

Bacterial Community Structure in Patagonian Andean Lakes Above and Below Timberline: From Community Composition to Community Function

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Abstract Lakes located above the timberline are remote systems with a number of extreme environmental conditions, becoming physically harsh ecosystems, and sensors of global change. We analyze bacterial community composition and community-level physiological profiles in mountain lakes located in an altitude gradient in North Patagonian Andes below and above the timberline, together with dissolved organic carbon (DOC) characterization and consumption. Our results indicated a decrease in 71 % of DOC and 65 % in total dissolved phosphorus (TDP) concentration as well as in bacteria abundances along the altitude range (1,380 to 1,950 m a.s.l.). Dissolved organic matter (DOM) fluorescence analysis revealed a low global variability composed by two humic-like components (allochthonous substances) and a single protein-like component (autochthonous substances). Lakes below the timberline showed the presence of all the three components, while lakes above the timberline the protein-like compound constituted the main DOC component. Furthermore, bacterial community composition similarity and ordination analysis showed that altitude and resource concentration (DOC and TDP) were the main variables determining the ordination of groups. Community-level physiological profiles showed a mismatch with bacteria community composition (BCC), indicating the absence of a relationship between genetic and functional diversity in the altitude gradient. However, carbon utilization efficiencies varied according to the presence of different compounds in DOM bulk. The obtained results suggest that the different bacterial communities in these mountain lakes seem to have similar metabolic pathways in order to be able to exploit the available DOC molecules.

Introduction

Mountain lakes include lakes located above (alpine lakes) and below the timberline. In particular, alpine lakes are remote systems for which the location determines a number of extreme environmental conditions, including a high irradiance of ultraviolet radiation (UVR), low temperature, and long periods of ice and snow cover, combined with a fluctuating hydrology [1, 2]. The chemistry is primarily determined by atmospheric deposition and the weathering of rocks, and these two processes also control the phosphorus, inorganic nitrogen, and dissolved organic carbon (DOC) supplies [3, 4]. The lack of terrestrial vegetation and steep slopes generate a low DOC concentration and consequently a high UVR penetration [5, 6]. Therefore, alpine lakes are considered physically harsh ecosystems and sensors of global change [2, 7, 8]. Since the 1990s, the microbial community structure in such special waters has attracted much attention from researchers, and considerable work has been performed in different mountain regions of the world [9–12].

Dissolved organic matter (DOM) in natural systems comprises a complex mix of organic compounds of different origin [10], which would result in variations in DOC availability for bacteria. Mountain lakes are distributed along a gradient of altitude, over a landscape with different geomorphologic characteristics where DOC sources and concentrations would be quite variable. Thus, mountain lakes represent interesting scenarios for the study of DOM lability. The DOC consumption by bacterioplankton represents a major pathway of organic carbon cycling [13, 14], and it is influenced by temperature, nutrient availability [15, 16], and organic matter characteristics [17–19]. In that sense, a gradient in DOC content was observed in relation to timberline [20], with the DOM bulk in lakes located above the timberline being dominated by autochthonous production, atmospheric inputs, and climatic

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controls [21], whereas the DOM below the timberline being influenced by the surrounding vegetation [22]. In addition, field [23, 24] and laboratory studies [3, 25] have shown that the quality of organic matter can induce changes in both the community composition and in various aspects of community metabolism. Consequently, the quantity and quality of DOM would play a major role in determining bacterioplankton dynamics in mountain lakes.

Nonetheless, the connections between microbial diversity and community functioning in aquatic bacterial assemblages remain a matter of intense discussion [26, 27]. Several studies indicate that taxonomic and functional diversity are positively related [28, 29] because the coexistence of species is partly dependent on niche differentiation and dissimilarity between life-history traits. However, this relationship would be weak if only a small subgroup of generalists controls most of the processes within a community [30]. Furthermore, functional diversity in mountain environments has been poorly analyzed, leaving a gap in the knowledge linking bacterial community structure and different DOM bulk consumptions in altitudinal gradients.

The North Patagonian Andes (approximately 41° S) does not exceed 2,500 m a.s.l., with a treeline located at the latitude of the studied lakes at 1,350 m a.s.l. in Chile [31] and in Argentina about 1,600 or higher [32]. When glaciers retreated from the North Patagonian Andean valleys during the last regional deglaciation, lakes were established both in the piedmont valleys [33] and in the cirques formed by the erosive activity of glaciers currently located above and below the timberline. Early studies on these lakes are mainly related to their low DOC and phosphorus concentration, high UVR penetration [2, 34], and the impacts on zooplankton [35–37]. A recent study noted extremely low levels of aerosol deposition in the area [4]; therefore, these lakes constitute scenarios in which solid atmospheric deposition would not be a significant source of nutrients. In this sense, the bacterioplankton structure and relationship between bacterioplankton genetic and functional diversity in these remote environments is intriguing. In the present study, we analyze the bacterial community composition and community-level physiological profiles in mountain lakes located along an altitudinal gradient in the North Patagonian Andes, including lakes above and below the timberline. In addition, we analyzed the DOM by fluorometric optical analysis (excitation–emission matrices) and the DOC consumption by bacterioplankton in the altitudinal gradient.

Materials and Methods

A list of the abbreviation and acronyms used in the text are presented in Table 1.

Table 1 Abbreviations and acronyms used in the text

ARISA	Automated ribosomal intergenic spacer analysis
AWCD	Average well color development
BCC	Bacterial community composition
CCA	Canonical correspondence analysis
Chl <i>a</i>	Chlorophyll <i>a</i> concentration
CLPPs	Community-level physiological profiles
DOC	Dissolved organic carbon
DOM	Dissolved organic matter
<i>E</i>	Evenness
EEMs	Excitation–emission matrices
<i>H</i>	Shannon–Wiener diversity index
<i>k</i>	First-order decay constant
LTCC	Long-term carbon consumption
LTL	Long-term labile pool
OTU	Operational taxonomic unit
<i>S</i>	Species richness
STCC	Short-term carbon consumption
STL	Short-term labile pool
TDP	Total dissolved phosphorus
TP	Total phosphorus
Zm	Maximum depth

Study Area and Sampling

We sampled eight lakes located at 41° S, 71° W in Nahuel Huapi National Park (Patagonia, Argentina) that correspond to the Glacial Lakes District of the Southern Andes (Fig. 1). The lakes are located within a distance of 40 km (Euclidian distance) between 1,380 and 1,950 m a.s.l. (Table 2).

At 40° S in northern Patagonia, the deciduous austral beech *Nothofagus pumilio* (Poepp. et Endl.) Krasser dominates altitudinal treelines, with erect trees decreasing in importance with increasing elevation, to krummholz that dominate the treeline ecotone [32]. We consider that the limit of erected trees (timberline) is around 1,650 m a.s.l., and at this limit, there is a mixture between rocks and *N. pumilio* krummholz [38]. Therefore, the surrounding lake environment varied among: (a) forest of pure stands of *N. pumilio* (Lakes Illón, Cab, Los Patos, Verde and Jakob), (b) predominately bare rock with sparse individuals of *N. pumilio* krummholz (Lake Toncek and Negra), and (c) bare rock (Lake Schmoll). The geology of the area is dominated by a mixture of crystalline igneous, volcanic, and plutonic rocks [39]. The climate is characterized by dry summers (December–March) and a period of precipitation during autumn–spring that includes snow falls mainly above 1,000 m [38]. Therefore, these lakes remain frozen for approximately 6–8 months every year.

