



Microbial Communities of High-Elevation Fumaroles, Penitentes, and Dry Tephra “Soils” of the Puna de Atacama Volcanic Zone

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Abstract

The aim of this study was to understand the spatial distribution of microbial communities (18S and 16S rRNA genes) across one of the harshest terrestrial landscapes on Earth. We carried out Illumina sequencing using samples from two expeditions to the high slopes (up to 6050 m.a.s.l.) of Volcán Socompa and Llullaillaco to describe the microbial communities associated with the extremely dry tephra compared to areas that receive water from fumaroles and ice fields made up of nieves penitentes. There were strong spatial patterns relative to these landscape features with the most diverse (alpha diversity) communities being associated with fumaroles. Penitentes did not significantly increase alpha diversity compared to dry tephra at the same elevation (5825 m.a.s.l.) on Volcán Socompa, but the structure of the 18S community (beta diversity) was significantly affected by the presence of penitentes on both Socompa and Llullaillaco. In addition, the 18S community was significantly different in tephra wetted by penitentes versus dry tephra sites across many elevations on Llullaillaco. Traditional phototrophs (algae and cyanobacteria) were abundant in wetter tephra associated with fumaroles, and algae (but not cyanobacteria) were common in tephra associated with penitentes. Dry tephra had neither algae nor cyanobacteria but did host potential phototrophs in the Rhodospirillales on Volcán Llullaillaco, but not on Socompa. These results provide new insights into the distribution of microbes across one of the most extreme terrestrial environments on Earth and provide the first ever glimpse of life associated with nieves penitentes, spire-shaped ice structures that are widespread across the mostly unexplored high-elevation Andean Central Volcanic Zone.

Keywords Water availability · Nieves penitentes · *Naganishia* · Snow algae · Astrobiology · Altiplano

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Introduction

In the last several decades, environments once thought to be devoid of life have been revealed to be populated by varying levels of biodiversity, supporting the almost inescapable notion that life, at least on Earth, always finds a way. Life in Earth's most extreme environments has been uncovered from the depths of the oceans [1] and terrestrial subsurface [2], to the high reaches of the stratosphere [3] and terrestrial mountains [4, 5], and from the freezing poles [6] to sweltering active geologic features [7]. Indeed, life has been found anywhere liquid water is at least occasionally available.

Investigations into microbial communities and water availability in extreme Earth environments serve an important role in determining habitability parameters not only for life on Earth but also assist in the search for extraterrestrial life [8]. The most extreme Earth environments, in terms of water

availability, are hot and cold deserts which make up as much as 40% of the Earth's terrestrial surface [9–11]. Investigations in Antarctica have shown that water availability, as soil moisture or relative humidity, may be the most limiting factor for life there [12–14]. In the Atacama Desert, potentially the world's driest and oldest desert [15], investigations into water availability are of particular interest. Studies in the warm, low-elevation, hyper-arid core of the Atacama have described dry limits to photosynthetic life [16] and potential non-phototrophic primary production [17] and the impact of water availability in concert with UV radiation on epilithic microbial communities [18]. How microbial communities utilize fog [19] and other atmospheric moisture [20, 21] has been explored in low-elevation areas of the Atacama as well.

In a review of the microbial research conducted in the Atacama region, Azua-Bustos et al. [19] suggested the following was a substantial unanswered question: “What is the minimum amount of water required for microbial life in this environment, where does it come from, and in which form is it useful?” While the lower elevations of the hyper-arid core provide excellent insight into questions of aridity, with a mean annual air temperature of ~ 16.5 °C [18], they do not provide for the combination of cold temperatures and limited water availability found in the barely studied highest elevations of the Andean Central Volcanic Zone (14°S to 28°S). This plateau stretches 1800 km from southern Peru into northern Argentina and Chile and is divided into two segments: the Altiplano in the north and the Puna in the south [22, 23]. The Puna's mean elevation (4400 m.a.s.l.) is higher than the Altiplano (3800 m.a.s.l.) [24], and the Puna de Atacama Desert is an area of extremes combining very dry (0–50 mm mean annual precipitation) and very cold (0 °C mean annual air temperature) conditions [25]. Furthermore, the Puna de Atacama features volcanoes that reach elevations approaching 7000 m.a.s.l. and are the highest elevation volcanoes on Earth [22]. Compared to lower-elevation areas of the Puna, in addition to colder and drier conditions, these volcanoes feature a thinner atmosphere and higher solar radiation [26], extremely low levels of atmospheric water [27], near undetectable levels of soil nutrients, and dramatic soil temperature fluctuations (-10 to 56 °C on a summer day) resulting in one of the most inhospitable ecosystems on Earth [28, 29]. Considering these conditions, along with the lack of coastal fog and atmospheric moisture found in the lower-elevation regions of the Atacama further west, the highest reaches of these volcanoes are one of the best earthly analogs for conditions on Mars [30, 31].

The study described here is a continuation of previous work on two volcanoes in this desolate region: Volcán Socompa (Fig. 1a) and Volcán Llullaillaco (Fig. 1d). Both volcanoes reach elevations above 6000 m.a.s.l. and are 50 km apart. The first scientific investigation of macroscopic communities on Socompa was conducted by Halloy [32]. More recently, Costello et al. [28] described the microbial

communities of soils associated with a fumarole (at 5825 m.a.s.l.) and compared them to non-fumarolic tephra (5235 m.a.s.l.) and showed the fumarolic soil supported a much more complex, higher biomass microbial community than the non-fumarolic tephra at lower elevation. On nearby Volcán Llullaillaco (where there are no active fumaroles), Lynch et al. [29] carried out the first modern molecular studies of terrestrial substrates above 6000 m.a.s.l. and described some of the lowest diversity microbial communities yet detected on Earth. Subsequent work has shown that the dominant 18S phylotype from soils above 6000 m.a.s.l. can grow during extreme diurnal freeze-thaw fluctuations (-10 to $+27$ °C) in both volcanic tephra substrate and in pure culture [33].

This study expands on the previous studies of Socompa and Llullaillaco by sampling tephra associated with penitentes in addition to fumarolic and barren (dry) tephra. Nieves penitentes are spire-shaped ice formations that are known to occur in the drier regions of the Andes [34, 35] and Pamir and Hindukush ranges of Central Asia [36, 37]. Penitentes are the only other potential semi-permanent sources of water (besides fumaroles) on these volcanoes and therefore are the areas where more complex microbial communities would be expected to develop compared to the hyper-arid tephra that covers well over 99% of the surface of these mountains. Surprisingly, there have been no previous studies of microbes associated with penitentes, despite their broad distribution in the southern Andes [38]. Here, we describe novel microbial communities that occur in soils within a penitente field on Socompa at the same elevation (5825 m.a.s.l.) as the fumarole described by Costello et al. [28] and compare that community to new samples of the fumarolic and dry soils at the same elevation. We further compare the microbial communities of these sites to a higher elevation fumarole and adjacent dry site near the summit of Socompa (6051 m.a.s.l.) and to an array of newly sampled (non-fumarolic and penitentes) sites on the west face of Volcán Llullaillaco (Fig. 1).

