Fumarole-Supported Islands of Biodiversity within a Hyperarid, High-Elevation Landscape on Socompa Volcano, Puna de Atacama, Andes^{∇†}

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Fumarolic activity supports the growth of mat-like photoautotrophic communities near the summit (at 6,051 m) of Socompa Volcano in the arid core of the Andes mountains. These communities are isolated within a barren, high-elevation landscape where sparse vascular plants extend to only 4,600 m. Here, we combine biogeochemical and molecular-phylogenetic approaches to characterize the bacterial and eucaryotic assemblages associated with fumarolic and nonfumarolic grounds on Socompa. Small-subunit rRNA genes were PCR amplified, cloned, and sequenced from two fumarolic soil samples and two reference soil samples, including the volcanic debris that covers most of the mountain. The nonfumarolic, dry, volcanic soil was similar in nutrient status to the most extreme Antarctic Dry Valley or Atacama Desert soils, hosted relatively limited microbial communities dominated by *Actinobacteria* and *Fungi*, and contained no photoautotrophs. In contrast, modest fumarolic inputs were associated with elevated soil moisture and nutrient levels, the presence of chlorophyll *a*, and ¹³C-rich soil organic carbon. Moreover, this soil hosted diverse photoautotroph-dominated assemblages that contained novel lineages and exhibited structure and composition comparable to those of a wetland near the base of Socompa (3,661-m elevation). Fumarole-associated eucaryotes were particularly diverse, with an abundance of green algal lineages and a novel clade of microarthropods. Our data suggest that volcanic degassing of water and ¹³C-rich CO₂ sustains fumarole-associated primary producers, leading to a complex microbial ecosystem within this otherwise barren landscape. Finally, we found that human activities have likely impacted the fumarolic soils and that fumarole-supported photoautotrophic communities may be exceptionally sensitive to anthropogenic disturbance.

Environments in which hydrothermal systems interact with the arid cryosphere are unique habitats for life on Earth and represent prospective analogs for habitable zones on Mars (45, 53). When these settings occur at high elevation, the accompanying thin atmosphere, intense UV radiation, and barren mineral soils further challenge life and approximate the physical characteristics of Mars. Such extreme environments are found within the remote desert landscape of the south-central Andean plateau, the Puna de Atacama (22 to 27°S), an area hosting some of the highest (>6,000 m) potentially active volcanoes in the world (17). Hydrothermal features, including hot springs, geysers, fumaroles, and fumarolic ground, occur throughout this exceptionally arid, high-elevation region (9) and may support distinctive biotic communities via the localized provision of water and warmth within a vast landscape of desiccation and cold. However, to our knowledge, except for in a single report (22), the biotic communities inhabiting south-central Andean volcanoes, their hydrothermal features, and the surrounding barren soils have not been the subject of study, likely due to the harshness and inaccessibility of the region.

Located at the southeast margin of the Atacama basin, the 6,051-m Socompa Volcano lies within the arid core of the Puna (24 to 25°S). Here, along the western slope of the Andes mountains, the hyperarid Atacama Desert extends up to 3,500 m in elevation, above which climate records for the volcanic peaks to the east, including Socompa, are scarce. In this region, summer precipitation generally occurs as transient snow or hail, winters are cold and dry, and vegetation is sparse and limited to between 3,500 and 4,600 m elevation (4, 22). Mean annual temperatures below −5°C and precipitation of <200 mm are likely for Socompa (4, 25), and the absence of glacial features or permanent snowfields on the mountain is indicative of the arid climate (23). The region is cloud-free throughout much of the year, which, along with the high elevation, contributes to extreme solar total and UV irradiances (39, 44). Socompa's slopes are barren for many square kilometers, as the highest vascular plants in the area are restricted to below 4,600 m elevation.

Fumaroles occur where steam and volcanic gases escape through Earth's crust as a result of magma degassing and/or geothermal heating of groundwater at a shallow depth. Although unlisted among active south-central Andean volcanoes (9, 17), Socompa indeed exhibits fumarolic activity near its summit (6, 22). Fumaroles on Socompa are weakly active and are not known to produce the sulfurous gases, acidic conditions, extreme high temperatures, or obvious plumes of venting steam that are characteristic of many volcanic fumaroles. As a

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FIG. 1. A map of the south-central Andes mountains and Atacama Desert region showing the location of Socompa Volcano on the border between Argentina and Chile. White areas represent major present-day salt deposits.

result, the most conspicuous surface manifestations of hydrothermal processes on Socompa are delicate, mat-like plant communities composed primarily of mosses and liverworts, which are sustained by areas of steam-warmed ground (22). These mat-like communities are isolated within an arid environment of rock and ice, up to 1,451 m above the highest vascular plants in the region, and hundreds of kilometers away from predicted sources of diaspores for many of the species (22). These fragile, carpet-like assemblages are biologically unique in the context of their surroundings and are thought to be the highest macroscopic, photoautotrophic communities on Earth. However, until now, rRNA-based molecular phylogenetic surveys of the communities inhabiting high-elevation, fumarolic soils and their barren, nonfumarolic counterparts have not been undertaken on Socompa or elsewhere.

The goal of the present study was a more comprehensive description of the biotic assemblages associated with Socompa's fumarolic and nonfumarolic soil environments. Samples were obtained from two sites within a fumarolic zone near Socompa's summit and two sites that were not part of the fumarolic system for comparison. Assessments of basic soil properties were coupled with measurements of fumarole-related soil CO_2 and CH_4 concentrations, soil photosynthetic and photoprotective pigments, and soil organic C stable isotope ratios. For each of the four soils, we determined a bacterial 16S rRNA gene sequence library and a eucaryotic 18S rRNA gene sequence library and assessed the phylogenetic structure of each community. With these data, we explored the relationships between environmental factors and the phylogenetic composition, diversity, evenness, and similarity of Socompa soil communities. Collectively, these data reveal the phylogenetic signature of communities experiencing extremely dry soil environments, and the unexpected diversity of communities

sustained by the localized provision of water, CO_2 , and CH_4 from deep within the Earth.

MATERIALS AND METHODS

Site location and sample collection. Socompa is a dacitic, composite-type stratovolcano located on the Chilean-Argentine border at coordinates $24^{\circ}24'S$ and $68^{\circ}15'W$ (Fig. 1). We accessed Socompa from the east via travel from Salta, Argentina. Samples were collected on 3 and 4 April 2005 during the austral autumn. Prevailing weather conditions were clear, cold, windy, and dry. At 5,824 m elevation, one of us (P. Sowell) and two other climbers ascending Socompa's southwestern flank encountered fumarolic ground hosting mat-like communities. This was determined to be "warmspot 2," the largest area of fumarolic ground previously described (22). Surprisingly, it appeared that recent foot traffic had heavily disturbed the mat communities. A soil sample (0 to 10 cm) was obtained from each of two sites within the fumarolic zone. The first site was located ~ 3 m away from the edge of the mat. We refer to this sample as "cold fumarole" (Fig. 2A and B). The second site was within the mat-covered zone where it appeared that the mat had been destroyed or removed by disturbance. We refer to this sample as "warm fumarole" (Fig. 2A and B). It should be noted that the mat itself was not directly sampled in this study because of its obvious sensitivity to disturbance. Both the cold-fumarole and warm-fumarole soils appeared barren at the time of sampling. Gas samples were obtained through the fumarolic ground and from two nearby steam vents by using a plastic chamber fitted with a rubber septum. The chamber was sealed onto the soil surface (or placed over a steam vent), and samples were collected via the septum. Each sample was injected into an evacuated 7-ml Vacutainer tube (Becton, Dickinson and Company, Franklin Lakes, NJ) and returned to the United States for analysis.

