proteobacteria, Cyanobacteria, Bacteroidetes, Actinobacteria, and Acidobacteria were the most abundant phyla and are common in glacier-fed ecosystems (Wilhelm et al., 2013; Ren et al., 2017).

Proteobacteria dominated the community all along the network and seasons. This phylum comprises phylotypes involved in biogeochemical processes of carbon, nitrogen, iron and sulfur cycling (Friedrich et al., 2005; Meyer et al., 2016). The most abundant genus were *Methylothenera*, *Massilia*, and *Rhodoferax*. In particular, *Massilia* has a soil origin since it is a root-colonizing bacteria (Ofek et al., 2012) while *Methylotenera* is very abundant in nature and common in lake sediments (Kalyuzhnaya et al., 2010). Finally, the purple nonsulfur bacteria *Rhodoferax* is widespread in environments affected by glacial recession (Garcia-Lopez et al., 2021; Modenutti et al., 2023).

We identified three different bacterial communities (sector 1, sector 2, and sector 3) along the network of decreasing glacial influence, suggesting that different mechanisms occur. Migration processes of bacteria from tributaries and the surrounding environment, as well as the strong environmental selection, have previously been proposed as factors that influence the assemblage of communities (Ruiz-González et al., 2015; Niño-García et al., 2016; Hassell et al., 2018). In glacier-fed streams, microbial communities differed longitudinally along the stream in relation to hydrological, biogeochemical, and physicochemical factors (Ren et al., 2017). The analyses carried out in our study suggest that communities of sector 1 would reflect the habitat of origin (closeness to Ventisquero Negro) while communities of sector 3 would result from downstream communities combined with the bacteria assemblages coming from forested tributaries. In addition, sector 2 showed an intermediate situation that reflects the transition from upstream to downstream communities. Furthermore, the obtained cluster and the NMDS and ANOSIM analyses of the entire network support this pattern of biofilm communities. In particular, the cryophilic *Polaromonas* (Proteobacteria) dominated sector 1 with a strong glacial influence that grouped the sampling sites close to the Ventisquero Negro glacier. On the contrary, *Scytonema* (Cyanobacteria) dominated sector 3 which grouped downstream sites and forested tributaries (Nalcas and Los Césares) with higher DOC concentration and comparatively low glacial influence. In this sense, forested tributaries may play

