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A close link between bacterial community composition and environmental heterogeneity in maritime Antarctic lakes

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Summary. Seven maritime Antarctic lakes located on Byers Peninsula (Livingston Island, South Shetland Islands) were surveyed to determine the relationship between planktonic bacterial community composition and environmental features. Specifically, the extent to which factors other than low temperature determine the composition of bacterioplankton assemblages of maritime Antarctic lakes was evaluated. Both deep and shallow lakes in the central plateau of the Peninsula, as well as a coastal lake, were studied in order to fully account for the environmental heterogeneity of the Peninsula's lakes. The results showed that shallow coastal lakes display eutrophic conditions, mainly due to the influence of marine animals, whereas plateau lakes are generally deeper and most are oligotrophic, with very limited inputs of nutrients and organic matter. Meso-eutrophic shallow lakes are also present on the Peninsula; they contain microbial mats and a higher trophic status because of the biologically mediated active nutrient release from the sediments. Diversity studies of the lakes' planktonic bacterial communities using molecular techniques showed that bacterial diversity is lower in eutrophic than in oligotrophic lakes. The former also differed in community composition with respect to dominant taxa. Multivariate statistical analyses of environmental data yielded the same clustering of lakes as obtained based on the DGGE band pattern after DNA extraction and amplification of 16S rRNA gene fragments. Thus, even in extremely cold lakes, the bacterial community composition parallels other environmental factors, such as those related to trophic status. This correspondence is not only mediated by the influence of marine fauna but also by processes including sediment and ice melting dynamics. The bacterial community can therefore be considered to be equally representative as environmental abiotic variables in demonstrating the environmental heterogeneity among maritime Antarctic lakes. [**Int Microbiol** 2010; 13(2):67-77]

Keywords: maritime Antarctic lakes · bacterioplankton · aquatic trophic status

Introduction

The maritime Antarctic region covers the western part of the Antarctic Peninsula and nearby islands. Its climate is less extreme than that of continental Antarctica, with an annual mean precipitation of 700–1000 mm per year [5] and an average temperature around 0ºC during the austral summer. These characteristics allow the appearance of many water bodies that are free of ice. Byers Peninsula, extending from the western end of Livingston Island (South Shetland Islands), is among the largest ice-free areas in the maritime Antarctic, and includes a large number of lakes [43]. It has been declared an Antarctic Special Protected Area (ASPA No. 126: Byers Peninsula, Livingston Island, South Shetland Islands) due to the importance of its biological communities

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as well as its archaeological and geological features. Previous studies in this area [7,14,43] as well as in other areas of the maritime Antarctic [40] have revealed a correspondence between certain aspects of the biological communities and the limnological characteristics of the water bodies in which they live.

In Antarctic lakes, the harsh environmental conditions explain the dominance of microorganisms [12]. However, the microbial communities inhabiting the lakes' planktonic and benthic environments consist of a low number of taxa, including viruses, bacteria, archaea, heterotrophic protists, algae, and metazooplankton [19,21,24,25]. In many polar lakes, the benthic environment of the lakes and their catchments also contain cyanobacterial mats, a type of microbial community with widespread occurrence on Earth [2,6]. Similar mats may have an important trophic function in the maritime Antarctic lakes of Byers Peninsula due to their high productivity and nutrient input into the water column [13,16,38,43,46]. Recent studies have used culture-independent molecular techniques to evaluate the main components of the microbial communities of maritime Antarctic lakes [31,32]. The results of these analyses suggested that changes in bacterial diversity are related to variations in nutrient concentrations [30,40] and supported the use of rRNA-gene-based molecular techniques to describe the environmental heterogeneity of microbial communities present in these lakes.

Here, we report the most relevant environmental variables of several contrasting water bodies of Byers Peninsula, together with the results of genetic fingerprinting of the associated planktonic bacterial communities. This survey included selected lakes within the central plateau of the Peninsula. Additionally, a coastal lake influenced by nutrient input from marine fauna (elephant seals, marine birds), and thus with a higher trophic status, was included. Byers Peninsula has been selected as an Antarctic reference site for the study of coastal, terrestrial, and limnetic ecosystems [39], as the maritime Antarctic is among the areas on Earth currently undergoing intense warming [37]. Increased knowledge of the Byers Peninsula's ecological features and biological communities is essential to model the possible effects of rising temperature occurring in the region [42] and may help to establish Byers Peninsula as a global climate change observatory.

Materials and methods

Study area. The studied area was located on Byers Peninsula (Livingston Island, South Shetlands Islands, Antarctica), between 62º34´35´´–62º40´35´´ S and 60º54´14´´–61º13´07´´ W. Compulsory permits for working in this ASPA were obtained from the Spanish Polar Committee (CPE) in coordination with the Scientific Committee on Antarctic Research. Byers Peninsula is characterized by the presence of a large number of water bodies that undergo melting during the austral summer. The thaw is accompanied by the intense development of microbial communities, both planktonic and benthic [12]. In this study, seven such lakes were selected in order to carry out a comparative study of their physical-chemical features and bacterioplankton assemblages. The selection was designed to cover the environmental heterogeneity of lakes within this area.

Lakes Limnopolar, Midge, Chester, Chica and Turbio are located on the central plateau of the Peninsula, with maximal depths ranging from 4 to 8.2 m. Lake Somero is also in the plateau but is very shallow (0.5 m maximum depth) and has an important coverage of microbial mats, mainly in shore areas. This lake shows a high abundance of fairy shrimps (*Branchinecta gaini*), which together with the wind-induced resuspension of sediments facilitates nutrient recycling and release to the water column. All of these lakes are only weakly influenced by the marine fauna. Finally, Lake Refugio is also shallow but is located at South Beaches, next to the sea, and, accordingly, receives strong inputs of nutrients by marine fauna. The lake's high mineral content is also due to its proximity to the sea. Additional details of the environmental features of the lakes and the studied area are provided in Toro et al. [43].