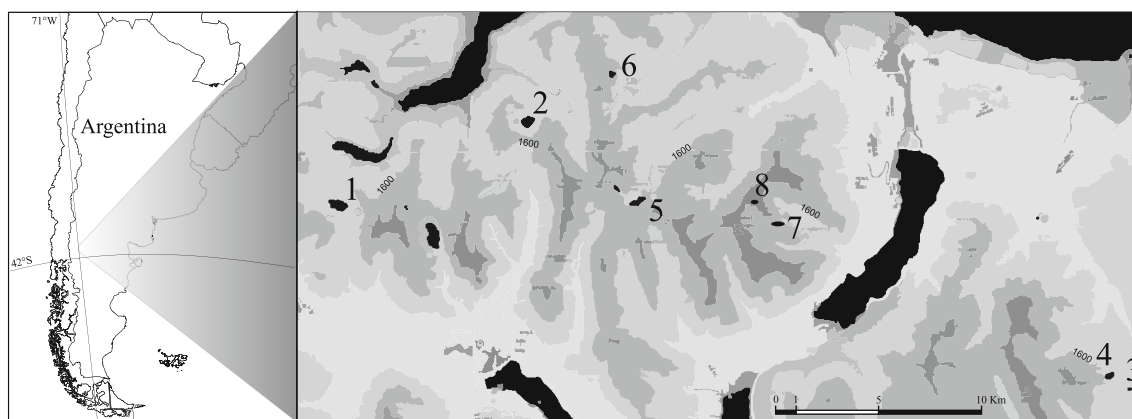


Fig. 1 Map of the Andean Patagonian high mountain lakes situated in North Patagonia, Argentina. 1 Illón, 2 CAB, 3 Los Patos, 4 Verde, 5 Jacob, 6 Negra, 7 Toncek, 8 Schmoll. Gray scale indicates altitude gradient, 1,600 m a.s.l. is indicated in the map, and black color indicates lakes

Sampling was performed in austral summer from 18 to 30 January 2010. Subsurface lake water samples were collected at a depth of 0.3 m in 500-mL sterile bottles (three bottles in each lake) and were transported immediately to the laboratory in thermally insulated containers. Additional samples of 2 L were obtained from five selected lakes distributed along the entire altitudinal gradient of this study (Table 2) for the analysis of DOC lability and bacterial functional diversity.

Laboratory Determinations

Total dissolved phosphorus (TDP) was determined using lake water filtered through GF/F filters, and total phosphorus (TP) was determined directly using unfiltered lake water. The samples for the TDP and TP determinations were digested with potassium persulfate at 125 °C and 1.5 atm for 1 h. The P

concentrations were obtained using the ascorbate-reduced molybdenum method [40].

Dissolved organic carbon concentration (DOC) was analyzed using lake water filtered through pre-combusted GF/F filters with a high-temperature combustion analyzer (Shimadzu TOC V-CSH), with potassium hydrogen phthalate as the standard.

Spectrophotometric scans (250–790 nm) were performed on filtered lake water (pre-combusted GF/F filters) using 10-cm path-length quartz cuvettes and a Shimadzu UV2450 double-beam spectrophotometer. The in situ absorption coefficients due to yellow substances [a_λ ; m^{-1}] were obtained by converting the measured base 10 values to base e logarithms, as follows [24]:

$$a_\lambda = \ln(10^A)/l$$

Table 2 Features of the studied lakes: geographic location (altitude and timberline position), Maximum depth (Z_m), catchment (area and features, *N. pumilio*=*Nothofagus pumilio*), water color (spectrophotometric measurement as a_{440}), dissolved organic carbon (DOC as mg L^{-1}) measured with a high-temperature combustion analyzer, total dissolved phosphorus

concentration (TDP as $\mu\text{g L}^{-1}$) estimated with the ascorbate-reduced molybdenum method, chlorophyll *a* concentration (Chl *a* as $\mu\text{g L}^{-1}$) estimated by extraction with 90 % ethanol and fluorometric readings, and bacterial abundance (as $\text{cell } 10^6 \text{ mL}^{-1}$) epifluorescence microscopy on DAPI stained samples

Lake	Altitude (m a.s.l.)	Z_m (m)	Catchment area (km^2)	Catchment features	Timberline location	DOC (mg L^{-1})	a_{440} (m^{-1})	TDP ($\mu\text{g L}^{-1}$)	Chl <i>a</i> ($\mu\text{g L}^{-1}$)	Bacterial abundance ($\times 10^6 \text{ cell mL}^{-1}$)
Illón	1,380	4	0.254	<i>N. pumilio</i> forest	Below	1.15±0.14	0.24±0.034	4.38±0.30	1.81±0.25	1.16±0.14
CAB	1,480	1.5	0.177	<i>N. pumilio</i> forest	Below	0.73±0.15	0.15±0.008	3.22±0.23	0.85±0.13	0.80±0.20
Los Patos ^a	1,500	3	0.001	<i>N. pumilio</i> forest	Below	0.47±0.03	0.16±0.002	5.63±0.27	2.93±0.48	1.07±0.12
Verde ^a	1,545	1.5	0.080	<i>N. pumilio</i> forest	Below	2.34±0.44	0.25±0.001	7.06±0.33	3.11±0.15	2.53±0.33
Jacob	1,570	25	0.131	<i>N. pumilio</i> forest	Below	0.36±0.01	0.12±0.0035	4.02±0.11	0.48±0.09	0.75±0.11
Negra ^a	1,620	10	0.116	Rocks with few <i>N. pumilio</i> krummholz	Above	0.56±0.11	0.15±0.0056	2.38±0.74	0.33±0.02	0.99±0.14
Toncek ^a	1,700	12	0.050	Rocks with few <i>N. pumilio</i> krummholz	Above	0.41±0.03	0.11±0.007	2.79±0.65	0.61±0.13	0.77±0.23
Schmoll ^a	1,950	5	0.027	Rocks	Above	0.33±0.02	0.02±0.004	1.55±0.72	0.31±0.03	0.67±0.09

^a indicates the lakes that were analyzed for bacterial functional diversity and DOC lability

where a_λ is the absorption coefficient (m^{-1}), A is the absorbance, and l is the path length (m). The water color was estimated according to a previous method [41] considering the absorbance at 440 nm a_{440} in a 100-mm cuvette.

The chlorophyll *a* concentration (Chl *a*) was determined by extraction with 90 % ethanol, according to Nusch [42], using a fluorometer (Turner Designs, 10-AU). The fluorometer was previously calibrated against spectrophotometric measurements.

