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ORIGINAL PAPER

Microbial community composition and trophic role along a marked salinity gradient in Laguna Puilar, Salar de Atacama, Chile

Cristina Dorador · Patrick Fink · Martha Hengst · Gonzalo Icaza · Alvaro S. Villalobos · Drina Vejar · Daniela Meneses · Vinko Zadjelovic · Lisa Burmann · Jana Moelzner · Chris Harrod

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Abstract The geological, hydrological and microbiological features of the Salar de Atacama, the most extensive evaporitic sedimentary basin in the Atacama Desert of northern Chile, have been extensively studied. In contrast, relatively little attention has been paid to the composition and roles of microbial communities in hypersaline lakes which are a unique feature in the Salar. In the present study biochemical, chemical and molecular biological tools were used to determine the composition and roles of microbial communities in water, microbial mats and sediments along a marked salinity gradient in Laguna Puilar which is located in the "Los Flamencos" National Reserve. The bacterial communities at the sampling sites were dominated by members of the phyla *Bacteroidetes*, *Chloroflexi*, *Cyanobacteria* and *Proteobacteria*. Stable isotope and fatty acid analyses revealed marked variability in the composition of microbial mats at different sampling sites both horizontally (at different sites) and vertically (in the different layers). The Laguna Puilar was shown to be a microbially dominated ecosystem in which more than 60% of the fatty acids at particular sites are of bacterial origin. Our pioneering studies also suggest that the energy budgets of avian consumers (three flamingo species) and dominant invertebrates (amphipods and gastropods) use minerals as a source of energy and nutrients. Overall, the results of this study support the

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C. Dorador · G. Icaza · A. S. Villalobos · D. Vejar · D. Meneses · V. Zadjelovic
Laboratorio de Complejidad Microbiana y Ecología Funcional, Instituto Antofagasta, Universidad de Antofagasta, Antofagasta, Chile

C. Dorador (✉)
Departamento de Biotecnología, Facultad de Ciencias del Mar y Recursos Biológicos, Universidad de Antofagasta, Angamos 601, Antofagasta, Chile
e-mail: cristina.dorador@uantof.cl

C. Dorador · M. Hengst · G. Icaza · D. Vejar · D. Meneses
Centre for Biotechnology & Bioengineering (CeBiB), Santiago, Chile

P. Fink · L. Burmann · J. Moelzner
Workgroup Aquatic Chemical Ecology, University of Cologne, Cologne Biocenter, ZülpicherStraße 47b, 50674 Cologne, Germany

M. Hengst
Laboratory of Molecular Ecology and Applied Microbiology, Departamento de Ciencias Farmacéuticas, Universidad Católica del Norte, Antofagasta, Chile

C. Harrod
Instituto de Ciencias Naturales Alexander von Humboldt, Facultad de Ciencias del Mar y Recursos Biológicos, Universidad de Antofagasta, Antofagasta, Chile

view that the Salar de Atacama is a heterogeneous and fragile ecosystem where small changes in environmental conditions may alter the balance of microbial communities with possible consequences at different trophic levels.

Keywords Halophiles · Stable isotopes · Fatty acids · Microbial mats · 16S rRNA gene sequencing · Flamingos

Introduction

The Salar de Atacama is the largest evaporitic basin of the Atacama Desert in northern Chile. This Salar occupies an area of 18,100 km^2 and it is located at 2300 masl between latitudes 22°20′ to 24°10′ S. The Salar has a surface area of 3000 km^2 and lagoon surface area that reaches 12.6 km^2 (Risacher et al. 1999). The origin of the Salar de Atacama Basin is tectonic, where in the southernmost part there are deposits of clastic and evaporitic sediments that mix with dendritic and saline materials at the borders.

The Salar de Atacama has been defined as a sedimentation basin where the central depression is occupied by a crust of halite (NaCl) of 1100 km^2 of surface and 900 m depth, which is surrounded by saline silt of 2000 km^2 of surface (Risacher et al. 1999). It has been said that because the purity of their salts, the nucleus of the Salar de Atacama is not from antique paleolakes, but from subterranean contributions. Precipitation in this area is concentrated in the austral summer, and reaches up to 25 mm/year: however, potential evaporation is ca. 2000 mm/year, resulting in a negative hydric water balance (Risacher and Alonso 1996). The differences of temperatures during day and night are broad and mean relative humidity is ca. 50%, median wind speed reaches 10 m/s at 0.4 m above the ground, and generally has a westerly direction (DGA 1991).

C. Harrod
Núcleo Milenio INVASAL, Concepción, Chile

A. S. Villalobos
Marine Microbiology, GEOMAR Helmholtz, Centre for Ocean Research Kiel, Düsternbrooker Weg 20, 24105 Kiel, Germany

The Salar de Atacama has been studied extensively regarding its geology, hydrogeology and mineralogy, reflecting its economic importance, as large deposits of lithium are found at the southern area of the Salar. The main superficial influents of the basin are the San Pedro and Vilama Rivers located at the north part of the Salar, where 40% of the total superficial discharge are estimated to be used by agriculture (Mardones 1997). Salar de Atacama receives important subterranean contributions of water from the East and South (springs) originated by the volcanic formations of the Andes. There several types of water bodies in the basin: (i) freshwater and saline springs, (ii) saline lagoons and (iii) rivers in the north west area. The dissolved components in the waters have two main origins: the alteration of volcanic rocks provides K, Li, Mg, B and a low concentration of Na and Ca, meanwhile the redissolution of antique salts (under volcanic formations) make an important contribution with Na, Cl, Ca and SO_4 in the more saline waters (Risacher and Alonso 1996).

Microbiological studies at the Salar de Atacama began in the early 1990s with descriptions of cyanobacterial communities at Laguna Tebenquiche (Zúñiga et al. 1991). Campos (1997) reported several isolates from different sites of the Salar de Atacama including moderate halophilic bacteria: *Marinomonas*, *Vibrio*, *Alteromonas*, *Marinococcus*, *Acinetobacter* and halotolerant bacteria: *Bacillus*, *Pseudomonas-Deleya*, *Micrococcus* and *Acinetobacter*. Studies in Laguna Tebenquiche have led to the isolation of *Halorubrum tebenquichense*, an extremely halophilic archaeon (Lizama et al. 2002) and *Chromohalobacter nigrandesensis*, a moderately halophilic bacterium member of the *Halomonadaceae* (Prado et al. 2006). This bacterium is closely related to *Chromohalobacter sarecensis*, previously isolated from the high altitude (4300 m) Saline Lake Laguna Verde located in south-west Bolivia (Quillaguamán et al. 2004).

In recent decades, the use of independent culture methods has revealed previously unexpected levels of microbial diversity in different water bodies of the Salar. Demergasso et al. (2004) using PCR-DGGE based techniques reported a dominance of *Proteobacteria*, *Bacteroidetes*, high-GC Gram positive bacteria and *Haloarchaea* in Laguna Cejar, Tebenquiche and Burro Muerto at Salar de Atacama. Further studies at Laguna Tebenquiche highlighted the novelty of

bacterial taxa, including Bacteroidetes and Gammaproteobacteria (Demergasso et al. 2008). Bacteroidetes has been largely described as the most frequent bacterial phyla in water bodies of the Salar de Atacama and other aquatic systems of the Altiplano (Dorador et al. 2010). Other studies have revealed methane production rates from microbial mats at Laguna Cejar and Laguna Chaxa at the Salar de Atacama, revealing a fluctuation between 7.8 and 30.4 nmol g/day (Kelley et al. 2014). Interestingly, King (2015) revealed the microbial production of CO from Laguna Cejar and lately reported the isolation of *Natronorubrum* strain C29, a halophilic archaeon capable of oxidizing carbon monoxide (McDuff et al. 2016). The use of functional genes (*pufM*) has allowed the identification of anoxygenic phototrophic bacteria from Laguna Tebenquiche and Laguna Chaxa, exhibiting the presence of phototrophs adapted to high salt concentrations, i.e., *Halochromatium*, *Thiohalocapsa*, *Ectothiorhodospira* and *Halorhodospira* (Thiel et al. 2010).

