Geographical Variability of Bacterial Communities of Cryoconite Holes of Andean Glaciers

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Abstract

Cryoconite holes, ponds full of melting water with a sediment on the bottom, are hotspots of biodiversity on glacier surface and host dynamic micro-ecosystems on these extreme environments. They have been extensively investigated in different areas of the world (e.g., Arctic, Antarctic, Alps, and Himalaya), but no study so far has described the bacterial communities of the glaciers in the Andes, the world longest mountain range. In this study, we start filling this gap of knowledge and describe the bacterial communities of Southern Andes in three small ($< 2 \text{ km}^2$) high elevation ($< 4200 \text{ m a.s.l.}$) glaciers of Central Andes (Iver, East Iver and Morado glaciers) and two large ($> 85 \text{ km}^2$) glaciers in Patagonian Andes (Exploradores and Perito Moreno glaciers) whose ablation tongues reach low altitude ($< 300 \text{ m a.s.l.}$). Results show that the bacterial communities were generally similar to those observed in the cryoconite holes of other continents. Indeed, the most abundant orders were Burkholderiales, Cytophagales, Sphingobacteriales, Actinomycetales, Pseudomonadales, Rhodospirillales, Rhizobiales, Sphingomonadales and Bacteroidales. However, the bacterial communities differed between glaciers and both water pH and O_2 concentration influenced the bacterial community composition.

Introduction

Glaciers and ice sheets have been recognized as a biome in their own right as they host viable populations of organisms [1]. Glacier ecosystems are mostly dominated by microorganisms that have been found in all parts of the glacier environment, including the englacial and the subglacial zones [1]. The supraglacial zone is the most biodiverse among glacier ecosystems [1] and, for practical reasons, is also the most studied. Here, small depressions of the glacier surface filled by meltwater, the cryoconite holes, form due to the atmospheric deposition of a fine-grained sediment, the cryoconite, which locally decreases the albedo and melts the underlying ice. The main source of this sediment is the surrounding environment, but a small part of it is deposited after long-range atmospheric transport [2]. In the extreme glacier environment, cryoconite holes are protected habitats where general conditions are more favourable for life than in the other parts of a glacier, as they provide liquid water which also acts as a protective barrier against the intense UV radiation characteristic of the glacier environment [3].

Cryoconite holes are present on glaciers in almost all areas of the world; so far they have been described in the Arctic, Antarctica, on the Alps, the Tien Shan, the Karakoram, and the Himalaya [2, 4–8]. However, they do not show the same features in all the different geographic areas where they occur. Polar cryoconite holes, in particular, are more stable than those that form on temperate mountain glaciers. On Himalayan glaciers, they can sometimes last for more than one year [7, 9]. However, on temperate mountain glaciers they are mostly ephemeral structures that can be destroyed by strong ablation that can quickly dismantle them washing away the sediment. The intense solar radiation can subsequently form new holes in a few days [10, 11].

Investigations of the temporal variability of cryoconite hole bacterial communities of a same glacier along one or even more ablation seasons were seldom conducted, mostly because of the difficulties and

the costs of visiting glaciers repeatedly, as they usually occur in remote areas. Most often, the description of the bacterial communities of cryoconite holes is conducted with snapshot studies [12, 13], which allowed a general description of their biotic communities, even if they cannot include the whole biodiversity of these environments [10, 14]. These studies showed that filamentous Cyanobacteria are the main responsible of cryoconite grain formation [7, 8] and the other most abundant bacterial phyla found in these microhabitats are Proteobacteria (Alpha and Beta), Actinobacteria, Chloroflexi, Acidobacteria and Bacteroidetes [5, 15–17], while the most typical orders are: Sphingobacteriales, Pseudomonadales, Rhodospirillales, Burkholderiales and Clostridiales [14, 18]. So far, snapshot studies have been used to describe bacterial communities inhabiting cryoconite holes on glaciers in the Alps [2, 12], Greenland [8], the Arctic [13], the Antarctica [19], the Himalaya [20], the Karakoram [4] and in Tien Shan [7].