Soil samples (0 to 10 cm) were also collected at a third and fourth site, away from the fumarolic area. The third site was located along a southwest-trending ridgeline at 5,235 m elevation and featured barren volcanic debris. Soils were collected at several locations spread out along a kilometer of ridgeline and combined for study. We refer to this composite sample as "nonfumarole" (Fig. 2A and C). The fourth site was located ~ 14 km southeast of Socompa at 3,661 m elevation. This site was chosen because it hosted a localized wetland community, apparently due to groundwater discharge into the area. We refer to this sample as "wetland" (Fig. 2A and D).

Latitude, longitude, and elevation data were collected using a handheld global-positioning-system device (Garmin, Olathe, KS). Soil and gas temperatures were measured using a bimetallic soil thermometer (Barnstead International, Du-

FIG. 2. Socompa sampling locations. (A) Google Earth imagery captured 13 April 2005, 10 days after field collections were made. White arrows indicate sampling locations. Image relief is exaggerated $\times 2$, and background objects are extracted for clarity. The inset image in panel A shows sampling locations as seen from above. (B to D) Photographs of soil-sampling sites. Panel B features the fumarolic ground and mat-like communities described previously as “warmspot 2” by Halloy (22). The inset image in panel B shows the mouth of a rock-tunnel steam vent covered by moss. cf, cold fumarole; wf, warm fumarole; nf, nonfumarole; w, wetland.

buque, IA). Soils were sampled into sterile plastic bags by using an aseptic metal trowel, packed on ice, and kept in the dark for transport to Boulder, CO (about 5 days). Soils were subsequently stored at -20°C , with an aliquot stored at -80°C for molecular analysis.

Soil and gas analysis. Soil pH was determined in a 1:5 soil-to-deionized water slurry ratio, using a digital pH meter (Fisher Scientific, Pittsburgh, PA). Soil moisture was measured by oven drying for 48 h at 70°C . Dry samples for total C and N analyses were ground to a fine powder, packaged into tin capsules, and analyzed using a Carlo Erba EA 1110 elemental analyzer (CE Elantech, Lakewood, NJ). Soil organic C stable isotope ratios were determined via mass spectrometry at the Stable Isotope Ratio Facility for Environmental Research (SIRFER) at the University of Utah (Salt Lake City, UT). Soil photosynthetic and photoprotective pigments were extracted and quantified as previously described (8, 30) via high-pressure liquid chromatography analysis at the USGS Southwest Biological Research Center (Moab, UT). Gas sample CO_2 and CH_4 concentrations were simultaneously measured on a Shimadzu 14-A gas chromatograph (Kyoto, Japan) equipped with a flame ionization detector (40°C), a thermal conductivity detector (110°C), and a Poropak N column (40°C ; Supelco, Bellefonte, PA). Using a series of CO_2 and CH_4 standards, the concentration of each sample was calculated in parts per million.

DNA extraction, PCR, and cloning. Soil genomic DNA was extracted according to a bead-beating method modified from Moré et al. (42). In brief, 0.5 g soil and 0.3 g each of 1.0-mm glass, 0.5-mm silica, and 0.1-mm silica beads (Biospec Products, Bartlesville, OK) were homogenized in 1.0 ml phosphate lysis buffer (100 mM NaPO_4 , 100 mM Tris-HCl, 100 mM NaCl, 10% sodium dodecyl sulfate [pH 8.0]) for 2 min on a bead mill (Biospec Products, Bartlesville, OK). DNA was purified via one extraction with ammonium acetate (7.5 M) and two extractions with phenol-chloroform-isoamyl alcohol (25:24:1) and precipitated with isopropanol. Three extractions were combined for each soil sample. Humic substances were removed using Sepharose 4D (Sigma-Aldrich, St. Louis, MO) columns according to Jackson et al. (28).

Bacterial 16S rRNA genes were amplified using the bacterial domain-specific primer 8F (5'-AGA GTT TGA TCC TGG CTC AG-3') and universal primer 1391R (5'-GAC GGG CGG TGG GTR CA-3'). Eucaryotic 18S rRNA genes were amplified using universal primer pair 515F (5'-GTG CCA GCM GCC GCG GTA A-3') and 1391R (5'-GAC GGG CGG TGG GTR CA-3'), with subsequent purification of the 18S rRNA gene amplicon. PCRs were performed with 2.5 mM MgCl_2 , 0.2 mM each deoxynucleoside triphosphate, 0.4 μM each primer, 1 U *Taq* polymerase (Promega, Madison, WI), and buffer supplied with the enzyme, using a range of template concentrations. Gradient thermal cycling was carried out for 25 cycles to minimize PCR bias. Amplicons from various reactions were pooled for cloning. PCR products were purified on agarose gels and extracted using spin columns (Qiagen, Valencia, CA). For amplifications using universal primers, only the larger eucaryotic 18S rRNA gene amplicons were isolated from the gel for cloning. Purified PCR products were ligated into TOPO TA cloning vectors (Invitrogen, Carlsbad, CA) and transformed into *Escherichia coli*, and transformants were randomly arrayed on 96-well plates. Cloned inserts were amplified using vector-targeted primers M13F and M13R. Prior to sequencing, amplified inserts were treated with exonuclease I and shrimp alkaline phosphatase (New England Biolabs, Ipswich, MA). Libraries were not screened prior to sequencing, and clones were sequenced bidirectionally. Functional Biosciences, Inc. (Madison, WI), performed the sequencing using vector-targeted T7 (5'-AAT ACG ACT CAC TAT AG-3') and M13R-9 (5'-GCT ATG ACC ATG ATT ACG-3') primers.

Sequence and phylogenetic analysis. Sequences were edited, assembled into contigs, and vector-trimmed in Sequencher (Gene Codes, Ann Arbor, MI). Approximate phylogenetic affiliation and related sequences were found using the basic local alignment search tool (BLAST) (2) and GenBank. We found that eucaryotic, bacterial, and several archaeal small-subunit (SSU) rRNA gene sequences were commingled within clone libraries derived from universal amplifications despite attempted isolation of the larger-sized 18S rRNA gene amplicon. The 515F-amplified bacterial 16S rRNA gene sequences were combined with the 8F-amplified sequences after determining that the relative abundances of phylogenetic groups were not different between the two libraries. Bacterial 16S rRNA gene sequences were aligned using the NAST alignment tool (15) and added to the Arb database (40) provided by the Greengenes project (16). Eucaryotic 18S rRNA gene sequences were aligned using the Arb autoaligner and added to an Arb database developed by Dawson and Pace (13). Putative chimeras were identified using Bellerophon (26), the Mallard program (5), and partial tree analysis in Arb. Alignments were manually fine-tuned, and assignment of nonchimeric sequences to their respective phylogenetic groups was based mainly on their position after parsimony insertion into the Arb phylogeny, with confirmation via BLAST and/or Greengenes classifier data.

Distance matrices and alignments were exported from Arb using the Lane mask (bacteria) (33) or euk-mask80-1391 (eucaryotes) (13) to remove ambiguously aligned hypervariable regions. Hypervariable regions were masked because they could not be unambiguously aligned across the large phylogenetic distances considered here. We used the program DOTUR to determine operational taxonomic units (OTUs) with the furthest-neighbor algorithm and a precision of 0.01 (50). For the purposes of this study, OTUs were defined at the minimum threshold of 99% sequence identity for masked alignments with distances corrected using a Jukes-Cantor model of sequence evolution. This level of masked sequence variation has been stated to correspond with the widely used 97% sequence identity for unmasked bacterial sequences (35); however, similar data were not available for eucaryotic sequences. DOTUR data were used to calculate a collector's curve, a Chao1 richness estimate, and Simpson's diversity index (*D*). Phylogenetic trees were inferred using parsimony-based (PAUP* version 4.0b10 for Unix) (58), Bayesian (MrBayes version 3.1.2) (47), and maximum likelihood (RAxML version 2.2.3, GARLI version 0.95) (56, 62) methods. When necessary, sequence evolution model selection and parameter estimation were performed using MODELTEST (version 3.5) and the Akaike information criterion (46).

