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Prokaryotic Community in Lacustrine Sediments of Byers Peninsula (Livingston Island, Maritime Antarctica)

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Abstract Byers Peninsula (Livingston Island, Antarctica), the largest seasonally ice-free region of the Maritime Antarctica, holds a large number of lakes, ponds, and streams. The prokaryotic structure and bacterial diversity in sediment samples collected during the 2008–2009 austral summer from five inland lakes, two coastal lakes, and an estuarine site were analyzed by Catalyzed Reporter Deposition Fluorescence In Situ Hybridization (CARD-FISH) and 16S rRNA 454 tag pyrosequencing techniques, respectively. Differently from inland lakes, which range around the oligotrophic status, coastal lakes are eutrophic environments, enriched by nutrient inputs from marine animals. Although the prokaryotic abundances (estimated as DAPI stained cells) in sediment samples were quite similar among inland and coastal lakes, *Bacteria* always far dominated over *Archaea*. Despite the phylogenetic analysis indicated that most of sequences were affiliated to a few taxonomic groups, mainly referred to *Proteobacteria*, *Bacteroidetes*, and *Actinobacteria*, their relative abundances greatly differed from each site. Differences in bacterial composition showed that lacustrine sediments were more phyla rich than the estuarine sediment. Proteobacterial classes in lacustrine samples were dominated by *Betaproteobacteria* (followed by *Alphaproteobacteria*, *Deltaproteobacteria*, and *Gammaproteobacteria*), while in the estuarine sample, they were mainly related to *Gammaproteobacteria* (followed by *Deltaproteobacteria*, *Epsilonproteobacteria*, *Alphaproteobacteria*, and *Betaproteobacteria*). Higher number of sequences of *Alphaproteobacteria*, *Cyanobacteria*, *Verrucomicrobia*, and *Planctomycetes* were observed in sediments of inland lakes compared to those of coastal lakes, whereas *Chloroflexi* were relatively more abundant in the sediments of coastal eutrophic lakes. As demonstrated by the great number of dominant bacterial genera, bacterial diversity was higher in the sediments of inland lakes than that in coastal lakes. *Ilumatobacter* (*Actinobacteria*), Gp16 (*Acidobacteria*), and *Gemmatimonas* (*Gemmatimonadetes*) were recovered as dominant genera in both inland and coastal lakes, but not in the estuarine sample, indicating that they may be useful markers of Antarctic lakes. The proximity to the sea, the different lake depths and the external or internal origin of the nutrient sources shape the bacterial communities composition in lacustrine sediments of Byers Peninsula.

Keywords Bacterial diversity · Maritime Antarctic lakes · 454 Pyrosequencing

This paper is dedicated to the memory of our wonderful colleague and friend, Dr. Luigi Michaud, who dramatically passed away in Antarctica.

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Introduction

The Maritime Antarctic region, which comprises the western side of the Antarctic Peninsula and the nearby Subantarctic

islands, is characterized by a less extreme climate with higher mean temperatures and more precipitation than southern Antarctic areas. Byers Peninsula, the largest seasonally ice-free region of the Maritime Antarctica, includes a large number of lakes, ponds, and streams [1]. Most lakes are shallow and unstratified, or the deepest ones (up to 9 m in Midge Lake), mainly inland lakes located in the central plateau, are cold monomictic, with a summer mixing ice-free period and winter stratification below the ice cap [2].

The harsh environmental conditions in Antarctic lakes explain the dominance of microorganisms [3]. Culture-independent molecular approaches, mainly based on PCR amplification of small subunit ribosomal RNA sequences, demonstrated that the microbial communities inhabiting the water column in Maritime Antarctic lakes consist of relatively low number of taxa, including a variety of viruses, *Bacteria*, *Archaea*, heterotrophic protists, and algae, compared to temperate lakes [4–6]. Bacterioplankton diversity of some Antarctic lakes, investigated by using genetic fingerprinting techniques, showed a close relationship between geographical (proximity to the sea), physical (depth), and chemical features (inorganic dissolved nutrients and chlorophyll-*a*), and the influence of lake's catchment processes on bacterial diversity [7, 8].

Even under the summer homogeneous physical conditions, the presence of benthic mosses (*Drepanocladus longifolius*) covering the bottom of the deepest lakes can be an important source of bacterial diversity in these aquatic systems [1, 9]. Benthic mosses may favor a "biological stratification," causing strong differences in the relative abundance of the dominant bacterial taxa within the water column [7, 10]. The development of distinct microbial populations in the deep part of the lakes of Byers Peninsula compared to surface waters has been previously reported [8, 11].

To gain more information on the microbiota of lacustrine ecosystems of Byers Peninsula, in our study, sediments were collected from five inland lakes located in the central plateau (Limnopolar, Somero, Domo, Chica, and Turbio), and from two coastal lakes (Maderos and Refugio). The studied lakes represent the lacustrine environmental heterogeneity in the Peninsula [7], since the trophic status of inland lakes ranges from ultraoligotrophic to mesotrophic, whereas coastal lakes display eutrophic conditions because marine animals enrich the waters with organic materials [1, 7]. Similar trophic conditions were described in other coastal lakes of the Maritime Antarctica (e.g., the Pingüi Pond, located in the Hope Bay) [4]. Lake sediments are one of the most complex microbial habitats, where prokaryotes give the main contribution to the transformation of organic carbon, sulfur, nitrogen compounds, and metals, and therefore, they play an important role in nutrient cycling and food webs. In studies of the structure and function of aquatic ecosystems, reliable estimates of microbial numbers, diversity, and activity are critical [12].

The aim of the present work is to describe, for the first time, the prokaryotic community structure and bacterial composition in the sediments from both inland and coastal Maritime Antarctic lakes, ranging from oligotrophic to eutrophic conditions, and to relate the *Bacteria* associated with sediments with the main ecological features of the studied environments, including the sediment biogeochemical features.

Materials and Methods

Study Area and Sampling

Byers Peninsula (Livingston Island, South Shetland Islands) is a 60.6-km^2 area that remains ice-free during the summer period. It is located between latitudes 62° 34′ 35″–62° 40′ 35″ S and longitudes 60° 54′ 14″–61° 13′ 07″ W and has been designed as the Antarctic Specially Protected Area (ASPA) no. 126 due to important natural and historical values [13]. In the framework of the International Polar Year and coexisting with a high number of parallel studies in the area [14], surface sediment samples were collected during the austral summer 2008–2009 from five oligotrophic, inland lakes located in the central plateau of the Peninsula, coded as previously designed [1] (L1, Lake Limnopolar; L2, Lake Somero; L8, Lake Domo; L11, Lake Chica; L15, Lake Turbio) and from two coastal lakes (L5, Lake Maderos, and L6, Lake Refugio) located at the President Beaches and South Beaches, respectively. An estuarine sediment sample (S1) was collected as well at the mouth of the Petreles stream into the ocean, whose sediments are regularly affected by tides (Fig. 1).