The microbial composition of different structures present in Salar de Atacama lakes has been examined. Recently, microbial mats, gypsum evaporites and carbonate microbialites have been analyzed using next generation DNA sequencing techniques with a reported dominance of *Proteobacteria* in Laguna Tebenquiche and Laguna La Brava, a hypersaline lake located at the southern part of Salar de Atacama and near the lithium extraction facilities (Farías et al. 2014). In Laguna Tebenquiche, the study of different microbial mats detected a high relative abundance of *Archaea* (order *Halobacteriales*) rather than *Bacteria*, nevertheless, though bacterial groups such as *Planctomycetes*, *Gammaproteobacteria* (*Chromatiales*) and *Firmicutes* are abundant in different samples (Fernandez et al. 2016). In Laguna La Brava, microbial diversity was dominated by *Euryarchaeota*, *Crenarchaeota* and *Firmicutes* in non-lithifying mats, meanwhile in microbialites, high proportions of *Planctomycetes* were reported (Farias et al. 2017). The bacterial diversity of microbial mats of Laguna Cejar was studied using pyrolibraries detecting a dominance of *Proteobacteria* (*Rhodospirillaceae*, *Desulfobacteraceae*), *Aminicenantes* (ex-OP8) and *Firmicutes* (Rasuk et al. 2016).

Despite recent increased efforts to characterize microbial diversity in different structures of lakes of Salar de Atacama, few studies have examined the functional role of microbes at an ecosystem level, e.g., what is their potential role in the provision of energy and nutrients to consumers. This is potentially important, given the role of the Salar de Atacama as key habitat for three species of flamingos which are of conservation concern (Andean flamingo *Phoenicoparrus andinus*, Chilean flamingo *Phoenicopterus chilensis* and James' flamingo *Phoenicoparrus jamesi*). In this study we characterized the composition of microbial communities associated with different key features of the Salar (mats, sediments and biofilms) along a marked salinity gradient at Laguna Puilar, part of the Soncor Hydrological system, located at the National Reserve Los Flamencos, a protected area and Ramsar site (Ortiz et al. 2013). We also characterized fatty acid profiles and C ($\delta^{13}C$) and N ($\delta^{15}N$) stable isotope values of the different features, as well as other potential primary producers and abundant consumers (amphipods and gastropods: FAA, SIA, flamingo: SIA) in order to examine the putative contribution of microbial-derived materials to the diet of these consumers. We also aimed to make a first examination of the utility of the two different biochemical approaches to characterize microbial community structure and activity in Salar ecosystems.

Materials and methods

Description of Laguna Puilar

The Soncor Hydrological system is formed by a series of four shallow permanent interconnected lakes: Burro Muerto (1 ha), Chaxa (37 ha), Barros Negros (103 ha) and Puilar, which is formed by a series of lacustrine mirrors (Fig. 1). During the austral winter of 2013 (June), we sampled the Laguna Puilar in the Salar de Atacama and conducted extra sampling in 2014 (May) for chemical analysis. The lagoon (Fig. 1) has a short but extreme salinity gradient ranging from mildly brackish waters (P01) to hypersaline conditions (P04) (Tables 1, 2). Colonial cyanobacteria (e.g., *Nostoc*) and extensive microbial mats are present throughout the lagoon. Macrophytes are present but rare and decrease in abundance with increasing salinity. Different birds species can be observed feeding on the submerged sediment surfaces of the lagoon and build nests and raise young in areas directly adjacent the lagoon together with other birds as Andean gull (*Larus*

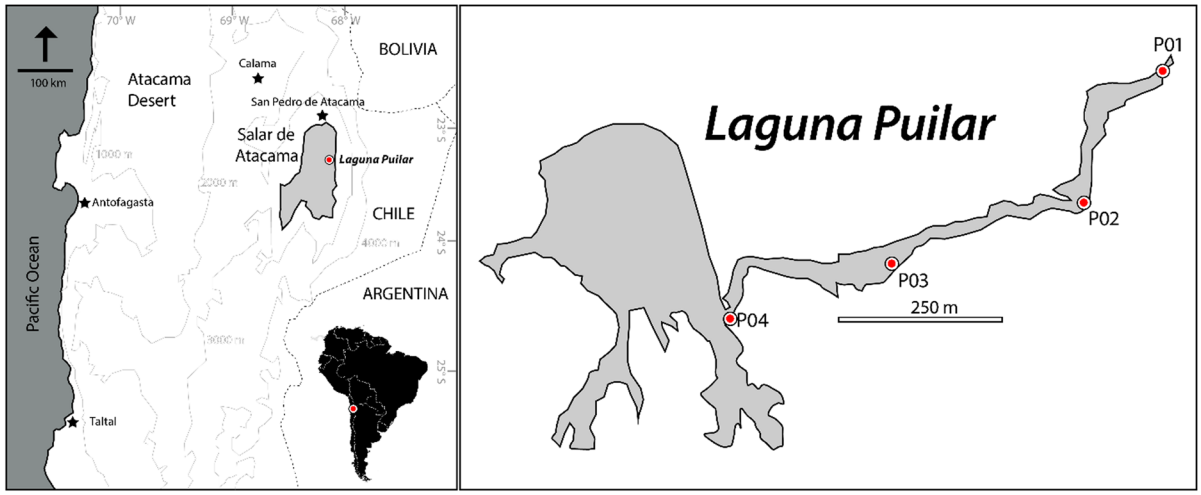

Fig. 1 Laguna Puilar at Salar de Atacama, northern Chile

Table 1 Physical and chemical characteristics of sampling sites at Laguna Puilar

Sites	Description	Water temperature (°C)	Salinity (mg/L)	pH	Location
P01	Spring	14.2	107	8	23°18.137′ S, 68°08.261′ W
P02	Channel (15 cm depth)	12.7	147	8.01	23°18.198′ S, 68°08.324′ W
P03	Channel	15.9	151	8.3	23°18.293′ S, 68°08.437′ W
P04	Main lake	18.6	162	8.11	23°18.354′ S, 68°08.643′ W

serranus) and Andean goose (*Chloehaga melanoptera*) (Information Sheet on Ramsar Wetlands 2006–2008).

Sampling

We sampled water, microbial mats, sediments, macrophytes, *Nostoc*, amphipods, gastropods and flamingo feathers from four sites (P01–4) located along a ca. 750 m salinity gradient (Fig. 1). Microbial mats and sediment samples were taken in sterile plastic tubes and stored at − 20 °C for further analysis. Samples were carefully obtained using sterile spatula and syringes from different layers of microbial mats following their vertical profile, taking into consideration the different coloured layers of the microbial mats (pink, green, purple). Other samples were removed to the laboratory and processed for analysis of $\delta^{13}C$ and $\delta^{15}N$ and fatty acids. Flamingo feathers were windblown and could not be reliably associated with a particular site along such a short transect. All samples were stored on ice and transported to the laboratories of the University of Antofagasta before being split for the various analyses; samples for DNA examination were stored frozen at − 80 °C for later analysis.