Overall, our results show that there are significantly different community types in dry, fumarolic, and penitentes sites and that elevation and which mountain the samples were from were not significant predictors of community structure. In addition, samples from dry sites (at all elevations on both mountains) had very low alpha diversity, had low beta diversity (clustered together), and did not contain cyanobacteria or algae. In contrast, wetter soils near penitentes contained algae, but no cyanobacteria, whereas fumarole soils contained moss, algae, and cyanobacteria. Taken together, our results paint a picture of life in the so-called permafrost belt [25] or aeolian zone [39] of the high elevation landscape of the Atacama region, showing that there are small pockets of photosynthetic life near rare sources of water (fumaroles and penitentes) in a vast, barren landscape that otherwise supports opportunistic

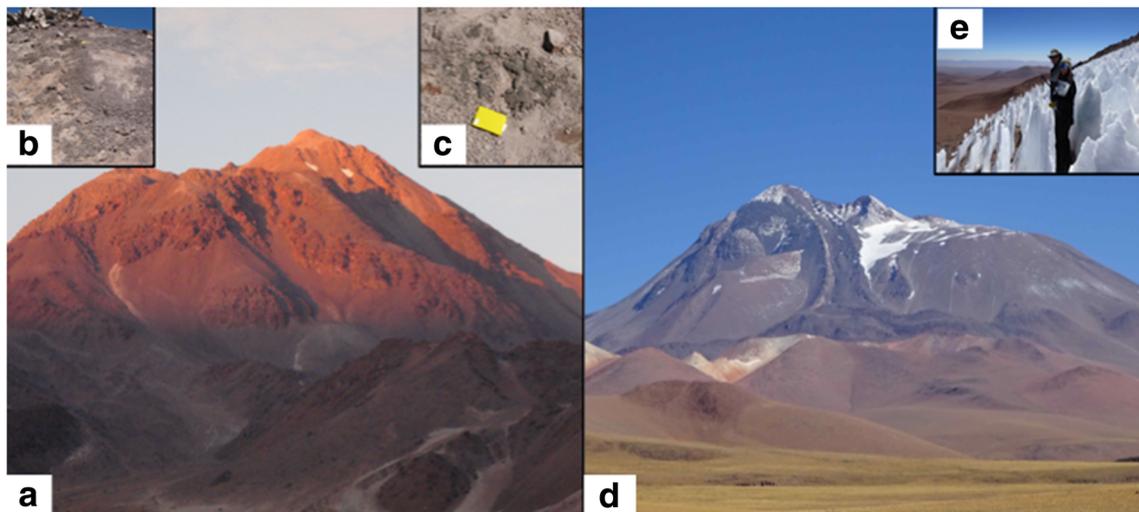


Fig. 1 **a** Photograph of Volcán Socompa (6051 m.a.s.l.) taken from Socompa Pass on February 8, 2009. **b** Photograph of the Socompa dry sample location near the summit at 6050 m.a.s.l. **c** Photograph of the Socompa fumarole near the summit at 6049 m.a.s.l., notice the moss next to the yellow notebook. **d** Photograph of Volcán Llullaillaco

(6723 m.a.s.l.) taken from northwest of Llullaillaco on March 6, 2016. **e** Photograph of the Llullaillaco penitentes at 5277 m.a.s.l., Jack Darcy and Lara Vimercati are pictured for scale. Photos by S.K. Schmidt and P. Sowell

heterotrophs that can likely only become active following rare snowfall events [33].

Materials and Methods

Site Description and Sampling

Volcán Socompa (6051 m.a.s.l., 24°23'S 68°14'W) and Volcán Llullaillaco (6723 m.a.s.l., 24°43'S 68°32'W), the second highest active volcano in the world, are complex strato-volcanoes on the Chile-Argentina border (Fig. 1). The geology of both volcanoes is described elsewhere [40–43].

Fifteen soil samples from Socompa were collected during the austral summer on February 11, 2009. Samples were collected to a depth of 4 cm at distances of ~5 m apart at each sampling site following the protocols of King et al. [44]. To factor out elevation as a variable, we first sampled three soil types: dry, fumarolic, and penitente at one elevation (5825 m.a.s.l.). The fumarolic ground at this site has been referred to as “warmspot 2” by previous researchers [28, 32]. In addition to this “natural” experiment at 5825 m.a.s.l., we also sampled fumarolic and dry soils (6049 m.a.s.l.) near the summit of Socompa on the same day. The fumarolic (Fig. 1c) soils are where hydrothermal gases (including water vapor) are passing through volcanic tephra resulting in increased temperature, soil moisture, and carbon levels [28]. The penitente soils (Fig. 1e) are areas where ice formations called penitentes (nieves penitentes) periodically release meltwater and cause the soils to be moist. Penitentes form when snowfields are subjected to the unique combination of high radiation and dry winds causing differential ablation that leads to formation of spire-like ice

pinnacles [45, 46]. The penitentes on Socompa were roughly 1–2 m in height. The dry soils sampled have soil moisture and nutrient levels near or below detection limits and serve as “controls” for the effects of fumaroles and penitentes. All soils were frozen in the field at $-10\text{ }^{\circ}\text{C}$ and transported to the lab where they were stored at $-70\text{ }^{\circ}\text{C}$ until DNA extractions were done (see below).

On Llullaillaco, soils were sampled during the austral summer of 2016 between March 8 and March 12, with the same protocol as on Socompa [44]. Ten samples were collected within and just downhill from a field of ice penitentes at 5277 m.a.s.l. (24°43'40" S, 68°34'20" W). Eighteen additional samples were taken from dry soils between 5100 and 5650 m.a.s.l. which we term “Llullaillaco dry.” There are no known active fumaroles on Llullaillaco. Also, it should be noted that while we use the word “soils,” the soils on these mountains are not “normal” soils that make up most terrestrial ecosystems in that there is very little to no organic matter (except near the fumaroles); these soils are made up of gravels and fine pyroclastic debris and are officially known as tephra.