α and β diversity. In order to further evaluate diversity within individual communities (α diversity), we used measures of phylogenetic richness and evenness. To assess phylogenetic richness, we measured phylogenetic diversity (PD) and the gain (*G*) in PD for each community (38). PD was calculated by summing the total branch length leading to taxa from a particular community when all other communities were removed from the tree. *G* was calculated by summing the total branch length remaining when taxa from a particular community were removed from the tree and then subtracting this sum from the total length of the tree. We calculated PD and *G* for 100 statistically equivalent Bayesian trees by using model estimates input into PAUP and report the means. To assess phylogenetic evenness within each community, we used the net relatedness index (NRI) and the nearest taxon index (NTI) (24, 61). The NRI measures overall phylogenetic clustering and is an index of the average branch length distance between all pairs of taxa within a focal community relative to the entire pool of taxa within the phylogeny. The NTI measures terminal phylogenetic clustering and is an index of the average branch length distance between pairs of nearest relatives within a focal community relative to the entire pool of taxa in the phylogeny. We calculated NRI and NTI for 100 statistically equivalent maximum likelihood trees by using the program Phylocom (<http://www.phylodiversity.net/phylocom/>) and report the means. Using a two-tailed test, communities were considered significantly clustered or overdispersed when their average rank was <25 or >975 , respectively, among values for 1,000 randomly assembled communities.

We also determined the degree to which lineages were shared between communities from different soils (β diversity). This phylogenetic similarity was assessed using the parsimony-based test of differentiation (phylo-test) described by Martin (41). The test was implemented in the program TreeClimber with 1,000 statistically equivalent Bayesian trees used as input (51). Phylo-test significance was assigned when more than 95% of 1,000 environment-randomized phylogenies had a greater number of parsimony changes than the average observed value. Using a single maximum likelihood tree as input, we also calculated the unweighted UniFrac metric to test for phylogenetic differentiation and to assess the hierarchical clustering (unweighted-pair group method using average linkages) of communities (37). UniFrac significance was assigned when 95% of 1,000 environment-randomized trees had UniFrac values greater than or equal to that of the observed tree. The robustness of the clustering analysis was determined using a jackknife resampling procedure.

By focusing these diversity analyses on measures that account for phylogenetic divergence, we attempted to bypass the process of choosing OTUs based on an arbitrary level of sequence identity prior to analysis. Also, by performing our analyses on 100 to 1,000 statistically plausible trees when possible, rather than on a single phylogenetic inference, we attempted to account for phylogenetic uncertainty in this study (29).

Nucleotide sequence accession numbers. The SSU rRNA gene sequences determined by this study were deposited in the GenBank database under accession numbers FJ592236 to FJ592937.

RESULTS

Soil and gas characteristics. The four Socompa soils differed in key ways relating to their habitability (Table 1). The nonfumigant soil was cold (-5°C), contained no detectable moisture or N, and had low levels of organic C (0.03%). We infer that

TABLE 1. Socompa Volcano site and soil characteristics^d

Soil ^e	Location	Elevation (m)	Vegetation	Disturbed	Soil temp (°C)	Enrichment of (ppm above ambient) ^a :		Texture	pH	% of:			δ ¹³ C-SOM (‰) ^b	Chl.a (μg g ⁻¹) ^c
						CO ₂	CH ₄			H ₂ O	C	N		
cf	24°23'57" S 68°14'53" W	5,824	Absent	—	−5	553 (± 13)	0.13 (± 0.34)	Sands and gravels	6.85	10	0.25	0.02	−23.6	2.25
wf	24°23'57" S 68°14'53" W	5,824	Absent	+	25	1,730 (± 282)	0.66 (± 0.31)	Sands with silt	6.73	<d.l.	0.03	<d.l.	−26.0	<d.l.
nf	24°24'09" S 68°15'53" W	5,235	Absent	—	−5	ND	ND	Sands and gravels	5.23	<d.l.	0.03	<d.l.	−25.9	<d.l.
w	24°30'31" S 68°10'51" W	3,661	Present	—	>0	ND	ND	ND	6.34	710	22.8	1.75	−27.3	10.70

^a Enrichment values are means (± SEM) of soil gas collected at 5-min intervals within a chamber. Ambient CO₂ and CH₄ concentrations were estimated at 380 and 1.59 ppm, respectively. Temperature and gas data were also obtained from two rock-tunnel steam vents near “warmspot 2.” The first vent was 35°C with CO₂ and CH₄ elevated 5,033 and 2.14 ppm, respectively. The second vent was 28°C and was enriched in CO₂ by 3,094 ppm.
^b SOM, soil organic matter. Isotopic values were referenced to those for PeeDee belemnite. Additionally, δ¹³C-SOM was found to be −26.6‰ for a 1,430-m-elevation forest soil collected 285 km east of Socompa near Salta, Argentina. Values are means of three sample replicates, and variability between replicates was low (SEM < 0.2).
^c Chl.a, chlorophyll a.
^d ND, not determined; <d.l., below detection limit.
^e cf, cold fumarole; wf, warm fumarole; nf, nonfumarole; w, wetland.

these characteristics were representative of the majority of Socompa’s nonfumarolic surface soil at the time of sampling. Within the fumarolic zone, the disturbed, warm-fumarole soil was geothermally heated (25°C) and enriched in CO₂ and CH₄ gases and yet was similar to the nonfumarole soil in that it was extremely dry and low in C and N. In contrast, the cold-fumarole soil (−5°C) contained higher levels of moisture (10% [wt/wt] H₂O) and approximately 10 times more organic C than either the nonfumarole or warm-fumarole soils. There was also evidence that the cold-fumarole soil received fumarolic CO₂

and CH₄, but to a lesser extent than the warm-fumarole soil. Finally, the lower-elevation wetland site was water-saturated and rich in C and N and supported the growth of vascular plants. Thus, we found that the nonfumarole and warm-fumarole soils were both extremely dry and nutrient limited, whereas the cold-fumarole and wetland soils were each locally supplemented with water from volcanic activity and ground-water discharge, respectively (Table 1).
In order to explore a potential signature of soil photoautotrophy, we examined organic C stable isotope ratios and con-

FIG. 3. Broad-level phylogenetic affiliation of Socompa soil SSU rRNA gene sequences. Gray bars show proportions of bacterial phyla (and proteobacterial subphyla), and black bars (inset graphs) show proportions of eucaryotic kingdoms (and fungal phyla) ordered by rank in relative abundance. Numbers of bacterial and eucaryotic sequences, respectively, are 105 and 124 (cold fumarole), 79 and 75 (warm fumarole), 120 and 33 (nonfumarole), and 119 and 45 (wetland). Bacterial-group abbreviations: Acido., *Acidobacteria*; Actino., *Actinobacteria*; Alphaprot., *Alphaproteobacteria*; Bacteroid., *Bacteroidetes*; Betaprot., *Betaproteobacteria*; Cyano., *Cyanobacteria*; Deltaprot., *Deltaproteobacteria*; Gemmat., *Gemmatimonadetes*; Plancto., *Planctomycetes*; Verruco., *Verrucomicrobia*. AD3, GAL15, OD1, OD2, OP10, OP11, SC3, SC4, SPAM, TM7, WPS-2, WS3, and WS5 are candidate phyla and have no cultured representatives. Eucaryotic-group abbreviations: Alveo., *Alveolates*; Asco., *Ascomycota*; Basidio., *Basidiomycota*; Cerco., *Cercozoa*; Chytridio., *Chytridiomycota*; Strameno., *Stramenopiles*. “Other fungi” are sequences lacking clear affiliation with an established fungal phylum. Libraries also yielded a C1 *Crenarchaeota* sequence from the cold-fumarole soil and a methanogen-related *Euryarchaeota* sequence from the wetland soil (not shown).

centrations of photosynthetic and photoprotective pigments. Soil C stable isotope ratios were measured via mass spectrometry for all of the Socompa soil samples, as well as for a reference forest soil sampled at a lower elevation near Salta, Argentina. All soil organic $\delta^{13}\text{C}$ values ranged from approximately -26 to -27‰ , with the exception of the cold-fumarole soil, which had a relatively ^{13}C -rich value of -23.6‰ (Table 1). Twelve photosynthetic and photoprotective pigments were also measured, all of which were detected within the wetland soil (data not shown). Of the three barren soils, only the cold-fumarole soil contained a detectable pigment, chlorophyll *a* (Table 1). Thus, two lines of evidence suggest that the cold-fumarole soil possessed a unique signature of photoautotrophy, namely, the presence of the photosynthetic pigment chlorophyll *a* and an enriched soil organic $\delta^{13}\text{C}$ value.