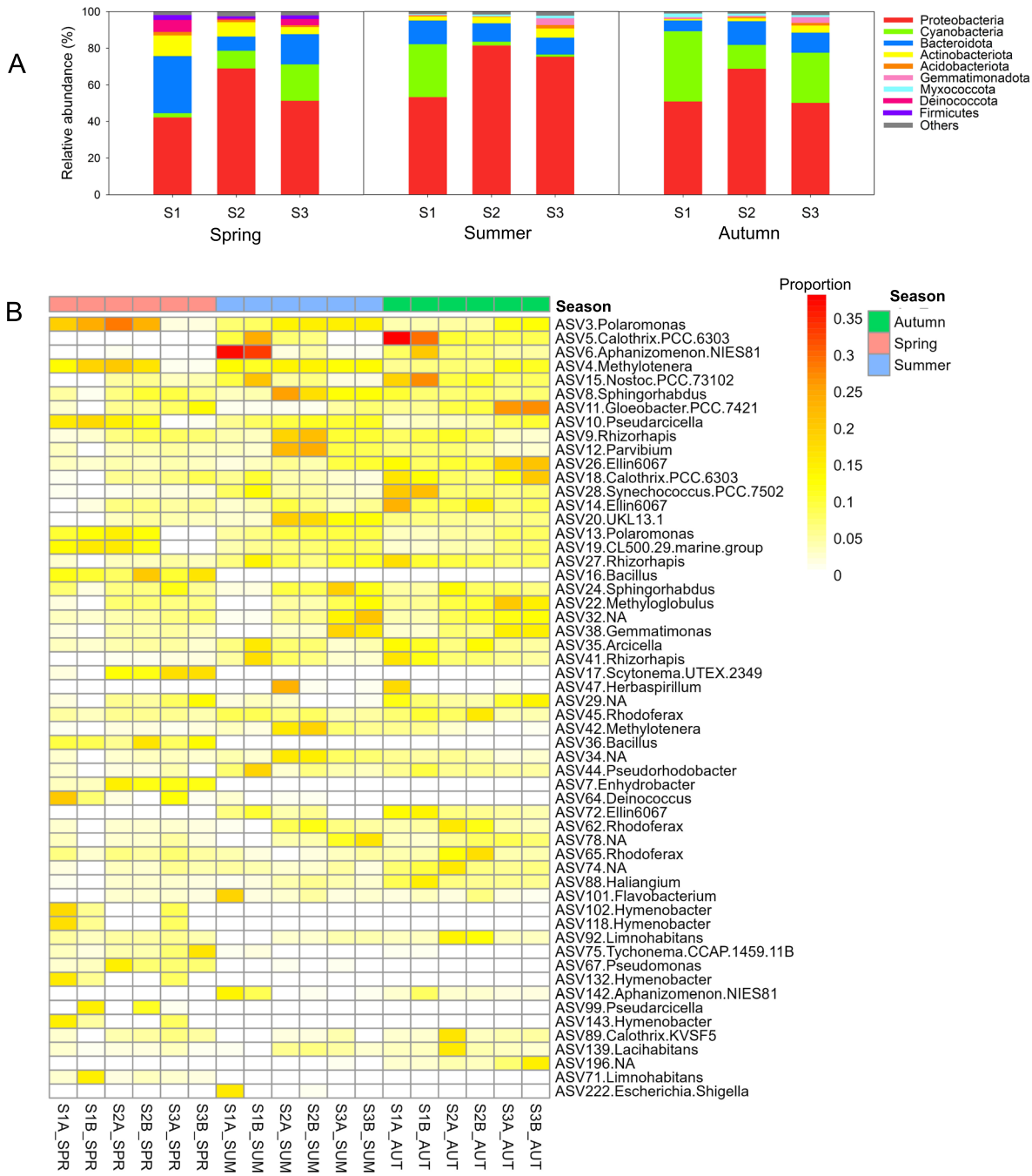

Fig. 6 **A** Relative abundances (proportion) of the main phyla in the three seasons. Phyla whose abundance was less than 1% are grouped in Others. **B** Heatmaps showing the relative abundance of the main ASVs in the studied sites by season. Letters (A and B) next to Sector 1 (S1), Sector 2 (S2), and Sector 3 (S3), indicate replicates for each sector

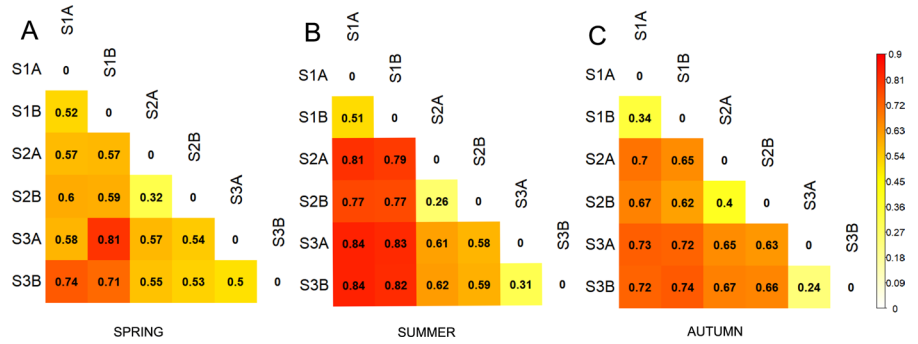

Fig. 7 Bray Curtis dissimilarity matrix of bacterial communities by season **A** Spring **B** Summer **C** Autumn. Letters (A and B) next to Sector 1 (S1), Sector 2 (S2), and Sector 3 (S3), indicate replicates for each sector. The color scale indicates the degree of dissimilarity, 0 and 1 are the minimum and the maximum, respectively