The bacterial abundance determination was achieved using 50-mL lake water samples fixed with filtered formaldehyde at a final concentration of 2 % v/v. The bacterial cells were stained with 4',6'-diamidino-2-phenylindole (DAPI) at a final concentration of 0.2 % w/v, following Porter and Feig [43]. The bacterial abundance was determined using 0.2- μm black polycarbonate filters (Poretics) under an epifluorescence microscope (Olympus BX50) at $\times 1,250$ magnification using a UV light (U-MWU filter).

An additional fluorometric optical analysis of dissolved organic matter (DOM) was performed for the selected five lakes (Table 2). Single measurements at specific excitation/emission wavelengths and excitation–emission matrices (EEMs) were performed with a PerkinElmer LS 45 fluorescence spectrometer equipped with a xenon discharge lamp. The excitation wavelength intervals were of 2 nm, between 240 and 450 nm, and the emission ranged between 300 and 550 nm, with 5-nm increments. The measurements were performed at a constant room temperature of 20 °C in a 1-cm quartz fluorescence cell; Raman scattering was corrected by subtracting the pure water (Milli-Q) EEM from the sample EEM.

Genetic Diversity

Extraction of Bacterial Assemblage DNA

A minimum of 100 mL and up to 500 mL of lake water was pre-filtered through a 20- μm mesh net and then through Nucleopore[®] filters (0.2- μm pore size, 25 mm diameter). The Nucleopore filters were then collected and stored at -20 °C in phosphate-buffered saline (50 mM Tris, 40 mM EDTA, 400 mM NaCl, and 0.75 M sucrose) for no longer than 2 months. Once thawed, each sample received 4 μL of 0.4 % (v/v) beta-mercaptoethanol and was incubated at 65 °C for 15 min. An equal volume of chloroform/isoamyl alcohol (24:1) was added to the vials and mixed for 20 min at room temperature. After centrifugation at 12,500 rpm for 15 min, the aqueous phase was extracted, and another volume of chloroform/isoamyl alcohol was added. The mixture was centrifuged for 5 min at 12,500 rpm, and the aqueous layer was mixed with 2:1 isopropanol: 5 M NaCl and stored at -80 °C for 1–2 h. Each tube was then centrifuged at 12,500 rpm for 30 min. The pellet was washed with 500 mL of 70 % ethanol, and the mixture was centrifuged for 5 min at

12,500 rpm. Following ethanol removal, the pellets were air-dried and suspended in 25 mL of sterile water on ice for 1–2 h, with gentle vortexing.

Community Fingerprinting via ARISA

Bacterioplankton diversity and community composition were assessed by automated ribosomal intergenic spacer analysis (ARISA), following the method of Fisher and Triplett [44], with the following modifications. PCR reactions contained 1X PCR buffer (consisting of 50 mM Tris [pH 8.0], 250 $\mu\text{g mL}^{-1}$ of bovine serum albumin, and 3.0 mM MgCl_2), 250 μM (final concentration) of each dNTP, 10 pmol of each primer, 1.25 U of Taq polymerase (Promega), and 1 μL of lake-extracted DNA in a final volume of 25 μL . The primers used were 1406f (universal, 16S rRNA gene; 5'-TGYACAC ACCGCC CGT-3') and 23Sr (bacterial specific, 23S rRNA gene; 5'-GGGTTBCCCCATTCTRG-3'); the 1406f primer was labeled at the 5' end with the phosphoramidite dye 6-FAM. The PCR was performed with an initial denaturation at 94 °C for 2 min, followed by 30 cycles of 94 °C for 35 s, 55 °C for 45 s, and 72 °C for 2 min, with a final extension at 72 °C for 2 min.

Based on estimates, a standardized amount (1 to 2 μL) of PCR product, along with 1 μL of the internal size standard GS 1200 LIZ (GeneScan-Applied Biosystems), was added to 20 μL of deionized formamide, and the mixture was denatured at 95 °C for 5 min, followed by 2 min on ice. The sample fragments were then discriminated using the ABI 3730xl genetic analyzer (Perkin-Elmer), comprising DNA electrophoresis in a capillary tube filled with the polymer POP-7 (Applied Biosystems). The data were analyzed using the Peak Scanner 1.0 software (Applied Biosystems). The program output consists a series of peaks (an electropherogram), the sizes of which are estimated by comparison to fragments of the internal size standard, allowing the calculation of the height and area of each peak, which are proportional to the amount of DNA present in each fragment.

Normalized peak heights and areas were calculated by dividing each peak's height and area by a scale factor that related the total signal strength of the peak's profile (in our case, the sum of all peak areas in the profile) to the total signal strength of one of the replicate profiles that was designated (arbitrarily) as a standard. Only peaks sized between 390 and 1,250 bp were considered, and all peaks within 1.2 bp of a higher peak (i.e., shoulder peaks) were eliminated from the profiles [45].

The ARISA fingerprints were manually binned to minimize spurious and non-replicated peaks in the electropherograms [46], and the percent area (integrated fluorescence) of each operational taxonomic unit (OTU) was calculated for all of the ARISA fingerprints.

Functional Diversity

The community-level physiological profiles (CLPPs) were assessed in the selected five lakes (Table 2) using Biolog EcoPlate™ (Biolog Inc., Hayward, CA), which contains three replicated wells of 31 carbon sources and three negative controls with no substrate. The metabolism of the carbon source results in the respiration-dependent reduction of a tetrazolium dye in formazan contained in each well, which induces the formation of a purple color and increases the optical density (OD) of the solution. The plates were inoculated with 150 µL of a lake water sample previously filtered through a sterile polycarbonate filter (Nucleopore, 2 µm pore size) to eliminate nanoflagellates. For one plate, three replicates of one condition were used and incubated at 20 °C in the dark. The OD_{590 nm} was measured every 24 h for approximately 10 days using a multiwell microplate reader (Chromate® Model 4300, Awareness Technology Inc.). The values of the respective control well were subtracted, and the negative values that occasionally found were set to zero. The overall color development of each plate was expressed as the average well color development (AWCD), as suggested by Garland and Mills [47], but adapted for the triplicate wells per substrate format of the Biolog EcoPlate to give a total of 93 response wells (containing carbon substrates). AWCD was then defined as $[\sum(R-C)]/93$, where R is the absorbance of each response well and C is the average of the absorbance of the control wells, at a specific time. We also analyzed the differences in the overall rate of color development between samples using the single-point reading approach: AWCD was computed at each reading, and the color development of each substrate was analyzed when the AWCD approached a reference value of 0.5 AWCD (± 0.2) [47]. The substrates were also assigned to guilds according to their chemical nature [48]: polymers, carbohydrates, carboxylic acids, phenolic compounds, amino acids, and amines. The color development of each substrate was used to estimate functional diversity [49, 50].

Lability Experiments

The lability of DOC was performed for the five selected lakes (Table 2) in 500-mL glass flasks previously washed with HCL and sterilized. The incubations were performed in two replicates over 28 days according Guillemette and del Giorgio [51]. The decrease in DOC during the first 2 days of the incubation was considered the short-term labile pool (STL), whereas the decrease in the next 26 days was considered the long-term labile pool (LTL). We also determined the bacterial carbon consumption rates associated with the two pools: the short-term C consumption (STCC) and the long-term C consumption (LTCC). The short-term dynamic of DOC was analyzed through respiration experiments lasting 2 days, and

the long-term dynamic was evaluated by determining the DOC (Shimadzu TOC VCSH) decay over 28 days of incubation. With the aim of minimizing the methodological disparities in the STCC and LTCC determinations, the same incubations and filtration methodologies were used in both experiments. Although we conducted this experiment during 28 days following Guillemette and del Giorgio [51], the asymptotic curve was obtained around 15 days.