Sampling was performed manually by using acid-washed, alcohol-sterilized polycarbonate corers (15 mm in diameter, 5 cm length) and 2-cm depth sediment cores were obtained. For each sample, several sediment cores were immediately placed in acid-washed plastic bags (Whirlpak) or in PE acid-washed bottles, then pretreated in situ within 5 h after sampling, as indicated below, and later shipped to our labs in Europe under the selected storage conditions. Subsamples for microbial abundance estimation were fixed with formaldehyde 2 % (*v*/*v*; final concentration), whereas subsamples for chemical analyses and DNA extraction were directly stored at −20 °C, and a replicate for DNA analysis was added by RNA-later stabilization reagent (Qiagen) until further processing.

Chemical Characterization of Water and Sediment Samples

Some variables were determined in situ, including maximum depth, temperature, pH, and conductivity, that were measured using the appropriate sensors, as previously described [2, 10]. The concentrations of dissolved nitrogen, nitrite plus nitrate, measured by the cadmium reductive method, and ammonium,

Fig. 1 Map of the Byers Peninsula (Livingston Island) and location of studied lakes and the Petreles stream estuary (S1) (modified from Toro et al., 2007). *L1*, Lake Limnopolar; *L2*, Lake Somero; *L5*, Lake Maderos; *L6*, Lake Refugio; *L8*, Lake Domo; *L11*, Lake Chica; *L15*, Lake Turbio

phosphorus (orthophosphate), and soluble silica compounds in water were determined in the lab on in situ-filtered samples following standard methods [15]. Total nitrogen (TN) and total phosphorus (TP) in water were determined on nonfiltered samples after digestion, using the same method described below for the sediments, but in this case on 20 ml water samples. All these analyses were performed on samples frozen immediately after sampling, then melt in the lab in Europe prior to the analyses. For the dissolved organic carbon (DOC) determinations, water was filtered in situ through 0.2-μm cellulose nitrate filters and then fixed with 0.2 ml of 1 N HCl; these samples were stored in acid-washed glass bottles at 4 °C and analyzed using a Shimadzu TOC-V CSN analyzer. Seston (that includes phytoplankton) samples were collected through filtration of a certain water volume on GF/F glass fiber filters (47 mm in diameter, Whatman) for the determination of chlorophyll-*a* concentration. Filters were kept frozen until the moment of the high-performance liquid chromatography (HPLC) analyses, which were performed on acetone extracts as previously described [16].

Moisture, organic matter, and carbonate content of the sediments were determined using gravimetric analysis [15]. Dry weight was determined after drying the sediments at 105 °C for 3 h. Subsequently, the weight of organic matter was obtained after ignition of the sample at 460 °C for 6 h by subtracting the weight loss from the previously determined dry weight. Then, the same procedure was followed to determine the carbonate content after ignition at 960 °C. For elemental (TN and TP) analyses, melted samples were first dried at 50 °C until obtaining a stable weight, and subsequently, they were ground to powder in a mortar. Total nitrogen was determined by adding NaOH–persulfate digestion reagent (6 g l^{-1} NaOH and 6 g l^{-1} $K_2S_2O_8$ final concentration), then digested for 2 h at 150 °C. The resulting nitrate was determined by the UV spectroscopy technique [17]. Total phosphorus was determined after a persulfatic-acid hydrolysis (0.072 N sulfuric acid and 12 g l^{-1} potassium peroxodisulfate final concentrations) of samples at 150 °C for 2 h. The obtained orthophosphate was determined using an ascorbic acid reduction of the phosphomolybdate complex following the Murphy and Riley method as for orthophosphate in water samples [15]. Chemical oxygen demand (COD) in the sediments was determined after oxidation of the organic matter in a strong sulfuric acid medium by $K_2Cr_2O_7$ at 150 °C, with back titration. The content of photosynthetic pigments in the sediments (i.e., chlorophylls and taxa-specific carotenoids) was assessed by HPLC [16]. Pigments were extracted from a suitable amount of sediment from each sample in pure acetone by vortexing and sonication. This procedure was repeated several times until the absorbance of the last extract did not exceed the 1 % of the first one. Peaks were identified by comparing them with those of pure standards purchased from DHI (Denmark). The potential hydrolytic capability in the sediments was explored by measuring fluorescently the degradative activity of endo-1,4-β-glucanase (cellulase). Accordingly, the fluorogenic molecule 4-methyl-umbelliferyl-beta-cellobiose served as substrate [18] for enzymatic activities determined at 4 and 14 °C, each in duplicates.

Prokaryotic Cells Abundance

Cell Detachment from Sediments by Density Gradient Centrifugation

To detach cells from the slurry sediment fixed with formaldehyde, chemical and physical treatments were performed as

previously described [19] with some minor modifications. Briefly, 1 g of wet slurry sediment was mixed with sodium pyrophosphate (0.1 %, *v*/*v*; Sigma-Aldrich) and Tween 20 (0.5 %, *v*/*v*; Research Organics Inc.), and then sonicated for 20 cycles of 30 s in ice by using a Brandelin SonoPlus HD 200 (Probe MS 72/D). Density gradient centrifugation was performed using Hystodenz™ as density nonionic gradient medium (Sigma-Aldrich). One volume in Histodenz concentration (1.310 g ml^{-1} of sterile distilled water) was carefully added to one volume of each slurry sediment by using a syringe needle with adequate length to reach the bottom of a tube. Samples were centrifuged (14,000*g* for 45 min at 4 °C) to allow the formation of four distinct layers (i.e., supernatant, cell layer, Histodenz cushion, and sediment pellet). The cell layer was carefully collected by an Eppendorf tip and used for subsequent analyses.

Abundance of Prokaryotic Cells Determined by DAPI Staining

To determine the abundance of total prokaryotic cells (total counts, TC), harvested cells were stained with 4′,6-diamidino-2-phenylindole (DAPI) fluorochrome (1 μg ml^{-1}, final concentration) and filtered through black polycarbonate membrane filters (0.2-μm pore size, 25-mm diameter, Nuclepore Corporation, Pleasanton, USA). TC were evaluated by using epifluorescence microscopy (Olympus BX-60M, at ×1000 magnification) [20].

Enumeration of Bacteria and Archaea by CARD-FISH

Catalysed Reporter Deposition-Fluorescence In Situ Hybridization (CARD-FISH) was used on harvested cells to estimate the abundance of microorganisms ascribed to *Bacteria* and *Archaea* domains, according to a protocol previously reported [21] with slight modifications. Probes EUB338 I, EUB338 II, and EUB338 III were combined in a mixture (EUB338 I–III) to enumerate bacterial cells, and ARCH915 probe [22] was used to evaluate archaeal cells. Oligonucleotides labeled with the cyanine dye Cy3 were purchased from ThermoHybaid (Interactiva Division, Ulm, Germany). Briefly, filter sections were prepared for hybridization by first embedding the cells with 0.2 % (*w*/*v*) low-gelling point agarose and drying at 37 °C, then inactivating the endogenous peroxidases by submerging filters 10 min in 0.01 M HCl. Permeabilization of cells was conducted in a lysozyme solution (10 mg ml^{-1} lysozyme; 0.05 M EDTA; 0.1 M Tris–HCl, pH 8) at 37 °C for 60 min, followed by washing in deionized sterile water and absolute ethanol. The hybridization was carried out at 46 °C for 2.5 h in a 300:1 mix of hybridization buffer (0.36 M NaCl; 8 mM Tris-HCl, pH 8; 40 mg ml^{-1} dextran sulfate; 35 % formamide; 0.4 % Roche Blocking Reagent; 0.08 % SDS) and horseradish peroxidase-conjugate probes (working solutions 50 ng μl^{-1}; Biomers, Ulm, Germany). For signal amplification with catalyzed reporter deposition, filter sections were incubated in the dark for 15 min at 37 °C in a mix of amplification buffer (1× PBS, 0.1 % Roche Blocking Reagent, 2 M NaCl and 0.1 g/ml dextran sulfate), H_2O_2 (0.015 %) and the fluorescently labeled tyramide. Filter sections were then counterstained with DAPI at a final concentration of 1 μg ml^{-1} and mounted onto microscope slides with a drop of a 4:1 mixture of Citifluor-VectaShield. For each sample, between 50 and 200 cells were then counted under epifluorescence, using Olympus BX60 microscope, equipped with an appropriate filter set for Cy3.