To the best of our knowledge, no published information is currently available about cryoconite hole bacterial communities from South American glaciers. Only one study by Tacheuki et al. (2001) described cryoconite characteristics of the Tyndall Glacier, but from a physico-chemical point of view, not reporting bacterial communities composition. Another study describing the gut microbiome of the glacier stonefly (*Andiperla willinki*) reported also some data about the bacterial communities of the glaciers surface because these insects likely feed on supraglacial bacteria [22], but no study specifically focussed on the bacteria inhabiting this environment in South America. This is a big gap of knowledge since Chilean and Argentinian Andes host many glaciers (~32558 km² of glacierized areas) along a latitudinal range of ~8,000 km from 11°N in Colombia and Venezuela to 55°S in Chile and Argentina [23, 24]. The Andes hosts the most diverse cryosphere on our planet [24].

The aim of this work is to provide the first description of the bacterial communities of cryoconite holes of South American glaciers, based on samples collected on four Chilean and one Argentinian glacier. Three of them (Morado, Iver and East Iver glaciers) are located on the Central Andes close to Santiago (Chile), while two (Exploradores and Perito Moreno) are located respectively in Chilean and Argentinian Patagonia.

Methods

Study areas

Morado Glacier is a valley glacier located about 60 km South-East from Santiago (Chile). It covers 1.1 km² and its altitudinal range is between 3535 m and 4604 m a.s.l. It is a small glacier, highly crevassed and partly debris-covered in the ablation area, terminating in a small lake.

Iver Glacier is a small mountain glacier located at Cerro El Plomo about 30 km North-West from Santiago (Chile). It has a surface area of 1.64 km² and its altitudinal range is between 4296 m and 5358 m a.s.l. It is a steep glacier, partly debris covered in the ablation area.

East Iver is a small mountain glacier located few hundred meters to the East of the Iver Glacier at Cerro El Plomo. Its surface is 0.3 km² and during the Little Ice Age (and in all probability also few years ago) it

was probably connected to Iver.

Exploradores Glacier is a valley glacier located in the Chilean Patagonia. It covers 85 km², it is 19 km long and its altitudinal range is between 158 m and 3735 m a.s.l. The frontal area of the ablation zone is debris-covered.

Perito Moreno Glacier is a valley glacier located in the Argentinian Patagonia. It covers 263 km², it is > 32 km long and its altitudinal range is between 190 m a.s.l and 2800 m a.s.l. The glacier snout ends in a lake, losing most of its mass through calving processes.

Sample collection

Cryoconite samples were aseptically collected in falcon tubes in 2017 and 2018 (Table 1). We collected samples from 15 cryoconite holes per glacier and, at each hole, we also recorded dissolved O₂ concentration and pH with a portable oximeter/pH meter (HACH LANGE HQ40D, Loveland, CO, USA).

Table 1
Date of sampling of each glacier.

Glacier	Date
Morado	18/02/2018
Iver	21/02/2018
East Iver	22/02/2018
Exploradores	01/03/2018
Perito Moreno	29-30/03/2017

DNA extraction and sequencing

DNA was extracted from 0.7 g of cryoconite with the FastDNA Spin for Soil kit (MP Biomedicals, Solon, OH, USA) according to the manufacturer's instructions. DNA sequencing was performed on the V5-V6 hypervariable region of the 16S rRNA gene as previously described in Pittino et al. [10]. Sequences were then demultiplexed according to the indices and clustered in Amplicon Sequences Variants (ASVs) with DADA2 [25]. ASVs were then taxonomically classified using the rdp classifier [26] keeping the full classification only of the taxa attributed with a confidence of 0.8 or higher. Cyanobacteria were classified at phylum level only, as the rdp classifier does not provide the full classification for them [27, 28].

Statistical analyses

Variation among glaciers in pH and [O₂] was investigated through ANOVA followed by Tukey post-hoc tests.