Sampling techniques and sample handling. Measurements of selected variables (electrical conductivity, pH, and dissolved oxygen) were conducted *in situ* using a YSI 556 MPS multiprobe. Subsurface water samples (i.e., 0.3 m depth for the shallow lakes Somero and Refugio, and 1 m for the others), were collected in acid-washed plastic containers of 5 and 15 liters. Since all lakes were free of ice and were not stratified at the time of sampling, the samples can be considered as representative and comparable for the whole set of lakes. Samples were obtained from the central zone of the lakes in January–February 2007. A fraction of the sample was quickly filtered at the lake shore through fiber-glass filters (Whatman GF/F). Both the filtered water in plastic bottles, used for nutrient analyses, and the filters for further analysis of photosynthetic pigments were stored at –20ºC. The remaining volume was brought to the laboratory at the camp site and processed within not more than 5 h as follows: For the bacterioplankton and virioplankton counts, sub-samples were fixed with 2% glutaraldehyde and stored at –20ºC and 4ºC, respectively. Another fraction was separated in acid-washed plastic containers and stored at –20ºC for quantification of total nitrogen and total phosphorus. For the analyses of dissolved organic carbon (DOC), water was filtered through 0.2-µm cellulose nitrate filters and kept in acid-washed glass bottles after fixation with 0.2 ml of 1 N HCl. For DNA analyses, water was pre-filtered through a 30-µm Nytal mesh and a 3-µm polycarbonate filter (Whatman) and then filtered by means of a 0.2-µm polycarbonate filter (Whatman) and stored frozen in cryovials with 1.5 ml of lysis buffer, as described by Casamayor et al. [8,10]. All samples were shipped under the selected storage conditions to our laboratories in Spain, where the analyses were performed as described below.

Physical, chemical, and biochemical analyses. Inorganic dissolved nutrients were quantified following standard methods [3]. Photosynthetic pigment analyses were carried out by HPLC as described by Fernández-Valiente et al. [14] and Pickney et al. [36]. Chlorophyll concentration was used as a marker for the trophic status of the lakes [22]. Phaeophytin is a chlorophyll degradation product that is commonly found in the sediment. Fucoxanthin was used as a marker of diatom (Bacillariophyceae) abundance and can likewise be used as a marker for chrysophytes (Chrysophyceae), which are significant members of the phytoplankton in Antarctic lakes. Lutein and other carotenoids, such as violaxanthin and antheraxanthin, were used as markers of chlorophytes (Chlorophyceae) abundance, and zeaxanthin as a pigment marker for the relevant presence of cyanobacteria [22].

Samples stored for DOC quantification were also used to quantify chromophoric dissolved organic matter (CDOM) by means of the excitation-emission matrix (EEM) method [41] using a F-7000 Hitachi fluorescence

spectrophotometer. Two main types of CDOM were detected. One was of protein type, tyrosine-like and tryptophan-like, with EE maxima at 280–344 and 275–344 nm, respectively. The other was of the humic type and could be further subdivided in two subtypes: humic acids of terrestrial origin (THA, EE maximum at 240–448 nm) and humic acids resulting from animal fertilization (FHA, EE maximum at 325–428 nm). Standards for protein-like CDOM were prepared with bovine serum albumin (BSA). The standard for humic-acid-type CDOM was prepared with quinine sulfate (QS).

Microbial enumeration. The virioplankton (virus-like particles, VLP) fraction was extracted by filtering 0.5 ml of sample water through 0.02-µm Anodisc filters (Whatman). The particles retained on the filters were then dyed with SYBR Green-I and quantified on a Zeiss III epifluorescence microscope as described by Noble et al. [29]. Bacterioplankton counts were carried out by flow cytometry. One-ml aliquots of water samples previously fixed in 2% glutaraldehyde were incubated for 20 min at room temperature and then stained with SYBR Green-I. The abundance of autotrophic picoplankton (APP) was also measured by flow cytometry by exploiting the natural fluorescence of chlorophyll. Samples were analyzed on a Beckman Coulter flow cytometer (Cytomics FC 500 MPL) with five fluorescent channels, following the procedures described by Gasol et al. [17]. Side-angle light scatter (SSC) served as a proxy for bacterial cell size [44]. Fluorescents beads of 0.5 and 1 µm were used as size markers. Clusters of bacteria were counted by logical gating from FL2 vs. SSC histograms. Total bacterioplankton abundance and the relative abundance of bacterioplankton groups I (low DNA) and II (high DNA) were obtained [26]. The active cell index (ACI) was calculated by dividing the number of HDNA bacterioplankton cells in each sample by the total abundance, and expressed as a percent [23]. The VLP per bacteria ratio (VBR) was calculated by dividing the number of virus-like particles by the number of bacteria in the sample.

Molecular techniques to describe bacterioplankton diversity. DNA was extracted by adding lysozyme, proteinase K, and SDS to filters containing the bacterial biomass that had been stored frozen with lysis buffer (40 mM EDTA; 50 mm Tris pH 8.3; 0.75 M sucrose), as described by Dumestre et al. [11]. DNA separation and purification were carried out using phenol:chloroform:isoamyl-alcohol (25:24:1, v/v) and chloroform:isoamyl-alcohol (24:1, v/v) followed by isopropanol precipitation according to Agogué et al. [1]. PCR amplification of 16S rRNA gene fragments was carried out with the universal bacterial primers 341fGC with GC clamp (5´-CGC CCG CCG CGC CCC GCG CCC GGC CCG CCG CCC CCG CCC C CCT ACG GGA GGC AGC AG-3´) and 907r (5´-CCG TCA ATT CCT TTG AGT TT-3´), following Casamayor et al, [8,10]. The quality of the PCR products was analyzed by electrophoresis on a 2% (w/v) agarose gel.

Denaturing gradient gel electrophoresis (DGGE) was run on a denaturing gradient of 40–70% (100% = urea 7 M and 40% deionized urea) at 120 V and 60ºC for 15 h in a DGGE D-Code system (Bio-Rad Laboratories, USA). Approximately 500 ng of PCR product were carried into the electrophoresis gel for each lane. The gels were dyed for 30 min with SybrGold. Digital images were obtained with the UVldoc gel documentation system (UVltec Limited, Cambridge, UK) and kept in digital archives.