Respiration experiments consisted of the incubation of lake water filtered through sterile polycarbonate filters (2 µm Nucleopore pore size) to exclude nanoflagellates. The incubations were performed in triplicate using 500-mL ground-stoppered Erlenmeyer flasks in an incubation chamber in the dark at 20 °C [51]. The concentration of dissolved oxygen was measured every 6 h with an optical-oxymeter (ODO). The ODO technology allows measurement from outside the container because the system works with non-invasive oxygen fluorescent sensors (PreSens) located inside the flasks, which can be read from outside the flasks. The O₂ consumption rates were estimated as the slope of the curve O₂ vs. time fitted to a least-squares regression. The oxygen consumption rates were converted to CO₂ production to provide short-term estimates of organic carbon consumption (STCC) using a respiratory quotient of 1 [52].

After 2 days of incubation, the sample was transferred to 500 mL-bottles, previously washed with HCl and sterilized. The DOC decay analysis was performed through samplings every 2–4 days up to 28 days by collecting 40 mL of sample in pre-combusted vials (450 °C) to which 40 µL of 5 M sulfuric acid had been added [51]. LTCC was calculated as the slope of the curve DOC vs. time fitted to a least-squares regression.

The size of short- and long-term labile DOC pools was calculated as the difference between the initial DOC concentration and the concentration after day 2 for STL and between days 2 and 28 for LTL.

Statistical Analysis and Calculations

The richness, Shannon–Weaver diversity index, and evenness were calculated for the OTUs and physiological profile data according to Pielou [53].

The bacterial abundance as a dependent variable and altitude, area, maximum depth, DOC, Chl *a*, TDP, and water color (a_{440}) as independent variables were analyzed through a forward stepwise regression using SigmaPlot 12 software.

Dendrograms were constructed from the OTUs matrix of ARISA (eight lakes, BCC) and the color development of the different substrates of the Biolog EcoPlate™ (five selected lakes, CLPP) using the Bray–Curtis dissimilarity index and grouped by the average linkage method. The comparison of the two distance matrices for the five selected lakes (BCC and CLPP) was performed applying a Mantel test.

In order to test if bacteria community composition (BCC) of lakes above and below the timberline were significantly different, a PERMANOVA test was carried out using a Bray–Curtis distance matrix. Previous to the PERMANOVA test, homogeneity of multivariate variance was checked. Analyses were carried out with Vegan package in R 3.0.2.

A canonical correspondence analysis (CCA) was used for revealing relationships between the bacterial community composition/functional groups and environmental variables. CCA was performed with the software CANOCO 4.53 (Scientia Software).

The fluorometric excitation–emission matrices (EEMs) were analyzed for surface decomposition with the Candecomp/Parafac analysis with Three-way package in R 3.0.2; resulting peaks were assigned to organic compounds through the “peak picking” technique [54, 55].

Significant differences in STCC and LTCC were tested by a one-way ANOVA, and a correlation analysis was performed through the Pearson or Spearman coefficient. The normality and homoscedasticity were evaluated, and transformations were applied when necessary. The statistics were performed using SigmaPlot 12.

STCC and LTCC were combined to generate a single decay curve to reconstruct the complete dynamics of C consumption. A first-order decay model based on the multiG model [56] with only one reactive member was applied to this single decay curve. In all experiments, there was a relatively large residual portion of the total DOC that was present at the end of the incubation. Thus, the equation was as follows:

$$G_T(t) = G_{\text{Lab}}[\exp(-kt)] + G_{\text{Res}}$$

where G_T is the total DOC concentration at time t ; G_{Lab} and G_{Res} are the labile and residual pools, respectively; k is the first-order decay constant; and t is the time of decomposition. The k value represents the shape of the overall DOC decline, thereby indicating the evolution of the initial consumption over time. High values of k represent a strong inflection in the DOC vs. time curve, suggesting the rapid exhaustion of a highly reactive pool and a rapid transition to refractory carbon. Conversely, low values of k imply relatively constant rates of DOC consumption over time, suggesting a more homogenous composition of the labile pool [51].

Results

The Lakes

The DOC varied between 0.33 and 2.34 mg L⁻¹ along the altitudinal gradient, with a significant decrease toward the highest altitudes ($\rho=-0.714$, $P=0.037$). We found a

concurrent significant decrease in chlorophyll a ($\rho=-0.614$, $P=0.048$) and TDP concentration ($\rho=-0.69$, $P=0.047$) (Table 2).

The bacterial cells consisted mainly of small cocci (~0.76 μm) and bacilli (~1 μm); filaments (>5 μm) were also observed in a proportion between 0.8 and 20 %. The bacterial counts ranged from 0.671×10^6 to 2.532×10^6 cell mL⁻¹ (Table 2). The forward stepwise multiple regression analysis showed that bacterial abundance was explained by the DOC and chlorophyll a concentrations, explaining 91 % of the variance (for DOC, $R^2=0.87$, $P<0.001$, and including chlorophyll a (cumulative), $R^2=0.91$, $P<0.001$).

Genetic and Functional Bacterioplankton Diversity

The BCC analysis revealed a total of 134 operational taxonomic units (OTUs). The richness and diversity differed substantially across the eight lakes (Table 3), showing a similar trend, with the lowest values in lakes located above or just at the timberline (lakes Negra and Schmoll) and the highest in Lake Verde. When considering all of the identified OTUs, 27.2 % were present in a single lake. The 540-bp OTU was the only one that appeared in all lakes and was inversely related to the DOC concentration (Pearson correlation $r=-0.794$, $P=0.018$).

The substrate utilization in the Biolog Ecoplates was studied in the five selected lakes, with the average well color development (AWCD) of 0.5 ± 0.2 (value of reference) reached by the lake samples after different times of incubation. We observed that three carbohydrates, two carboxylic acids, one polymer, and one amine (in total seven substrates) were the most utilized by all the communities. The other substrates (25) provided by the Biolog Ecoplates were not utilized or exhibited a very low color development that pooled resulted in less than half of the total color development. The CLPP richness and diversity exhibited the lowest values in the Lakes Verde and Schmoll (Table 3).

The bacterial community composition (BCC) of the eight lakes revealed two different groups: (1) include lakes located above the timberline: Toncek, Schmoll, and Negra; (2) include lakes below the timberline: Illón, CAB, and Los Patos; and finally Lake Verde and Lake Jakob appeared as two isolated groups (Fig. 2). With the PERMANOVA analysis, we obtained significant differences for the first group (lakes above the timberline) ($F=1.69$, $P=0.03$), while the second group did not exhibited significant differences ($P>0.05$).