DNA Extraction and Sequencing

Total DNA was extracted from ~0.3 g of soil subsamples with a Power Soil DNA Isolation Kit (MOBIO Laboratories, Carlsbad, CA, USA) and used for amplification and subsequent sequencing of 16S (Bacteria and Archaea) and 18S (Eukaryota) gene markers. Each sample was amplified twice with standard primers (515f/806r for 16S and Euk_1391f/EukBr for 18S) as described by the Earth Microbiome Project (<http://www.earthmicrobiome.org/emp-standard-protocols/>). For multiplexing, all forward and reverse

primers were modified to include a unique 12 nucleotide barcode. PCR reaction mixtures contained 0.5 μL of forward primer, 0.5 μL of reverse primer, 1 μL of template, and 12.5 μL of MM Gotaq Hot Start Colorless Master Mix (Promega Corporation, Madison, WI, USA). The reaction volume was adjusted to a total of 25 μL with ultrapure DNase/RNase free water. Thermal cycles consisted of a step at 94 °C for 3 min, followed by 35 cycles of 94 °C for 45 s, 57 °C for 60 s, and 72 °C for 90 s, with a final elongation step of 72 °C for 10 min. Prior to sequencing, amplicons were purified and normalized with Invitrogen SequelPrep Normalization Kit (Invitrogen Inc., CA, USA). All amplicons were combined into a single pool and sequenced using the Illumina MiSeq platform (BioFrontiers Institute, Boulder, CO) using pair-end 2 \times 150 bp chemistry.

Bioinformatics

The Illumina raw sequencing data were processed through QIIME 1.9 [47]. The dataset was subjected to the QIIME 454 default pipeline except for those differences noted below. OTUs (97% identity level) were clustered using UCLUST [48], and all singleton OTUs were removed. Taxonomic assignments utilized the SINA aligner and Silva database version SSU NR 97_119 [49, 50] (accessible at <http://www.arb-silva.de/download/arb-files/>) and the BLAST assigner [51]. FastTree [52] was employed to build the phylogenetic tree. Alpha rarefaction was used to determine the mean richness of the taxa in each of the different sampling habitats with the observed species (OTUs) estimator. The 16S communities were subsampled (rarefied) at a sequence depth of 4710 sequences per sample, and the 18S communities were subsampled to a depth of 7704 sequences per sample.

Beta diversity (as defined by the difference in community composition among sampling units) was calculated through weighted UniFrac [53]. Weighted UniFrac uses phylogenetic distances in addition to shared and unique OTUs between samples to determine the dissimilarity among samples and takes into account relative abundances of each OTU. To visualize the level of clustering in the distance matrices, Principal Coordinate Analysis (PCoA) plots were created using EMPERor [54]. These analyses are standard for comparing microbial community assembly across samples and ecosystems, but caution is warranted in attempting to assign function and absolute abundance based on measures of relative abundances as discussed thoroughly elsewhere [10, 11, 33, 52, 53].

Biogeochemical Analysis

Soil biogeochemical parameters including percent organic carbon, organic nitrogen, dissolved organic carbon, total dissolved nitrogen, pH, and various enzyme assays were measured for the Socompa samples at 5825 m using procedures as described

elsewhere [29, 44, 55]. Soil moisture for all samples was measured using gravimetric procedures as described elsewhere [28].

Statistical Analyses

Statistical significance of beta diversity clustering between samples was determined with permutational multivariate analysis of variance (PERMANOVA) with function ADONIS; test of homogeneity of multivariate dispersion (PERMDISP) was determined with function BetaDisper; and alpha diversity significance was determined by ANOVA with function AOV. The subsequent test of homogeneity of dispersion was used to parse out whether the difference supported by ADONIS was due to differences between clusters or differences between the variations within clusters. All statistical work were performed using the Vegan package of *R* [56]. To analyze the 2009 data from Socompa, two main factors were considered in analyzing the data-habitat type (fumarole, penitente, dry) and elevation (5825 or 6049 m). A separate analysis was done to compare the data from Socompa (2009) to the data from Llullaillaco (2016). For this analysis, three factors were considered in analyzing the data-habitat type (fumarole, penitente, dry), elevation (5100–6049 m), and site (Socompa, Llullaillaco). It should be noted that site is confounded with elevation and time as all samples on Socompa were 5825–6049 m and all samples on Llullaillaco were 5100–5650 m, and all Socompa samples were from 2009 and all Llullaillaco samples were from 2016.

Results

The first question we asked was whether fumaroles and penitentes significantly affect the diversity of microbes on Volcán Socompa compared to dry sites at the same elevations (5825 or 6049 m.a.s.l.). Figure 2a shows fumaroles at both 5825 and 6049 m.a.s.l. had significantly higher ($p < 0.0001$, $F = 33.7$) 18S OTU richness (alpha diversity) than dry or penitente samples from the same elevations. Likewise, 16S OTU richness was also significantly higher ($p < 0.002$, $F = 15.8$) in the fumarole soils (Fig. 2b). Fumaroles contained 587 and 803 16S OTUs at 5825 and 6049 m, respectively (Fig. 2b), while all the non-fumarole sites had many fewer OTUs, i.e., 61, 147, 224 OTUs, for the dry (6050 m), penitentes, and dry (5825 m) sites, respectively. For 18S communities, both fumarole sites contained over 300 observed OTUs (fumarole 5825 m—326 OTUs; fumarole 6049 m—319 OTUs), whereas all the non-fumarole sites had 107 or fewer (dry 5825 m—101 OTUs; dry 6050 m—83 OTUs; and penitentes—107 OTUs) (Fig. 2a).

Using the same “natural experiment” on Volcán Socompa, our data also show that despite the fact that penitentes did not increase alpha diversity compared to dry sites (Fig. 3), they did significantly affect community structure (beta diversity) of