DNA extraction and sequencing

Microbial mats were collected at sites P01–4 (Table 1). P01 samples correspond to a hard biofilm layer covering rock; samples from P02–4 represent different layers of microbial mats. DNA from sediment samples was extracted using the DNA Isolation Kit PowerSoil (MoBio Laboratories, CA, USA), according to the manufacturer's instructions. The quantity and purity were tested in a Nanodrop2000 (Thermo Scientific, Wilmington, DE) and quality was checked by 0.8% p/v agarose gel electrophoresis. 16S rDNA gene was amplified with primers 27F and 519R (V1–3 regions). Sequencing was performed at MR DNA (http://www.mrdnalab.com) on a 454 Pyrosequencing device (Roche) following the manufacturer's guidelines. Sequencing results were obtained as fasta-qual and pipeline files.

Table 2 Chemical analysis of water samples from Laguna Puilar, Salar de Atacama

Elements	Sampling sites		
	P01	P02	P03
Nitrite (μg/L)	ND	ND	ND
Nitrate (μg/L)	118.04	90.27	134.62
Ammonium (μg/L)	34.95	35.32	65.33
Phosphate (μg/L)	376.09	405.56	398.94
Sulphate (mg/L)	389.37	293.28	361.93
Silica (mg/L)	253.11	266.82	265.03
Total nitrogen (μg/L)	177.78	302.23	405.65
Total phosphorus (μg/L)	1074.16	1032.49	953.32
Calcium (mg/L)	156.23	141.49	146.5
Magnesium (mg/L)	444.93	580.41	623.11
Sodium (mg/L)	4021.57	5172.66	5244.38
Potassium (mg/L)	743.84	965.71	1004.23
Chloride (mg/L)	24,996.3	29,686.66	29,936.66
Hardness (mg/L)	1600	2300	2250
Total alkalinity (mg/L)	3.55	3.8	3.4
Carbonate (mg/L)	9	9	18
Bicarbonate (mg/L)	216.55	231.8	207.4
Conductivity (μS/cm)	15,000	17,600	17,411

ND not detected

Sequences were analyzed in QIIME v1.9.1 (Caporaso et al. 2010). The single-end sequencing reads were quality-checked using the default settings for the split_libraries_fastq.py function in QIIME. Sequences were aligned against the data base SILVA (release v128) and clustered into representative bacterial OTUs using the sortmerna/sumaclust implementation of open-reference OTU-picking at 97% sequence similarity.

Stable isotopes analysis

Samples for stable isotope analyses were washed in distilled water, and then dried in an oven at 60 °C for at least 24 h. Due to issues of contamination with inorganic C, some samples (gastropods, sediments, microbial mats) were split, with one half treated with 1 N hydrochloric acid to remove inorganic carbon (e.g., in gastropod shells), while the second half of the sample was retained untreated for the estimation of $\delta^{15}N$ (as acid treatment can affect $\delta^{15}N$ values). Samples were ground in an agate mortar and weighed into tin capsules. Isotope-ratio mass spectrometry (performed by www.oealabs.com) was used to determine the stable carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotope composition with a precision of ± 0.1‰ and expressed in δ notation in ‰ relative to the internationally established standards.

Fatty acids analysis

Water samples were filtered on precombusted glass fibre filters immediately upon sampling and frozen at − 20 °C to minimize the oxidation of highly unsaturated fatty acids. Biofilm and sediment samples were freeze-dried prior to the extraction and stored at − 20 °C until processed. Total lipids were extracted from 2 to 5 mg (dry mass) of lyophilized samples with 10 mL CH_2Cl_2/MeOH (2:1 v/v) and dried under a stream of nitrogen gas. Hydrolysis of the lipids in the residue and subsequent methylation of fatty acids were achieved by incubation in 5 mL of 3 N methanolic HCl (Supelco) at 70 °C for 20 min. Fatty acid methylesters (FAMEs) were extracted from the acid with 3 mL iso-hexane and the solvent evaporated under a stream of nitrogen gas. Final FAME residues were redissolved in 100 μL of iso-hexane, from which 1 μL was subjected to gas chromatographic analyses. Analyses were performed on an Agilent 6890N GC system equipped with a flame ionization detector with helium as the carrier gas. FAMEs of eukaryotic origin were separated on a DB-225 capillary GC column (J&W Scientific, Folsom, USA) and identified by comparison of retention times to those of a FAME standard (Supelco CRM47885). For details on the column and oven temperature gradients see Ghomi et al. (2014). BAMEs (bacterial FAMEs) were separated on a HP-5 capillary GC column (J&W Scientific, Folsom, USA) and identified by comparison of retention times to those of a BAME (Supelco 47080-U) standard mixture. The GC oven program for the separation of BAMEs was as follows: 1 min at 60 °C, 30 °C/min to 160 °C, 4 °C/min to 200 °C, 20 °C/min to 280 °C with a final hold for 1 min at 280 °C. Relative FA amounts (per cent of total peak area) were standardized between the FAME and BAME analyses using palmitic acid (present in the FAME and BAME standards) as a reference peak.

Statistical analysis

Variation in the community composition associated with the different sites (P01–4), sample type (microbial mat, sediment, biofilm) and the relative strata depth of microbial mats (surface layer = L1, deepest layer = L4) was examined using principal coordinate analysis (PCoA) ordination of Bray–Curtis dissimilarities of square-root transformed data through PRIMER and PERMANOVA 7.0.13 (Anderson et al. 2008). This method allows rapid visual assessment of the relative similarity of microbial community composition between samples, as data that are located closely in ordination space are more similar (Paliy and Shankar 2016). We also overlaid multiple correlation vectors to show the direction and strength of the relationship between phyla abundance and the two canonical axes, in order to provide an indication of which phyla were likely driving the patterns seen in the ordination.

We used permutational ($n_{permutations}$ = 9 999) multivariate analysis of variance (PERMANOVA: Anderson 2001; Anderson et al. 2008) to examine whether microbial community composition varied between microbial mats and biofilms, and for microbial mats, between sites P02, P03 and P04. Fatty acid data were also treated in a similar manner, with the exception of the use of Euclidean distance matrix, as recommended for such data (Happel et al. 2017). Again, PCoA ordinations were generated and multiple correlation vectors superimposed to show which fatty acids were associated with the patterns in the data. Where we had both fatty acid and molecular data for the same sample, we also examined correlations between fatty acid data and the abundance of different microbial phyla between sites and layer depth, by superimposing correlation vectors of molecular data (relative phyla abundance) on a PCoA of fatty acid data from microbial mat samples. We focussed on microbial mat samples as the comparability between these samples was highest. We used PERMANOVA to examine whether microbial mat fatty acid profiles differed between sites P02, P03 and P04.

Stable isotope data were plotted following convention ($\delta^{15}N$ vs. $\delta^{13}C$) allowing the characterisation of the origin and flow of energy (C) and nutrients (N) from putative primary producers (biofilm, macrophytes, microbial mats) to consumers. We used PERMANOVA to examine how stable isotope data (based on Euclidean distances) differed between different taxa and sites. Different primary production pathways have distinctive $\delta^{13}C$ values, allowing them to be identified and their contribution to a food web estimated (Peterson and Fry 1987). Generally, there is a predictable isotopic shift (trophic discrimination) of ca. + 1‰ in C and 3+ ‰ in N between a consumer's food and its tissues (McCutchan et al. 2003), resulting in consumers being isotopically heavier than their long-term assimilated diet. We used PERMANOVA to test for isotopic differences between different putative sources of primary production and to examine whether there were spatial differences in isotope values within (a) microbial mats, and (b) biofilm between sites.