Singletons (ASVs present once in one sample only) were removed from the analyses because they can inflate the variance explained by multivariate tests [29]. Alpha-diversity was measured using the Shannon diversity index, which accounts for both the richness and the evenness of the species [30], and the Gini inequality index, which is an index of inhomogeneity largely used in economics [31]. Gini index ranges from 0 to 1 and low values indicate an homogeneous distribution of the species in the community, while values close to one indicate an heterogeneous distribution [31]. These indices were calculated on a dataset rarefacted to 7500 sequences per sample, i.e. slightly less than the minimum number of sequences in a sample (see results).

Beta diversity analyses were conducted on a non-rarefied dataset, and were based on the Hellinger distance, which depends on the differences in the ASV proportion among samples, decreases the importance of ASV abundance over occurrence and avoids the double-zero problem when comparing ASV composition among samples [32, 33]. We performed a redundancy analysis (RDA) and a variation partitioning (VP) to quantify the variation of community structures among glaciers (five-level factor), and according to dissolved oxygen concentration and pH of the water above the sediment. The last two variables were mean-centred within glacier before the analyses (i.e. we subtracted from the values recorded at each glacier their mean value). Hereafter, centred variables will be called ΔpH and $\Delta[\text{O}_2]$, respectively. Post-hoc tests (Tukey method) were also performed to assess pairwise differences in the structure of bacterial communities between glaciers, correcting P-values for multiple testing according to the false discovery rate (FDR) procedure [34].

Generalized least-square (GLS) models accounting for heterogeneity of variance among glaciers were also used to investigate changes in alpha diversity indices according to ΔpH , $\Delta[\text{O}_2]$, and glacier (five-level factor). Variation in the abundance of the nine most abundant orders (see below) according to the variables that significantly affected the structure of bacterial communities identified by the RDAs was investigated by generalized linear models (GLMs) assuming a Poisson distribution and correcting for overdispersion. Also in these cases, P-values were corrected using the same FDR procedure as above. Analyses were performed with R 3.5.1 (R Core Team, 2014) with the VEGAN, BIODIVERSITYR, MULTTEST, and MULTCOMP packages.

Results

Both pH ($F_{4,70} = 11.87$, $P = < 0.001$) and oxygen concentration ($F_{4,70} = 281.9$, $P < 0.01$) in cryoconite holes differed significantly among glaciers. Post-hoc tests highlighted that glaciers could be divided into two groups with respect of pH values, with Exploradores and Morado showing higher values than Perito Moreno, Iver, and East Iver ($|t_{70}| \geq 0.147$, $P \leq 0.035$; Fig. 2a), while all glaciers differed to one another in their oxygen concentrations ($|t_{70}| \geq 3.480$, $P \leq 0.011$; Fig. 2b).

We obtained 7600 - 93425 sequences per sample. Orders with more than 24000 sequences were considered as the most abundant ones. They were: Burkholderiales, Cytophagales, Sphingobacteriales, Actinomycetales, Pseudomonadales, Rhodospirillales, Rhizobiales, Sphingomonadales and

Bacteroidales. Together these orders include 76.9% of sequences. The RDA performed on all the samples, showed that bacterial communities differed significantly among glaciers and varied according to ΔpH and $\Delta[\text{O}_2]$ (Table 2; Fig. 4). The biplot also suggests that cryoconite hole bacterial communities of the three small glaciers in Chilean Central Andes (Iver, East Iver and Morado) vary mostly according to oxygen concentration, while those of the Exploradores glacier in Patagonian Andes vary according to pH. Post-hoc tests also revealed that the structure of the bacterial communities of all the five glaciers were significantly different from one another ($F_{62} \geq 21.527$, $P_{\text{FDR}} \leq 0.001$).

Table 2
RDA of Hellinger-transformed bacterial ASVs abundances of all the samples according to the glacier, pH and oxygen concentration centred according to their mean value per glacier.

