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Article

Differences in bacterial community-level physiological profiles between deep and shallow North-Patagonian Andean lakes

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With 4 figures and 5 tables

Abstract: The utilization of carbon substrates by bacteria results in a process of great ecological significance for aquatic ecosystems. Bacteria are capable of consuming a wide range of organic molecules, but despite the highly diverse functional abilities, environmental availabilities can influence the composition of the community. Thus, differences in dissolved organic matter (DOM) and nutrient bulk between shallow and deep lakes would affect bacterial metabolic capacities. Here, we used the metabolic profiles obtained with Biolog EcoPlates® as a proxy of bacterial processing of DOM, and compared the bacterial community-level physiological profile (CLPP) from 20 North-Andean Patagonian lakes, including shallow (piedmont and high altitude, $Z_{max} < 15$ m) and deep piedmont lakes. In addition, we carried out an incubation experiment of bacterial communities from one shallow lake to assess the response of the CLPP enriched with algal exudates or leaf leachates. Our results show that the lakes have contrasting limnological features relatable to the obtained CLPP. Shallow lakes have higher nutrient and dissolved organic carbon concentrations than deep lakes and high-altitude shallow lakes. Accordingly, bacterial CLPP differed between piedmont shallow lakes and deep lakes, with a higher ability of using carboxylic acids in deep lakes. The incubation experiment shows that bacteria can develop different metabolic capacities depending on the DOM (leachates versus algal exudates) offered during incubation, increasing the consumption of the carbohydrate D-cellobiose in the algal exudate treatment. Our results show that resource availability (concentration and origin) is an important metabolic-capacity driver of bacterial communities.

Keywords: dissolved organic matter; nutrients; Biolog EcoPlates; functional approach

Introduction

Dissolved organic matter (DOM) is utilized by bacteria and thereby transferred to higher trophic levels of aquatic food webs (Azam et al. 1983; Berggren et al. 2010). In particular, the dissolved organic carbon (DOC) as substrate for bacteria has a great importance for ecosystem functioning because it influences the nature, persistence and fate of carbon in the biosphere (Battin et al. 2008). Dissolved organic matter is a pool of highly heterogeneous and complex mixture of organic compounds, including different chemical functional groups and molecular sizes with distinctive bioavailability for bacterial consumption (Benner 2003; Leehneer & Croué 2003; Cory & McKnight 2005; Jaffé et al. 2008). According to its origin, DOM can be classified as autochthonous and allochthonous; while the former derives from internal sources, such as algae (Baines & Pace 1991; Pérez & Sommaruga 2006), bacteria (Anesio et al. 2000; Guillemette & del Giorgio 2011) and also from zooplankton (Nelson et al. 2004), the latter enters from the terrestrial environment, for

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example, leachates from soil and plants (Leehneer & Croué 2003).

The bacterial ability to process and assimilate the different molecule types would be associated to the DOM pool to which bacteria are exposed (Ruiz-González et al. 2015). The ability of bacteria to consume algal-derived DOM is higher than that from DOM of an allochthonous origin (Attermeyer et al. 2013; Guillemette et al. 2013). Furthermore, the availability of different C compounds (carbohydrates, aminoacids, etc.) seems to affect the metabolic profiles of bacterial communities (Sala et al. 2006; Dickerson & Williams 2014; Freixa & Romaní 2014), with differences in bacterial metabolic capacities observed in a wide range of freshwater ecosystems with different C supply (Comte & del Giorgio 2009; Ruiz-González et al. 2015).

The study of functional diversity in bacterial communities has often been carried out with artificial substrates, such as the Biolog EcoPlate® (Zak et al. 1994), which provides a community-level physiological profile (CLPP) of the bacterial community's ability to utilize specific carbon sources. Although Biolog EcoPlates can detect considerable variation in the ability of microbial communities to metabolize different carbon compounds, different methodological limitations have been indicated (Kirk et al. 2004). However, a CLPP is very useful since the pattern of substrate used reflects a functional response of that ambient community, which is pre-adapted by the distribution of the target functional capacities within the initial community (Ruiz-González et al. 2015). In addition, the method is relatively easy to use, reproducible, and produces a large amount of data reflecting metabolic characteristics of the bacterial communities.