Results

Physical and chemical characteristics of Laguna Puilar

Laguna Puilar exhibited a salinity gradient of 107–162 mg/L of salinity between sites P01 and P04 (Table 1). The system is dominated by sodium (40.2–52.4 g/L) and chloride ions (25–30 g/L). In some sectors of the lake there is total saturation of salts (Table 2). In increasing order, cation concentrations are: $Na^+ > K^+ > Mg^{2+} > Si^+ > Ca^{2+}$ and anion are: $Cl^- > SO_4^{2-} > HCO_3^-$. For nutrients, total nitrogen values ranged between 177.8 and 405.7 µg/L and total phosphorus ranged between 953.3 and 1047.6 µg/L.

Invertebrates

Few multicellular animals were found in the system, the most common being amphipods (*Hyalella* spp.), gastropods (*Heleobia* spp.) and brine flies (*Ephydridae*), albeit not at all sampling stations. The density of gastropods and amphipods decreased markedly between P01 and P03 and were entirely absent at the hypersaline site P04. Although large volumes of water (> 150 L) were filtered through a phytoplankton net (20 µm), we did not encounter any *Artemia*, the putative diet of flamingos which are frequently observed in the lagoon. These birds can be observed feeding on the submerged sediment surfaces of the

lagoon and build nests and raise young in areas directly adjacent the lagoon. *Hyalella* amphipods exhibited body sizes ranging from 1.5 to 4.5 mm, but neither stable isotopes nor fatty acid biomarkers indicated size-specific patterns in resource use between amphipod size classes (data not shown). The shell height of *Heleobia* gastropods decreased significantly (Kruskal–Wallis ANOVA on ranks, $H = 69.6$, $df = 2$, $p < 0.001$) along the salinity gradient from P01 to P03 (no snails were found at P04), although the mechanisms underlying this pattern remain unclear (Table 3).

Microbial communities

The number of operational taxonomic units (OTUs) ranged between 113–301 and Shannon diversity index between 3.86–6.86 in samples P01-1 and P04-1 respectively (Table 3). The rock biofilm at site P01 comprised members of 14 bacterial phyla. This site was dominated by *Chloroflexi* (22–40%) and *Cyanobacteria* (31–43%) followed by *Proteobacteria* (13.3–23.3%) and *Bacteroidetes* (6.3–21.3%; Figs. 2, 3). Specifically, in the sample P01-1 the 40% of the relative abundance corresponded to the genera *Roseiflexus* and 19% to *Leptolyngbya* (Table 4). *Planctomycetes* were recorded in minor proportion (0.8–5.3% relative abundance) and *Verrucomicrobia* (0.7–1.8%). Regarding their relative abundance, seven phyla were considered as semi-rare (0.1–0.5%) and rare (> 0.1%) (Pedrós-Alió 2012) (Fig. 4).

Members of nineteen bacterial phyla were detected from four different depth layers of microbial mats from site P02. In general, bacterial diversity was dominated by *Proteobacteria* (25.6–73.6%) and *Firmicutes* (8.6–35.2%; Figs. 2, 3). Specifically, the whole community was dominated by three families: *Ectothiorhodospiraceae* (*Halorhodospira*), *Halomonadaceae* (*Salicola*) and *Halanaerobiaceae* (*Halanaerobium*). At this site, most of the phyla (13) were considered to be semi-rare and rare (Fig. 4). According to the different layers, an increase in the relative abundance of *Bacteroidetes* (2.8–25.8%) and *Chloroflexi* (1.4–3.3%) was apparent with increased depth. For the case of *Proteobacteria*, the dominant taxon was *Gammaproteobacteria* (65.9–9.3%) which decrease with depth.

Two samples of microbial mats from site P03 were collected. Both exhibited a high relative abundance of *Cyanobacteria* (53.9–30.7%) and *Proteobacteria* (22–32.7%), followed by *Bacteroidetes* (10–20.7%; Figs. 2, 3). Members of 20 phyla were detected in these samples of which 18 were semi-rare or rare (Fig. 4). The Bacteroidetes include the presence of nine families, highlighting the predominance of *Flavobacteriaceae*. Other frequent families were

Table 3 Alpha diversity index and metrics using 454 pyrosequences of the V4 region of the 16S rRNA from environmental samples of Laguna Puilar, Salar de Atacama

Samples	Raw reads	Post QC filtered reads	OTUs	Alpha diversity index				
				Shannon	Chao1	Simpson	ACE	Good's coverage
P01-1	6023	5728	113	3.86	135	0.81	149	0.98
P01-2	5310	4940	211	5.14	314	0.90	311	0.95
P01-3	6731	6198	218	5.63	295	0.93	310	0.95
P01-4	3302	2712	154	5.14	186	0.92	180	0.98
P02-1	2163	1817	117	4.95	139	0.94	142	0.98
P02-2	6799	6369	136	4.95	196	0.93	203	0.97
P02-3	7481	7118	127	4.65	196	0.90	208	0.97
P02-4	3149	2915	274	6.97	362	0.98	331	0.95
P03-1	4354	3950	256	6.17	386	0.96	403	0.93
P03-2	4476	4284	158	4.00	194	0.72	204	0.97
P04-1	4454	3749	301	6.83	397	0.98	434	0.93
P04-2	6803	5435	291	6.79	369	0.98	401	0.94
Orange mat	6034	5578	253	6.32	375	0.97	411	0.93

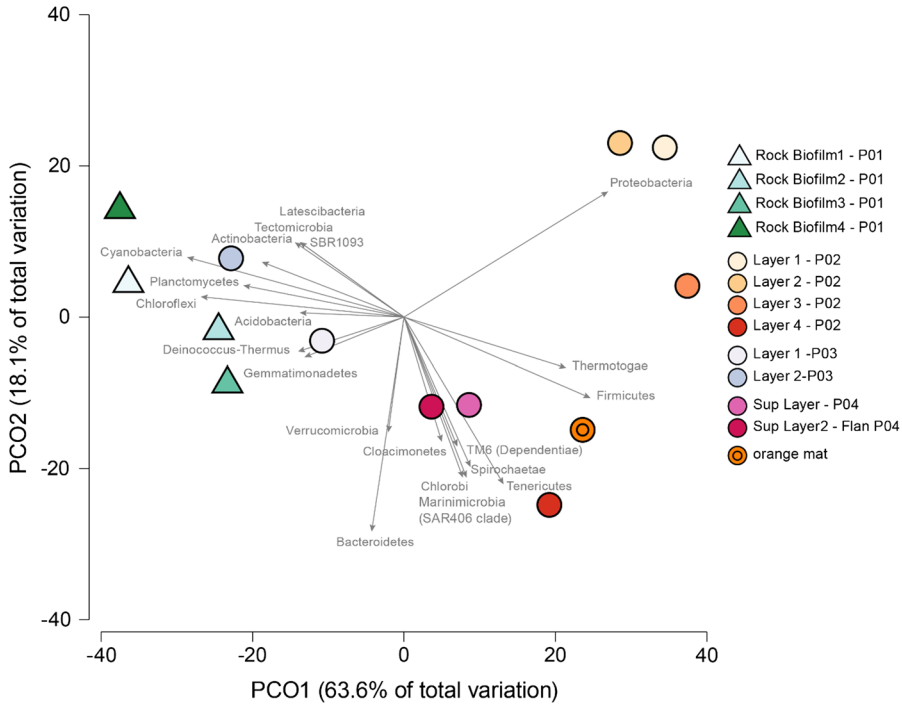

Fig. 2 PCoA ordination of 454 sequence data showing variation in microbial community composition (phyla level) associated with biofilm, microbial mat and surface sediment samples. Vectors show correlations between the relative abundance of the different phyla and the two canonical axes