Prominent bands in the DGGE gel were excised and resuspended in 25 µl of Milli-Q (Millipore) ultrapure water and stored at 4ºC overnight, as described by Casamayor et al. [9]. A 2- to 5-µl aliquot of supernatant together with the original primer set was used for PCR re-amplification. The PCR product was purified and then sequenced at external sequencing facilities (www.macrogen.com), and the sequences submitted to a BLAST search [4] for a preliminary identification of the closest relative in the database. The ARB program package (www.arb-home.de) was used for sequence alignment. Partial sequences were inserted into the optimized and validated tree available in ARB (derived from complete sequence data) by using the maximum-parsimony criterion and a special ARB parsimony tool that did not affect the initial tree topology. Chimeric sequences were checked by CHECK_CHIMERA from the Ribosomal Database Project [http://rdp.cme.msu.edu] and by visual inspection of sequence alignments. Nucleotide sequences from the 18 main sequences were submitted to the EMBL database, where they were assigned the accession numbers FN398055 to FN398072.

Data analyses. DGGE bands identified in the fingerprinting of the seven lakes were classified in 19 different band types (from A to S). A binary data matrix was created, considering the presence or absence of the individual bands. A dissimilarity matrix based on the Jaccard coefficient (Sj) was then calculated, and a dendrogram built using the UPGMA method (unweighted pair group average linkage method). A correspondence analysis (CA) was carried out using the binary matrix to also group the lakes by bacterioplankton fingerprint similarity. Finally, principal components analysis (PCA) was carried out using data from selected physical, chemical, and biological variables other than bacterioplankton diversity. All of the statistical analyses were done using the MVSP program [15].

Results

Physical and chemical features. The selected lakes differed markedly in their chemistries, with respect to both salt and nutrient content (Table 1). The low mineralization of the plateau was evident by their low conductivity (40–70 µS/cm) whereas the marine influence on coastal Lake Refugio resulted in a higher conductivity (131 µS/cm). The pH values reflected the catchment geology as well as the differences in productivity among the lakes, with the highest values in the most productive lakes. The concentrations of nutrients, mainly nitrogen and phosphorus compounds, identified three levels of nutrient abundance: (i) low concentrations, measured in all deep lakes from the central plateau; (ii) higher N and P concentrations, but mostly in the particulate form, in the shallow Lake Somero, also located in the central plateau; (iii) very high N and P concentrations, both in the particulate and dissolved forms, in coastal Lake Refugio, reflecting the direct influence of marine fauna.

Fluorometric analyses of CDOM were carried out by means of EEM. The results of Fig. 1A showed that in most of the plateau lakes protein-type CDOM values did not exceed 0.02 mg/l BSA, while even lower values were measured for the humic acid type. Nevertheless, in Lake Somero, the concentration of protein-like CDOM was almost triple those of the other studied lakes of the plateau, and that of humic acid THA-CDOM almost four times higher. Remarkably, Lake Refugio presented a more equilibrated abundance of the four CDOM subtypes and, among the lakes studied, was the sole lake in which FHA was detected, again due to the influence of marine animals along the sea shore.

Photosyntetic pigments characterization. Chlorophyll *a* concentrations (Table 1) indicated that the trophic rank of most of the lakes ranged from ultraoligotrophic to oligotrophic. The exception was eutrophic Lake Refugio. Relatively high levels of the chlorophyll-a degradation product

Table 1. Values of the lakes' environmental variables in samples corresponding to those from which bacterioplankton DNA samples were obtained. Data on catchment areas are as given by Toro et al. [43]

Variables	Limnopolar	Somero	Midge	Chester	Chica	Turbio	Refugio
Catchment size (km^2)	0.58	0.06	0.27	0.09	0.01	0.58	0.12
Maximum depth (m)	5.5	0.5	8.2	5	4	7.8	0.5
Conductivity (µS cm^{-1} 25°C)	66	70	68	52	40	58	131
pH	7.2	7.2	6.9	6.9	7.0	6.6	7.7
NH4 (µM)	0.42	0.62	0.68	0.43	<0.1	<0.1	0.5
NOx (µM)	0.15	0.14	0.64	0.21	1.144	1.04	8.36
SRP (µM)	0.06	0.15	0.04	0.03	0.066	0.06	5.27
SRSi (µM)	71.84	64.87	10.23	46.52	21.95	59.55	62.88
Total Nitrogen (µM)	2.56	4.5	0.97	1.50	2.03	1.32	37.15
Total Phosphorus (µM)	0.73	0.98	0.23	0.22	0.52	2.86	23.10
Chl *a* (µg/l)	0.04	0.47	0.10	0.07	0.21	0.15	18.92
Fucoxanthin (µg/l)	0.001	0.101	0.013	0.002	0.001	0.007	ND
Lutein (µg/l)	0.001	0.033	0.009	0.002	0.011	0.008	2.122
Violaxanthin (µg/l)	0.001	ND	0.002	0.001	0.003	0.002	0.456
Antheraxanthin (µg/l)	ND*	0.025	ND	ND	0.003	ND	0.242
Zeaxanthin (µg/l)	ND	0.065	ND	ND	ND	ND	ND
Phaeophytin (µg/l)	ND	0.196	ND	ND	ND	ND	ND

*ND: Not detected

phaeophytin-a [22], which accumulates in sediments following the death of phototrophic organisms, were detected in Lake Somero, possibly due to sediment resuspension processes.

Bacterioplankton and virioplankton abundance. Total bacterioplankton abundances in the subsurface of the lakes (Fig. 1B) was in the range of 1–12 × 10^6 cells/ml. Lake Refugio had by far the highest bacterial abundance, in agreement with its high trophic status and abundance of organic matter. The opposite was observed for APP, which was essentially absent from the lakes. Flow cytometry analysis divided bacterioplankton into two groups (Fig. 1B). The first corresponded to the LDNA fraction, which had a low fluorescence (FL2) signal and was located in the lower part of the flow cytometer plot, and the second to the HDNA fraction, corresponding to a high fluorescence(FL2) signal and located in the upper part of the plot. The ratio HDNA vs. total bacterioplankton, expressed as the ACI, was lower in the most oligotrophic lakes (Limnopolar, Midge, Chester, Chica, and Turbio) and higher for lakes with a higher trophic status (Somero and Refugio).