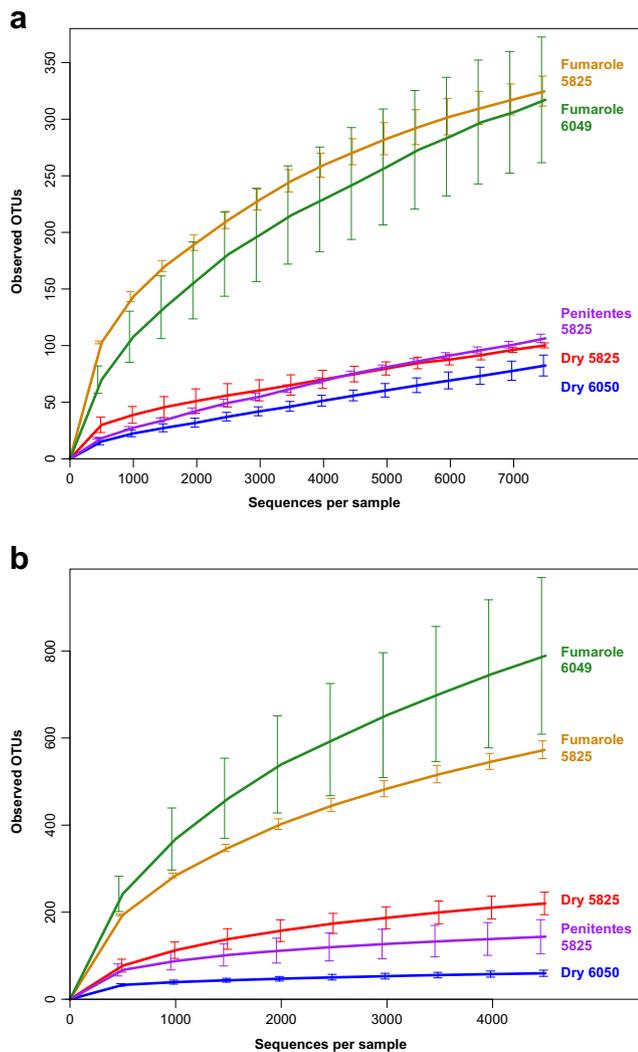


Fig. 2 Alpha rarefaction curves based on the number of OTUs observed for fumaroles, penitentes, and dry sites on Socompa: **a** 18S and **b** 16S. The fumarole communities had 3× the number of 18S and 16S OTUs compared to the penitente fields and the dry soils (18S ANOVA $p < 0.0001$, $F = 33.7$), (16S ANOVA $p < 0.002$, $F = 15.8$)

the 18S communities (Fig. 3, $p < 0.001$, $R^2 = 0.483$). In contrast, 16S microbial communities did not show significant clustering by habitat type across the three habitats (Supplemental Fig. S1).

In 2016, we expanded our analyses to include penitentes and dry sites on Volcán Lullaillaco. In general, microbial communities in the penitente fields on Lullaillaco were more diverse than those in any sites on Socompa for both 18S (ANOVA $p < 0.005$, $F = 5.548$) and 16S (ANOVA $p < 0.0002$, $F = 10.11$) (Fig. 4a, b), but it should be noted that the sites on Lullaillaco were at lower elevations and sampled in a different year than those on Socompa. Nonetheless, analyses of 18S beta diversity showed clustering of communities based on habitat (dry vs. penitentes) (ADONIS $p < 0.001$, $R^2 = 0.35$), and a significant interaction between habitat and mountain or elevation (ADONIS $p < 0.001$, $R^2 = 0.12$)

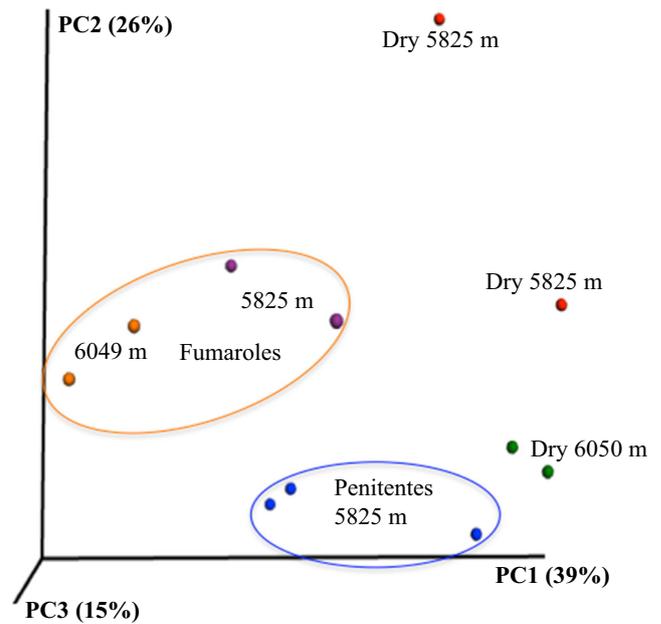


Fig. 3 PCoA plot depicting clustering of 18S microbial communities on Socompa. The plot is a visualization of a weighted UniFrac distance matrix. Fumarole 6049 m = orange, Fumarole 5825 m = purple, Penitentes 5825 m = blue, Dry 5825 m = red, and Dry 6050 m = green. The blue oval surrounds samples from the penitente field. The orange oval surrounds samples from the two fumarole sites. (ADONIS $p < 0.001$, $R^2 = 0.483$)

(Fig. 5). The test for homogeneity of dispersion was not significant (PERMDISP: $p < 0.091$, $F = 3.13$), indicating that the main factor driving community signatures was the difference between habitat types rather than that within habitat types (Fig. 5). In contrast, 16S beta diversity showed significant clustering by site/elevation (site, $p < 0.001$, $R^2 = 0.19$; elevation, $p < 0.001$, $R^2 = 0.19$) and not habitat type (penitentes vs. dry) (Supplemental Fig. S2). The test of homogeneity of dispersion was not significant (PERMDISP: $p < 0.948$, $F = 0.004$), indicating that the difference in composition was by site/mountain (Supplemental Fig. S2).

The most conspicuous difference between penitente and dry microbial communities was the presence of photosynthetic eukaryotes (algae) in tephra near penitentes and their absence in dry tephra on both Socompa and Lullaillaco (Fig. 6). Dry sites were dominated by a basidiomycete yeast related to *Naganishia friedmannii* (formerly *Cryptococcus friedmannii*). No cyanobacteria or other traditional photosynthetic bacteria were found in either dry or penitente-associated tephra on either volcano, but some potential photosynthetic bacteria in the Rhodospirillales and genus *Sphingomonas* were found in dry and penitente sites on Lullaillaco (Fig. 7).