Bacterial Community Composition Analysis

DNA Extraction

DNA was extracted from 5 g of sediment by employing the MoBio PowerMax Soil DNA isolation kit (MoBio Laboratories, Carlsbad, CA, USA) according to the manufacturer's instructions. DNA concentrations and purity were quantified by using a NanoDrop ND-1000 UV-vis Spectrophotometer (NanoDrop Technologies, USA).

Amplification of 16S rRNA Genes and Pyrosequencing

The V3-V4 region of the 16S rRNA genes was amplified by PCR. In order to reduce bias in massive sequencing, the "two-step PCR" protocol was applied [23], consisting in a first step of 20 PCR cycles with conventional PCR primers and then using 1 μl of first reaction amplicon for 5 cycles PCR with barcoded primers. For each sample, triplicate PCR reactions were set up at 0 °C under a PCR cabin by using 2.5 U of Taq Fast Start High Fidelity PCR System (Roche) in a reaction buffer containing 25 mM $MgCl_2$, 10 mM dNTPs, 10 mg ml^{-1} BSA (New England Biolabs), and 0.4 μM of the two universal bacterial primers Bact341F (5′-CCTACGGGAGGCAGCAG-3′) and Bact805R (5′-GACTACCAGGGTATCTAAT-3′). The three reactions were pooled and used for the second PCR with the same conditions. Amplicons were then purified using a 0.8 % agarose gel (*w*/*v*) and a QIAquick Gel Extraction Kit (Qiagen) following manufacturer's instructions. The following thermal cycling scheme was used: 94 °C for 3′, 20 cycles (5 cycles for the second PCR) of 94 °C 15″, 55 °C 45″, 72 °C 60″, final extension at 72 °C for 8′. The sequences of the partial 16S rRNA genes were determined by using a Roche GS-FLX 454 pyrosequencer (Roche, Mannheim, Germany), following the instructions of the manufacturer for amplicon sequencing.

Postrun Analysis

All the raw reads were treated with the Pyrosequencing Pipeline Initial Process [24] of the Ribosomal Database Project (RDP) in order to sort those exactly matching the specific barcodes into different samples, to trim off the adapters, barcodes and primers using the default parameters, and to remove sequences containing ambiguous "N" or shorter than 150 bp [25]. Selected reads were denoised using the "pre.cluster" command in Mothur platform [26] to remove sequences that are likely due to pyrosequencing errors [27]. The average length of all bacterial sequences without the primers was 515 bp. Chimeric sequences were excluded by using USEARCH (http://www.drive5.com/usearch/) [28].

Analyses of Bacterial Communities

To generate taxonomic profiles, sequences were assigned to taxonomic groups by using the Naïve Bayesian classifier v.2.1 [29] from RDP, with a bootstrap cutoff of 80 %. Reads were clustered in operational taxonomic units (OTUs) at 97 % pairwise identities using UCLAST [28]. Diversity analyses were conducted using the software program Mothur (http://www.mothur.org/wiki/Download_mothur) [26]. The observed richness (OTUs at 97 % similarity level), the nonparametric estimators of richness (abundance-based coverage estimator, ACE) and Chao1, and the Shannon diversity index (H′) were computed for all recovered high-quality sequences from each sample.

To compare the bacterial community compositions across groups of samples, distinguished as inland and coastal lakes, Bray–Curtis similarity analyses were performed and similarity matrices were used to obtain dendrograms and nonmetric multidimensional scaling (NMDS) plots by using PRIMER 6.1.12 (Primer-E, Ltd).

Principal component analyses (PCAs) were also performed on data from selected physical and chemical properties of sediments and lake waters, and the relative abundance of significant bacterial groups. Environmental variables used in these analyses were as follows: lake depth (Depth), water electrical conductivity (Cond), and, for sediments, concentrations of chlorophyll-*a* (Chl-*a*), phaeophytin (Phaeo), organic matter (OM), total nitrogen (TN), and total phosphorus (TP), as well as the evaluation of 1,4-β-glucanase activity at 4 °C (Cellu4). Nonparametric Mann–Whitney tests were performed to evaluate differences in the relative abundance of bacterial groups. Differences were considered significant when p value was less than 0.05. Prior to this analysis, the data were log-transformed to linearize the relationships and avoid the influence of magnitude.

Results

Chemical Characterization of the Main Limnological Variables

The water of all the systems has low mineralization, with coastal lakes (L5 and L6) showing higher saline content though they are still freshwater lakes (Table 1).

Trophic status of inland lakes generally ranges around ultraoligotrophic (L8) to oligotrophic conditions, whereas coastal lakes are eutrophic (L5 and L6), as shown by the chlorophyll-*a*, COD, and nutrient (N and P) concentrations (Table 1).

Sediment features (Table 2) appeared in consonance with the trophic status registered on lakes' waters. The higher abundances of TN, TP, and organic matter (COD and % OM) were recorded in sediments from the coastal lakes (L5 and L6) and from the shallowest inland Lake Somero, as well as in the Petreles river mouth where a slight accumulation of materials occurred. Sediments of Lake Somero, which are partly covered by microbial mats, also showed the highest pigment (chlorophyll-*a* and phaeophytin) content, while in the coastal lakes, where microbial mats were not so extensive, relatively high pigment concentrations were observed in comparison with those of the rest of the inland lakes. Chlorophyll-*a* to phaeophytin ratios were higher for the coastal lakes (L5 and L6) and the lowest for the estuarine S1 site. N/P molar ratios in the sediments ranged from 4.6 to 13.7, and they were always below the Redfield ratio (16:1). 1,4-β-glucanase (cellulase) activity, a measure of the degradative capacity of the sediment's microbiota, always yielded higher rates for both the coastal lakes and the estuarine sample compared to the inland lakes, regardless the temperature at which the enzymatic activity was determined, although this activity consistently increased at 14 °C compared to 4 °C.

Prokaryotic Abundance

The abundance of total prokaryotic cells (TC) in the analyzed sediments, obtained after DAPI staining, ranged from 64.8 (L8, the ultraoligotrophic lake) to 184.0×10^5 cells g^{-1} (L6, coastal eutrophic lake), and was lower in all cases in comparison to those values retrieved from the estuarine S1 site (Table 3).