The VLP counts (Fig. 1C) ranged between 0.2 and 5.8 × 10^7 VLP/ml. Again, higher abundances were found in Lake Somero and, mainly, in Lake Refugio, with concentrations in the latter being one order of magnitude higher than those of the remaining lakes. These two lakes also had higher VLP/bacteria ratios (VBR), with the highest ratio in Lake Somero.

Molecular analysis of bacterioplankton community. Overall, up to 80 bands were observed in the DGGE fingerprints from 19 different positions (A–S, Fig. 2). Sequencing of the prominent bands yielded 18 different sequences that were subsequently assigned to different operative taxonomic units (OTUs). The sequences belonged to the following band types: type A (18, 26, 34), type B (1, 15), type C (2, 19, 22, 27), type D (16), type J (28), type K(29), type L (23), type M (17, 30), type N (21, 36), and type Q (33), with the phylogenetic affiliations given in Fig. 3. The sequences were ordered in two main differentiated groups, clustering within the α-subclass of Proteobacteria and within the phylum Bacteroidetes (Fig. 3). Among those belonging to α-Proteobacteria, sequences clustering with members of the genus *Sphingomonas* dominated in the fingerprints of Lake Limnopolar and Lake Somero, which are physically connected by a short stream. Within the Bacteroidetes group, sequences closer to the genus *Flavobacterium* were present in most of

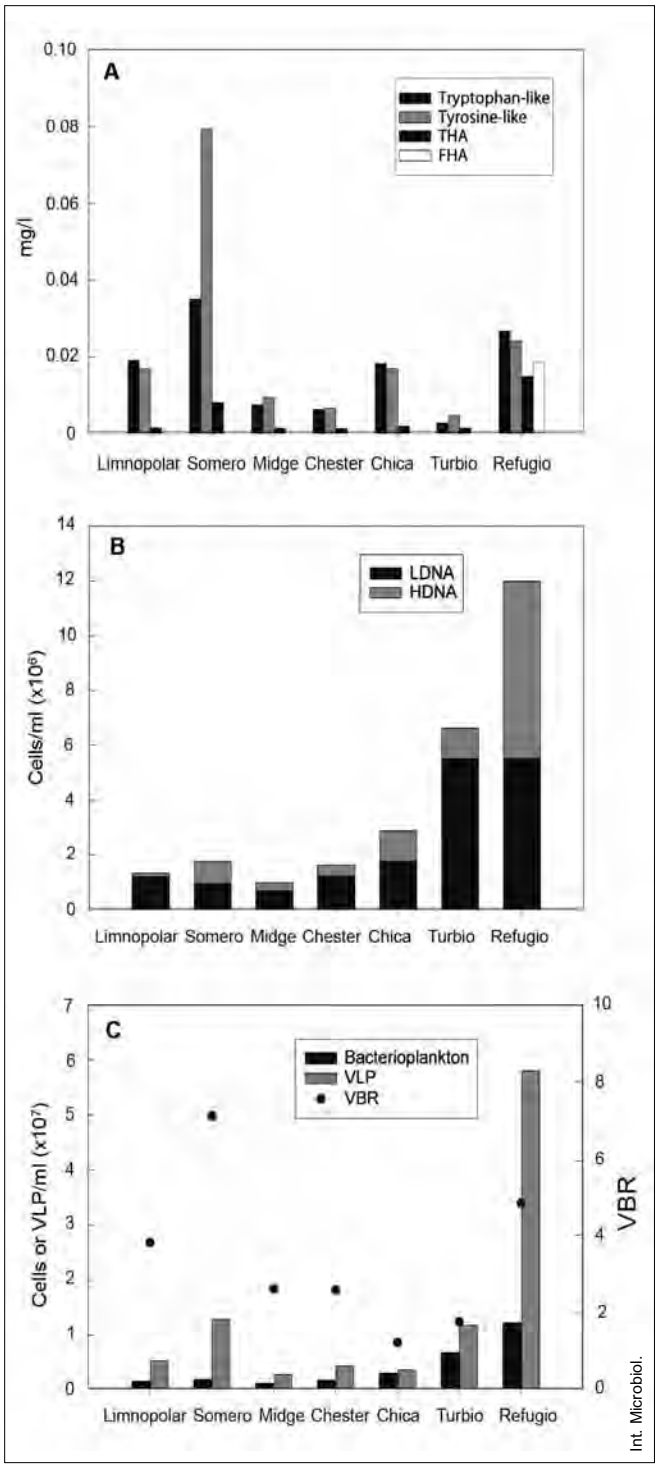

Fig. 1. (**A**) Fluorescent dissolved organic matter concentration (CDOM) for protein-like CDOM (tryptophan-like and tyrosine-like), reported in mg/l bovine serum albumin, and humic-like CDOM (terrestrial origin "THA" and fertilization origin "FHA") in mg/l quinine sulfate. (**B**) Total bacterioplankton abundance (cell/ml × 10^6) was analyzed by flow cytometry, which resolved two groups based on low and high DNA content (LDNA and HDNA, respectively). (**C**) Bacterioplankton densities (cell/ml) and virioplankton (VLP) densities (VLP/ml), with VBR as the ratio between virioplankton and bacterioplankton.

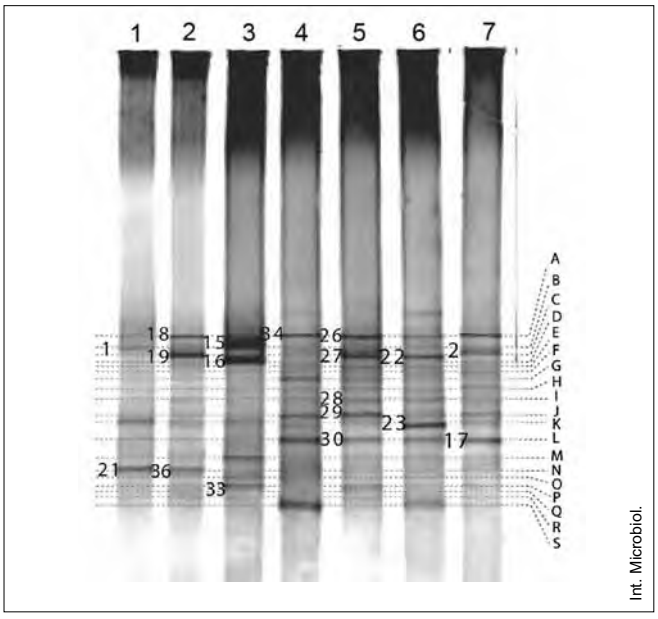

Fig. 2. Fingerprinting from 16S rRNA gene fragments for samples of the seven lakes identified 19 different types (A-S) of DGGE bands, 18 of which were successfully sequenced. The lakes (number codes in the upper part of the figure) are: 1-Somero, 2-Limnopolar, 3-Refugio, 4-Turbio, 5-Midge, 6-Chica, and 7-Chester.

the lakes, and those closer to *Flectobacillus* only in Lake Refugio.