The determination of water availability through gravimetric soil moisture analysis revealed the Socompa penitentes fields to contain the highest moisture (mean = 24.5%, SE = 1.9), with the Lullaillaco penitentes (mean = 11.6%, SE = 2.3) and Socompa fumaroles (mean = 11.5%, SE = 2.7) close in moisture, and the

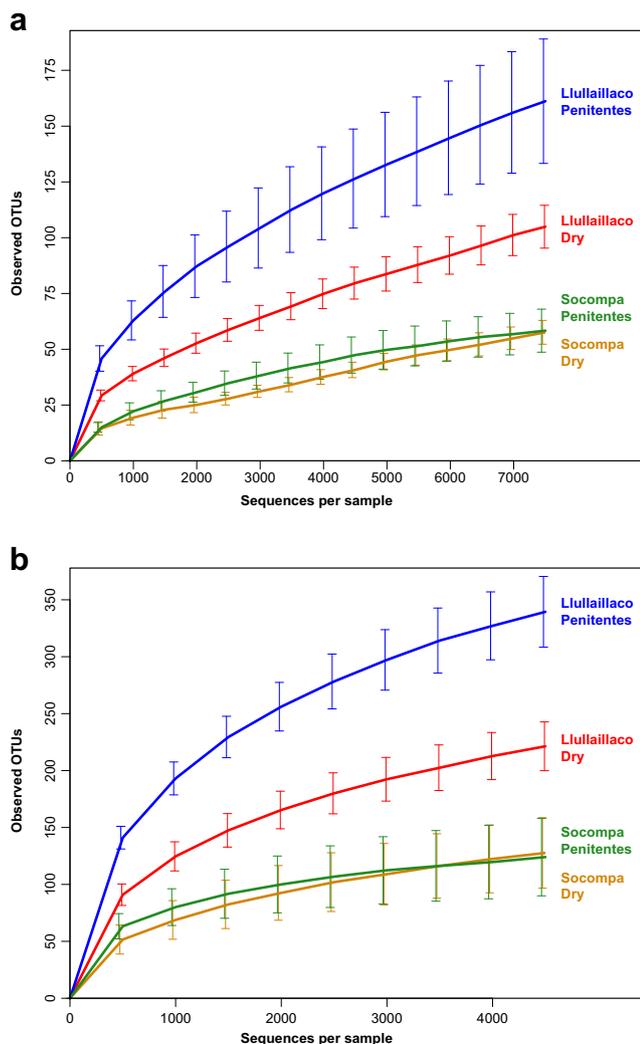


Fig. 4 Alpha rarefaction curves based on OTUs observed for penitentes and dry sites on both Socompa and Lulluailaco: **a** 18S and **b** 16S. The penitentes fields of Lulluailaco had 3× the number 18S and 16S OTUs compared to the penitentes and dry soils on Socompa. 18S (ANOVA $p < 0.005$, $F = 5.548$); 16S (ANOVA $p < 0.0002$, $F = 10.11$)

two dry habitats Socompa Dry (mean = 2.8%, SE = 1.3) and Lulluailaco Dry (mean = 1.2%, SE = 0.6) near lower detection limits (Fig. 8). Statistical tests showed the difference in means to be significant (ANOVA $p < 0.0001$, $F = 16.81$). The remaining biogeochemical analyses for the Socompa sites at 5820 m.a.s.l. are included in Supplemental Table S1. The only statistical differences ($P < 0.05$) in these data were that penitente tephra had higher soil moisture than either the dry or the fumarolic tephra and the fumarolic tephra had significantly higher organic matter content than the dry or penitente tephra.

Discussion

This study combines results of two expeditions to Socompa and Lulluailaco and builds on the findings from a 2005 expedition

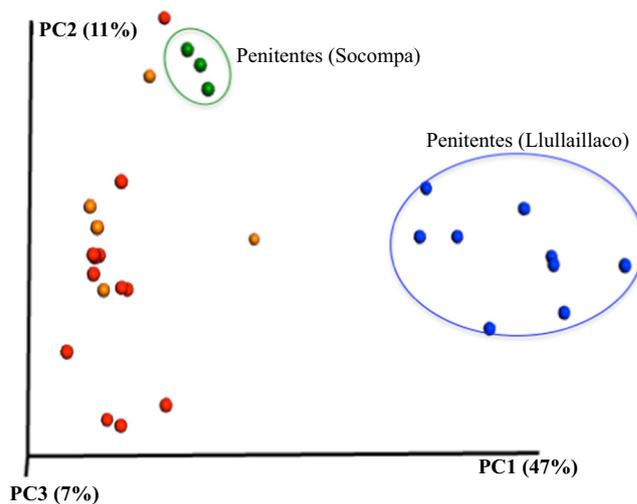


Fig. 5 PCoA plot depicting clustering of 18S microbial communities in the penitentes fields and dry soils from Socompa and Lulluailaco. The plot is a visualization of a weighted UniFrac distance matrix. Socompa dry = orange, Lulluailaco dry = red, Lulluailaco penitentes = blue, Socompa penitentes = green. The green oval surrounds the Socompa penitente samples. The blue oval surrounds the Lulluailaco penitente samples which were clearly different from all other samples. (ADONIS $p < 0.001$, $R^2 = 0.35$)

that focused on fumaroles [28] and a preliminary survey of dry tephra materials (“soils”) on Lulluailaco [29]. During the 2009 expedition to Socompa, we sampled dry, fumarole, and penitente sites at 5825 m.a.s.l. and fumaroles and dry sites at 6050 m.a.s.l. This “natural experiment” allowed us to compare fumaroles and penitentes to dry sites at the same elevations in order to determine if increased water availability (at fumaroles and penitentes) affected microbial diversity. The presence of fumaroles resulted in much higher species richness (18S and 16S) and significantly different communities than dry and penitente sites regardless of the elevation at which samples were taken (Figs. 2 and 3). This finding was not surprising because of the increased temperature and water provided by the fumaroles (Table S1) and corroborates the findings of Costello et al. [28] who compared dry sites at 5235 m.a.s.l. to the fumarole at 5825 m.a.s.l. The high species richness in the fumarolic soils (587 to 803 observed 16S OTUs) puts them on a par with many soils from mesic environments at much lower elevations. For example, Ferrenberg et al. [57] found about 500 OTUs in sub-alpine forest soils and Shen et al. [58] found 572 OTUs in subtropical forest soils. But higher OTU numbers have been observed in agricultural soils (829–955 OTUs) and wetlands (820–1101 OTUs) [59, 60]. The 16S OTU richness within fumarole soils is higher than that in soils of the Dry Valleys in Antarctica (e.g., < 500 [61] and 105–312 16S OTUs [62]). In contrast, the dry sites on Socompa supported the most depauperate communities yet observed. In fact, 16S OTU richness (61 OTUs) at 6050 m.a.s.l. stands, to our knowledge, as the lowest value for OTU richness yet reported for a terrestrial habitat in the high-throughput sequencing literature.