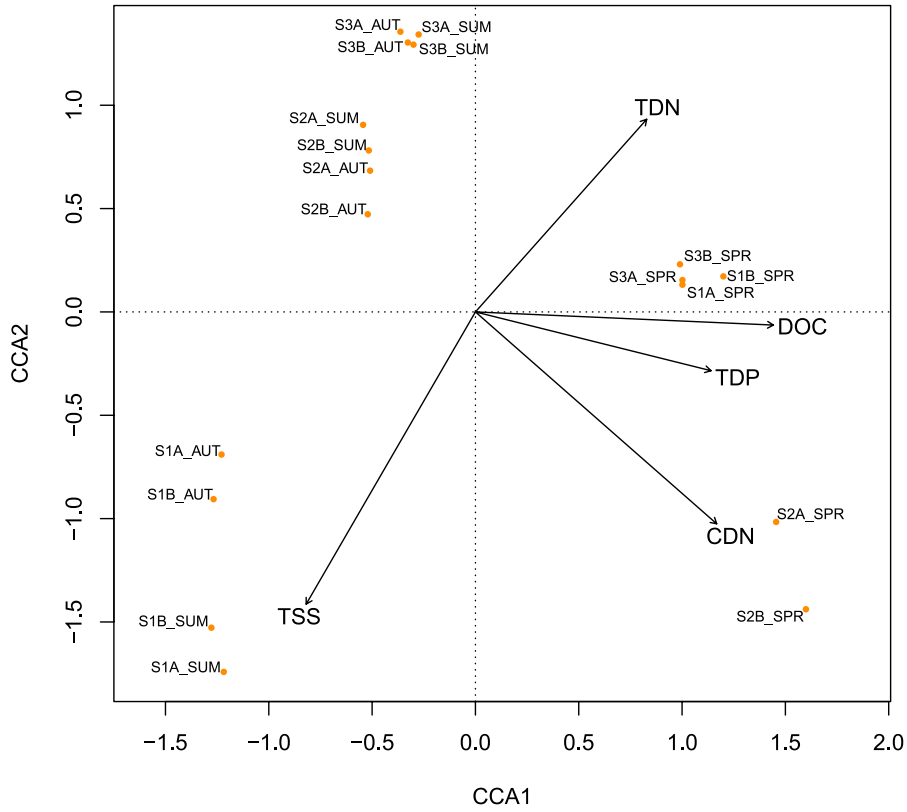

Fig. 8 Canonical correlation analysis (CCA) of bacterial community composition by season, related to environmental variables. References: *TSS* total suspended solids, *CND* conductivity, *DOC* dissolved organic carbon, *TDP* total dissolved phosphorus, and *TDN* total dissolved nitrogen. *S1* Sector 1, *S2* Sector 2, and *S3*: Sector 3. *SUM* summer, *SPR* Spring, and *AUT* autumn

an important role in supplying DOC from the surrounding environment. This subsidy of terrestrial DOC may promote the growth of heterotrophic biofilm bacteria (Battin & Sengschmitt, 1999; Risse-Buhl et al., 2012). Accordingly, previous work carried out in this network found that the enzymatic activity of the biofilm is limited by carbon in the headwaters, while downstream sites are limited by phosphorus (Martyniuk et al., 2022).

Studies in glacier-fed streams in the Alps and Asia have found an increase in downstream diversity (Milner et al., 2001; Wilhelm et al., 2013; Ren & Gao, 2019). We also observed a similar trend, and this pattern has been associated with increased channel

stability and water temperature (Milner et al., 2001), and distance from the glacier (Ren et al., 2017), indicating the important role of hydrological factors and the cumulative increase in catchment size on the microbial communities of these environments. The glacier-fed stream ecosystems are subjected to marked seasonal changes (Busi et al., 2022). As was observed in Manso river, studies have reported changes in the composition and diversity of communities in stream biofilms associated with a strong seasonal dynamic (Wilhelm et al., 2014; Ren et al., 2017). Here, we found a clear differentiation in bacteria community composition among seasons. In particular, there was a shift in Cyanobacteria (although with different genera) from downstream sites in spring to upstream in summer. In autumn, cyanobacteria showed a more even distribution in the three sectors. Although TSS varied among seasons the pattern of decreasing TSS along the main course of Manso River was consistent in the three seasons. In summer and autumn in sector 1, we observed higher TSS concentration with an increase in the relative contribution of cyanobacteria. Light is an important factor affecting biofilm primary producers in Patagonia (Martyniuk et al., 2016). Light attenuation by glacial clay can have great ecological importance affecting biofilm communities that are unable to change their position on the stone surface (Martyniuk et al., 2014).

In glacial ecosystems, Cyanobacteria have an important role as atmospheric nitrogen fixers (Quesada & Vincent, 2012). In this sense, the N:P relationship emerges as an important driver of Cyanobacteria abundance (Noges et al., 2007; Ipek & Jeyasingh, 2021). The chemical reactions involved in N fixation process are highly endergonic and phosphorus-demanding, since, reducing one molecule of N_2 demands 16 molecules of ATP (Paerl, 2017). In addition, this metabolic path uses iron as a cofactor (Kim & Rees, 1994). Glacial runoff transports P-rich sediments (Chillrud et al., 1994; Martyniuk et al., 2022), and is a potential source of iron (Hodson et al., 2017; Raiswell et al., 2018; Kanna et al., 2020). Thus, the observed increase in the abundance of Cyanobacteria in the upstream sector with higher glacial influence can be also due to an increase in P availability relative to Nitrogen (N:P atomic ratio: spring 12, summer: 5.5, autumn: 6.4) and the higher availability of Fe (Martyniuk and Modenutti unpublished data) interacting with light as discussed previously.

Summarizing, our results indicate that in the glacial network of Manso River bacteria community composition changes longitudinally and seasonally. The variables that have a higher contribution to the observed variation are highly related to glacial influence (TSS concentration /light and phosphorus) or the effect of the input of forested tributaries (DOC). In this sense, for certain phylotypes of Proteobacteria (*Polaromonas*, *Methylotenera*, *Rhodoferax*) and Cyanobacteria we observed substantial changes along the main course of Manso River and also among seasons that have important consequences for ecosystem functioning. Glacier-fed streams are environments subjected to constant change due to glacial runoff, showing a high environmental heterogeneity as was observed in our study. Consequently, identifying patterns of biodiversity and bacterial composition in biofilms of such dynamic habitats constitutes a valuable tool to understand the effect of climate change on mountain glaciers, which, in North Patagonia, are at risk to disappear.

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Data availability All the data generated during and/or analyzed during the current study will be available at the data repository of Universidad del Comahue http://rdi.uncoma.edu.ar/. The sequencing data have been deposited in NCBI BioProject PRJNA901989 with the Biosample accessions SAMN31743636-SAMN31743660.

Declarations

Conflict of interest The authors have not disclosed any competing interests.

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