Cluster analysis based on the Jaccard coefficient (Sj) (Fig. 4) grouped together the five oligotrophic lakes from the central plateau (Limnopolar, Midge, Chester, Chica, and Turbio). Lake Somero, although also located in the plateau and connected to Lake Limnopolar by a short stream, did not cluster tightly with the plateau lakes. As expected based on its location, Lake Refugio was clearly separated from these lakes.

Comparative ordination analysis: principal component analysis vs. correspondence analysis. Physical, chemical, and biological data were used to conduct a PCA, the results of which (Fig. 5) showed a separation of the lakes into three types, with the two main components explaining close to 90% of the variance. Deep lakes of the plateau (Limnopolar, Midge, Chester, Chica, and Turbio) grouped together and were distinct from Lake Somero because of its shallowness as well as the dominance of diatoms (high fucoxanthin to lutein ratio), high ACI, and high nutrient and organic matter content. Lake Refugio was strongly separated from the others, with the highest factor score for the principal component I, which explained 71.5% of the variance. This axis can be interpreted as representing a combination of the variables related to higher trophic status,

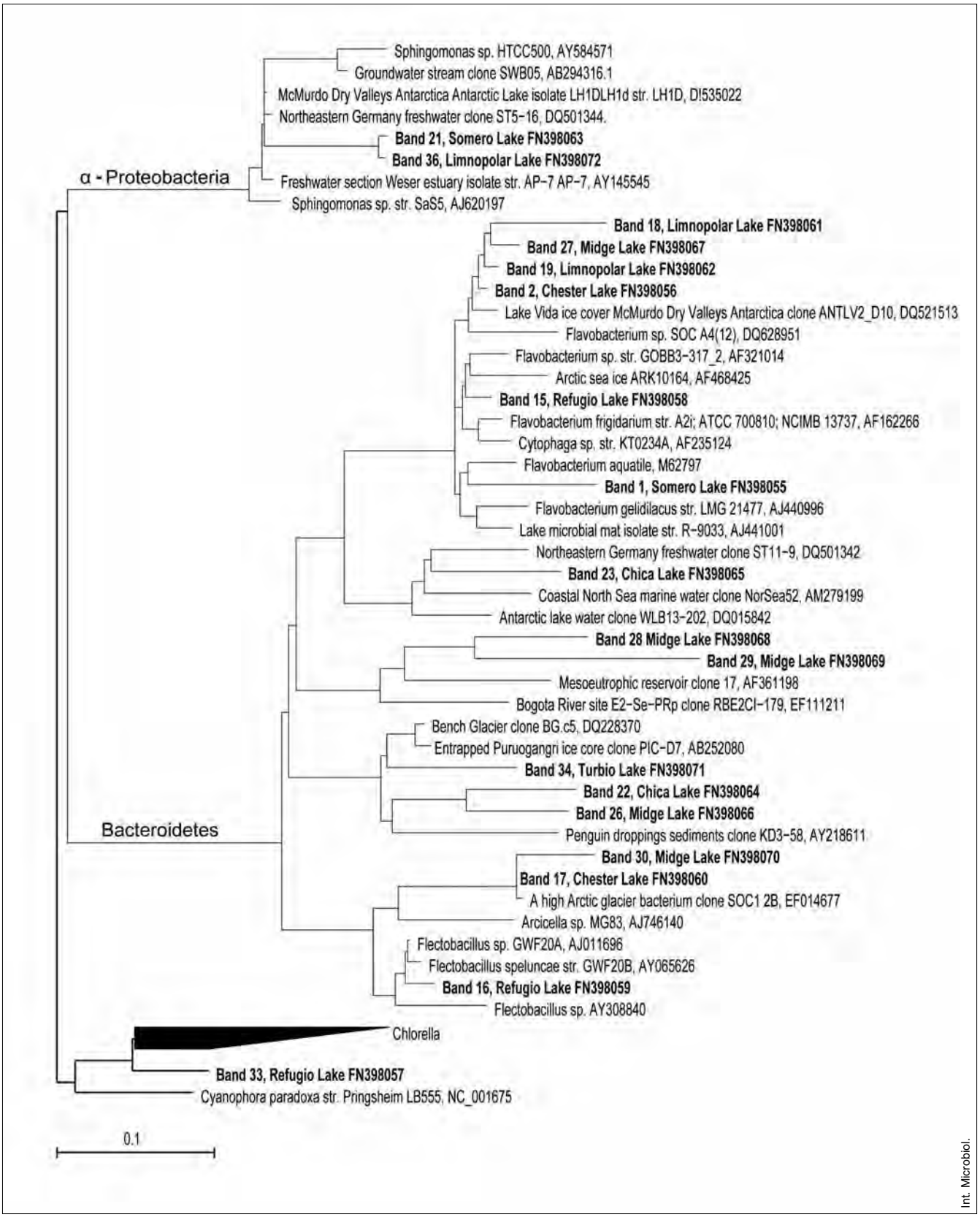

Fig. 3. Phylogenetic tree with the affiliation of the dominant bacterioplankton 16S rRNA gene fragments sequences (in bold) found in the seven lakes. Band 33 corresponds to plastid DNA.