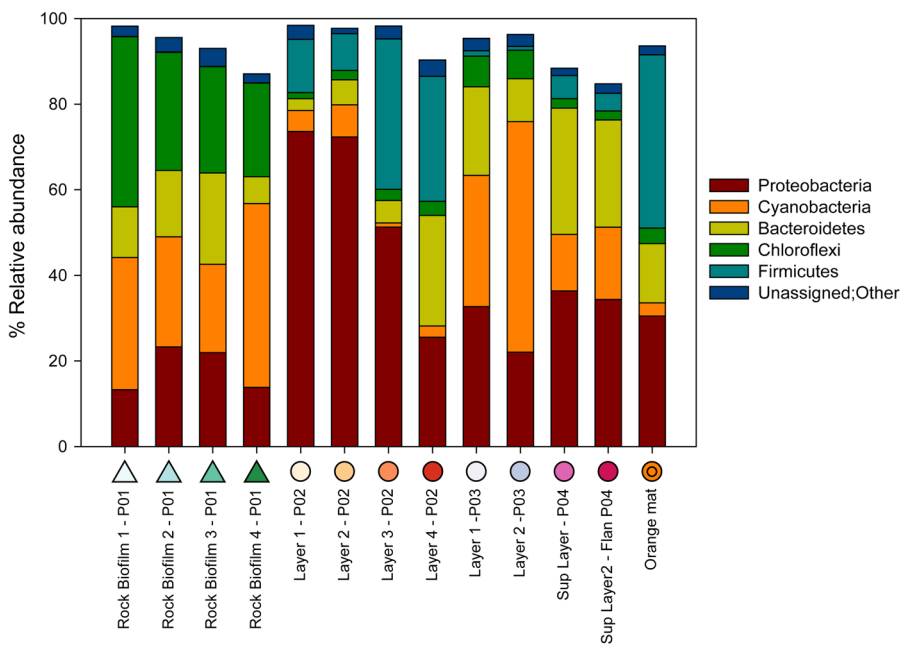

Fig. 3 Most frequent bacterial phyla in different sites and samples of Laguna Puilar, Salar de Atacama

Table 4 Most frequent bacterial genera at Laguna Puilar, Salar de Atacama

Genera	Phyla	Maximal values of total relative abundance in samples (%)
Pleurocapsa	Cyanobacteria	51.5
Halorhodospira	Proteobacteria	45.7
Roseiflexus	Chloroflexi	39.8
Halanaerobium	Firmicutes	33.7
Salicola	Proteobacteria	19.8
Leptolyngbya	Cyanobacteria	18.5
Idiomarina	Proteobacteria	11.5
Truepera	Deinococcus-Thermus	10.8
Rhodomicrobium	Proteobacteria	8.3
Spiribacter	Proteobacteria	8.2
Thiohalocapsa	Proteobacteria	8.1
Ca. Alysiosphaera	Proteobacteria	6.9

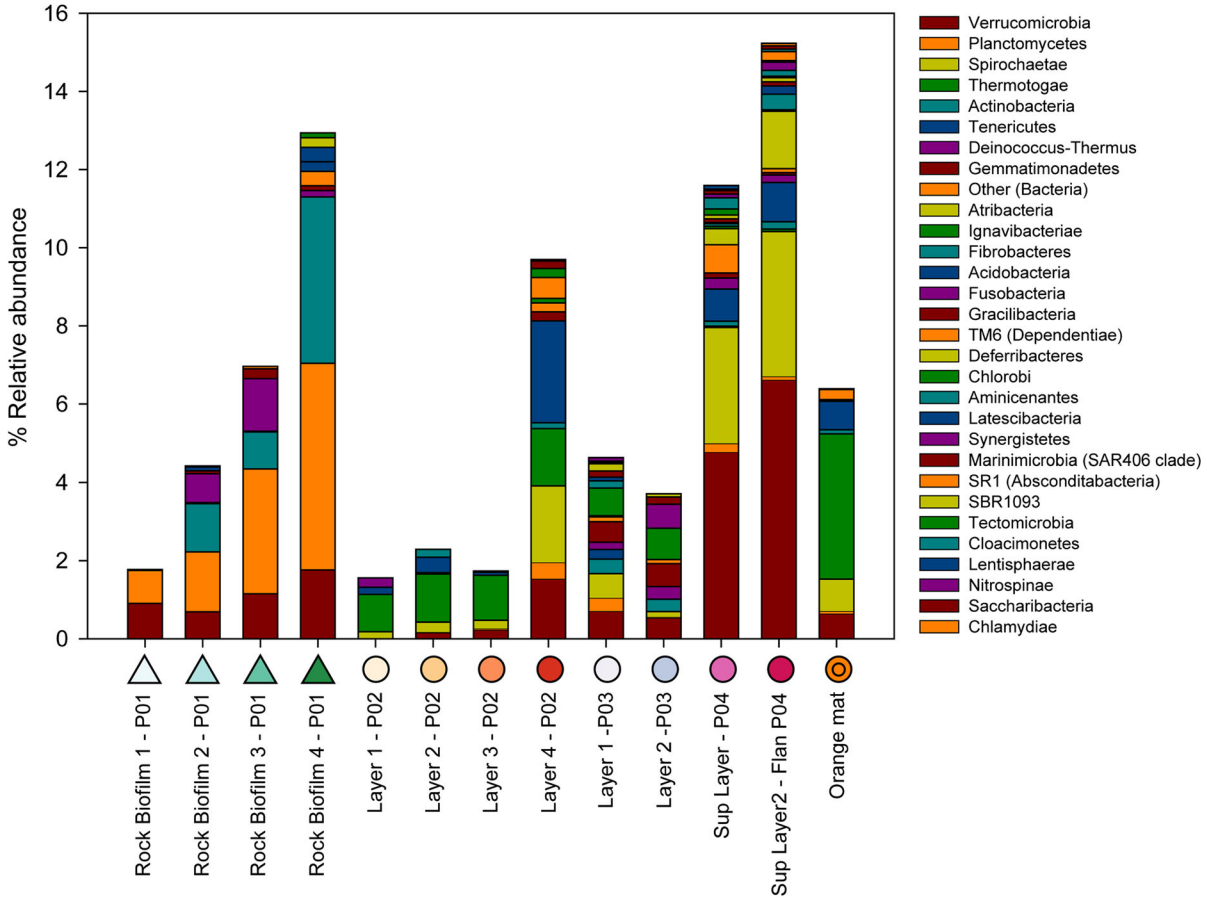

Fig. 4 Rare and semi-rare bacterial phyla in different sites and samples of Laguna Puilar, Salar de Atacama

Rhodobacteraceae and Chromatiaceae (*Thiohalocapsa*).

Samples from site P04 correspond to the superficial layer of the microbial mat. This site exhibited the highest number of bacterial phyla (28) in Laguna Puilar (Fig. 3), 20 of which were semi-rare or rare (Fig. 4). Finally, we analyzed an isolated sample called 'orange mat', detecting that 33% of the relative abundance was related with the family *Halanaerobiaceae* (*Firmicutes*) and 12% to the genus *Ectothiorhodospira* (*Gammaproteobacteria*).