Community composition and coverage. Socompa soil bacterial and eucaryotic communities were evaluated using SSU rRNA-based surveys, and community composition was determined via assignment of SSU rRNA gene sequences to known groups, using comprehensive phylogenetic analyses. The results of these analyses are summarized as proportions of major phylogenetic groups within communities (Fig. 3).

Bacterial assemblages from the extremely dry nonfumarole and warm-fumarole soils were each composed of 11 groups (phyla or subphyla) and dominated by members of *Acidobacteria*, *Actinobacteria*, *Bacteroidetes*, and *Verrucomicrobia* (Fig. 3). However, while *Actinobacteria* were most abundant (33%) in the nonfumarole soil, *Verrucomicrobia* dominated (44%) the warm-fumarole soil. We found that the *Verrucomicrobia*-related lineages from the warm-fumarole and nonfumarole soils were phylogenetically novel and exclusively affiliated with the subphylum *Spartobacteria* (Fig. 4). Nonfumarole and warm-fumarole bacterial assemblages also shared representatives of *Alpha*- and *Betaproteobacteria*, *Gemmatimonadetes*, and candidate phylum SC4. The eucaryotic assemblages from the extremely dry soils were limited to just three or four major phylogenetic groups (Fig. 3). Nonfumarole soil was heavily dominated by *Fungi* (97%) and included basidiomycete yeasts (*Cryptococcus* spp.) and ascomycetes (*Cladosporium* sp. and *Ulocladium* sp.) (see Table S1 in the supplemental material). In contrast, close relatives of the cercoconad *Heteromita globosa* were most abundant (87%) in the warm-fumarole eucaryotic community (see Table S1 in the supplemental material). Despite these large differences in the relative abundances of cercoconads and basidiomycetes between the nonfumarole and warm-fumarole soils, the species represented were highly similar.

We found that the cold-fumarole soil hosted communities that were radically different from those hosted by the extremely dry soils (Fig. 3). Bacterial phylum-level representation was higher, falling out into 19 groups dominated by *Ac-*

idobacteria (28%), *Alphaproteobacteria* (13%), *Cyanobacteria* (11%), and *Chloroflexi* (9%). Cold-fumarole *Alphaproteobacteria* sequences were related to methanotrophs and N-fixing isolates, and among the *Cyanobacteria*, phototrophic *Nostoc* relatives and algal plastids were abundant (see Table S2 in the supplemental material). Other cold-fumarole bacterial lineages that were not found in the extremely dry soils included members of the *Deltaproteobacteria*, *Nitrospira*, *Chlorobi*, and *Deinococcus* groups and the uncultivated candidate phyla GAL15, SPAM, WPS-2, AD3, and OP10. Surprisingly, this high degree of bacterial phylum-level representation was on par with that of the wetland soil community, which hosted 18 major groups but was instead dominated by members of the phyla *Proteobacteria* (38%) and *Bacteroidetes* (13%) (Fig. 3). The cold-fumarole eucaryotic community also exhibited unexpected composition and was dominated by photoautotroph-related lineages, including moss, liverwort, and a particularly abundant (59%), diverse array of green algae (Fig. 3 and 5). In contrast, wetland photoautotroph-related sequences were mainly from vascular plants, and although both soils contained moss-derived sequences, they were from different genera. *Metazoa*-related sequences were also surprisingly common (14%) in the cold-fumarole soil and comprised the following two groups: (i) bdelloid rotifers and (ii) a clade of phylogenetically novel, putative microarthropods, neither of which were found in the wetland soil (Fig. 3 and 6). The novel metazoan sequences exhibited low similarity (80 to 82%) with their closest database matches and a weak but consistent phylogenetic affiliation with lineages of mites within the phylum *Arthropoda*. We did not detect eucaryotic photoautotrophs or metazoan lineages within the extremely dry nonfumarole and warm-fumarole soils.

We employed collector's curves to determine the sampling coverage of bacterial and eucaryotic "species-level" taxa from each soil (Fig. 7). In this analysis, taxa were defined at 99% sequence identity. Within each soil, sampling coverage was higher for eucaryotic taxa than for bacterial taxa. Sampling coverage was also higher within extremely dry nonfumarole and warm-fumarole soils than within cold-fumarole and wetland soils for both bacterial and eucaryotic taxa. Species-level sampling coverage was incomplete, as the observed number of taxa was less than the estimated number of taxa (e.g., Chao1 richness [data not shown]) for each community.

α diversity. Diversity within individual soils (α diversity) was assessed using measures of phylogenetic richness and evenness. To measure richness, we calculated the PD and PD gain (*G*) for each community. PD represents the total amount of branch length leading to sequences from a particular community, while *G* represents only those branch lengths that are unique (i.e., not shared with other communities). We plotted the PD and *G* of each community against the organic C con-

FIG. 4. Phylogeny of *Verrucomicrobia* from Socompa Volcano soils. The Bayesian consensus phylogenetic tree includes 16S rRNA gene sequences from Socompa soils, their closest GenBank BLAST matches, and various representatives of the bacterial phylum *Verrucomicrobia*. Nodes with <0.50 posterior probability are collapsed. Posterior probabilities for "key" nodes are shown. "Key" nodes generally demonstrate support for affiliation of Socompa soil lineages within established groups. Established subphyla are indicated at the right. Taxon color code is as follows: blue, cold fumarole (cf); red, warm fumarole (wf); yellow, nonfumarole (nf); green, wetland (w); and black, reference taxa with GenBank accession numbers. The scale bar corresponds to 0.10 substitutions per site. Sparto., *Spartobacteria*; Verruco., *Verrucomicrobia*; str., strain.

tent of the corresponding soil (Fig. 8A and B). Bacterial and eucaryotic PD and *G* were lowest in the nonfumarole and warm-fumarole soils, which had the lowest soil C contents. Bacterial PD and *G* were highest in the wetland soil (highest soil C) and intermediate in the cold-fumarole soil, which also had an intermediate, although still relatively low, soil C content. In contrast, eucaryotic PD and *G* were highest in the cold-fumarole soil and intermediate in the wetland soil. The large discrepancy between PD and *G* exhibited by the cold-fumarole eucaryotic community indicates that although phylogenetic richness was high, much of it, in terms of branch length within the phylogeny, was shared with other communities. OTU-based estimates of richness, such as Chao1 and Simpson's diversity index (*D*), further support the observed trends in phylogenetic richness (data not shown).