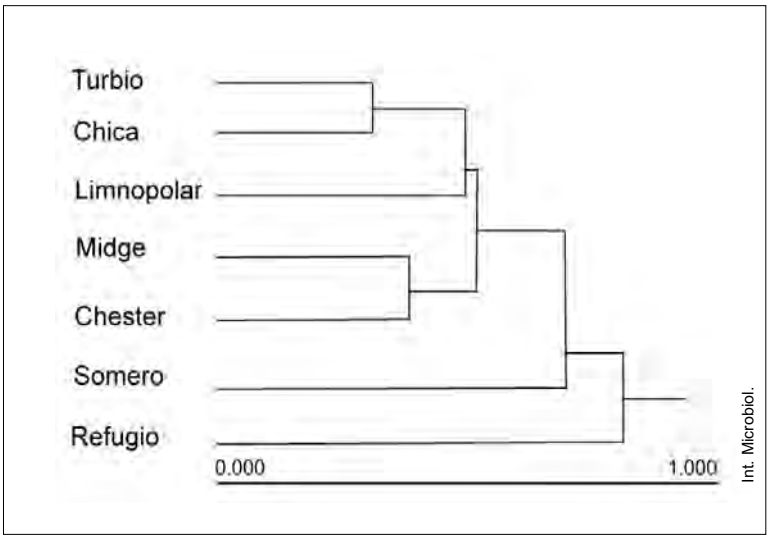

Fig. 4. UPGMA dendrogram resulting from cluster analysis of the data reporting the presence/absence of DGGE bands in the seven studied lakes.

i.e., soluble reactive phosphorus (SRP), DIN, and Chl-*a* concentration, with a contribution, in this case, also by the conductivity because high trophic status is also related to the proximity of the sea, with its influence of marine animals.

The presence/absence binary matrix of the genetic fingerprinting results was used in a CA (Fig. 6), which grouped lakes with the same band patterns. The two first axes were found to explain 75% of the variance, forming a homogeneous group that included the deep lakes from the plateau (Limnopolar, Midge, Chester, Chica, and Turbio), whereas Lake Somero appeared in the upper part of the graph and Lake Refugio highly separated from the others.

A similar distribution pattern of the lakes' features was obtained when the two ordination analyses were compared. Lakes Somero and Refugio were highly separated from each other whereas the remaining lakes (Limnopolar, Midge,

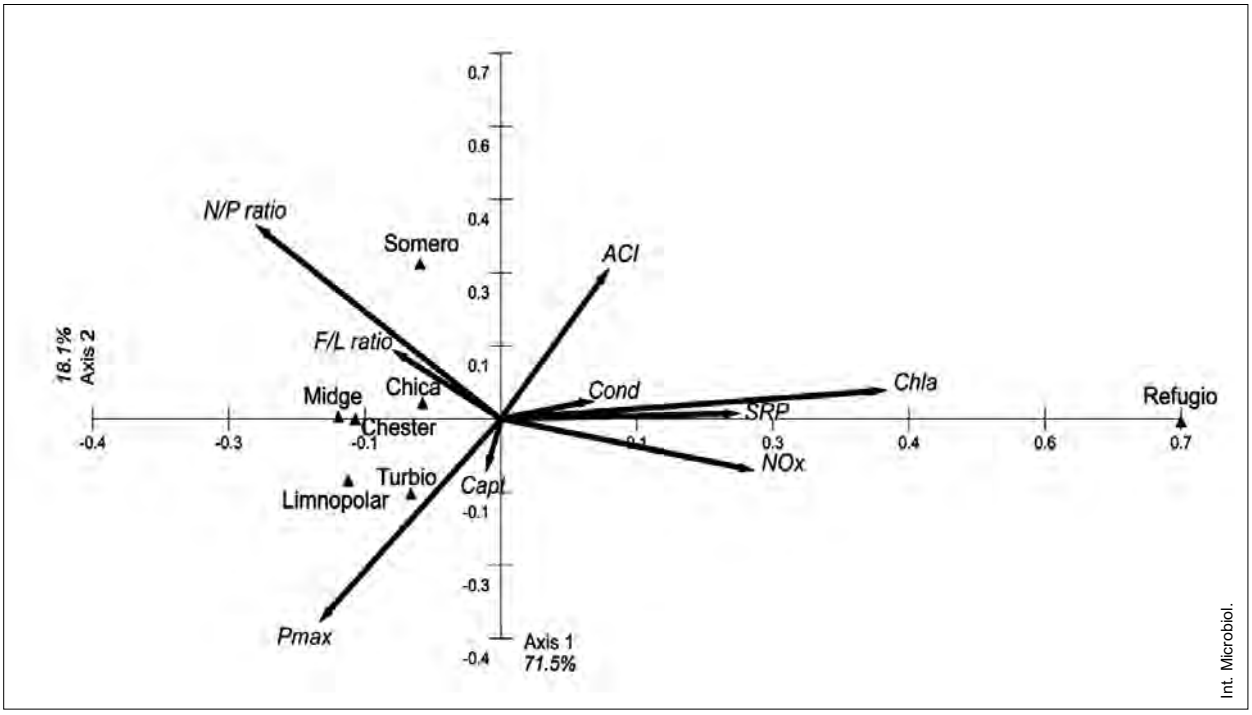

Fig. 5. Biplot corresponding to the PCA for the seven lakes (black triangles) and the most significant environmental variables: Capt (catchment size), Pmax (maximum depth), Cond (electrical conductivity), Chla (chlorophyll concentration), SRP (soluble reactive phosphorus), NOx (oxidized inorganic nitrogen forms), N/P ratio (total nitrogen/total phosphorus ratio), ACI (active cell index), and F/L ratio (fucoxanthin/lutein ratio).