The Biolog data for the five selected lakes showed the highest dissimilarity between the Lakes Verde and Negra (46 %) and the lowest one between the Lake Los Patos and Lake Toncek (58.6 %) (Fig. 3). In general, the five bacterial communities reacted with carbohydrates as the most common substrate, followed by polymers, particularly the communities of the Lakes Verde and Negra. Nitrogen compounds (amino

Table 3 Bacteria community richness, diversity, evenness (S_{Gen} , H_{Gen} , and E_{Gen}), and number of unique OTUs based on ARISA OTU relative abundance, and carbon substrates (Biologs Ecoplate™) used for functional diversity (S_{Func} , H_{Func} , and E_{Func}) of the studied North Patagonian Andean lakes. For abbreviations see Table 1

Lake	S_{Gen}	H_{Gen}	E_{Gen}	N° of unique OTUs	S_{Func}	H_{Func}	E_{Func}
Illón	40	4.29	0.80	2	–	–	–
C.A.B.	38	3.92	0.74	4	–	–	–
Los Patos	35	3.61	0.70	2	29	3.06	0.63
Verde	80	5.84	0.92	15	19	2.49	0.58
Jacob	66	4.25	0.70	8	–	–	–
Negra	24	1.57	0.37	1	27	2.81	0.59
Toncek	38	3.66	0.70	1	22	2.81	0.63
Schmoll	18	2.59	0.62	1	19	2.58	0.61

acids and amines) were in general less utilized, becoming important in the Lakes Negra, Toncek, and Schmoll with the lowest DOC concentrations (Fig. 3).

When analyzing the two obtained matrix (BCC and CLPPs) of the selected five lakes through a Mantel test, we obtained no significant differences (Mantel statistic $r=0.078$, $P=0.23$) suggesting an absence of relationship between the functional capacity and community composition values.

Different patterns of grouping were observed between both datasets. For the OTU data, the first two principal components explained 49.6 % (Z_m , Chl a , and DOC) and 23.6 % (TDP, Chl a , DOC, and altitude) of the variance, respectively. The first axis separated Lake Jacob from the remaining lakes based on its higher maximum depth; the remaining lakes were distributed along the altitude-resource concentration (DOC and TDP) gradient (Fig. 4a). However, CLPPs showed a clear segregation of three groups of lakes: Lakes Los Patos and Toncek were characterized by a narrow substrate reaction (carbohydrates), while Lakes Schmoll and Verde showed the widest metabolic pathways, since they were able to profit from polymers, organic acids, and amines. Finally, Lake Negra showed an intermediate range, reacting with carboxylic acids and amino acids (Fig. 4b).

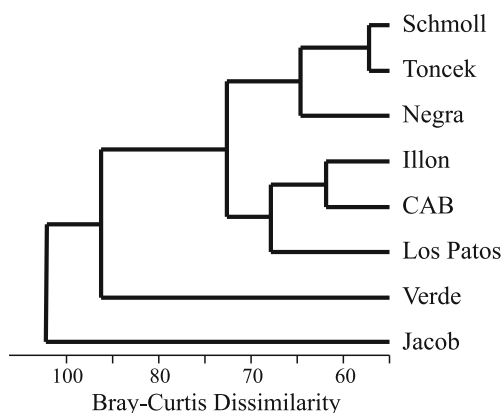


Fig. 2 Cluster analysis of the bacterioplankton communities in eight North-Patagonian Andean mountain lakes, as assessed through ARISA and based on the Bray-Curtis dissimilarity index

Dissolved Organic Matter and Physiological Responses

We detected one to three different peaks based on excitation–emission matrices (EEMs) (Fig. 5). The Candcomp/Parafac analysis showed the presence of three components (first peak, $Ex_{max}=230–260$ nm, $Em_{max}=380–460$ nm; second peak, $Ex_{max}=320–360$ nm, $Em_{max}=420–480$ nm, and a third one $Ex_{max}=260–280$ nm, $Em_{max}=300–340$ nm). The two former peaks were originally designated as A and C, respectively, representing terrigenous humic acids [54]. The third one matches a protein-like fluorescence peak corresponding to the tyrosine fluorescence of Coble [54]. Three peaks were observed in the lakes located below the timberline (Los Patos and Verde), while in Lakes Schmoll and Negra, only the protein-like peak was observed (see peak B in Fig. 5), and in Lake Toncek, this peak B was dominant with an incipient humic-like peak (Fig. 5).

The results obtained from the metabolic experiments were found to be in good correspondence with the abovementioned DOC characterization (Fig. 6). The lakes located below the timberline (Los Patos and Verde) showed STL pool values that were significantly lower than the LTL pool (t test, $P<0.001$ for both lakes). Thus, a small portion of the carbon labile pool in these lakes was consumed during the first 2 days, with most of the carbon consumed during the remaining incubation time. In contrast, a high percentage of carbon (~49 %) was consumed during the first 2 days of incubation in the lakes at or above the timberline (Negra, Toncek, and Schmoll), and there were no significant differences between STL and LTL (t test, $P=0.411$ for Lake Negra, $P=0.144$ for Lake Toncek, and $P=0.115$ for Lake Schmoll) (Fig. 6).

The integration of the STCC and LTCC measurements within the same DOC time course and fitted to a one-reactant multi-G model also showed differences among the lakes located below and at or above the timberline (Fig. 7a, b). The k constant ranged from 0.083 ± 0.024 days $^{-1}$ (Lake Verde) to 0.415 ± 0.057 days $^{-1}$ (Lake Toncek) and showed a significant relationship with altitude (Spearman correlation $\rho=0.665$, $P=0.033$) (Fig. 7c, filled dots). STCC showed a decreasing pattern from Lake Los Patos to Lake Schmoll (Fig. 7c). Lakes above the timberline showed smaller values