Next, we explored the phylogenetic evenness of Socompa soil bacterial and eucaryotic communities using the relatedness indices developed by Webb (61). The NRI and the NTI, respectively, measure overall and terminal clustering of taxa from a particular soil with respect to the total pool of taxa from all soils within a phylogeny. We calculated NRI and NTI for each community and plotted the results against the organic C content of the corresponding soil (Fig. 8C and D). We found that soils with the lowest C content (warm fumarole and non-fumarole) hosted bacterial and eucaryotic communities that were significantly clustered at the tips and throughout the phylogeny ($P < 0.05$). This excess of closely related lineages is inferred to be the signature of a selective sweep favoring just a few species. Conversely, soils with higher C contents (cold fumarole and wetland) hosted bacterial and eucaryotic communities that were considerably more even or overdispersed in structure than the ones hosted by the extremely dry, low-C-content soils. These trends toward an abundance of distantly related species suggest selection for the maintenance of diversity.

β diversity. The amount of diversity shared between soils (β diversity) was assessed using measures of phylogenetic differentiation, including the parsimony-based phylogenetic test (phylo-test) (41) and the UniFrac test (36). According to pairwise phylo-tests, each Socompa soil harbored phylogenetically distinct bacterial ($P < 0.0001$) and eucaryotic ($P < 0.0001$) communities. However, the UniFrac test contradicted the phylo-test in one case by indicating that the bacterial communities from extremely dry nonfumarole and warm-fumarole soils were not significantly different in overall composition,

FIG. 5. Phylogeny of plants and green algae from Socompa Volcano soils. Bayesian consensus tree including 18S rRNA gene sequences from Socompa soils, their closest GenBank BLAST matches, and various representative plant and green-algal taxa. Nodes with <0.50 posterior probability are collapsed. Posterior probabilities for "key" nodes are shown. "Key" nodes generally demonstrate support for affiliation of Socompa soil lineages within established groups. Major groups are shown to the right. Taxon color code is as follows: blue, cold fumarole (cf); green, wetland (w); and black, reference taxa with GenBank accession numbers. Plant and green-algal sequences were not found in warm-fumarole or nonfumarole soils. The scale bar corresponds to 0.10 substitutions per site. The arrow leads to the outgroup. vasc., vascular plants; liver., liverworts; Klebs., *Klebsormidiales*; gr. alg., green algae.

FIG. 6. Phylogeny of Metazoa from Socompa Volcano soils. Bayesian consensus tree including 18S rRNA gene sequences from Socompa soils, their closest GenBank BLAST matches, and various representative metazoan taxa. Nodes with <0.50 posterior probability are collapsed. Posterior probabilities for “key” nodes are shown. “Key” nodes generally demonstrate support for affiliation of Socompa soil lineages within established groups. Major groups are shown to the right. Taxon color code is as follows: blue, cold fumarole (cf); green, wetland (w); and black, reference taxa with GenBank accession numbers. Metazoan

despite large differences in the relative abundances of particular lineages. This discrepancy may be caused by the phylo-test being more likely than UniFrac to yield significance when a community harbors many closely related sequences—a phylogenetic structure we have observed for the extremely dry Socompa soils. We also clustered Socompa soil communities based on overlap in the phylogenetic lineages they contained using the UniFrac metric (Fig. 9). Bacterial and eucaryotic communities exhibited the same clustering pattern when based on environment. Extremely dry nonfumarole and warm-fumarole soils showed a well-supported phylogenetic similarity between both their bacterial and their eucaryotic communities. Wetland and cold-fumarole soil communities also demonstrated some phylogenetic overlap, but this association was not as well supported as it was for the drier soils.

DISCUSSION

Fumarolic ground near the 6,051-m summit of Socompa Volcano was previously shown to host localized areas of mat-like photoautotrophic vegetation within an otherwise barren, high-elevation landscape (22). The goal of our current study was to expand the scope of this earlier work and to provide a glimpse into the characteristics of microbial communities inhabiting Socompa’s fumarolic and nonfumarolic soils and the environmental factors that shape them. To our knowledge, we have conducted the first rRNA-based survey of microbial assemblages from high-elevation fumarolic soils and the surrounding barren landscape within the hyperarid south-central Andean volcanic region. In addition to cataloging the unique and diverse types of organisms that we found, we have revealed the distinct phylogenetic structure of bacterial and eucaryotic communities inhabiting several extremely dry soil environments. We have also shown that mild fumarolic activity likely mitigates stress and supports microbial communities that are markedly different in composition and structure from those inhabiting nearby drier soils. While acknowledging that our conclusions must be limited in scope based on our small number of samples, the following discussion suggests some potential relationships between microbial community characteristics and habitat variables on Socompa and attempts to place our findings within the context of other studies.

Extreme soils below the dry limit of photoautotrophy?

Among soil environments, the Atacama Desert and Antarctic Dry Valleys are often considered the harshest, primarily for their low soil moisture and nutrient content. Equally harsh are some soils found on Socompa, such as the nonfumarole and warm-fumarole soils studied here, which contained no detectable moisture and had organic C contents on par with those of Atacama Desert and Antarctic Dry Valley soils (1, 19, 21). Accordingly, nonfumarole and warm-fumarole microbial com-

sequences were not found in warm-fumarole or nonfumarole soils. Reference taxa from the *Deuterostomia*, *Cnidaria*, *Ctenophora*, *Porifera*, and *Lophotrochozoa* and the choanoflagellate outgroup were removed for clarity of presentation. The scale bar corresponds to 0.10 substitutions per site. The arrow leads to the outgroup. Arth., *Arthropoda*; Nem., *Nematoda*; Rotif., *Rotifera*; Platy., *Platyhelminthes*; Gast., *Gastrotricha*.

FIG. 7. Collector's curves for Socompa soil bacterial and eucaryotic taxa (OTUs) defined at 99% SSU rRNA gene sequence identity. cf, cold fumarole; wf, warm fumarole; nf, nonfumarole; w, wetland.

munities exhibited the phylogenetic signatures of extreme habitat filtering, including relatively low diversity and comparatively clustered lineages that were phylogenetically similar (e.g., *Spartobacteria* [Fig. 4]). These extreme soil environments

likely select for closely related suites of organisms sharing evolved ecological strategies for survival under harsh conditions. Such strategies may include desiccation resistance, freeze-thaw tolerance, UV-protective pigment production, and

FIG. 8. Phylogenetic richness and evenness within Socompa soil bacterial (A and C) and eucaryotic (B and D) communities. (A and B) PD and the PD gain (G) plotted against soil organic C for each of the four soils. Units of branch length are in the number of substitutions per site (SPS). (C and D) NRI and NTI plotted against soil organic C for each of the four soils. Relatedness indices near zero (dotted line) are considered unstructured (i.e., even). PD, G , NRI, and NTI were each calculated for 100 statistically equivalent phylogenetic trees, and the means are shown. Standard deviations were small and are therefore not shown.

FIG. 9. Phylogenetic similarity between bacterial (upper dendrogram) and eucaryotic (lower dendrogram) Socompa soil communities. Hierarchically clustered (unweighted-pair group method using average linkages) relationships are based on the unweighted UniFrac metric. Jackknife support for nodes is indicated (1,000 replicates). A distance of zero indicates that the soils contain identical lineages, and a distance of 1 indicates that the soils contain mutually exclusive lineages.

the formation of resting stages or spores (7, 12). Indeed, the dominant groups found within the extremely dry Socompa soils are related to organisms that exhibit such characteristics, including rapid cyst formation by the heterotrophic microflagellate *Heteromita globosa* (27), spore formation and pigment production by saprophytic fungi, pigment production by *Spartobacteria* (phylum *Verrucomicrobia*) (48), and spore formation and UV-B repair by *Actinobacteria* (20). Notably, several recent studies on the survival of microbes under Mars-like conditions have focused on *Escherichia coli*, *Deinococcus radiodurans*, and *Bacillus* spp. (e.g., see references 18 and 52), yet close relatives of these bacterial species were not detected in our rRNA-based surveys of several extremely dry, high-elevation soils. Our results point to a number of other microbes that would make appropriate candidates for exobiological studies.