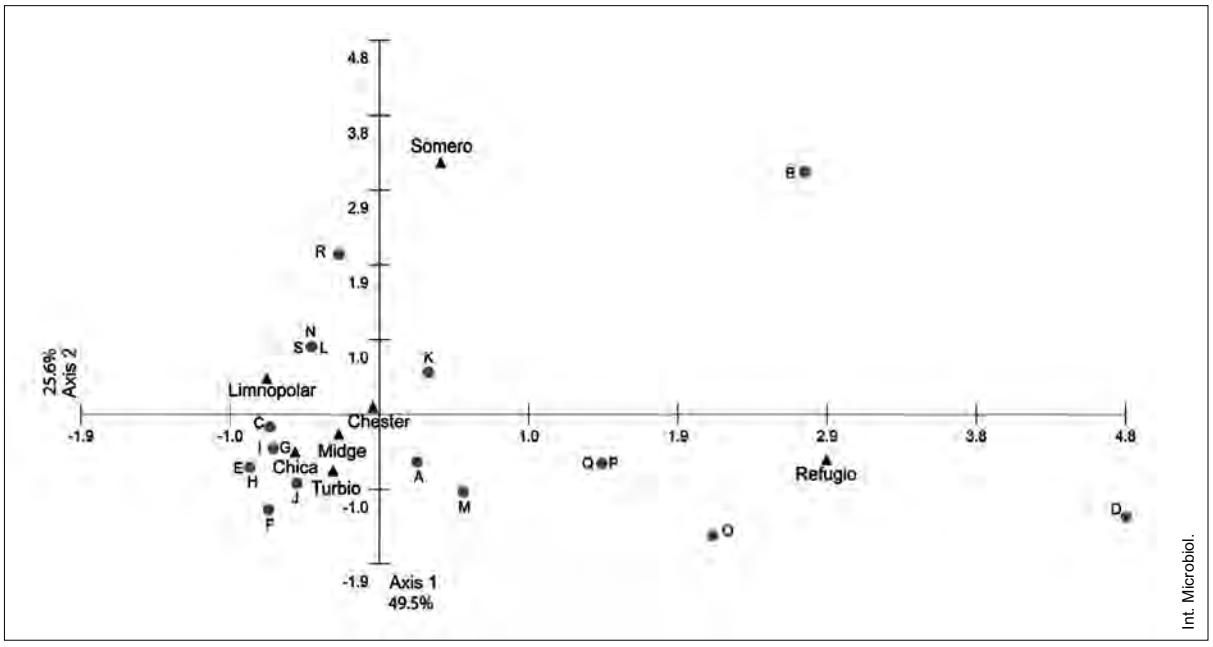

Fig. 6. Biplot resulting from the CA using the presence/absence matrix of the 19 different types (A–S) of DGGE bands (gray circles) of the seven studied lakes (black triangles).

Chester, Chica, and Turbio) clustered together. These results matched those obtained with the cluster analyses performed using the binary matrix data, thus suggesting athe close relationship between the environmental features of the lakes and the composition of the bacterioplankton assemblages.

Discussion

Antarctic lakes are often perceived, like many other terrestrial Antarctic ecosystems, as very unproductive, in which physical limitations such as low temperature and low light (energy) availability restrict biological productivity [7]. Lakes from maritime Antarctica, however, are located in the less harsh area of the continent. Especially during the austral summer, conditions are less extreme, allowing a limited variety of life forms to flourish [7]. Under these circumstances, in which light availability is relatively high and temperatures are not too low, other factors, such as nutrient availability, play a major role in controlling the productivity of maritime Antarctic lakes.

The chemical and biological features of the lakes included in our study were indicative of a contrasting trophic status very much dependent on the influence of external inputs but also modulated by nutrient recycling within the lake basins, which, in turn, is somewhat linked to the lakes' morphology and ice dynamics. Sediment removal, as occurs in shallow lakes such as Lake Somero or in the deeper Lake Turbio (in this case because of ice dynamics), can promote greater development of bacterioplankton since both organic and inorganic nutrients released from the sediments become partly available for heterotrophs, thus supporting a greater bacterioplankton abundance (Fig. 1). The trophic pattern reflected by the nutrient concentrations was confirmed by other chemical and biological variables. For instance, concentrations of chlorophyll and carotenoids, which serve as markers of phytoplankton abundance (and, in shallow lakes, also of tychoplankton abundance), were very low in lakes from the plateau. This was not the case for Lake Somero, where pigment concentrations were higher, including that of the chlorophyll degradation product phaeophytin, which accumulates in the sediment. In the oligotrophic lakes from the plateau, the amounts of both protein-like and humic-type CDOM were also relatively low, which indicated that inputs of organic matter were less than in richer lakes [41]. In Lake Somero, however, because of its shallowness, both the removal of nutrients from the sediments and their recycling are favored by physical (e.g., wind resuspension) and biological (sediment turn over by fairy shrimps) processes, in contrast to the deep lakes of the plateau. This was also confirmed by the much higher abundance of autochthonous protein-like CDOM in the plateau lakes than in the other inland lakes.

At the other trophic extreme, the coastal shallow Lake Refugio was characterized by higher nutrients and chlorophyll *a* concentrations, but in contrast to Lake Somero the

main reason for its high trophic status was its proximity to the coast, where seals and penguins are an important source of nutrients. This lake also had high concentrations of humic-type CDOM and was the only one with significant amounts of FHA CDOM, clearly indicating animal inputs [41]. In this lake, phytoplankton composition switched to a dominance of chlorophytes, as indicated by the strong increase in the abundance of the specific carotenoids lutein and violaxanthin, which also evidenced the influence of nutrient status on protist community structure. Additionally, lake productivity was also reflected by other physical and chemical variables; for example, the pH of the plateau lakes varied between 6.64, at Lake Turbio, to 7.21, at Lake Somero, and was 7.65 at coastal Lake Refugio. In low buffered waters of relatively similar mineralization, this indicates an increase in primary production with increasing pH. Variability in the trophic status of the lakes close to the sea was previously described for lakes located in maritime Antarctica. Commonly, lakes that are far from the sea have much lower nutrient concentrations than coastal lakes [21], as the latter, unlike the more inland lakes, usually have high nutrient inputs from marine fauna, especially seals and penguins [18,19,20,21,27]. This is also the case for Byers Peninsula, although, as our results highlight, other processes (e.g., sediment suspension and nutrient recycling) govern the differences among lakes with low external nutrient inputs.