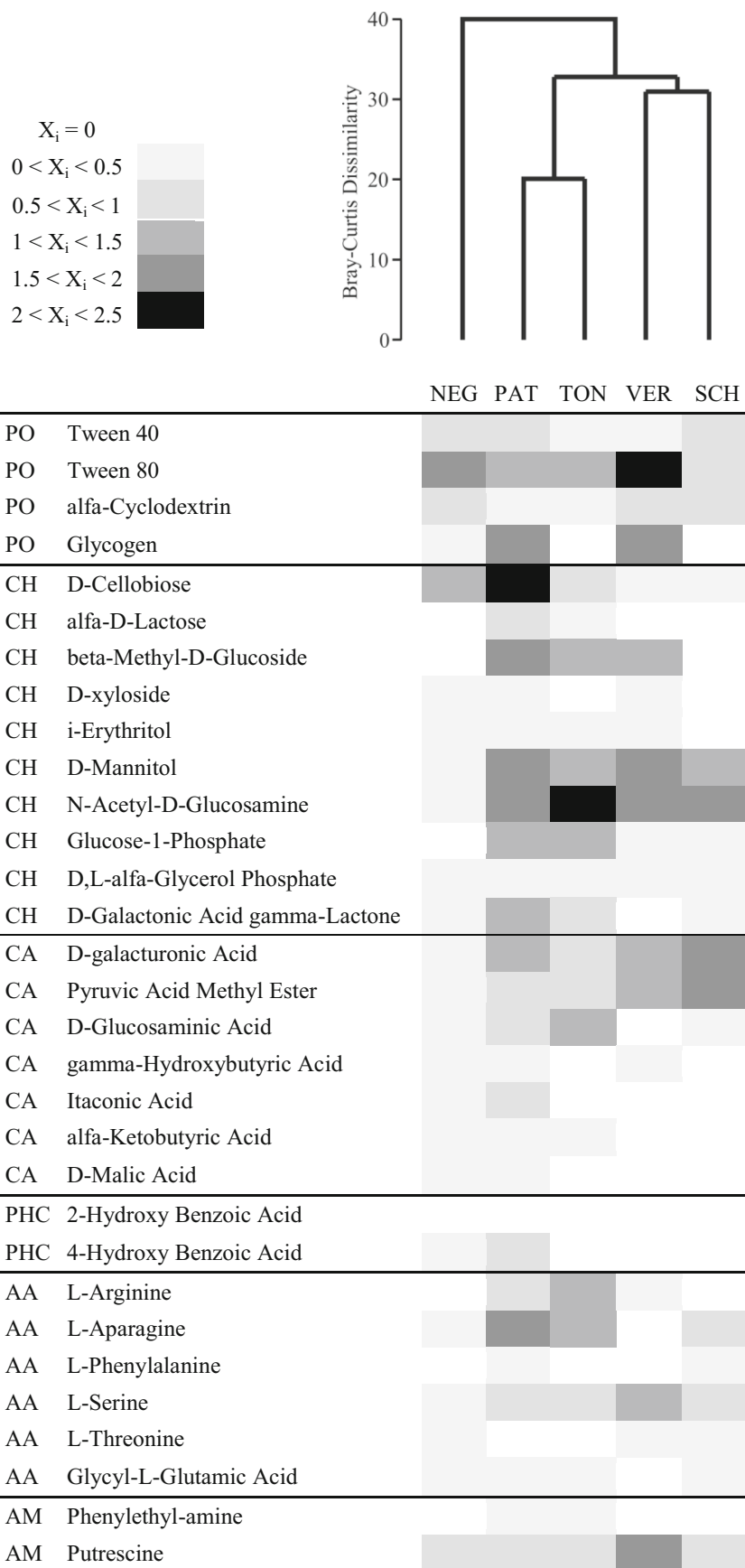


Fig. 3 Cluster analysis of the community-level physiological profiles in five selected North-Patagonian Andean mountain lakes. X_i corresponds to the absorbance at 595 nm of each well divided by the plate's respective AWCD. The classes of substrates are indicated in the first column, as follows: *PO* polymers, *CH* carbohydrates, *CA* carboxylic acids, *PHC* phenolic compounds, *AA* amino acids, *AM* amines

of STCC, without significant differences among them, but with significant differences with Verde (one-way ANOVA, $P < 0.001$ in all cases) and between Verde and Los Patos (one-way ANOVA, $P > 0.001$) (Fig. 7c). The LTCC values were, on average, 34.5 % of STCC in the lakes below the timberline (t test, $P < 0.001$ in all cases) and 7.7 % in the other group of lakes (t test, $P < 0.001$ in all cases) (Fig. 7c, white and black bars).

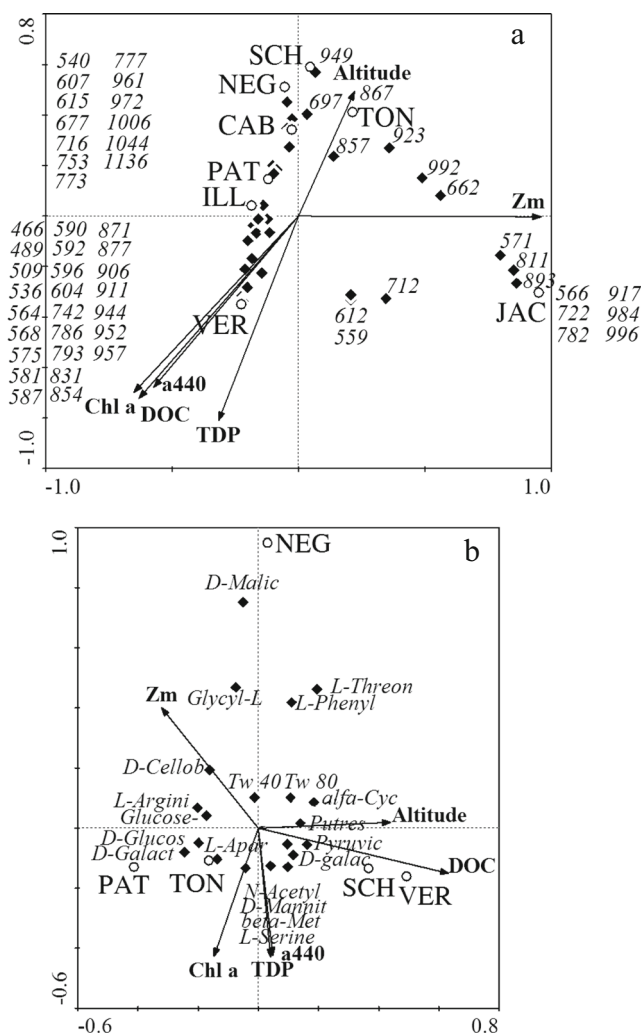


Fig. 4 **a** CCA analysis biplot showing OTUs assessed through ARISA and their relationship with environmental variables in eight North-Patagonian Andean mountain lakes. **b** CCA analysis biplot showing the result of the different substrates from Biolog Ecoplate™ and their relationship with environmental variables in five selected North-Patagonian Andean mountain lakes. The length of each arrow indicates the degree of correlation with the ordination axes