Variable	Df	Variance	F	P
Glacier	4	0.445	38.15	0.001
ΔpH	1	0.006	2.110	0.010
$\Delta[\text{O}_2]$	1	0.006	2.204	0.001
Residuals	62	0.181		
Overall model: $F_{6,62} = 26.105$, $P = 0.001$, Adjusted $R^2 = 0.689$				

GLS models showed that both the alpha diversity indices varied significantly among glaciers (Shannon index: $F_{4,62} = 39.76$, $P < 0.001$; Gini index: $F_{4,62} = 23.01$, $P < 0.001$). Post-hoc tests showed that Shannon index was higher in Exploradores samples, intermediate in Perito Moreno and Morado ones, and lower in Iver and East Iver ones, while Gini index was higher in Iver and in East Iver samples, intermediate in Perito Moreno and Morado ones, and lower in Exploradores ones (Fig. S1). No trends were observed according to ΔpH ($F_{1,62} < 0.449$, $P_{\text{FDR}} > 0.5$) and $\Delta[\text{O}_2]$ ($F_{1,62} < 0.449$, $P_{\text{FDR}} > 0.5$).

GLMs performed on the most abundant orders showed that the abundances of Burkholderiales, Cytophagales, Sphingobacteriales, Actinomycetales, Rhodospirillales, Rhizobiales, Pseudomonadales, Sphingomonadales and Bacteroidales varied among glaciers ($F_{4,64} \geq 4.207$, $P_{\text{FDR}} < 0.010$), without showing any consistent pattern, Iver was the glacier with the highest relative abundance of Burkholderiales, Morado the one with the highest relative abundance of Pseudomonadales, East Iver showed the highest abundance of Cytophagales, and Perito Moreno had more Rhodospirillales, but less Burkholderiales, Pseudomonadales and Cytophagales than the other glaciers. Differences in Rhizobiales and Sphingobacteriales were even less marked (Fig. 5).

The five glaciers we considered showed different pH and $[\text{O}_2]$ values, and the RDA biplot suggested that the communities of different glaciers were affected by different environmental variables. We therefore investigated the relations between each of the most abundant orders and pH and O_2 concentration on

each glacier separately. Similarly, we analysed the relations between alpha diversity indices pH and $[O_2]$ separately per glacier.

On the Perito Moreno Glacier, only Actinomycetales decreased according to $[O_2]$ ($F_{1,12} = 16.714$, $P_{FDR} = 0.0383$) (Fig. S3e). On the Exploradores Glacier, Burkholderiales decreased with pH ($F_{1,12} = 13.762$, $P_{FDR} = 0.0397$), while Bacteroidales increased ($F_{1,12} = 13.582$, $P_{FDR} = 0.0397$) (Fig. S2e-f). Cytophagales was the only order that increased with $[O_2]$ ($F_{1,12} = 20.999$, $P_{FDR} = 0.016$) (Fig. S3d). On the Iver Glacier abundance of no order varied according to either pH or $[O_2]$. On the East Iver Glacier, abundance of Burkholderiales increased with pH ($F_{1,12} = 11.414$, $P_{FDR} = 0.049$), while that of Rhizobiales ($F_{1,12} = 14.476$, $P_{FDR} = 0.049$) and Sphingomonadales ($F_{1,12} = 11.257$, $P_{FDR} = 0.049$) decreased (Fig. S2a-b-c). The abundance of no order changed significantly with $[O_2]$. On the Morado Glacier, only Rhizobiales increased with pH ($F_{1,12} = 16.449$, $P_{FDR} = 0.049$) (Fig. S2d). Actinomycetales ($F_{1,12} = 17.695$, $P_{FDR} = 0.0155$), and Rhizobiales ($F_{1,12} = 23.874$, $P_{FDR} = 0.0096$) instead decreased with $[O_2]$, while Rhodospirillales increased ($F_{1,12} = 15.662$, $P_{FDR} = 0.016$) (Fig. S3a-b-c). Shannon and Gini indices respectively increased and decreased significantly according to pH ($F_{1,12} = 9.943$, $P = 0.008$) on East Iver only (Fig. S4), while on the other glaciers no trend was observed ($F_{1,12} \leq 1.244$, $P \geq 0.307$). No trend in alpha diversity indices with $[O_2]$ was detected on any glacier ($F_{1,12} \leq 1.970$, $P \geq 0.186$).