The abundances of cells hybridized with probes for *Bacteria* (EUB338 I-III) and *Archaea* (ARCH915) are reported in Table 3. The recovery of *Bacteria* by CARD-FISH related to TC (ranging from 65.27 to 74.47 % of TC) was higher in the eutrophic coastal lakes and in S1 site than that retrieved from the oligotrophic inland lakes (46.3–61.2 % of TC) (Table 3). Archaeal contribution to TC in the lakes ranged from 0.4 (L11) to 1.2 % (L8) and was 1.1 % in S1 site. *Archaea* to *Bacteria* ratio was higher (3:100) in the

Table 1 Physical and chemical characteristics in water collected from lakes of the Byers Peninsula and in the estuarine site, during the sampling period (austral summer 2009)

	Site	Lake	Max depth (m)	Temp[a] (°C)	pH	Conductivity (μS cm^{-1})	Chl-*a* (μg l^{-1})	DOC (mg l^{-1})	NH_4 (μM)	NO_2+NO_3 (μM)	Total N (μM)	SRP-PO_4 (μM)	Total P (μM)	SiO_2 (μM)
Inland lakes	L1	Limnopolar	5.5	4.5	7.5	67.0	0.1	1.1	0.4	0.0	3.0	0.0	0.2	93.2
	L2	Somero	0.5	5.4	7.2	70.0	0.5	2.6	0.8	0.9	12.1	0.1	0.9	92.7
	L8	Domo	4.5	2.8	7.0	26.0	0.1	0.5	0.1	0.1	2.4	0.0	0.1	19.2
	L11	Chica	2.5	5.7	6.8	45.0	0.2	0.6	0.6	0.8	4.4	0.1	0.3	47.5
	L15	Turbio	7.8	5.1	7.0	93.0	0.2	0.6	0.5	1.0	3.0	0.1	0.5	51.2
Costal lakes	L5	Maderos	0.5	3.0	7.4	257.0	12.8	3.7	9.3	6.2	81.1	0.2	5.8	14.7
	L6	Refugio	0.5	4.4	8.5	130.0	18.9	3.5	12.9	8.7	44.9	0.1	7.4	26.6
Estuarine site	S1	Petreles	0.3	5.8	6.8	275.0	n.d.	n.d.	0.8	18.7	n.d.	0.9	n.d.	76.2

[a] Mean values

DOC dissolved organic carbon, *n.d.* not determined

Table 2 Main characteristics of sediments collected from lakes of the Byers Peninsula and in the estuarine site, during the sampling period (austral summer 2009) and endo-1,4-β-glucanase (cellulase) activity in sediments at 4 and 14 °C

	Site	Lake	Chl-*a* (μg g^{-1} dw)	Phaeophytin (μg g^{-1} dw)	Chl/Phaeo	COD (mg O_2 g^{-1} dw)	Organic matter (% of dw)	Carbonate content (% of dw)	TN (mg-N g^{-1} dw)	TP (mg-P g^{-1} dw)	N/P (molar ratio)	Cellulase activity 4 °C (nmol g^{-1} dw h^{-1})	Cellulase activity 14 °C (nmol g^{-1} dw h^{-1})
Inland lakes	L1	Limnopolar	0.8	0.4	2.1	13.6	3.4	1.7	3.4	0.6	11.8	0.2±0.1	0.4±0.1
	L2	Somero	6.2	3.1	2.0	13.1	3.7	2.2	6.8	1.3	11.6	0.3±0.1	0.5±0.1
	L8	Domo	0.04	0.02	2.0	1.8	1.0	1.8	1.6	0.4	10.4	0.1±0.1	0.3±0.2
	L11	Chica	0.4	0.2	2.0	2.6	1.7	2.4	1.0	0.5	4.6	0.1±0.01	0.3±0.1
	L15	Turbio	0.1	0.03	1.6	6.4	2.0	1.6	2.0	0.6	7.4	0.1±0.02	0.4±0.1
Coastal lakes	L5	Maderos	2.4	0.3	7.5	19.8	5.0	2.2	6.2	1.3	10.5	0.3±0.03	0.8±0.1
	L6	Refugio	3.4	0.5	6.9	26.1	7.0	2.1	10.9	1.8	13.7	0.3±0.1	0.8±0.1
Estuarine site	S1	Petreles	2.1	1.8	1.2	14.9	5.0	2.7	4.8	0.9	12.2	0.2±0.1	1.0±0.1

dw dry weight, *COD* chemical oxygen demand, *TN* total nitrogen, *TP* total phosphorus

Table 3 Abundances ($\times 10^5$ cells g^{-1}) of total prokaryotic cells (TC) and of hybridized cells with probes for *Bacteria* (EUB338 I-III) and *Archaea* (ARCH915) in sediments from inland lakes (L1–L15), coastal lakes (L5 and L6), and from the estuarine site (S1)

	Site	Lake	TC	*Bacteria*	*Archaea*	*Archaea/Bacteria*
Inland lakes	L 1	Limnopolar	117.0±12.1	71.5±7.4 (61)	1.2±0.3 (1.1)	0.02
	L 2	Somero	97.3±14.6	53.3±14.0 (55)	0.9±0.5 (0.9)	0.02
	L 8	Domo	64.8±11.9	30.3±11.0 (47)	0.8±0.5 (1.2)	0.03
	L 11	Chica	133.0±19.1	81.6±25.8 (61)	0.5±0.3 (0.4)	0.01
	L 15	Turbio	134.0±13.9	71.0±14.6 (53)	0.7±0.4 (0.5)	0.01
Coastal lakes	L 5	Maderos	170.0±21.6	126.0±18.8 (74)	1.1±0.6 (0.6)	0.01
	L 6	Refugio	184.0±22.9	122.0±33.3 (66)	2.0±0.4 (1.1)	0.02
Estuarine site	S1	Petreles	223.0±18.6	173.0±27.7 (78)	2.4±0.7 (1.1)	0.01

In brackets, the percentage of cells recovered by FISH with respect to TC

ultraoligotrophic inland Lake Domo than in the other lakes, and was also higher than that observed in the estuarine sediment (1:100).

Phylogenetic Diversity of *Bacteria*

Pyrosequencing Reads

The pyrosequencing-based analysis of the V3-V4 region of the 16S rRNA genes for *Bacteria* produced 335,477 total sequences. After quality check within the RDP pyrosequencing pipeline and removing chimeras, 287,297 high-quality sequences were obtained. The highest number of high-quality reads was in sediment from lake L11 (39,661 reads), whereas the lowest value was found in the estuarine sediment (29,915 reads) (Table 4). H′ reached the highest value (4.0) in the inland L15 site and the lowest (1.9) in the coastal L6 site. Chao1 and ACE estimators predicted that the highest richness was in L1 and the lowest in L6 site. Total coverage (TCov) ranged from 47.6 (L15) to 52.7 % (L8) as estimated by Chao1, and from 31.3 (L1) to 69.4 % (L2) by ACE. The diversity index and the observed richness (OTUs) did not show a general symmetric pattern, since L15, with the highest H′ showed the lowest number of OTUs, even though L6, with the lowest H′, displayed the lowest number of OTUs.