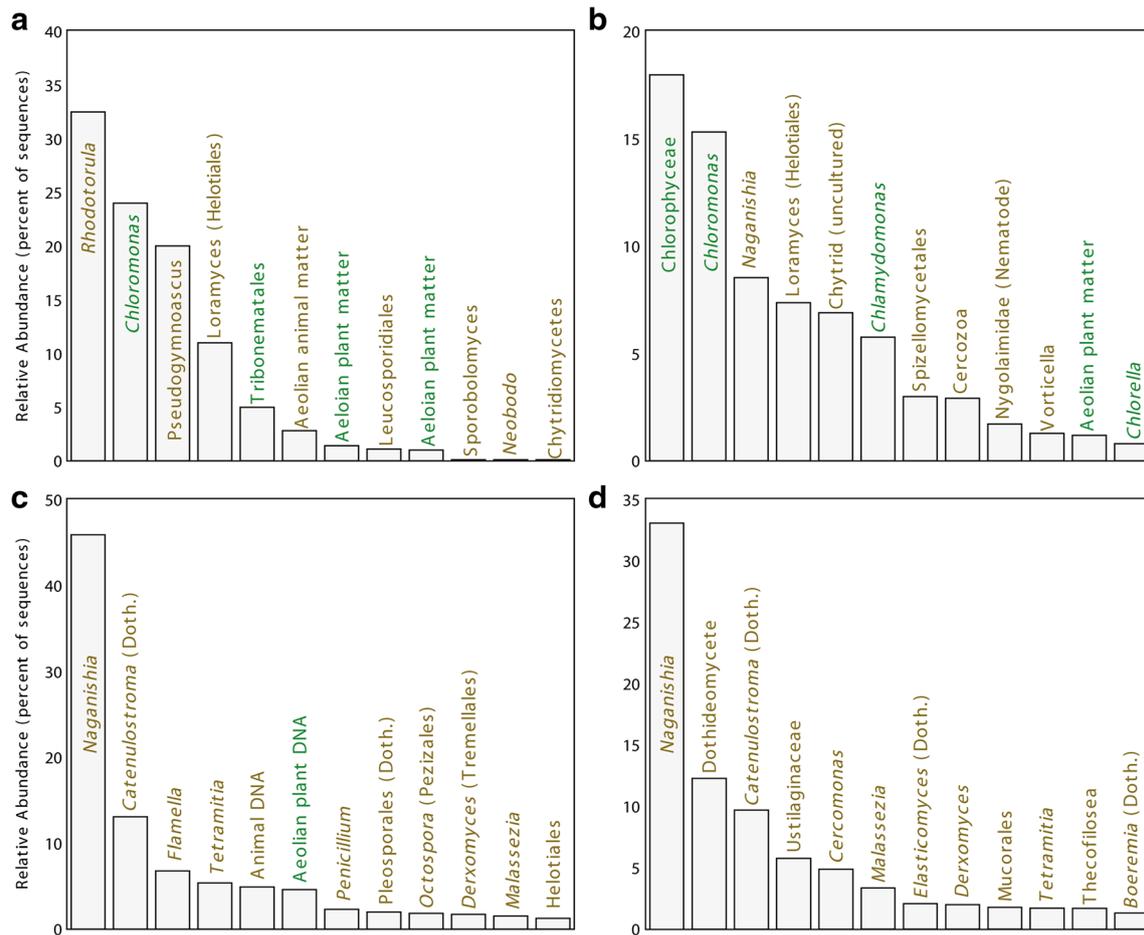


Fig. 6 Rank abundance plots of the mean relative abundance (percentage) of the top twelve 18S OTUs for each habitat: **a** Socompa penitentes, **b** Llullaillaco penitentes, **c** Socompa Dry, and **d** Llullaillaco Dry

Our finding that water alone (from penitentes) did not increase diversity on Socompa differs from the work of Pointing et al. [9] who found that water but not temperature increased microbial diversity across a range of cold and warm deserts in China. It should be noted however that our study sites are much higher in elevation and colder than any previously studied sites, and therefore, higher temperatures (from fumaroles) are likely more important than water availability per se in determining alpha diversity. Despite the fact that penitentes did not increase alpha diversity compared to dry sites at the same elevation on Socompa (Fig. 2), penitentes did significantly affect beta diversity (Fig. 3). The differences were particularly evident for eukaryotic communities providing the first evidence that meltwater from penitentes may be supporting previously un-studied microbial communities at these high-elevation sites. The most striking feature of soil microbial communities receiving penitente meltwater was the presence of green algae (*Chloromonas*) (Fig. 6), whereas no algae or cyanobacteria were found in the dry sites at the same elevation (Supplemental Table S2 and S3). Our results (Fig. 8) are similar to those from studies in the Dry Valleys of Antarctica where soils with less than 5% soil moisture did not

support phototrophic communities [63] and studies in low elevation areas of the Atacama where very dry soils were devoid of phototrophs [19, 64].

To further test the idea that penitentes allow for the development of phototrophic communities, we sampled both penitentes and dry sites above 5100 m.a.s.l. in Llullaillaco National Park (Chile) in 2016. Due to weather conditions, we were unable to sample sites above 5650 m.a.s.l., but nonetheless, these samples constituted the first soil samples associated with penitentes on Volcán Llullaillaco. Overall, the results from Llullaillaco support our results from Socompa in at least two ways. Firstly, based on 18S community structure (beta diversity), the samples near penitentes grouped together to the exclusion of all of the samples from dry sites on Llullaillaco (Fig. 5). And secondly, just as on Socompa, green algae were only detected in soils affected by penitentes (Fig. 6), again supporting the idea that phototrophs can only subsist on these mountains if soil moisture levels are above a certain threshold. As mentioned above, the low level of alpha diversity in the penitente soils on Socompa (Fig. 2) may be related to their elevation (5825 m.a.s.l.) relative to the lower-elevation (5277 m.a.s.l.) penitentes sampled on Llullaillaco,