Discussion

In this study, we provide the first description of the bacterial communities of cryoconite holes from Southern Andean glaciers, in particular from both small high elevation glaciers of the Central Andes in the Santiago Metropolitan Region (Chile), and from the tongues of two large glaciers in Patagonian Andes that reach low altitudes. This information fills a large geographical gap in our knowledge of glacier environments because it is the first description of the microbial communities of supraglacial environments in South America, a continent with about 30000 km² covered by ice corresponding to about 5% of the glacierized area of the whole planet outside the Antarctica [36]. Results showed that the large Patagonian glaciers (Exploradores and Perito Moreno) had the highest oxygen concentrations, while Iver and East Iver had the lowest ones and Morado an intermediate value. This pattern seems related to the different altitude of the glaciers. Jacobsen et al. [37] calculated that at 4000 m a. s. l. oxygen availability in water is one fifth of that at sea level also because the kinematic viscosity of water plays an important role and depends on the elevation. This result is consistent with $[O_2]$ values we found in our samples. Indeed, Exploradores and Perito Moreno are located in Patagonia at low altitude (< 200 m a.s.l.), while Iver and East Iver are the highest ones among those we investigated (samples were collected at about 4000 m a.s.l.) and Morado is at an intermediate value (3400 m a.s.l.).

pH values seem to follow a different pattern: the highest values were recorded on Exploradores and Morado, while the lowest ones on East Iver and Iver. However, all the mean pH values of the five glaciers were basic (between 8.57 and 10.47). Differences in pH among glaciers are not easy to explain and may

be due to differences in lithology of the surrounding environments and also by aerosol transport from Santiago that act as sources of cryoconite. Interestingly, a high percentage of black carbon deposition on ice in the Central Andes close to Santiago have been associated to emissions from the city (>40%), whereas mining is also an additional important black carbon source [38]. We note, however, that we measured water pH rather than that of the cryoconite, because the first one was already reported to influence bacterial communities in cryoconite holes [4]. However, water pH may also be partially affected by the metabolic activities of the same bacterial communities of cryoconite holes, so it is still unclear whether water pH affects cryoconite hole bacterial communities or, at least, partially, also the reverse occurs [39].

The most abundant orders were (in decreasing order): Burkholderiales, Cytophagales, Sphingobacteriales, Actinomycetales, Pseudomonadales, Rhodospirillales, Rhizobiales and Sphingomonadales. These orders are typical of cryoconite holes and dominate bacterial communities in these environments in all the geographical areas investigated so far: Arctic [13], Antarctica [5], Europe [10, 12], Asia [16]. Therefore, cryoconite holes of Andean glaciers seem to host bacterial communities that are quite typical of these environments worldwide. Interestingly, diverse psychrotolerant Proteobacteria of the orders Pseudomonadales (*Pseudomonas*) and Xanthomonadales (*Stenotrophomonas*, *Xanthomonas*), Actinobacteria of the order Actinomycetales (*Brachybacterium*, *Curtobacterium*, *Pseudoarthrobacter*) and Bacteroidetes from the order Sphingobacteriales (*Pedobacter*) have been isolated at the Central Andes in Valparaíso Region at 3149 m a.s.l. from native flora, showing interesting biotechnological applications such as plant growth promotion and protection from cold stress [40].