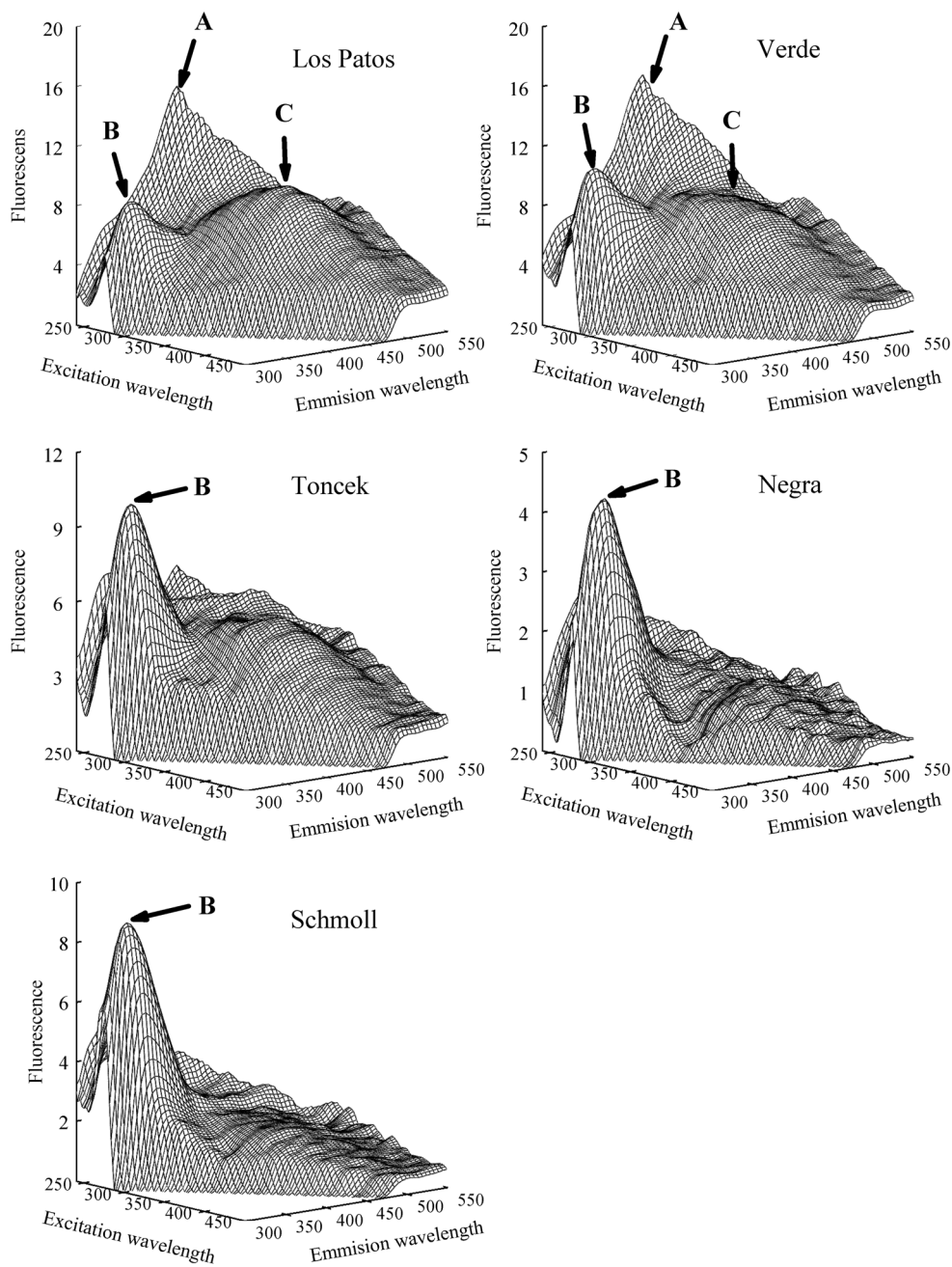
Discussion

Dissolved Organic Matter and Nutrients Along the Altitudinal Gradient

In the present study, we observed changes in the dissolved organic matter (mainly in components) and TDP in mountain Andean lakes in an altitudinal gradient from 1,380 to 1,950 m a.s.l. The decrease in the number of components of DOC (as revealed by EEMs) suggests a higher contribution of dissolved organic matter of autochthonous origin in the lakes situated at higher altitudes. In contrast, allochthonous sources appear to be more important in lakes at lower altitudes, e.g., lakes Los Patos and Verde. The fluorescence characteristics of the identified components in the EEMs analysis can be related to previously identified peaks in DOM from marine and freshwater environments [57–60]. Three main components contributed to CDOM in the mountain lakes of the Patagonian Andes, namely, two humic-like components and a protein-like component, revealing a rather low global variability in DOM composition in the area. The two humic-like peaks detected in our study (designated as A and C in Fig. 5) represent terrigenous humic-like substances or microbial degradation products of biological exudates [54, 61]; these peaks were only observed in lakes situated below the timberline, confirming the influence of the surrounding soil and vegetation to the DOM bulk in these lakes. However, the protein-like fluorescence peak (B in Fig. 5) was present in all of the studied lakes and was the only peak present in lakes above the timberline Schmoll and Negra and clearly dominant in Toncek. This peak has been described as autochthonous and labile DOM, as it has been linked to exudates from phytoplankton [57, 58]. Thus, in the altitude gradient of the studied North-Patagonian Andean lakes, the presence of surrounding vegetation (*N. pumilio* erected trees) appears to strongly influence the DOM composition, determining lakes with a dominance of allochthonous (below the timberline) or autochthonous (above the timberline) DOM, with a concomitant consequence on bacterial consumption dynamics (Figs. 5 and 7).

Further evidence of the differential DOM composition across lakes was found through the bacterial respiration and lability experiments. The shape of the DOC consumption dynamics, as reflected in the k constant and in the relative sizes of the short- and long-term labile pools, determines the potential of this carbon to fuel bacterial metabolism at different temporal and spatial scales [51]. In that sense, Del Giorgio and Pace [62] found, in the Hudson River, that short-term bacterial consumption rates target a highly reactive DOC, whereas the longer-term measures target a DOC pool that decrease in reactivity. Our results showed a lower proportion of short-term labile (STL) pool of carbon than the long-term labile (LTL) pool in the lakes located below the

Fig. 5 Surface graphs of the fluorometric excitation–emission matrix (EEM) for five selected North-Patagonian Andean mountain lakes. The fluorescence intensity is in Raman units (ru, nm^{-1}). Peaks were determined with the Candecomp/Parafac analysis and assigned to organic compounds through the “peak picking” technique. *A* and *C* terrigenous humic acid peaks; *B* protein-like fluorescence peak



timberline (Lakes Los Patos and Verde). It is likely that the LTL pool in these lakes is constituted mainly by humic-like substances revealed by peaks A and C in the EEMs. In these lakes, the pattern obtained in DOC bioassays showed that the DOC remained decreasing up to at least 25 days, suggesting the existence of DOC pool that is decreasingly reactive as was indicated by [63]. Several studies have suggested that bacteria do consume terrestrially derived DOC in lakes [25, 52], including humic and fulvic acids, and that terrestrial organic carbon significantly supports bacterial metabolism [19, 22]. The shape of the DOC consumption dynamics was influenced by these humic components, since the k constant was lower in

lakes below the timberline; sites where humic-like fractions may fuel a low but rather continuous level for bacterial activity and becomes increasingly important as the other pools are exhausted.

Conversely, the lakes without the influence of surrounded forest (erected *N. pumilio* trees) showed a relative more importance of the STL over LTL pools. The protein-like peak detected in these lakes includes molecules that could be consumed in the first hours (up to 2 days) [51, 58, 64], then in the absence of other component, the consumption reaches an asymptotic value in the remaining time of incubation (from 4 to 28 days). This result suggests that proteinaceous

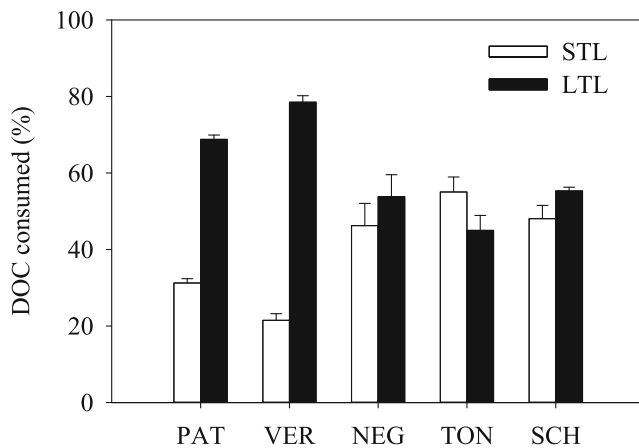


Fig. 6 Result of the lability experiments carried out in five selected North-Patagonian Andean lakes. Proportion of DOC consumed during both short- and long-term incubations (*STL* and *LTL*). Each bar represents the average recorded in each lake, and the *error bars* correspond to the standard deviation

components (peak B) can play a central role in determining the overall DOC lability, particularly in lakes located above the timberline. Hence, the number of components in the bulk DOC would determine the ratio between *STL* and *LTL* carbon consumption. In our study, we identified one to three different components, while Guillemette and del Giorgio [51], studying a more complex drainage basin in Canada, found five. Within this range (1 to 5), the *STL* decreases its relative importance and the *LTL* increases not only the importance but also the lasting time of it, from 2 days in lakes with only one component to 15 days with three components and 3 weeks with five components [51].