Fatty acids analysis

A total of 49 FAMEs and BAMEs were recorded from the different producer and consumer taxa (Table S1). Fatty acid profiles were dominated by both saturated (mean contribution = 42.3%) and monounsaturated fatty acids (42.8%), with lesser contribution by polyunsaturated fatty acids (12.4%). On average, 34.3% of all fatty acids recorded from producers (biofilm, macrophytes and algae, microbial mats), and consumers (amphipods and snails) were of microbial origin. PCoA ordination (Fig. 5) revealed considerable variation in fatty acid profiles both within and between different sample types, with microbial mat samples being most distinct (especially from P02). This observation was supported by statistical analyses comparing fatty acid profiles of the different producers (PERMANOVA: Pseudo-$F_{7,59}$ = 3.4, P = 0.0001). Microbial mat fatty acid profiles differed between sites (Pseudo-$F_{3,34}$ = 9.6, P = 0.0001) and even between the different layers of a mat sampled from one location (P02 layers 1–4: Pseudo-$F_{3,10}$ = 3.8, P = 0.003). Biofilm samples had similar fatty acid profiles between sites (Pseudo-$F_{2,8}$ = 1.08, P = 0.39).

Given the marked differences in both microbial communities and fatty acid profiles observed from microbial mats, we examined whether there was any association between the two measures. Comparison of microbial mat samples in PCoA ordinations based on molecular and fatty acid data show close similarities (see patterns shown by circle markers in Figs. 5, 6), with the following six phyla being highlighted: the *Bacteroidetes*, *Cyanobacteria*, *Firmicutes*, *Proteobacteria*, *Tenericutes* and *Verrucomicrobia*. It is therefore possible that the distribution and availability of particular fatty acids in the Salar is driven by the presence and activity of different microbial groups. Bacterial fatty acids made a large contribution to our amphipod sample (47.8% of total fatty acids), while

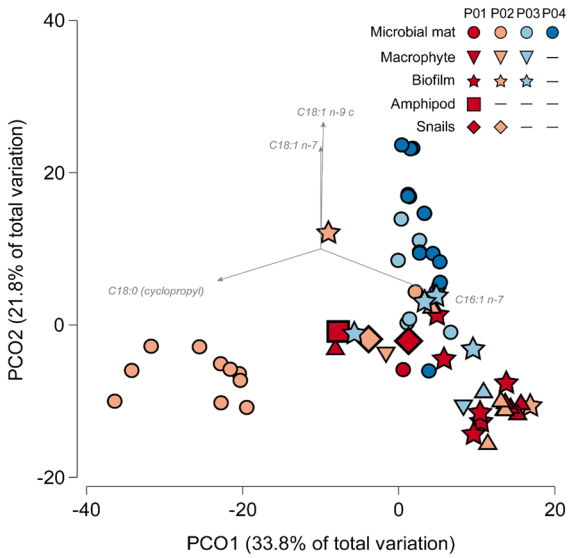

Fig. 5 *PCoA* ordination of fatty acid data recorded from a range of producer and consumer taxa collected from four sites at Laguna Puilar. The vectors reflect the direction and strength of multiple correlations between the percentage contribution of individual fatty acids and the two canonical axes

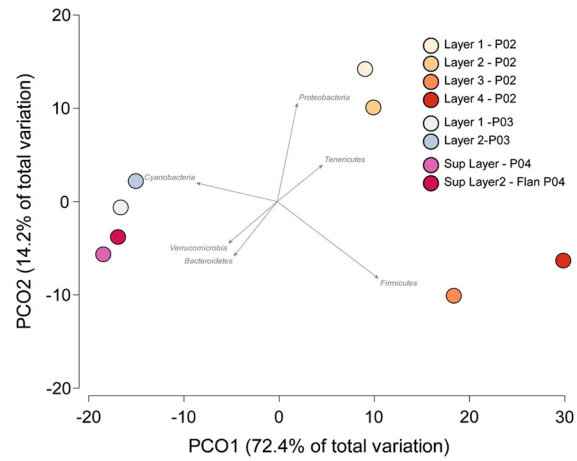

Fig. 6 PCoA ordination showing variation in microbial mat fatty acid profiles (based on mean values where more than one sample was taken per site/layer). Vectors show the strength and direction of correlations between the relative abundance of different microbial phyla and the two canonical axes shown, indicating the likely bacterial influence on fatty acid profiles. Note the overall similarity between this ordination and the pattern shown by microbial mat samples in Fig. 2

contribution to the gastropods varied between 22 and 27% (Table S1).

Stable isotope analysis

Given that the survey area was relatively small, isotopic variation was considerable both in terms of the range of $\delta^{15}N$ (− 0.3 to 13.1‰) and $\delta^{13}C$ (− 26.4 to − 1.2‰) values displayed by the different taxa examined (Fig. 7). Different sources of primary production were isotopically distinct (PERMANOVA $\delta^{15}N$–$\delta^{13}C$: Pseudo-$F_{2,53}$ = 22.14, P = 0.0001). Both microbial mats (Pseudo-$F_{3,27}$ = 345.6, P = 0.0001) and macrophytes (Pseudo-$F_{2,11}$ = 101.6, P = 0.0001) varied isotopically by site. Biofilm also varied, but to a lesser degree (Fig. 7a: biofilm: Pseudo-$F_{2,15}$ = 3.43, P = 0.05).

The three consumer taxa examined here were isotopically distinct (Pseudo-$F_{2,55}$ = 54.89, P = 0.0001) with snails and amphipods being ^{13}C and ^{15}N depleted relative to flamingos. Both amphipods (Pseudo-$F_{2,26}$ = 3.74, P = 0.007) and snails (Pseudo-$F_{1,6}$ = 21.16, P = 0.028) differed isotopically between sites with individuals from P01 being depleted in ^{13}C but enriched in ^{15}N relative to individuals from P02. Amphipods did not show any consistent pattern in terms of their position to producers in the three sites where they were found (P01–3). They overlapped with macrophytes at P01, but were enriched in both ^{13}C and ^{15}N relative to macrophytes at P02 and P03, and overlapped with microbial mats at both of these sites. Snails at P01 overlapped with macrophytes, but snails at P02 were not obviously linked to any source of energy at P02. Flamingo feathers were extremely variable in terms of both $\delta^{13}C$ and $\delta^{15}N$, and were typically ^{13}C and ^{15}N enriched relative to producer or other consumer taxa.

Discussion

Microorganisms were long considered as a 'black box' with regard to their ecological role in aquatic environments. Their functional role, frequently associated with the processing of organic matter has been largely underestimated.

Recent advances in DNA sequencing techniques have allowed an increased understanding of the functional role, diversity and distribution of microbial communities in different ecosystems. Aquatic ecosystems of Salar de Atacama are unique environments,