Despite this general similarity of the dominant orders of bacterial communities at a global scale, a closer inspection at ASV level revealed that the glacier bacterial communities of the different glaciers differed to one another. In addition, the RDA biplot showed that the three small and high elevation glaciers clustered quite close to one another, whereas Perito Moreno and Exploradores were separate from one another and from the other glaciers. This pattern may be due to the geographic distance, the different altitude, or to other ecological and environmental differences among glaciers. Indeed, bacterial communities in high elevation glaciers are exposed to an higher UV radiation and, therefore, to high oxidative stress [3]. Patagonian glaciers are 440 km to one another and c.ca 2000 km from the other glaciers we sampled, which, in turn, are less than 60 km to one another. In addition, it has been demonstrated that the main source of cryoconite bacterial communities is the local environment surrounding the glacier [2]. The high elevation glaciers we sampled are small and above the tree line, and generally in areas with few vegetation. In contrast, the Patagonian glaciers are huge and their tongues (where we collected the samples) is well below the tree line and therefore exposed to very different potential sources of microorganisms with respect of the other glaciers.

The RDA also showed that ΔpH and $\Delta[\text{O}_2]$ significantly explained the variability of bacterial communities of cryoconite holes. We stress that these variables represent the difference between pH and oxygen concentration of each cryoconite hole from the respective mean value of all the holes of that glacier. They therefore do not account *per se* for the difference in mean pH and oxygen concentration among glaciers

highlighted in the analyses discussed above, because in this analysis this effect was accounted by the glacier factor, which accounts for any difference between glaciers. Thus, this analysis suggests that bacterial communities vary consistently according to pH and oxygen concentration gradients present on each glacier, even if different glaciers show on average different pH and oxygen concentration values. In other words, for example, an increase of two pH units seem to determine a similar variation in bacterial communities independently from the absolute pH value, at least in the range of variability of pH values recorded in this study. In addition, no effect of ΔpH on alpha diversity resulted from GLSs.

Analyses on the most abundant orders within each glacier revealed that some order varied according to both pH and $[\text{O}_2]$. pH is known to affect bacterial communities of cryoconite holes [4], but little is known about the mechanisms underlying these relationships. In particular, it is unknown whether and to what extent the bacterial communities contribute to determine water pH. Different studies already investigated the effect of both water and sediment pH on bacterial communities composition, proving that they influence the communities of different types of river sediments [41]. Nonetheless, so far only the decrease of Acidobacteria at basic pH values appears from literature data [41], but was not detected in our study, probably because the pH values of our samples varied from 7.25 to 12.71 i.e. in a range where the abundance of Acidobacteria is always low. Abundance of Rhizobiales showed opposite patterns of variation on different glaciers. On East Iver, they decreased when pH values increased from 8 to about 9, while on Morado they increased with pH from 9 to 12. In contrast, Burkholderiales increased with pH on the East Iver, while on the Exploradores they decreased for higher values. These patterns may suggest that the pH values around 9 limit the abundance of Rhizobiales and promote that of Burkholderiales. Interestingly, the 61% of the Burkholderiales ASVs belonged to the genus *Polaromonas* that normally shows an optimum growth pH of c.ca 6-7 [42], therefore the decline of Burkholderiales with pH on the Exploradores may be because pH valued on that glacier varied between 9 and 12. On the other side any conclusion on the Rhizobiales is even more difficult because the majority of their ASV sequences was unclassified at genus level and this order is quite heterogeneous. In addition, other ecological variables not considered in this study may also concur in determining these patterns.

Cryoconite holes are mostly aerobic environments, particularly in the water, thanks to the high O_2 solubility in cold environments, to the release of air bubbles from the ice that melts because of the presence of the dark sediment and to photosynthesis [43]. On these glaciers, we observed on average lower oxygen concentrations in high altitude cryoconite holes probably because water oxygen depends on the equilibrium with the atmospheric oxygen. In addition, variation in oxygen concentration seems to play a role in explaining bacterial community structures. In particular, at order level, Actinomycetales decreased at increasing values of oxygen concentrations on both Morado and Perito Moreno glaciers, thus showing similar trends on both high and low elevation glaciers, whit large differences of absolute values of $[\text{O}_2]$. Interestingly, no differences in Cyanobacteria abundance were observed, indeed it would be expected that their abundance would have increased with $[\text{O}_2]$ because of their photoautotrophic activity, but no evidence emerged about it. Furthermore, a previous study found that their abundance was higher when also $[\text{O}_2]$ was higher [14], and in this study an effect of oxygen concentration on the whole bacterial

community of cryoconite holes was reported. Anyway, the mechanisms that link oxygen concentration in the water and bacterial community structure in the sediment are not easy to explain.