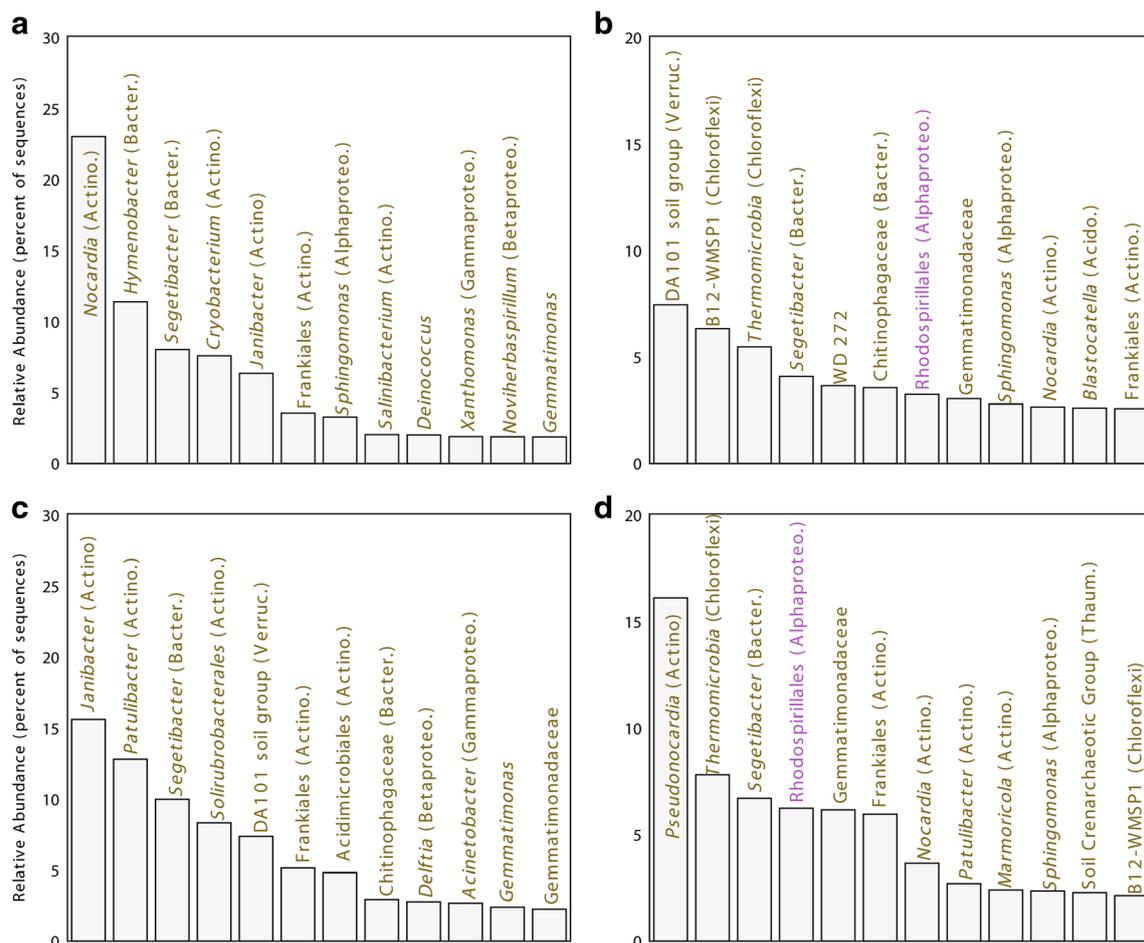


Fig. 7 Rank abundance plots of the mean relative abundance (percentage) of the top twelve 16S OTUs for each habitat: **a** Socompa penitentes, **b** Lulllaillaco penitentes, **c** Socompa Dry, and **d** Lulllaillaco Dry

where the alpha diversity was much higher (Fig. 4). The effects of elevation in the present data set cannot be separated from possible effects of cold and UV radiation, both of which increase with elevation [18, 29, 65]. In the future, more intensive sampling of penitente fields and nearby dry areas across a range of elevations will be required to resolve questions relative to the effects of temperature and moisture as have been done in cold and dry deserts at much lower elevations [9]. Manipulative experiments may also be needed to determine how the change in UV radiation with elevation affects microbial communities.

The presence of algae in soils associated with penitente fields on both Socompa and Lulllaillaco is the first evidence of phototrophy occurring above 5200 m.a.s.l. on these mountains at sites not associated with fumarolic activity. Previous work on both Socompa and Lulllaillaco failed to detect signs of traditional phototrophs (cyanobacteria and algae) at dry sites on either mountain [28, 29, 66] although an analysis by King et al. [55] and Fig. 7 suggest that bacteria in the Rhodospirillales (Alphaproteobacteria) at dry sites on Lulllaillaco may indicate that alternate forms of phototrophy

may occur there. Some Rhodospirillales are “purple photosynthetic bacteria” that can utilize light in the infrared range (e.g., 875 nm) [67] and therefore may be able to grow at deeper soil depths than cyanobacteria or algae (which require shorter wavelengths of light). However, some species in Rhodospirillales are not photosynthetic; therefore, more research is needed to determine if the members of this group in dry soils on Lulllaillaco are actually capable of photosynthesis.

While the presence of algae in soils associated with penitentes suggests that photosynthesis may occur in these soils, it by no means proves it. It is possible that the phototrophs we detected in these soils are “snow algae” that spend part of their life cycle in the soil and part in the ice and snow of the penitentes themselves. We observed scattered patches of red color in penitentes on Lulllaillaco in 2016. This would constitute the first evidence of snow algae (or any algae) in penitentes but requires microscopic or sequencing results from red snow patches to confirm this hypothesis. However, the *Chloromonas* sp. found in tephra soils associated with penitentes on Socompa (Fig. 6) is closely related to an