Bacterial Taxa

A total number of 23 different bacterial phyla were retrieved, of which 16 were common to all samples. The estuarine sediment was less phylum rich than those from lacustrine sites, since four taxa (i.e., *Clamydiae*, OP10, BRC1, and *Lentisphaerae*) were absent in S1 (Table S1 in the supplemental material). Overall, sequences of the dominant taxonomic groups (abundance ≥1 %) across all sediment samples were mostly affiliated with *Proteobacteria* (range 15.6–40.0 % of high-quality sequences), *Actinobacteria* (range 8.6–27.8 %), *Bacteroidetes* (range 7.5–38.0 %), and *Verrucomicrobia* (1.2–6.1 %). However, the relative abundance at phylum level varied considerably across the different samples, determining different bacterial assemblages (Fig. 2; Table S1). *Proteobacteria* was the predominant phylum in L1, L2, L11, L5, and S1, whereas *Actinobacteria* was the predominant group in L8, L15, and L6. Differences in relative

Table 4 Information on sequences obtained by 454 pyrosequencing from sediments collected from inland (L1–L15) and coastal lakes (L5 and L6), and in the estuarine site (S1)

	Inland lakes					Coastal lakes		Estuarine site
	L1	L2	L8	L11	L15	L5	L6	S1
Number of reads	45,328	38,932	39,577	47,140	46,177	47,989	38,916	31,418
Number of high quality reads	39,077	34,092	33,798	39,661	39,314	37,954	33,486	29,915
OTUs at 97 % genetic similarity	4687	3578	4321	4896	3247	3684	2998	4178
Shannon H'	3.02	2.14	2.67	3.14	4.00	3.57	1.89	3.68
Chao1 (TCov%)	9375 (50.0)	7258 (49.3)	8794 (52.7)	9354 (52.3)	6823 (47.6)	6987 (51.0)	5879 (49.1)	8456 (49.4)
ACE (TCov%)	14,987 (31.3)	10,459 (69.4)	12,687 (63.0)	14,598 (64.1)	10,487 (65.1)	11,054 (65.7)	8947 (69.3)	12,654 (66.8)

TCov total coverage

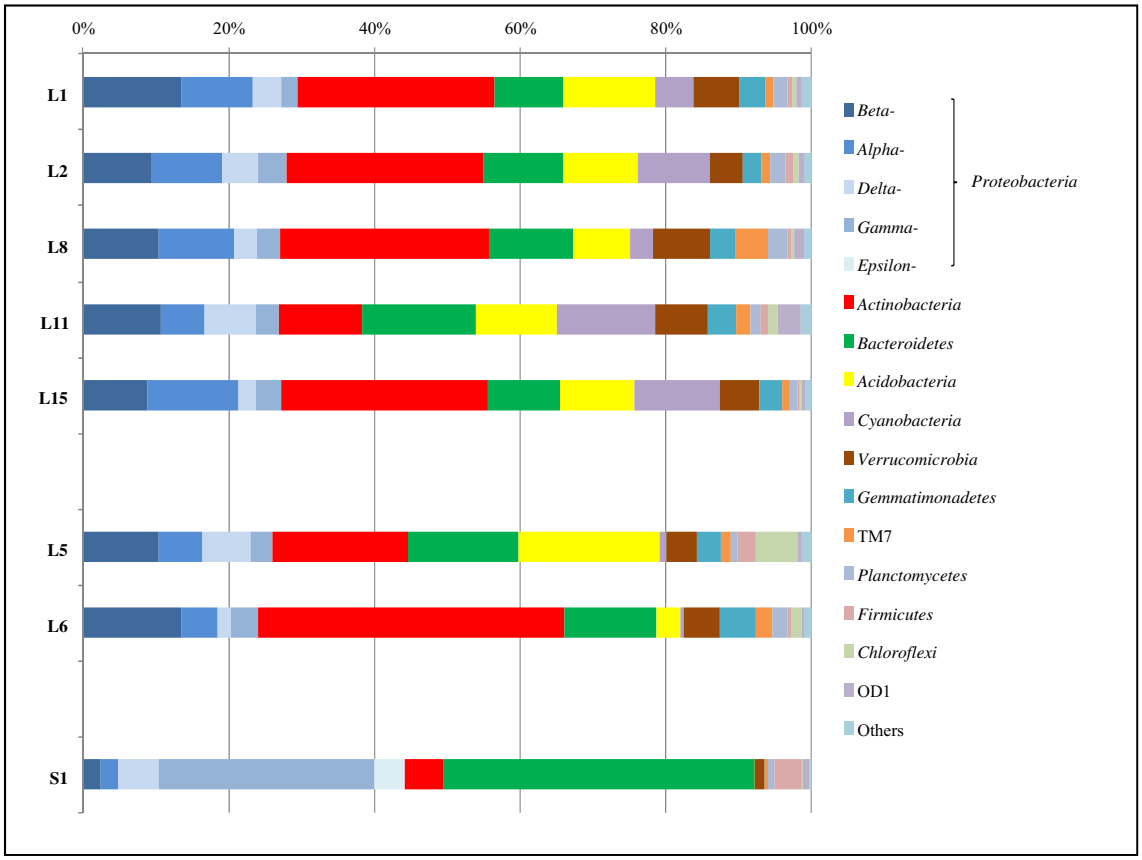

Fig. 2 Comparison of bacterial community composition in sediments collected from inland lakes (*L1*, *L2*, *L8*, *L11*, and *L15*), coastal lakes (*L5* and *L6*), and in the estuarine sediment (*S1*). Others included the following taxa: *Nitrospira*, WS3, *Spirochaetes*, SR1, *Fusobacteria*, *Chlamydiae*, OP10, BRC1, *Lentisphaerae*, *Fibrobacteres*, and *Deinococcus-Thermus*

abundances were also observed for sequences affiliated to proteobacterial classes: in the lacustrine samples, they were mainly referred to *Betaproteobacteria* (followed by *Alphaproteobacteria*, *Deltaproteobacteria*, *Gammaproteobacteria*, and *Epsilonproteobacteria*), while in the estuarine samples, they were mainly related to *Gammaproteobacteria* (followed by *Deltaproteobacteria*, *Epsilonproteobacteria*, *Alphaproteobacteria*, and *Betaproteobacteria*).

A very different community composition was observed in the sediment from S1 site, since sequences affiliated to *Proteobacteria*, *Bacteroidetes*, and *Actinobacteria* covered almost all classified bacterial sequences (Fig. 2). *Firmicutes* were more abundant (3.4 %) in the estuarine S1 site than in lacustrine ones (<1.8 %). *Acidobacteria* (6.5–20.9 %), *Gemmatimonadetes* (2.2–3.8 %), and *Verrucomicrobia* (3.0–6.1 %) were abundant in the lacustrine sediments, while they constituted a minor component in the estuarine sample. Exception was Lake Chica, where the lowest abundance of *Actinobacteria* was retrieved (Fig. 2).

The bacterial community composition of sediments of both coastal lakes differed from those determined for inland lakes, and also greatly differed each other (Fig. 2). In fact, *Proteobacteria* were there less abundant than in the inland lakes, but they resulted slightly more abundant than the *Actinobacteria* and *Bacteroidetes* in L5. Conversely, *Actinobacteria* resulted more abundant than the *Proteobacteria* and *Bacteroidetes* in L6.