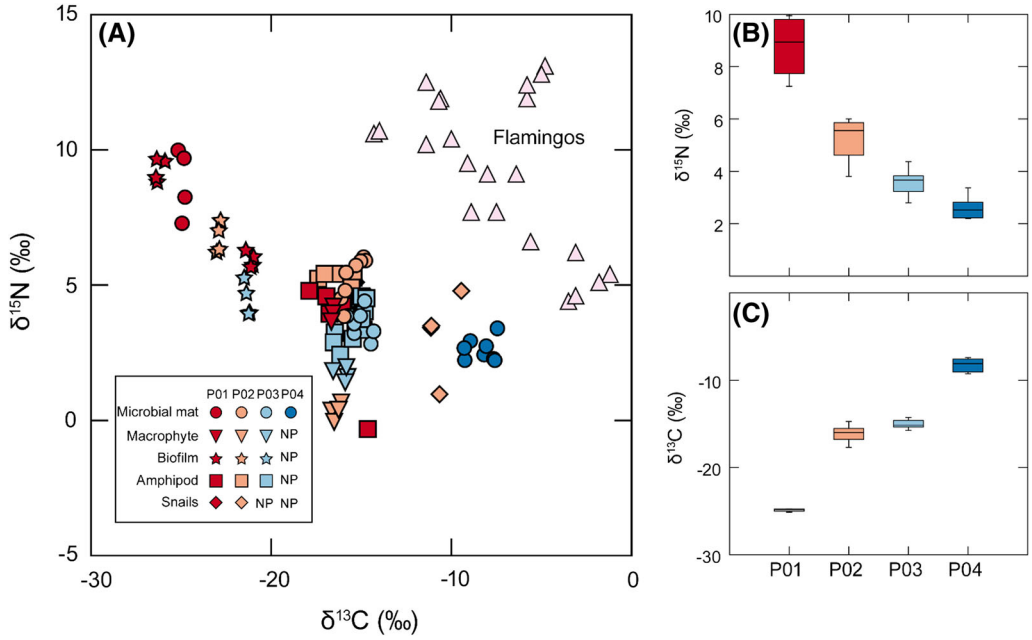

Fig. 7 Stable isotope values varied considerably (**a**) between the different putative sources of production (biofilm, macrophytes and microbial mats), and consumer taxa. The spatial variation was also extremely marked within the different producers [e.g., microbial mats (shown **b**, **c**)]

where microbial life dominates. Microbial diversity studies in the Salar de Atacama have repeatedly revealed an important number of new bacterial and archaeal clusters (e.g., Demergasso et al. 2008; Dorador et al. 2009; Thiel et al. 2010), nevertheless the description of new strains is infrequent.

The lakes at Salar de Atacama are fragile microbial ecosystems, the existence of which directly depends directly on water availability. Therefore, current practices of water and brine extractions for mining activities represent important and pressing threats against the maintenance of these ecosystems.

Beyond the microbial diversity reported from microbial mats, it is apparent that these structures play a key functional role in the system. Fatty acids profiles revealed both high spatial variability, and a close relationship with key bacterial taxa. For instance, fatty acids profile of microbial mat in site P02 showed a markedly differentiation and a specific associated with certain groups. Layer 1 was related to *Proteobacteria* (*Ectothiorhodospira* and *Halomonadaceae*) and layer 2 to *Idiomarinaceae* and *Ectothiorhodospira*. In turn, layer 3 was related to *Firmicutes* (*Halanaerobium*). *Ectothiorhodospira* species have shown to show responses to changes in salinity and temperature, with temperature changes having a marked effect on their fatty acid composition (Imhoff and Thiemann 1991).

The fatty acid analyses focussed on the microbial mats, as the comparability between samples was highest for this sample type. Similar to the results for the stable isotope analyses, the microbial mats from the four sampled subsites with different salinity differed markedly in their fatty acid biomarkers. Interestingly, palmitoleic acid ($C_{16:1}$ n-7) was most strongly associated with the less saline subsite P01 near the freshwater inflow into the Salar. This suggests that diatoms (for which palmitoleic acid is a commonly used biomarker fatty acid, see, e.g., Dalsgaard et al. 2003) dominated in the freshwater part, while they were suppressed by the increasing salinities. At the more saline sites, monounsaturated C_{18} fatty acids such as oleic ($C_{18:1}$ n-9) and vaccenic acid ($C_{18:1}$ n-7) dominate, which are most probably associated with phototrophic prokaryotes such as cyanobacteria (Dalsgaard et al. 2003). The use of fatty acid biomarkers adds a new dimension to the picture of the Salar de Atacama food web; this is the first such from a high altitude Salar. As the fatty acid composition of consumers typically reflects that of their diet, fatty acid biomarkers are a useful tool to gain more extensive information than that obtained from stable isotopes alone. They hence add to a deepened understanding of trophic structure, in particular when fatty acid and stable isotope biomarkers are combined.

With respect to the putative role of microbial taxa as a source of energy and nutrients to invertebrate and vertebrate consumers in the Salar, our initial data indicate that both amphipods and gastropods likely rely heavily (20–50%) on bacterial-derived fatty acids in the Salar. Stable isotope data were less clear, reflecting the large amount of isotopic variation apparent within the very short salinity gradient (Fig. 7). Stable isotope data showed that at the site with the most extensive microbial mats (P02) amphipods and gastropods closely resembled microbial mat values but diverged from microbial mats at P01 and P02.

The isotopic variation apparent in the samples of flamingo feathers is of a similar scale to that seen in the microbial mats and other potential sources of C and N. This can be partly attributed to the fact that the feathers originate from three different flamingo species, with different age structures, feeding preferences and histories as well as geographical distributions. However, if flamingo feathers are corrected for trophic fractionation [mean ($\pm$ SD) trophic discrimination factors = δ^{13}C 3.6 $\pm$ 0.6; δ^{15}N 5.6 $\pm$ 0.3] (Mizutani et al. 1992), it suggests that they are consuming microbial components in sediments.

So far, our data demonstrate a remarkable variability in all measured parameters (salinity, stable isotopes, fatty acids, microbial community composition). This applies to the samples from the presumed primary producers, and consumers as well as decomposers along a small scale but steep salinity gradient. Our results suggest that the basal processes underlying the ecosystem functions of the Salar are probably complex and highly variable in space and time. This highlights that the Salares are much more complex ecosystems than previously thought and that further, more detailed studies are required to understand their biology. Future work from our group will make use of compound specific stable isotope analysis of fatty acids and amino acids to fingerprint the origin of these compounds and their importance to consumers (Evershed et al. 2007; Larsen et al. 2009; Middelburg 2014).

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References

Anderson MJ (2001) A new method for non-parametric multivariate analysis of variance. Austral Ecol 26:32–46. https://doi.org/10.1111/j.1442-9993.2001.01070.pp.x

Anderson MJ, Gorley RN, Clarke KR (2008) PERMANOVA+ for PRIMER: guide to software and statistical methods. PRIMER-R, Plymouth

Campos V (1997) Microorganismos de ambientes extremos: Salar de Atacama, Chile. In: Charrier R, Aceituno P, Castro M, Llanos A, Raggi LA (eds) El Altiplano: ciencia y conciencia de los Andes. Actas del segundo Simposio internacional de Estudios Altiplánicos, Santiago, pp 143–147

Caporaso JG, Kuczynski J, Stombaugh J et al (2010) QIIME allows analysis of high-throughput community sequencing data. Nat Methods 7:335–336. https://doi.org/10.1038/nmeth.f.303

Dalsgaard J, St John M, Kattner G et al (2003) Fatty acid trophic markers in the pelagic marine environment. Adv Mar Biol 46:225–340

Demergasso C, Casamayor EO, Chong G et al (2004) Distribution of prokaryotic genetic diversity in athalassohaline lakes of the Atacama Desert, northern Chile. FEMS Microbiol Ecol 48:57–69. https://doi.org/10.1016/j.femsec.2003.12.013

Demergasso C, Escudero L, Casamayor EO et al (2008) Novelty and spatio–temporal heterogeneity in the bacterial diversity of hypersaline Lake Tebenquiche (Salar de Atacama). Extremophiles 12:491–504. https://doi.org/10.1007/s00792-008-0153-y

Dirección General de Aguas, DGA (1991) Catastro General de usuarios de aguas de los cauces afluentes al Salar de Atacama. Informe final. Tomo 1 - 4

Dorador C, Meneses D, Urtuvia V et al (2009) Diversity of *Bacteroidetes* in high altitude saline evaporitic basins in northern Chile. J Geophys Res 114:G00D05. https://doi.org/10.1029/2008JG000837