Bacterial Community: Genetic and Functional Diversity and Its Relationship

In our study, bacterial composition was uneven among lakes, since only one OTU was shared by all of the lakes (540 bp) and it was related to the DOC concentration. The community similarity and ordination analysis also showed that the altitude and resource concentration (DOC and P) were the main variables determining the ordination of groups. Although this pattern was observed in other mountain lakes [11], a recent study showed a strong positive correlation between bacterial abundance and altitude driven by nutrient fertilization of atmospheric inputs such as dust [4]. Our results showed an inverse trend for North Patagonian alpine lakes: the bacterial abundance decreased with increasing altitude that would result from the decrease in phosphorus and DOC concentration above the timberline playing an important role in shaping microbial communities. Such evidence, and the low levels of dry deposition in North Patagonia [4] suggests that environmental features in alpine lakes are important factors that prevent the homogenization of bacterial communities.

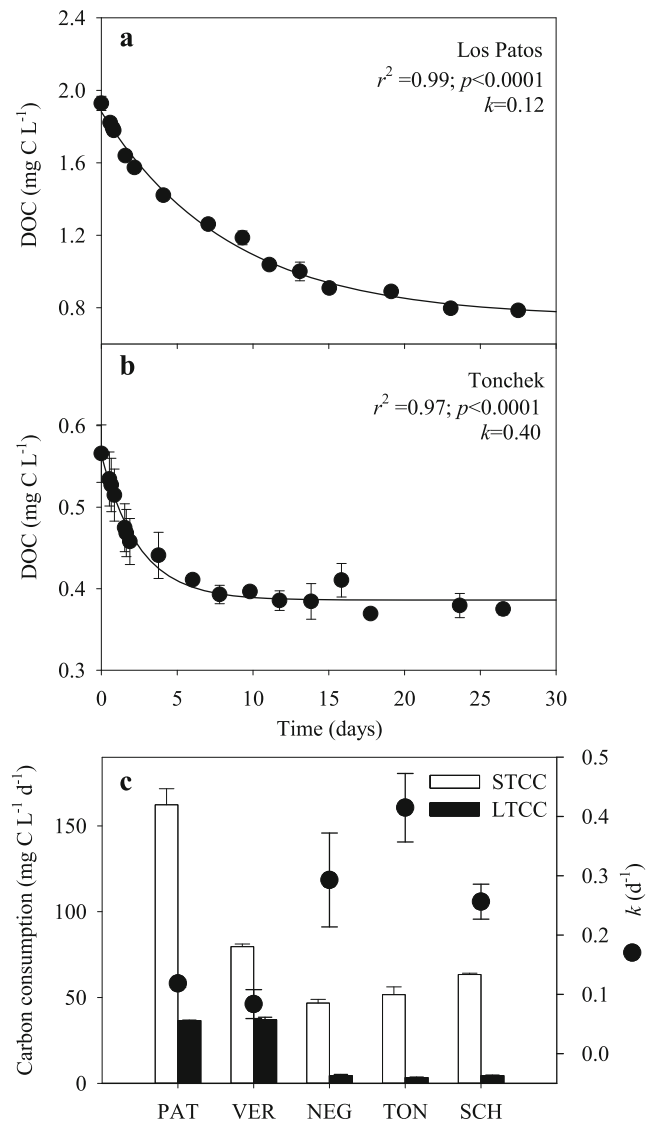


Fig. 7 Result of the lability experiments in one lake below the timberline (Lake Los Patos) and one lake located above timberline (Lake Tonček). Integrated carbon consumption time course of lakes Los Patos (**a**) and Tonček (**b**), combining the short-term bacterial respiration experiment (the first 2 days) and the DOC consumption experiment (from days 2 to 28) of the same samples. A first-order decay model was then fitted to the data (*black line*) to derive a global first-order decay constant, *k*. **c** The average and range for the study lakes with regard to the short-term bacterial carbon consumption (*STCC*) (0–2 days), long-term bacterial carbon consumption (*LTCC*) (2–28 days) and *k* values

The use of ARISA allowed us to identify differences in richness and diversity across the eight lakes and was used to screen the bacterial community in a large number of lakes [65]. However, the method has been shown to have limitations in the species-level taxonomic resolution, as with other methods used to identify bacterial communities [66]. For example, ARISA has biases such as preferential amplification of abundant organisms [44] and different 16S rDNA fragments have the same internal transcribed spacer (ITS) length [67]. Therefore, the data of absolute values of bacterial community richness should

be taken with caution since methods are inherently limited in their ability to detect the many numerically minor constituents of microbial communities [66].

The metabolic capacities of communities (estimated with Biolog) showed a mismatch with the bacterial genetic composition (revealed by the non-significant result in the Mantel test), suggesting the development of differential taxonomic diversity. Biodiversity–ecosystem functional relationships are often positive but saturating, indicating some degree of functional redundancy [21, 68, 69] and implying the ability of more than one taxon to perform the same process [27, 70]. However, other studies have noted that species identity plays an important role in maintaining ecosystem multifunctionality [29, 71], with a reduction in bacterial diversity leading to a loss of functions (e.g., extracellular enzyme activity). In these sense, functional redundancy implies that although the community composition change, the community maintains the same function [27] and this would result from a high metabolic plasticity that was mentioned as an intrinsic property of aquatic microbial communities [70]. In our study, different bacterial assemblages (of the different lakes) used the same group of substrates, indicating that the different assemblages carry out the same functions. The substrates used by the bacteria assemblages (provided in the Biolog plate) was assumed as an index of functional diversity (e.g., [49, 65, 72]); however, this approach is far to provide information of the dynamics of each microbial population that are necessary for detailed functional redundancy studies [27]. Nevertheless, the apparently redundancy observed in North Patagonian mountain lakes in the Biolog experiments seems to reflect the common metabolic pathways of these bacteria assemblages in order to be able to exploit the available DOC molecules.

In conclusion, this study highlights the importance of the quality of the DOC pool for bacteria assemblages along an altitudinal gradient in mountain lakes of North-Patagonian Andes. The obtained results suggest that the different bacterial communities in these mountain lakes seem to have similar metabolic pathways in order to be able to exploit the available DOC molecules.

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