Cyanobacterial abundance varied considerably between estuarine and lacustrine sediments, where they were more abundant in inland (range 2.5–10.2 %) than in coastal lakes (range 0.3–0.7 %). Cyanobacterial sequences were particularly abundant in sediments from L2, L11, and L15, whose bed was partly covered by microbial mats as lake L2 or presented partial mat coverage in the shores and higher picocyanobacterial abundance in the water column (L11 and L15). In lacustrine samples, relatively high abundant phyla also included TM7, OD1, *Planctomycetes*, and *Chloroflexi*. All the latter were particularly scarce in the estuarine site.

Among the low abundant phyla (abundance <1 %), *Nitrospira*, WS3, *Spirochaetes*, and SR1 occurred across all sediment samples, even if at different relative abundances. Sequences related to *Chlamydiae* and OP10 were recovered in lacustrine but not in the estuarine sediments. Sequences affiliated with *Fibrobacteres* and *Deinococcus-Thermus* occurred in all samples but were not retrieved in L6 and L2,

respectively. Sequences related to *Fusobacteria* were present only in samples from L5 and S1. BRC1 and *Lentisphaerae* were absent in samples L1, L8, and S1, while *Lentisphaerae* were only retrieved in L11 (Table S1). NMDS diagram, representing similarities in the bacterial community composition (phyla and proteobacterial classes) of the Antarctic lacustrine sediment samples, grouped all inland lakes except Lake Chica, whereas the coastal lakes (L5 and L6) did not cluster together because of the big differences among their respective bacterial communities (Fig. 3).

PCA based on selected physico-chemical properties of lake sediments and waters, as well as on the relative abundance of the main bacterial groups (*Alphaproteobacteria*, *Betaproteobacteria*, *Deltaproteobacteria*, *Bacteroidetes*, *Actinobacteria*, *Acidobacteria*, *Cyanobacteria*, *Verrucomicrobia*, and *Chloroflexi*), was performed to identify groups of samples with similar community compositions and to find their relationships with the environmental variables (Fig. 4). The PCA showed that the bacterial communities from inland lakes differed from those of coastal lakes, confirming results obtained by NMDS analysis that examined only the bacterial composition (Fig. 3). The two main components explained 76.1 % of the total variance. Axis 1 (explaining 55.6 % of the variance) was strongly associated with the relative abundance of *Acidobacteria* and with a combination of physical and chemical variables (TP, Cellu4, OM, depth, Chl-*a*, and Cond) that are strictly related to the trophic status of the lakes. Instead, Axis 2 (20.5 % of the variance) was mainly related to the abundances of *Bacteroidetes*, *Deltaproteobacteria*, and *Chlorofexi* and to TN concentrations.

The two coastal lakes (L5 and L6), with high factor scores for the Axis 1 and Axis 2, appeared clearly separated from the other lakes by Axis 1. Although distantly, the shallowest inland L2 appeared closer to the coastal lakes in Axis 1, mainly due to the influence of the high values of its nutrient concentrations and chlorophyll-*a* content (Table 2), which are markers of higher trophic status. The negative side of Axis 1 was associated to *Verrucomicrobia* and *Cyanobacteria*, and that of Axis 2 to *Actinobacteria* and *Betaproteobacteria*. Deeper inland lakes L1, L15, and L8 grouped together, as occurred in the NMDS plot (Fig. 3), and were distinct from L11, which showed the highest factor score for Axis 2 (Fig. 2).

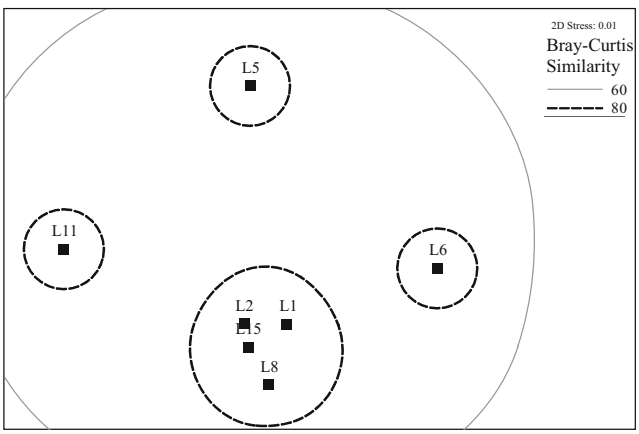

Fig. 3 Nonmetric multidimensional scaling (NMDS) diagram representing the bacterial community composition (phyla and proteobacterial classes) of Antarctic lacustrine sediment samples. The *circles* represent similarity boundary from the cluster diagram

Bacterial Genera

Of the total high-quality bacterial sequences, about 64 % were not classified at genus level. A total of 1107 genera were resolved from the rest, 40 of which were ubiquitous to all samples. The dominant bacterial genera (41), which occurred in ≥1 % of the total bacterial sequences at least in one of the eight samples, are shown in Table 5. Almost all the dominant genera retrieved from the estuarine sediment (14/15) were not retrieved in lacustrine sediments, with the only exception of OD1 that was also collected in L8 and L11 sites. Differently from lacustrine sediments, genera identified in the estuarine sediment were characteristically related to *Gammaproteobacteria*, *Epsilonproteobacteria*, and *Bacteroidetes*. Genera within *Alphaproteobacteria* were only retrieved from inland lacustrine sediments, among which *Sphingomonas* was particularly abundant in L8 and L15 sites. *Ilumatobacter* (*Actinobacteria*), Gp16 (*Acidobacteria*), and *Gemmatimonas* (*Gemmatimonadetes*) were recovered in all inland and coastal lakes, but not in the estuarine sample. *Ferruginibacter* (*Bacteroidetes*) was retrieved in all inland lakes and only in one of the two coastal lakes (L6). Nine genera were retrieved only in one of the seven lakes and may be considered distinctive of each lake. *Prolixibacter* (*Bacteroidetes*) and GpI (*Cyanobacteria*) were present only in L2 site. Within *Actinobacteria*, genera referred to *Marmoricola* and *Cryobacterium* were unique in L8, as well as *Conexibacter* in L15, and Gp3 (*Acidobacteria*) in L5. *Hymenobacter* (*Bacteroidetes*) was only retrieved in L15, as *Verrucomicrobium* in L6, and *Longilinea* (*Chloroflexi*) in L5.

Discussion

The water bodies studied in the present work are representative of the main lake types from Maritime Antarctica, differing in their trophic status, morphological features, and their distance to the sea, which in turn is linked to the relative influence of sea animals causing lake eutrophication [7]. Limnological properties (evaluated as chlorophyll-*a* and nutrient concentrations), recorded in water and sediment samples, distinguished those inland lakes as oligotrophic, with two opposite exceptions (L2 and L8), and the coastal ones as eutrophic.