The aim of this study was to compare the metabolic capacities (by means of CLPPs) of the bacterial communities in deep and shallow lakes located in the Andean North-Patagonian region. Deep and shallow lakes in the area have different optical characteristics (Pérez et al. 2002) and different dissolved organic carbon (DOC) and nutrient concentrations, exhibiting extremely low values in the deep lakes (Morris et al. 1995) and a prevailing DOM of terrestrial origin in shallow piedmont lakes (Bastidas Navarro et al. 2009). Based on these features, we hypothesized that the bacterial CLPP differs between these shallow and deep lakes. We analysed the substrate utilization profiles generated by Biolog EcoPlates exposing bacteria to a fixed set of organic compounds, and expected the bacterial communities from deep lakes to react more slowly and consume fewer substrates than bacteria from shallow lakes because of the lower organic carbon and nutrient concentrations in deep lakes. Additionally, we carried out an experiment to test if the bacteria of one of these shallow lakes incubated with different DOM sources (algal exudates and leaf leachates) developed different metabolic capacities.

Materials and methods

Study area

The North-Andean Patagonian lake district (41° S) includes a series of deep lakes (up to 460-m depth) located 700–800 m above sea level (a.s.l.), which are highly transparent environments, with very low extinction coefficients ($K_{d(PAR)}$ = 0.1–0.16 m^{-1} , Morris et al. 1995) and very low nutrient concentration (Modenutti et al. 2010). The region also includes shallow lakes located in an altitudinal gradient (from 700 to 1700 m a.s.l.), which are less transparent and have higher nutrient concentrations than the deep ones (Modenutti et al. 2000; Bastidas Navarro et al. 2014). The climate is cold temperate, with a mean annual temperature of 8 °C and rainfall around 1700 mm $year^{-1}$ in mainly autumn, with snow during the winter (Paruelo et al. 1998).

We studied a total of 20 lakes located in the Nahuel Huapi National Park, North Patagonia, Argentina (between 40° 41′ S, 71° 42′ W and 41° 31′ S, 71° 32′ W, Fig. 1). These lakes can be divided into the following categories: 1) deep (70–460-m depth) located at 700–800 m a.s.l.; 2) shallow (0.5–15-m depth) piedmont lakes, located below the timberline (770 and 1600 m a.s.l.); 3) high-altitude lakes (also shallow, 5–15 m depth) located above 1600 m a.s.l. (Table 1). Lakes located below 1000 m a.s.l. are surrounded by forests of the perennial austral beech *Nothofagus dombeyi* (Mirb.) Blume, while those lakes located from 1000–1500 m a.s.l. are surrounded by the deciduous *Nothofagus pumilio* (Poep. and Endl.) Krasser (Table 1). Finally, high-altitude lakes are located above the timberline and surrounded by rocks. Deep lakes exhibited very narrow littoral zones with macrophyte colonization only in a few areas (Modenutti et al. 1998). In contrast, the littoral zones of shallow lakes are colonized by aquatic weeds, such as the emergent *Schoenoplectus californicus* (Meyer) Soják and the submersed *Potamogeton linguatus* Hangstrom (Bastidas Navarro et al. 2009), and the bottom can be covered by green filamentous algae of the genera *Spirogyra* and *Zygnema* (Cuassolo et al. 2011).

The study was carried out during the austral summer 2013 (January–March). Subsurface lake water samples were collected in a central sampling point located in the deepest part of the lake at a depth of 0.3 m. Samples were collected in 500-mL polypropylene acid-washed sterile bottles (three bottles in each lake), and transported immediately to the laboratory in thermally-insulated conditions.

Laboratory analysis

Total dissolved phosphorus (TDP) was determined in lake water filtered through GF/F filters, and total phosphorus (TP) was determined directly in 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, and P concentra-