The Iver and East Iver glaciers are the two geographically closest glaciers (only 1.2 km apart), and they show also similar pH and $[O_2]$ values (Fig. 1a-b) and similar alpha diversity values. In addition, their bacterial communities, although significantly different, were close to one another in the RDA biplot. Interestingly, also the Morado glacier clustered close to them in the RDA biplot, even if it is about 60 km from them. In contrast, the two Patagonian glaciers are separated to one another and from the other glaciers on the same plot. These results, on the one side, support the hypothesis that altitude and the geographic position play an important role in defining bacterial community composition. On the other side, however, the three small glaciers in central Andes are also at similar elevation and they are surrounded by very similar environments (R. Ambrosini and F. Pittino, personal observation). These three high mountain glaciers have also an important input of black carbon of Santiago [38]. Their similarity can therefore derive also from being exposed to the same general ecological conditions, including high UV radiation and oxidative stress, and probably to similar sources of bacterial communities. These results therefore highlight that correlative studies like the present ones can hardly disentangle the effects of geographical positions and ecological conditions on the structure of cryoconite hole bacterial communities, and further studies should be designed to add insight into this still open question.

Analyses of alpha diversity indicated that cryoconite holes on Exploradores glacier showed the highest richness and evenness. Samples on the Exploradores were collected close to the glacier terminus, surrounded by a rich evergreen broadleaf vegetation, and in an area with abundant supraglacial debris and frequented by tourists. The higher biodiversity of this large, low altitude glacier, compared to that than of the small, high altitude Iver and East Iver glaciers is not surprising, as the rich evergreen broadleaf forest that surrounds the glacier tongue can be the source of a richer and more diverse bacterial community than the bare ground surrounding the other glaciers. However, it is more surprising that the alpha biodiversity of the large, low altitude Perito Moreno was intermediate and similar to that of Morado glacier. Interestingly, Perito Moreno was the southernmost glacier among those we collected, and was surrounded by a less diverse forest, dominated by southern beeches, *Nothofagus* ssp. than that of Exploradores, while Morado was the glacier where samples were collected at the lowest altitude among the three glaciers near Santiago. We may therefore speculate that a general gradient related to general altitude and climate conditions of the area surrounding the glacier may somehow affect its biodiversity. For instance, among the most abundant orders, Cytophagales and Burkholderiales were more abundant on high than on low elevation glaciers (Fig. 5a-d). A similar pattern was observed for the Actinomycetales (Fig. 5b) with the only exception that on the Morado Glacier, that is at a lower elevation than Iver and East Iver, their abundance was intermediate between low and high elevation glaciers. Burkholderiales are a quite heterogeneous order, but diverse members showed resistance to stressful conditions including oxidative stress and also degradation of aromatic compounds [44]. Interestingly, 61.6% of Burkholderiales sequence in our samples belong to the genus *Polaromonas*, a bacterium well adapted to glacier environments [45]. Probably, *Polaromonas* was more abundant on high altitude glaciers because

it well adapted to these harsh environments where competition is low, also because of its ability to survive long range atmospheric transport and thanks to its versatile metabolism (can use both organic and inorganic electron donors) [45]. Cytophagales are Gram negative bacteria [46], that are known to be less resistant to UV radiation than Gram positive bacteria [47]. In addition, 99% of Cytophagales sequences belong to Cytophagaceae (data not shown), an heterotrophic family composed of mostly aerobic bacteria able to use cellulose as carbon source [48]. This result is unexpected and difficult to explain because their relative abundance is higher in high elevation glaciers that are located in an area poor in vegetation. The order Actinomycetales, on the other hand, is composed by Gram positive bacteria [49], and their higher relative abundance in high elevation glaciers is consistent with their resistance to UV radiation and the fact that they have been isolated at 3149 m a.s.l. in the Central Andes mountains in Valparaiso Region [40].