Trophic status was also observed to influence bacterioplankton abundances, with a much higher abundance found for the coastal Lake Refugio than for other eutrophic Antarctic lakes [40]. However, as discussed, the modulation by trophic status of bacterioplankton abundance may be influenced not only by external inputs, but also by sediment release, benthic production, and ice-cap dynamics. In addition to trophic status, a role for predation could also be claimed to explain the control of bacterioplankton abundance in plateau lakes, since trophic cascades involving metazooplankton and bacteriophagous protists, releasing grazing pressure on bacterioplankton, have been suggested to occur in oligotrophic polar lakes [7]. In addition to these top-down effects, viral impacts could influence bacterioplankton abundance and growth. Thus, among all the studied lakes, Lake Somero presented the highest VBR (Fig. 1C). Virioplankton proliferation can result in a high mortality in bacterioplankton but, when nutrients are available, as typical of Lake Somero (compared to the other plateau lakes), the bacterioplankton community can enhance growth, thus compensating for the viral effect [35]. Although we did not directly measure bacterial growth or bacterial production, the ACI, defined as the percentage of HDNA bacterioplankton as a function of the total bacterioplankton population, can be used to some extent and together with other variables, e.g., CDOM-type concentrations, as an indicator of bacterial activity and growth [17,23]. Among the plateau lakes, both the ACI and the amount of protein-like CDOM were highest in Lake Somero, indicating more active bacterial growth dynamics. Both parameters were also much higher in Lake Refugio, consistent with the common pattern of a high correlation of HDNA cell abundance with chlorophyll-a concentration and productivity, as reported by Li and co-workers [26].

The limitations of the DGGE method to describe microbial diversity are well known [8,34]. However, since this technique selectively amplifies the dominant taxa of bacterioplankton, band number serves as a good indication of the minimum number of dominant phylotypes present [30,40]. Although not necessarily conclusive, our DGGE results (Fig. 2) showed a higher number of dominant bands, and consequently a high OTU richness in the deep oligotrophic lakes, with 11–16 different bands in the DGGE gel, compared to the lower richness in the shallow lakes Somero and Refugio (8 and 9 OTUs, respectively). As a general ecological pattern, eutrophic conditions can produce a strong competitive exclusion, reducing diversity. Pearce et al. [30] found a similar pattern of reduced bacterioplankton diversity with increasing trophic status in lakes from the nearby Signy Island, although other studies performed in lakes of the maritime Antarctic failed to find a clear pattern [40].

Sequencing of the dominant bands in the DGGE fingerprints showed that most of them corresponded to uncultivated bacteria (Fig. 3). The majority of the dominant OTUs detected in this study were affiliated with α-Proteobacteria and Bacteroidetes, both of which are dominant in other maritime Antarctica lakes, together with β-Proteobacteria and Actinobacteria [30–32], although in a recent revision Pearce and Galand [33] stated that β-Proteobacteria might be the most abundant group in polar freshwater ecosystems. However, recent studies performed in other lakes of the maritime Antarctic, demonstrated the dominance of bands whose sequences clustered within the Bacteroidetes [40], which is consistent with our results.

Most of the detected bands clustered with sequences coming from high-latitude or glacier habitats—typical of samples of freshwater Antarctic bacterioplankton [40]—with many of the taxa showing a bipolar distribution [32]. Cluster analysis defined three main groups of organisms. Within those grouped with α-Proteobacteria, sequences clustering with members of the genus *Sphingomonas* (bands 21 and 36) corresponded to some of the dominant bands in lakes Limnopolar and Somero. Despite their marked differences, these lakes are connected by a short stream, which would explain their shared dominant taxa. *Sphingomonas* has been

found in diverse environments, including aquatic (both fresh- and seawater) and terrestrial habitats from Antarctica [45] and temperate zones. The second group clustered within the Bacteroidetes, specifically, with sequences present in cold habitats, including sequences from uncultivated bacteria of the genus *Flavobacterium*. Species of this genus (e.g. *Flavobacterium antarticum*, *F. hibernum*), some of which are psychrophilic, are usually found in cold aquatic habitats with low salinity, typically in polar lakes [45], although also in rivers, springs, or soils. The third group, also clustering within the Bacteroidetes, included a sequence with high similarity to three *Flectobacillus* sequences (band 16 from Lake Refugio). This genus is commonly found in Arctic and Antarctic aquatic cold environments [28] but also in the lakes and springs of temperate zones. Within the third group, but of low similarity to the above-mentioned sequences of *Flectobacillus*, were bands 17 and 30, from lakes Chester and Midge, which are located quite close to one another but are not connected. These sequences clustered with a sequence retrieved from an Arctic glacier.

Cluster analysis (Fig. 4) gave a preliminary indication of the similarity among the bacterial assemblages dominating the different types of lakes. The most homogeneous cluster was that associated with oligotrophic deep lakes from the plateau. These lakes shared many of the dominant bands and many environmental features. Among them, the tight clustering of sequences from lakes Midge and Chester, which are located quite close to one another, reflected the high similarity of the dominant bacterioplankton taxa in these lakes. The same was true of lakes Chica and Turbio, which also share specific environmental features, such as the presence of ice dams that suddenly break down, which also occurs in Lake Limnopolar.

The existence of three differentiated types of lakes was shown by PCA of the environmental variables (Fig. 5), confirming the results of the cluster analysis. The first group included the deep oligotrophic lakes from the plateau (Limnopolar, Chester, Midge, Chica, and Turbio). Within the first axis, which explained a high percentage of the variance (71.5%) and can be interpreted as related to trophic status, lakes with more active suspension of the sediments because of ice dynamics, such as Chica and Turbio, were located closer to another plateau lake, Somero, whose sediments dynamics are more active. However, Lake Somero was segregated by axis 2, in which several variables, such as lake depth, strongly scored, thus separating this lake from the other plateau lakes. Finally, Lake Refugio was extremely separated from all other lakes, with its appearance at the higher end of axis 1 indicating its much higher trophic status. The same pattern of similarity among lakes was obtained in a comparison of environmental and fingerprinting analyses, regardless of whether they were performed with data from environmental variables (PCA, Fig. 5) or from determination of dominant bacterioplankton diversity, and either after classification (cluster techniques, Fig. 4) or ordination (CA, Fig. 6) methods were applied. Thus, the composition of the bacterioplankton assemblages matched very well with the environmental diversity among the studied lakes.

We have shown that the trophic status of the lakes of Byers Peninsula mediates both the abundance and the composition of bacterioplankton assemblages. Trophic status, in turn, is regulated not only by external inputs from marine fauna, as often reported [21,40], but also, as demonstrated here for the first time for maritime Antarctic lakes, by lake morphometry and sediment dynamics, with the latter influenced by physical and biological processes.

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