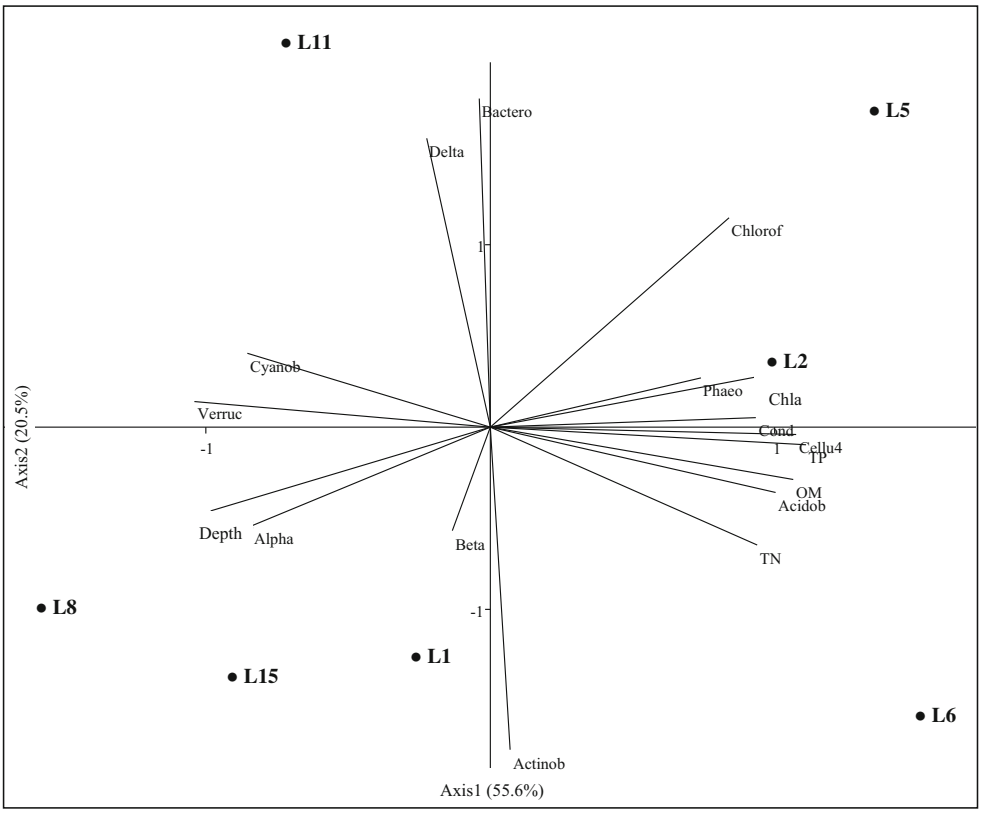

Fig. 4 Principal component analysis based on selected physico-chemical variables and the most significant bacterial populations associated with sediment samples from inland lakes (*L1*, *L2*, *L8*, *L11*, and *L15*) and coastal lakes (*L5* and *L6*). Variable examined were as follows: lake depth (Depth), water electrical conductivity (Cond) and, for the sediments, chlorophyll-*a* (Chla) and phaeophytin (Phaeo) concentrations, relative organic matter content (OM), total nitrogen (TN), total phosphorus (TP), and 1,4-β-glucanase (cellulase) activity at 4 °C (Cellu4). Bacterial phyla/classes: *Alphaproteobacteria* (Alpha), *Betaproteobacteria* (Beta), *Deltaproteobacteria* (Delta), *Acidobacteria* (Acidob), *Actinobacteria* (Actinob), *Bacteroidetes* (Bactero), *Cyanobacteria* (Cyanob), *Chloroflexi* (Chlorof), and *Verrucomicrobia* (Verruc)

The estuarine S1 site represented a totally different environment, since sediments are directly exposed to the effects of the sea, such as the diel fluctuations in the salinity. Among the inland lakes, the shallowest Lake Somero, partly covered by biofilms and microbial mats and surrounded by mosses, showed limnological features more similar to those of eutrophic coastal lakes (L5 and L6) than the other inland lakes. In contrast, the inland Lake Domo was considered ultraoligotrophic, since very low chlorophyll concentrations were always recorded, and inorganic nutrients were almost undetectable. Chlorophyll-*a* to phaeophytin ratios, which are indicative of the physiological status and photosynthetic capacity of primary producers, were also higher for the coastal lakes, in concordance with their much higher external nutrient inputs. Differently, this ratio was the lowest for the estuarine sample, indicating higher degradation in the populations of photosynthetic organisms or even a major abundance of detritus. The external sources of organic matter (mainly from sea animals) greatly influenced the trophic status of the coastal lakes sediments. When considering N/P ratios in the studied lakes, a relative low abundance of N was observed, especially in L11 and L15 sites, indicating that the sediments could represent a relatively better source of phosphorus than of nitrogen. According to the organic matter availability in sediments, the 1,4-β-glucanase (cellulase) activity (i.e., a measure of the degradative capacity of the sediment's microbiota) was higher in the coastal lakes and the estuarine sample than in the inland lakes, with the only exception of the shallow Lake Somero, which showed intermediate characteristics among both types of lakes. The cellulase activity at 14 °C was higher than that at 4 °C, and it was positively correlated ($p<0.01$) with bacterial abundances, suggesting that mesophilic microorganisms could be, at least partially, responsible for this activity.

Microorganisms may greatly influence the functioning of environments, including those characterized by very harsh conditions as the Antarctic lacustrine sediments. Beside similar prokaryotic abundance in inland and coastal lakes, *Archaea* represented 1–3 % of the total community. In comparison with the other lakes, the sediment from the ultraoligotrophic Lake Domo displayed the lowest prokaryotic abundances and the highest *Archaea* to *Bacteria* ratio, which may suggest that bacterial and archaeal populations were differently affected by the trophic conditions. It is consistent with data reported a negative correlation between total archaeal rRNA gene levels and chlorophyll *a* concentration from other environments in Antarctica [30].

Despite the high-throughput sequencing efforts, the values for the ACE and Chao1 estimators indicated that the diversity was not totally well covered for the bacterial community associated with the studied sediment samples (Table 4). Sequence reads unclassified at phylum level greatly varied among samples (10 % of total reads in the estuarine site, about 20 % in inland lakes and >30 % in coastal lakes), and they

Table 5 Bacterial genera retrieved in sediments from Antarctic inland lakes (L1–L15) and coastal lakes (L5 and L6), as well as in the estuarine site (S1)

Phylum	Class	Genus	Inland lakes					Coastal lakes		Estuarine site
			L1	L2	L8	L11	L15	L5	L6	S1
Proteobacteria	*Beta-*	*Methylibium*								
		Novosphingobium								
	Alpha-	*Rhodobacter*								
		Sphingomonas								
	Gamma-	*Granulosicoccus*								
		Leucothrix								
		Psychrobacter								
	Delta-	*Desulforhopalus*								
		Geobacter								
	Epsilon-	*Arcobacter*								
Actinobacteria		*Marmoricola*								
		Conexibacter								
		Ilumatobacter								
		Janibacter								
		Mycobacterium								
		Cryobacterium								
Bacteroidetes		*Actibacter*								
		Ferruginibacter								
		Flavobacterium								
		Arenibacter								
		Bizionia								
		Cellulophaga								
		Lutimonas								
		Maribacter								
		Hymenobacter								
		Polaribacter								
		Prolixibacter								
		Winogradskyella								
		Pseudozobellia								
Acidobacteria		Gp16								
		Gp3								
		Gp4								
		Gp6								
		Gp7								
Cyanobacteria		GpI								
Verrucomicrobia		*Verrucomicrobium*								
		Luteolibacter								
Gemmatimonadetes		*Gemmatimonas*								
TM7		TM7 *genus incertae sedis*								
OD1		OD1 *genus incertae sedis*								
Chloroflexi		*Longilinea*								

> 8% > 5% > 3% > 2% > 1 % < 1 %

may be considered as representatives of novel, not yet described bacterial taxa.