Bacterial communities of cryoconite holes show seasonal variation on temperate mountain glaciers [10], therefore it may be argued that the differences we observed in the community structures among glaciers may be due, at least partly, to differences in the stages (sera) of the seasonal ecological succession present on each glacier when we collected the samples. In other words, one may argue that the bacterial communities were identical on all glaciers at the beginning of the melting season and changed seasonally according to identical ecological succession, but they appear different in our samples because we collected them at different stages. Despite we acknowledge that this process may contribute to the observed variability among glaciers, we consider this effect minor, and we confidently suggest that the differences we observed depend mostly on spatial variation and on variation in the general ecological conditions of the glacier and of the surrounding environments (mostly altitude). Furthermore, there may be an effect due to the proximity of high elevation glaciers to the city of Santiago that is a highly polluted and urbanized area and may have an important impact on the bacterial communities composition, since it is well known that pollutants can be subjected to atmospheric transport and the high rate of precipitation facilitate their deposition on glaciers surfaces [50].

In summary, we provide the first-ever description of the bacterial communities of cryoconite holes of glaciers in South America, specifically in the Southern Andes. This study thus fills an important gap of knowledge as almost no information was previously available on the cryoconite holes of South America, and opens the possibility of future biogeography analyses including samples from almost every important glacial area of the world. The five glaciers we investigated are still a too small sample for thoroughly assessing the ecological processes that control cryoconite hole bacterial communities, but it can be the basis for further investigations aiming at a deeper understanding of these extreme environments.

Declarations

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Competing interests

The authors declare that they have no conflict of interest.

Author Contributions

FP participated to sampling, molecular analyses and statistical analyses, RA participated to sampling and statistical analyses, RSA, PA and AF participated to sampling, all the authors discussed the results and contributed to the final manuscript.

Data availability

Sequences were submitted to NCBI (<http://www.ncbi.nlm.nih.gov/bioproject/745072>).

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Figures

Figure 1

Location Map of Andean glaciers located in Chile and Argentina analysed in this report. Glacier limits and morphological parameters were obtained from the Randolph Glacier Inventory 6.0 (RGI Consortium 2017).

Figure 2

Boxplots of pH (a) and oxygen concentration (b) of cryoconite holes in the investigated South American glaciers. The thick lines represent the median, boxes upper and lower limits the 25th and the 75th percentiles respectively, whiskers the data that go beyond the 5th and the 75th percentile, dots represent the outliers and different letters indicate significant differences between glaciers at post-hoc tests.

Figure 3

Barplot showing the relative abundance of bacterial orders in the cryoconite holes on each glacier. Orders representing < 1 % of sequences in each sample were grouped in “others”. Cyanobacteria are reported as phylum because the rdp classifier does not provide their classification at order level.

Figure 4

Correlation biplot from RDA on Hellinger-transformed bacterial ASVs abundances of all the samples according to glacier (five level factor), pH and oxygen concentration centred to their mean value per glacier. The green arrow indicates increasing values of oxygen concentration in each glacier, while the violet one increasing pH values in each glacier. The percentage of variance explained by each axis and their significance (***: $P < 0.001$) is reported. r_M is the Mantel correlation coefficient between the Hellinger distances between samples and the Euclidean distances between the corresponding symbols in the graph. Values close to one indicate that the graph correctly represents the distance between samples.

Figure 5

Boxplots of the relative abundances of Burkholderiales (a), Actinomycetales (b), Pseudomonadales (c), Cytophagales (d), Rhizobiales (e), Sphingobacteriales (f), Bacteroidales (g), Sphingomonadales (h) and Rhodospirillales (i) on the five glaciers where we collected cryoconite samples. The thick lines represent the median, boxes upper and lower limits the 25th and the 75th percentiles respectively, whiskers the data

that go beyond the 5th percentile (lower whisker) and the 75th percentile (upper whisker), dots represent the outliers and different letters indicate differences between the mean values of different groups.

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