Dorador C, Vila I, Remonsellez F et al (2010) Unique clusters for Archaea in Salar de Huasco, an athalassohaline evaporitic basin of the Chilean Altiplano. FEMS Microbiol Ecol 73:291–302. https://doi.org/10.1111/j.1574-6941.2010.00891.x

Evershed RP, Bull ID, Corr LT et al (2007) Compound-specific stable isotope analysis in ecology and paleoecology. In: Michener RH, Lajtha K (eds) Stable isotopes in ecology and environmental science, II edn. Blackwell, Oxford, pp 480–540

Farias ME, Rasuk MC, Gallagher KL et al (2017) Prokaryotic diversity and biogeochemical characteristics of benthic microbial ecosystems at La Brava, a hypersaline lake at Salar de Atacama, Chile. PLoS ONE 12(11):e0186867. https://doi.org/10.1371/journal.pone.0186867

Farías ME, Contreras M, Rasuk MC et al (2014) Characterization of bacterial diversity associated with microbial mats, gypsum evaporites and carbonate microbialites in thalassic wetlands: Tebenquiche and La Brava, Salar de Atacama, Chile. Extremophiles 18:311–329. https://doi.org/10.1007/s00792-013-0617-6

Fernandez AB, Rasuk MC, Visscher PT et al (2016) Microbial diversity in sediment ecosystems (evaporites domes, microbial mats, and crusts) of hypersaline Laguna Tebenquiche, Salar de Atacama, Chile. Front Microbiol 7:1284. https://doi.org/10.3389/fmicb.2016.01284

Ghomi MR, Von Elert E, Borcherding J et al (2014) Fatty acid composition and content of round goby (*Neogobius melanostomus* Pallas 1814) and monkey goby (*Neogobius fluviatilis* Pallas 1814), two invasive gobiid species in the lower Rhine River (Germany). J Appl Ichthyol 30(3):527–531. https://doi.org/10.1111/jai.12312

Happel A, Czesny S, Rinchard J et al (2017) Data pre-treatment and choice of resemblance metric affect how fatty acid profiles depict known dietary origins. Ecol Res 32:757–767. https://doi.org/10.1007/s11284-017-1485-9

Imhoff JF, Thiemann B (1991) Influence of salt concentration and temperature on the fatty acid compositions of *Ectothiorhodospira* and other halophilic phototrophic purple bacteria. Arch Microbiol 156:370-375. https://doi.org/10.1007/BF00248713

Kelley C, Nicholson BE, Beaudoin CS et al (2014) Trimethylamine and organic matter additions reverse substrate limitation effects on the ^{13}C values of methane produced in hypersaline microbial mats. Appl Environ Microbiol 80(23):7316–7323. https://doi.org/10.1128/AEM.02641-14

King G (2015) Carbon monoxide as a metabolic energy source for extremely halophilic microbes: implications for microbial activity in Mars regolith. Proc Natl Acad Sci USA 112(14):4465–4470. https://doi.org/10.1073/pnas.1424989112

Larsen T, Taylor DL, Leigh MB et al (2009) Stable isotope fingerprinting: a novel method for identifying plant, fungal, or bacterial origins of amino acids. Ecology 90:3526–3535. https://doi.org/10.1890/08-1695.1

Lizama C, Monteoliva-Sanchez M, Suárez-García A et al (2002) *Halorubrum tebenquichense* sp. nov., a novel halophilic archaeon isolated from the Atacama Saltern, Chile. Int J Syst Evol Microbiol 52:149–155. https://doi.org/10.1099/00207713-52-1-149

Mardones L (1997) Flux et evolution des solutions salines dans les systemes hidrologiques des salars d' Ascotan et d' Atacama. These de Doctorat en Sciences de la Terre, Universitè et Paris

McCutchan JH, Lewis WM, Kendall C et al (2003) Variation in trophic shift for stable isotope ratios of carbon, nitrogen, and sulfur. Oikos 102:378–390. https://doi.org/10.1034/j.1600-0706.2003.12098.x

McDuff S, King GM, Neupane S et al (2016) Isolation and characterization of extremely halophilic CO-oxidizing

Euryarchaeota from hypersaline cinders, sediments and soils and description of a novel CO oxidizer, *Haloferax namakaokahaiae* Mke2.3T, sp. nov. FEMS Microbiol Ecol 92(4):fiw028. https://doi.org/10.1093/femsec/fiw028

Middelburg JJ (2014) Stable isotopes dissect aquatic food webs from the top to the bottom. Biogeosciences 11:2357–2371. https://doi.org/10.5194/bg-11-2357-2014

Mizutani H, Fukuda M, Kabaya Y (1992) ^{13}C and ^{15}N enrichment factors of feathers of 11 species of adult birds. Ecology 73:1391–1395. https://doi.org/10.2307/1940684

Ortiz C, Aravena R, Briones E et al (2013) Sources of surface water for the Soncor ecosystem, Salar de Atacama basin, northern Chile. Hydrolog Sci J 59:336–350. https://doi.org/10.1080/02626667.2013.829231

Paliy O, Shankar V (2016) Application of multivariate statistical techniques in microbial ecology. Mol Ecol 25:1032–1057. https://doi.org/10.1111/mec.13536

Pedrós-Alió C (2012) The rare bacterial biosphere. Ann Rev Mar Sci 4:449–466. https://doi.org/10.1146/annurev-marine-120710-100948

Peterson BJ, Fry B (1987) Stable isotopes in ecosystem studies. Annu Rev Ecol Syst 18:293–320. https://doi.org/10.1146/annurev.es.18.110187.001453

Prado B, Lizama C, Aguilera M et al (2006) *Chromohalobacter nigrandesensis* sp. nov., a moderately halophilic, Gram-negative bacterium isolated from Lake Tebenquiche on the Atacama Saltern, Chile. Int J Syst Evol Microbiol 56:647–651. https://doi.org/10.1099/ijs.0.63983-0

Quillaguamán J, Delgado O, Mattiasson B et al (2004) *Chromohalobacter sarecensis* sp. nov., a psychrotolerant moderate halophile isolated from the saline Andean region of Bolivia. Int J Syst Evol Microbiol 54:1921–1926. https://doi.org/10.1099/ijs.0.63153-0

Rasuk MC, Fernández AB, Kurth D et al (2016) Bacterial diversity in microbial mats and sediments from the Atacama Desert. Microb Ecol 71:44–56. https://doi.org/10.1007/s00248-015-0649-9

Risacher F, Alonso H (1996) Geoquímica del Salar de Atacama, parte 2: evolución de las aguas. Rev Geol Chile 23(2):123–134. https://doi.org/10.5027/andgeoV23n2-a02

Risacher F, Alonso H, Salazar C (1999) Geoquímica de Aguas en Cuencas Cerradas: I, II y III Regiones - Chile. Volumen III: Estudio de Cuencas de la II Región. Convenio de Cooperación DGA – UCN – IRD (S.I.T. N° 51)

Thiel V, Tank M, Neulinger SC et al (2010) Unique communities of anoxygenic phototrophic bacteria in saline lakes of Salar de Atacama (Chile): evidence for a new phylogenetic lineage of phototrophic *Gammaproteobacteria* from *pufLM* gene analyses. FEMS Microbiol Ecol 74:510–522. https://doi.org/10.1111/j.1574-6941.2010.00966.x

Zúñiga LR, Campos V, Pinochet H et al (1991) A limnological reconnaissance of lake Tebenquiche, Salar de Atacama, Chile. Hydrobiologia 210:19–24. https://doi.org/10.1007/BF00014320