As estimated by the Shannon H′ index, bacterial diversity showed a large variation among the examined sites (Table 4). As generally accepted [31], moderately disturbed conditions often result in high diversity of communities, like those in the estuarine S1 site, where the large fluctuations of the key physico-chemical parameters may allow for the coexistence of more diverse bacterial assemblages. The proximity to the sea may also differently affect the bacterial diversity and richness, as observed in the coastal Maderos and Refugio lakes. This was sustained by the multivariate analysis, since variables other than total phosphorous and nitrogen content in sediments, such as lake depth and salinity, were also important determinants of bacterial diversity. Differently from L5, the extreme eutrophic conditions registered at L6 site can

selectively favor numerically fewer bacterial taxa, best dealing with these features. Among inland lakes, bacterial community with the highest degree of diversity and the lowest richness was from the deepest lake Turbio (L15). This was substantiated by taxonomic analyses of the different bacterial phylogenetic lineages that yielded the greatest number of genera. Compared to the other inland lakes, Lake Turbio is highly turbid because of a high wind fetch, which can be a selective factor, but also shows a relatively higher level of disturbance by the stronger wind effects.

Even if the phylogenetic analysis indicated that most of sequences were affiliated with few taxonomic groups dominated by *Proteobacteria*, *Bacteroidetes*, and *Actinobacteria*, their relative abundances greatly differed from each site. The same groups have been previously reported as the major phyla in the planktonic bacterial communities in both Arctic and Antarctic lakes [32–34]. However, sequences related to these three phyla covered ~83 % of total bacterial sequences in the estuarine sediment, while only ~50 % in lacustrine sediments, indicating that more phylotypes are involved in the lacustrine than in the estuarine bacterial composition. In addition, sequences referred to *Acidobacteria*, *Cyanobacteria*, and *Verrucomicrobia* constituted a relevant fraction of total retrieved sequences from lacustrine samples. Representative sequences of *Verrucomicrobia* and *Bacteroidetes* phyla were relatively more abundant in sediments of the lakes, and both groups were also observed in the surface waters of Lake Limnopolar [8]. *Acidobacteria* are commonly retrieved from some Antarctic terrestrial ecosystems [35]. *Acidobacteria* were not found in the clone libraries obtained from the deep water sample collected from the same lake in a previous study [8].

Although *Proteobacteria* was the largest phylum, its major classes and their proportions varied greatly among the different sites. Proteobacterial classes in lacustrine samples were dominated by *Betaproteobacteria* (followed by *Alpha*-, *Delta*- and *Gammaproteobacteria*), while in the estuarine samples, they were mainly related to *Gammaproteobacteria* (followed by *Deltaproteobacteria*, *Epsilonproteobacteria*, *Alphaproteobacteria*, and *Betaproteobacteria*). The presence of *Gammaproteobacteria*, traditionally associated with polar marine sediments [36, 37] in the lacustrine sediments, might suggest that these bacteria are transported into inland Antarctic lakes in aerosols moved by winds, and/or by fecal pellets of seabirds [8, 10], whereas the more direct marine influence by tides most probably accounts for the dominance of *Gammaproteobacteria* in the estuarine site.

Concerning the affiliation of the sequences from lacustrine sediments to lower taxonomic levels, dominant retrieved phyla contained genera that have been described for polar freshwater environments. Sequences related to the genera *Ilumatobacter* (*Actinobacteria*), *Gemmatimonas* (*Gemmatimonadetes*), and Gp16 (*Acidobacteria*) were recovered as dominant in both inland and coastal lakes, but not in the estuarine sample, indicating that they may represent markers of sediments from Antarctic lakes. Several sequences were affiliated with the *Flavobacterium* genus (within the phylum *Bacteroidetes*) that is considered a common heterotrophic member of the Antarctic bacterial communities in both aquatic and terrestrial environments [7, 38]. Novel *Flavobacterium* species related to the genus have been often reported from Antarctica [39–42], with interesting biotechnological properties [35]. On the other hand, the detection of sequences affiliated with the *Geobacter* genus (*Deltaproteobacteria*), previously retrieved in a wide variety of pristine environments [35], in Antarctic inland lakes might reflect their still pristine conditions.

Sequences related to the nonphotosynthetic *Longilinea* genus (within the phylum *Chloroflexi*), including new cultured mesophilic, strictly anaerobic, heterotrophic filamentous bacteria in the class of *Anaerolineae* [43], were only retrieved in the coastal L5 site.

Only in the sediments of Lake Somero which are partly covered by microbial mats, dominant sequences were assigned to GpI (*Cyanobacteria*), which includes species capable of fixing nitrogen, suggesting an inner load of nutrients to fed phototrophic and heterotrophic organisms. The microbial mats represent a common characteristic of vast areas in Byers Peninsula where they play important trophic functions also for lakes, as its relatively high rate of primary productivity may convert them in sources of nutrients to the microorganisms in the water column, regardless they are located in the own lake bed or in the lake's catchment area [44, 45].

Some sequences that distinguished bacterial populations of lakes L1, L8, and L15 from L11 were affiliated to potentially photosynthetic members belonging to the order *Rhodobacterales* (*Alphaproteobacteria*). Other sequences were affiliated with the genus *Sphingomonas*, which is a widely distributed group in aquatic (both freshwater and marine) and terrestrial environments in Antarctica and in temperate zones. This genus was also previously reported to be a dominant genus in water samples of Limnopolar and Somero lakes [1]. Among *Betaproteobacteria*, the most abundant group in polar freshwater ecosystems [46], several sequences were assigned to the genus *Methylibium*, which includes highly specialized bacteria able to use C1 compounds as a carbon source. This genus, retrieved in most of the examined lakes, was also detected in sediments from Arctic lakes by using pyrosequencing techniques [47].

Other highly abundant phyla (≥1 % of total reads) retrieved in Antarctic lacustrine sediments included also *Planctomycetes* members, previously found in Antarctic soils [48], suggesting that they may come from the surrounding terrestrial habitat in the highly dynamic lake catchments. At the tail of bacterial diversity, comprising <70 reads in the samples, members of the *Nitrospira*, *Fibrobacteres*,

Fusobacteria, and of several other candidate divisions (WS3, OP10, BRC1) originally detected in other extreme, low-diversity environments were also found in the present study. Considerable numbers of sequence reads related to candidate divisions included TM7 and OD1, that are found in a wide range of environments [49, 50], but of which no cultivated representatives exist to date. Thus, these Antarctic sites represent interesting environments to perform culture efforts to recover more species and diversity.

Our results demonstrated that in sediments of Byers peninsula, a close relationship exists between bacterial community compositions and the limnological characteristics. Geographical location and particularly the proximity to the sea, where marine animals provide the main external nutrient source, as well as the lake depths, which are related to the higher nutrient remobilization from sediments in the shallower lakes (internal load), may greatly affect their bacterial community composition.

Particular attention is required to inland Maritime Antarctic lakes, that as remote environments in a relatively mild polar climate may be considered as sentinels of climate change, and that as a main stressor, can modify the bacterial community structure and composition.

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