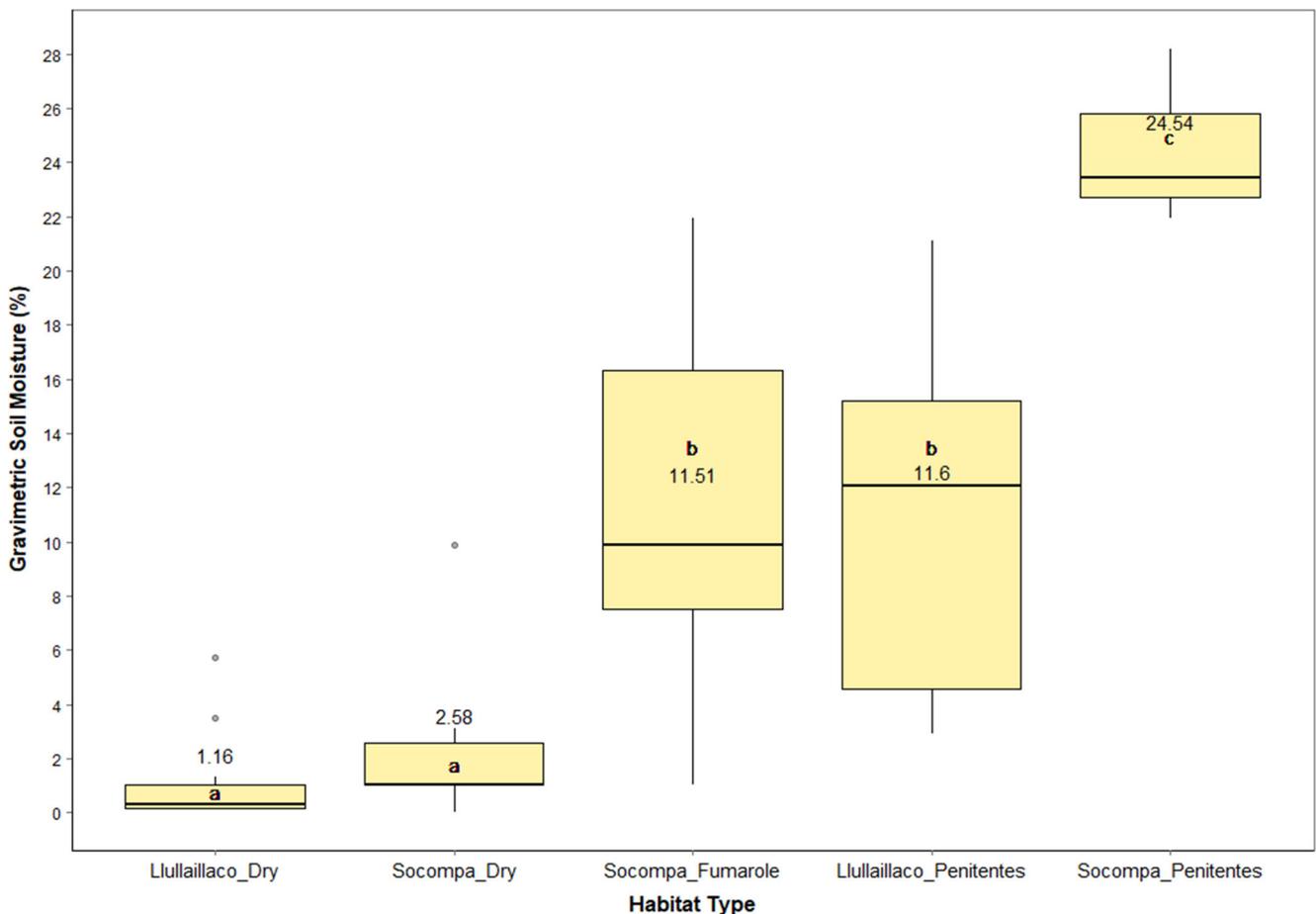


Fig. 8 Soil moisture (%) for each habitat type. The dark horizontal black line inside the box represents the median, with the number located above indicating the mean soil moisture. The long vertical black lines for each

box represent the maximum and minimum moisture recordings and the circles represent outliers. (ANOVA $p < 0.0001$, $F = 16.81$). Boxes with the same letter are not significantly different

Antarctic (Langhovde) snow alga (accession no. AB903007), while the *Chloromonas* sp. found in tephra soils associated with penitentes on Lullllaillaco (Fig. 7) is closely related to the snow alga *Chloromonas brevispina* (accession no. KF683610).

The present study also lends strong support to the idea that yeast in the genus *Naganishia* (Basidiomycota) are the dominant eukaryotic life form found in the dry sites that cover more than 99% of the land above 5100 m.a.s.l. on both Lullllaillaco and Socompa [68]. *Naganishia* phylotypes made up over 45% of all 18S phylotypes found in dry soils on Socompa and 32% of all phylotypes in similar sites on Lullllaillaco (Figs. 6 and 7). This is even more amazing because the dry sites sampled in this study ranged in elevation from 5100 to over 6000 m.a.s.l. and were sampled in two different years (2009 for Socompa and 2016 for Lullllaillaco). In addition, previous studies using different primers and Sanger rather than Illumina sequencing also showed a strong dominance of *Naganishia* phylotypes at elevations ranging from 5285 m.a.s.l. on Socompa to over

6300 m.a.s.l. on Lullllaillaco [28, 29]. Evidence from recent ecophysiological studies show that the *Naganishia friedmannii* from Lullllaillaco can grow exponentially during diurnal freeze-thaw cycles (-10 to $+30$ °C) in the lab, indicating that it has the potential to grow during the extreme freeze-thaw dynamics that occur year-round at elevations above 5100 m on Socompa and Lullllaillaco [33].

Besides the Rhodospirillales (discussed above), there was little evidence of phototrophic bacteria in dry tephra or tephra associated with penitentes on either Socompa or Lullllaillaco (Fig. 7). Instead, the bacterial communities of these two areas on Socompa were dominated by Actinobacteria and Bacteroidetes (Fig. 7a, c), whereas the penitente community on Lullllaillaco was dominated by Verrucomicrobia (DA101 soil group) and Chloroflexi (B12-WMSP1) (Fig. 7b). Finding DA101 in tephra associated with penitentes is consistent with the worldwide distribution of this group in soils [69] and related phylotypes being among the dominant 16S phylotypes in fumarolic and dry tephra soils on Socompa [28]. In addition, the high relative abundance of B12-WMSP1 (a

ctedonobacterium) at this site is consistent with the previous description of this group in cold, water-saturated, high-elevation soils [70] and as the dominant phylotype at the highest elevation ever sampled (6330 m.a.s.l.) on Llullaillaco [29]. In contrast, the dominant phylotype at dry sites in the present study was an actinobacterium related to *Pseudonocardia*, consistent with previous work showing its abundance at 6034 m.a.s.l. [29]. Subsequent metagenomic evidence [66] indicates that this *Pseudonocardia* sp. has the metabolic machinery to subsist on trace levels of carbon monoxide and other gases from the atmosphere or from fumaroles.

Finally, it is interesting to note that on Llullaillaco, the 16S community was structured largely by site/elevation rather than by habitat type, whereas the 18S community structure could be explained by habitat type (dry vs. penitente). Our working hypothesis for this difference is that the 18S community is largely structured by the micro-eukaryotes that make up unique snow algae communities of the penitentes, whereas the 16S community may not be controlled by the presence of ice, but very little is known about bacterial snow communities, especially in high-elevation ecosystems [71, 72]. It is also possible that the 16S community is largely made up of dormant or dead wind-transported cells and that they are being sorted by elevation and site due to differential settling rates from the atmosphere. Obviously, much more work is required to determine which organisms are actually active in these sites [66, 68].

Conclusions

The present study builds on past studies of high elevation sites on Llullaillaco and Socompa and suggests for the first time that penitentes are an intermittent source of water that supports photosynthetic organisms not present in drier soils on these mountains. Future investigations are needed to confirm whether the algae found in tephra soils near penitentes are snow algae that spend part of their life cycle in the penitentes or whether they just reside in the soils dampened by runoff from penitentes. This study also broadens the evidence for yeast related to *Naganishia friedmannii* being the dominant heterotrophic organism across large areas of dry soils on these high volcanoes [28, 29] and on other volcanoes such as Volcán Saírecabur further to the north [65]. Future studies will focus on determining the relative contributions of aeolian deposited organic matter and photosynthesis by purple photosynthetic bacteria to the functioning of these extreme high elevation ecosystems.

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