We found scant evidence of primary production within the extremely dry nonfumarole and warm-fumarole soils, and they may represent environments below the dry limit of photoautotrophy. In Antarctic Dry Valley soils, this dry limit was found for eucaryotic primary producers at 1.3% soil moisture, below which fungi dominated (21). In Antarctic soils with moistures above 1.3%, eucaryotic primary producers were commonly detected (21, 34). A dry limit for bacterial photoautotrophs in Antarctic soils was not apparent via rRNA-based surveys, as extremely dry soils were sometimes found to contain *Cyanobacteria* (1, 55). Atacama Desert bacterial rRNA-based surveys have found soils dominated by *Actinobacteria* (11) and generally lacking *Cyanobacteria* except in small, isolated niches (60). rRNA gene surveys of microbial eucaryotes from Atacama Desert soils were not available for comparison, but a cultivation-based study detected numerous fungal lineages (10). Overall, rRNA-based surveys of Antarctic Dry Valley and Atacama Desert soils support the notion that *Actinobacteria* and *Fungi* dominate communities below the dry limit of pho-

toautotrophy, as observed for Socompa's nonfumarole soil. On the other hand, the high abundance of *Spartobacteria* (phylum *Verrucomicrobia*) and the cercomonad *Heteromita globosa* within the warm-fumarole soil is a unique finding for an extremely dry soil and may relate to the relative warmth or recent disturbance of the site. Together, these extremely dry soils may represent truly aeolian ecosystems. In aeolian zones, nutrients and organisms (including all forms of fixed C) are wind transported and deposited, with some microbial lineages surviving to bloom during transient pulses of water and nutrients (57).

Fumarole-supported soil photoautotrophic communities?

Socompa's cold-fumarole soil harbored a surprisingly diverse, yet cryptic, likely primary-producing microbial assemblage that exhibited community-wide phylogenetic diversity, structure, and composition more akin to a nutrient-rich wetland than to the other barren soils in its vicinity. Although not fumarole-warmed at the time of sampling, this soil was enriched in water, CO₂ gas, and possibly CH₄ gas. The cold-fumarole soil was richer in organic matter than its extremely dry counterparts, contained chlorophyll *a*, and displayed a ¹³C-rich soil organic C signature. Possibly, this community signal was derived from wind- or water-deposited material dislodged from upslope or adjacent mats. However, because we detected fumarolic inputs into the soil as well, we suggest that the cold-fumarole community may actually be in the early stages of mat development within a shifting fumarolic landscape or, alternatively, that gradients in fumarolic activity result in cryptic outer-ring communities that surround the central mats, which may develop only where fumarolic activities are highest. It is important that future studies accurately map fumarolic zones and their associated biotic communities, as well as their potential changes over time.

We further sought to explain the unique stable isotope signature of the cold-fumarole organic C, which had a relatively enriched δ¹³C value of −23.6‰. The other soil organic δ¹³C values measured in this study ranged from approximately −26 to −27‰, including the warm-fumarole, nonfumarole, and wetland soils and an additional forest soil sampled near Salta, Argentina, all of which reflect average δ¹³C values for plant-fixed biomass across a range of elevations (32, 49). We suggest that C fixed by fumarole-supported primary producers may have a ¹³C-rich CO₂ source. Indeed, the average isotopic signature of mantle C, with δ¹³C values around −5‰, is slightly heavier than atmospheric CO₂, which has an isotopic value of −8‰ (14). The δ¹³C of CO₂ emanating from degassing volcanoes often reflects the magmatic value (14). Therefore, because cold-fumarole organic matter was enriched in ¹³C by about 3‰, we propose that isotopically heavy volcanic CO₂ may supplement C fixed by fumarole-associated autotrophs, a phenomenon that has been recorded in plants from other volcanic areas (43). We suggest that Socompa's high-elevation, fumarole-associated photoautotrophic communities are not only buffered against cold and desiccation but may also be fertilized by volcanic carbon from the degassing of magma. However, because we did not directly measure the δ¹³C of Socompa's CO₂ emissions or dissolved inorganic C and because we cannot completely rule out stress or species effects, including different carbon isotope fractionation pathways by photoautotrophs, our inference must remain tentative. For example, photoautotrophic *Chloroflexi* using the 3-hydroxypro-

pionate pathway may also contribute to a relatively ^{13}C -rich biomass (59).

The majority of Socompa's cold-fumarole soil photoautotrophic lineages were eucaryotic and related to free-living, unicellular coccoid green algae of the class *Trebouxiophyceae*. Other cold-fumarole photoautotrophs included those related to the alga-like basal land plants *Klebsormidium* spp.; the liverwort *Jamesoniella autumnalis*; and the "copper" moss, *Mielichhoferia elongata* (Fig. 5). Our study suggests that the local soils we examined were unlikely sources for most cold-fumarole bacterial and eucaryotic species, supporting Halloy's conclusion that the mat ecosystems were colonized by species from afar (22). Indeed, most of the cold-fumarole community members, including the photoautotrophs, were closely related to species capable of dispersing widely from other terrestrial ecosystems (e.g., see reference 54). Green algae from the cold-fumarole soil were most closely related to those isolated from globally distributed environments, including building facades, desert soils and crusts, tree bark, and rhizosphere soils. Many of these isolates produce UV-absorbing sunscreens, which are a likely necessity for life at high elevation (31).

Finally, we also found molecular evidence for genetic novelty potentially due to the geographic isolation of Socompa's fumarole-supported communities. Over half of the metazoan sequences from the cold-fumarole community were phylogenetically unique and exhibited a weak but consistent affiliation with microarthropod mite species (Fig. 6). These novel sequences may represent a previously unknown animal lineage that is endemic to this highly insular and relatively harsh, high-elevation fumarolic ecosystem. However, microarthropod mites are also likely capable of wind-borne dispersal (57). Therefore, it may be that these novel microbial animals are simply awaiting discovery in more ubiquitous habitats.

Disturbance and a role for conservation on Socompa? Finally, the potential effect of disturbance on Socompa's unique and delicate fumarole-supported communities must be considered. The fumarolic area studied here was previously described to host $\sim 200\text{ m}^2$ of continuous, carpet-like vegetation (22). Natural events such as extreme weather, seasonal changes, and shifts in fumarolic activity must certainly act to disrupt these communities from time to time. However, our field observations suggest that the mat-like assemblages have been disturbed by human activities in the form of recent foot traffic. The mats are easily detached from the ground, and once detached, could be easily blown away by high winds. We examined soil sampled directly from a patch of fumarolic ground that was likely recently disturbed. This soil was shown to be warm (25°C) and enriched in volcanic gases but also extremely dry, low in nutrients, and lacking evidence for photoautotrophy. We suggest that organic C in this soil, including perhaps the microbes that were present, was also wind deposited and therefore did not exhibit the ^{13}C -rich signal seen at the nearby cold-fumarole site. Accordingly, its microbial communities exhibited extremely low diversity and, also, phylogenetic structure and composition similar to those of Socompa's barren nonfumarolic soil. Taken together, these data suggest that water is the most important limiting factor to life in this environment and that when the mat is removed, the underlying soil may quickly become desiccated and impacted by UV radiation. This implies that the presence of the mat may create a positive

feedback on the habitability of the soil by trapping moisture and retaining nutrients. Under the otherwise harsh conditions at 5,824 m elevation, mat reestablishment and growth may be exceedingly slow. We therefore propose that disturbance by human activities may present a risk to Socompa's unique fumarole-associated communities.

As in many remote places on Earth, the frequency of human access to Socompa and the south-central Andes is increasing. The southern portion of the Monturaqui-Negrillar-Tilopozo aquifer underlies the area northwest of Socompa and was recently tapped by copper-mining operations in the region (3). Roads built to reach drill sites, pumping stations, and permanent camps have greatly increased accessibility from the west. Undoubtedly, this spectacular and unexplored landscape will continue to attract adventurers, archaeologists, volcanologists, and biologists alike. Our hope is that by bringing attention to the biological uniqueness of Socompa's diminutive and fragile mat-like communities, perched precariously on the life-sustaining breath of a volcano amid one of the harshest landscapes on Earth, we will encourage others and remind ourselves to step carefully when we go.

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REFERENCES

1. Aislabie, J. M., K. L. Chhour, D. J. Saul, S. Miyauchi, J. Ayton, R. F. Paetzold, and M. R. Balks. 2006. Dominant bacteria in soils of Marble Point and Wright Valley, Victoria Land, Antarctica. *Soil Biol. Biochem.* **38**:3041–3056.
2. Altschul, S. F., W. Gish, W. Miller, E. W. Myers, and D. J. Lipman. 1990. Basic local alignment search tool. *J. Mol. Biol.* **215**:403–410.
3. Anderson, M., R. Low, and S. Foot. 2002. Sustainable groundwater development in arid, high Andean basins. *J. Geol. Soc. London* **159**:133–144.
4. Arroyo, M. T. K., F. A. Squeo, J. J. Armesto, and C. Villagran. 1988. Effects of aridity on plant diversity in the northern Chilean Andes—results of a natural experiment. *Ann. Mo. Bot. Gard.* **75**:55–78.
5. Ashelford, K. E., N. A. Chuzhanova, J. C. Fry, A. J. Jones, and A. J. Weightman. 2006. New screening software shows that most recent large 16S rRNA gene clone libraries contain chimeras. *Appl. Environ. Microbiol.* **72**:5734–5741.
6. Beorchia Nigris, A. 1975. El Volcan Socompa, p. 22–24. In A. Beorchia Nigris (ed.), Centro de investigaciones arqueológicas de alta montaña, vol. 2. San Juan, Argentina.
7. Billi, D., and M. Potts. 2002. Life and death of dried prokaryotes. *Res. Microbiol.* **153**:7–12.
8. Bowker, M. A., S. C. Reed, J. Belnap, and S. L. Phillips. 2002. Temporal variation in community composition, pigmentation, and f-v/f-m of desert cyanobacterial soil crusts. *Microb. Ecol.* **43**:13–25.
9. Casertano, L. 1963. General characteristics of active Andean volcanoes and a summary of their activities during recent centuries. *Bull. Seismol. Soc. Am.* **53**:1415–1433.
10. Conley, C. A., G. Ishkhanova, C. P. McKay, and K. Cullings. 2006. A preliminary survey of non-lichenized fungi cultured from the hyperarid Atacama Desert of Chile. *Astrobiology* **6**:521–526.
11. Cannon, S. A., E. D. Lester, H. S. Shafaat, D. C. Obenhuber, and A. Ponce. 2007. Bacterial diversity in hyperarid Atacama Desert soils. *J. Geophys. Res.* **112**:G04S17. doi:10.1029/2006JG000311.
12. D'Amico, S., T. Collins, J. C. Marx, G. Feller, and C. Gerday. 2006. Psychrophilic microorganisms: challenges for life. *EMBO Rep.* **7**:385–389.
13. Dawson, S. C., and N. R. Pace. 2002. Novel kingdom-level eukaryotic diversity in anoxic environments. *Proc. Natl. Acad. Sci. USA* **99**:8324–8329.
14. Deines, P. 1992. Mantle carbon: concentration, mode of occurrence and isotopic composition, p. 133–146. In M. Schildowski, S. Golubic, M. M. Kimberley, D. M. McKirdy, and P. A. Trudinger (ed.), Early organic evolu-

- tion: implications for mineral and energy resources. Springer-Verlag, New York, NY.
15. DeSantis, T. Z., P. Hugenholtz, K. Keller, E. L. Brodie, N. Larsen, Y. M. Piceno, R. Phan, and G. L. Andersen. 2006. NAST: a multiple sequence alignment server for comparative analysis of 16S rRNA genes. *Nucleic Acids Res.* **34**:W394–W399.
 16. DeSantis, T. Z., P. Hugenholtz, N. Larsen, M. Rojas, E. L. Brodie, K. Keller, T. Huber, D. Dalevi, P. Hu, and G. L. Andersen. 2006. Greengenes, a chimera-checked 16S rRNA gene database and workbench compatible with Arb. *Appl. Environ. Microbiol.* **72**:5069–5072.
 17. de Silva, S. L., and P. W. Francis. 1991. Volcanoes of the central Andes. Springer-Verlag, New York, NY.
 18. Diaz, B., and D. Schulze-Makuch. 2006. Microbial survival rates of *Escherichia coli* and *Deinococcus radiodurans* under low temperature, low pressure, and UV-irradiation conditions, and their relevance to possible Martian life. *Astrobiology* **6**:332–347.
 19. Drees, K. P., J. W. Neilson, J. L. Betancourt, J. Quade, D. A. Henderson, B. M. Pryor, and R. M. Maier. 2006. Bacterial community structure in the hyperarid core of the Atacama Desert, Chile. *Appl. Environ. Microbiol.* **72**:7902–7908.
 20. Ensign, J. C. 1978. Formation, properties, and germination of actinomycete spores. *Annu. Rev. Microbiol.* **32**:185–219.
 21. Fell, J. W., G. Scorzetti, L. Connell, and S. Craig. 2006. Biodiversity of micro-eukaryotes in Antarctic Dry Valley soils with <5% soil moisture. *Soil Biol. Biochem.* **38**:3107–3119.
 22. Halloy, S. 1991. Islands of life at 6000 m altitude—the environment of the highest autotrophic communities on Earth (Socompa Volcano, Andes). *Arct. Alp. Res.* **23**:247–262.
 23. Haselton, K., G. Hilley, and M. R. Strecker. 2002. Average pleistocene climatic patterns in the southern central Andes: controls on mountain glaciation and paleoclimate implications. *J. Geol.* **110**:211–226.
 24. Horner-Devine, M. C., and B. J. M. Bohannan. 2006. Phylogenetic clustering and overdispersion in bacterial communities. *Ecology* **87**:S100–S108.
 25. Houston, J., and A. J. Hartley. 2003. The central Andean west-slope rain shadow and its potential contribution to the origin of hyper-aridity in the Atacama Desert. *Int. J. Climatol.* **23**:1453–1464.
 26. Huber, T., G. Faulkner, and P. Hugenholtz. 2004. Bellerophon: a program to detect chimeric sequences in multiple sequence alignments. *Bioinformatics* **20**:2317–2319.
 27. Hughes, J., and H. G. Smith. 1989. Temperature relations of *Heteromita globosa* stein in Signy Island fellfields, p. 117–122. In R. B. Heywood (ed.), University research in Antarctica. Proceedings of British survey Antarctic special topic award scheme symposium, 9–10 November 1988. British Antarctic Survey, Natural Environment Research Council, Cambridge, United Kingdom.
 28. Jackson, C. R., J. P. Harper, D. Willoughby, E. E. Roden, and P. F. Churchill. 1997. A simple, efficient method for the separation of humic substances and DNA from environmental samples. *Appl. Environ. Microbiol.* **63**:4993–4995.
 29. Jones, R. T., and A. P. Martin. 2006. Testing for differentiation of microbial communities using phylogenetic methods: accounting for uncertainty of phylogenetic inference and character state mapping. *Microb. Ecol.* **52**:408–417.
 30. Karsten, U., J. Maier, and F. Garcia-Pichel. 1998. Seasonality in UV-absorbing compounds of cyanobacterial mat communities from an intertidal mangrove flat. *Aquat. Microb. Ecol.* **16**:37–44.
 31. Karsten, U., T. Friedl, R. Schumann, K. Hoyer, and S. Lembecke. 2005. Mycosporine-like amino acids and phylogenies in green algae: *Prasiola* and its relatives from the Trebouxiophyceae (Chlorophyta). *J. Phycol.* **41**:557–566.
 32. Korner, C., G. D. Farquhar, and Z. Roksandic. 1988. A global survey of carbon isotope discrimination in plants from high altitude. *Oecologia* **74**: 623–632.
 33. Lane, D. J. 1991. 16S/23S rRNA sequencing, p. 115–175. In E. Stackebrandt and M. Goodfellow (ed.), *Nucleic acid techniques in bacterial systematics*. John Wiley & Sons Ltd., New York, NY.
 34. Lawley, B., S. Ripley, P. Bridge, and P. Convey. 2004. Molecular analysis of geographic patterns of eukaryotic diversity in Antarctic soils. *Appl. Environ. Microbiol.* **70**:5963–5972.
 35. Ley, R. E., J. K. Harris, J. R. Wilcox, J. R. Spear, S. R. Miller, B. M. Bebout, J. A. Maresca, D. A. Bryant, M. L. Sogin, and N. R. Pace. 2006. Unexpected diversity and complexity of the Guerrero Negro hypersaline microbial mat. *Appl. Environ. Microbiol.* **72**:3685–3695.
 36. Lozupone, C., and R. Knight. 2005. Unifrac: a new phylogenetic method for comparing microbial communities. *Appl. Environ. Microbiol.* **71**:8228–8235.
 37. Lozupone, C., M. Hamady, and R. Knight. 2006. Unifrac—an online tool for comparing microbial community diversity in a phylogenetic context. *BMC Bioinformatics* **7**:371. doi:10.1186/1471-2105-7-371.
 38. Lozupone, C. A., and R. Knight. 2007. Global patterns in bacterial diversity. *Proc. Natl. Acad. Sci. USA* **104**:11436–11440.
 39. Luccini, E., A. Cede, R. Piacentini, C. Villanueva, and P. Canziani. 2006. Ultraviolet climatology over Argentina. *J. Geophys. Res.* **111**:D17312. doi: 10.1029/2005JD006580.
 40. Ludwig, W., O. Strunk, R. Westram, L. Richter, H. Meier, Y. Kumar, A. Buchner, T. Lai, S. Steppi, G. Jobb, W. Forster, I. Brettske, S. Gerber, A. W. Ginhart, O. Gross, S. Grumann, S. Hermann, R. Jost, A. König, T. Liss, R. Lussmann, M. May, B. Nonhoff, B. Reichel, R. Strehlow, A. Stamatakis, N. Stuckmann, A. Vilbig, M. Lenke, T. Ludwig, A. Bode, and K. H. Schleifer. 2004. Arb: a software environment for sequence data. *Nucleic Acids Res.* **32**:1363–1371.
 41. Martin, A. P. 2002. Phylogenetic approaches for describing and comparing the diversity of microbial communities. *Appl. Environ. Microbiol.* **68**:3673–3682.
 42. Moré, M. I., J. B. Herrick, M. C. Silva, W. C. Ghiorse, and E. L. Madsen. 1994. Quantitative cell-lysis of indigenous microorganisms and rapid extraction of microbial DNA from sediment. *Appl. Environ. Microbiol.* **60**:1572–1580.
 43. Pasquier-Cardin, A., P. Allard, T. Ferreira, C. Hatte, R. Coutinho, M. Fontugne, and M. Jaudon. 1999. Magma-derived CO₂ emissions recorded in C-14 and C-13 content of plants growing in Furnas Caldera, Azores. *J. Volcanol. Geoth. Res.* **92**:195–207.
 44. Piacentini, R. D., A. Cede, and H. Barcena. 2003. Extreme solar total and UV irradiances due to cloud effect measured near the summer solstice at the high-altitude desertic plateau Puna of Atacama (Argentina). *J. Atmos. Sol. Terr. Phys.* **65**:727–731.
 45. Pirajno, F., and M. J. Van Kranendonk. 2005. Review of hydrothermal processes and systems on Earth and implications for Martian analogues. *Aust. J. Earth Sci.* **52**:329–351.
 46. Posada, D., and K. A. Crandall. 1998. Modeltest: testing the model of DNA substitution. *Bioinformatics* **14**:817–818.
 47. Ronquist, F., and J. P. Huelsenbeck. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* **19**:1572–1574.
 48. Sangwan, P., X. L. Chen, P. Hugenholtz, and P. H. Janssen. 2004. *Chthoniobacter flavus* gen. nov., sp. nov., the first pure-culture representative of subdivision two, *Spartobacteria* classis nov., of the phylum *Verrucomicrobia*. *Appl. Environ. Microbiol.* **70**:5875–5881.
 49. Schlesinger, W. H. 1997. Biogeochemistry: an analysis of global change, 2nd ed. Academic Press, San Diego, CA.
 50. Schloss, P. D., and J. Handelsman. 2005. Introducing DOTUR, a computer program for defining operational taxonomic units and estimating species richness. *Appl. Environ. Microbiol.* **71**:1501–1506.
 51. Schloss, P. D., and J. Handelsman. 2006. Introducing TreeClimber, a test to compare microbial community structures. *Appl. Environ. Microbiol.* **72**:2379–2384.
 52. Schuerger, A. C., J. T. Richards, D. A. Newcombe, and K. Venkateswaran. 2006. Rapid inactivation of seven *Bacillus* spp. under simulated Mars UV irradiation. *Icarus* **181**:52–62.
 53. Schulze-Makuch, D., J. M. Dohm, C. Fan, A. G. Fairen, J. A. P. Rodriguez, V. R. Baker, and W. Fink. 2007. Exploration of hydrothermal targets on Mars. *Icarus* **189**:308–324.
 54. Sharma, N. K., A. K. Rai, S. Singh, and R. M. Brown. 2007. Airborne algae: their present status and relevance. *J. Phycol.* **43**:615–627.
 55. Smith, J. J., L. A. Tow, W. Stafford, C. Cary, and D. A. Cowan. 2006. Bacterial diversity in three different Antarctic cold desert mineral soils. *Microb. Ecol.* **51**:413–421.
 56. Stamatakis, A. 2006. RAXML-VI-HP: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics* **22**:2688–2690.
 57. Swan, L. W. 1992. The aeolian biome. *Bioscience* **42**:262–270.
 58. Swofford, D. L. 2003. PAUP*. Phylogenetic analysis using parsimony (*and other methods), 4th ed. Sinauer Associates, Sunderland, MA.
 59. van der Meer, M. T. J., S. Schouten, B. E. van Dongen, W. I. C. Rijpstra, G. Fuchs, J. S. Sinninghe Damsté, J. W. de Leeuw, and D. M. Ward. 2001. Biosynthetic controls on the ¹³C contents of organic components in the phototrophic bacterium *Chloroflexus aurantiacus*. *J. Biol. Chem.* **276**:10971–10976.
 60. Warren-Rhodes, K. A., K. L. Rhodes, S. B. Pointing, S. A. Ewing, D. C. Lacap, B. Gomez-Silva, R. Amundson, E. I. Friedmann, and C. P. McKay. 2006. Hypolithic cyanobacteria, dry limit of photosynthesis, and microbial ecology in the hyperarid Atacama Desert. *Microb. Ecol.* **52**:389–398.
 61. Webb, C. O. 2000. Exploring the phylogenetic structure of ecological communities: an example for rain forest trees. *Am. Nat.* **156**:145–155.
 62. Zwickl, D. J. 2006. Genetic algorithm approaches for the phylogenetic analysis of large biological sequence datasets under the maximum likelihood criterion. Ph.D. dissertation. The University of Texas at Austin.