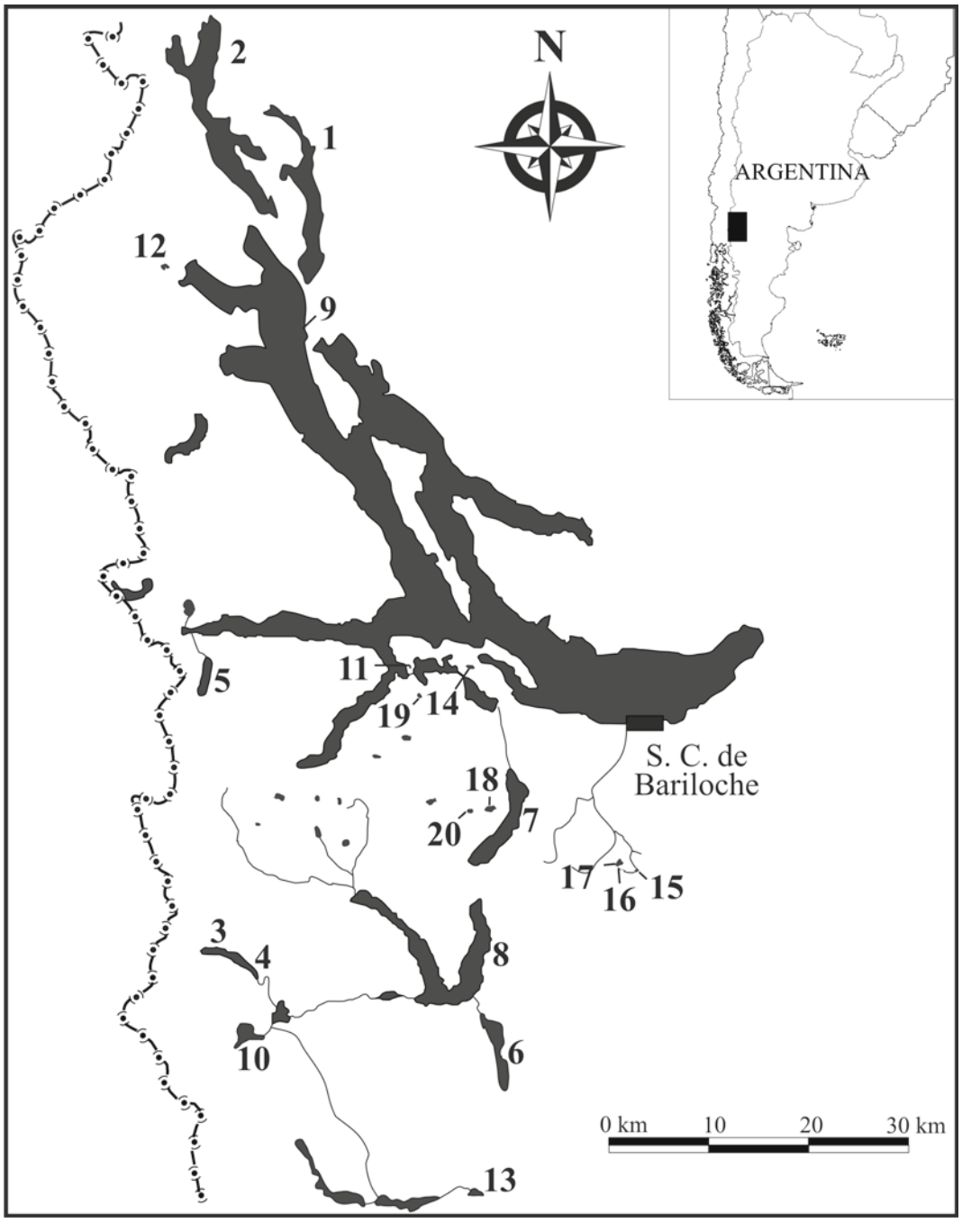

Fig. 1. Map of the 20 studied lakes in the Nahuel Huapi National Park, Argentina. For lake names see the numbers in Table 1.

tions were obtained using the ascorbate-reduced molybdenum method (APHA 2005).

Dissolved organic carbon concentration (DOC) and total dissolved nitrogen (TDN) were measured in lake water filtered through pre-combusted GF/F filters with a high-temperature combustion analyser (Shimadzu TOC V-CSH), with potassium hydrogen phthalate as the standard, and the TN-M1 unit on the Shimadzu TOC V-CSH, respectively.

Spectrophotometric scans (250–790 nm) were performed in filtered lake water (pre-combusted GF/F filters) using 10-cm path-length quartz cuvettes on a Shimadzu UV2450 double-beam spectrophotometer. The absorption coefficients (a_λ; m^{-1}) were obtained by converting the measured base-10 values to base-e logarithms as (Kirk et al. 2004)

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

where a_λ is the absorption coefficient (m^{-1}), A is the absorbance, and l is the cuvette path length (m). The water colour was estimated considering the absorbance at 440 nm (a_{440}) (Pace & Cole 2002). The specific ultra-violet absorbance at 254 nm, divided by the DOC concentration ($SUVA_{254}$), was calculated, as this index is associated with the aromatic content (Weishaar

Table 1. Morphometric characteristics of the studied lakes: altitude, surface area, maximum depth (Z_{max}), and dominant riparian vegetation. The numbers correspond to lake position in the map of Fig. 1.

Lake type	Name	Altitude (m a.s.l.)	Surface (ha)	Z_{max} (m)	Dominant riparian vegetation
Deep lakes	Correntoso (1)	777	1950	120	*Nothofagus dombeyi*
	Espejo (2)	788	3000	245	
	Fonk (3)	780	4820	145	
	Fonk chico (4)	780	59	25	
	Frías (5)	782	400	75	
	Guillelmo (6)	826	550	107	
	Gutiérrez (7)	785	1640	110	
	Mascardi (8)	750	7840	220	
	Nahuel Huapi (9)	770	55700	460	
	Roca (10)	725	3820	80	
Shallow lakes	Escondido (11)	778	8	8	*N. dombeyi*
	Piré (12)	770	15	15	
	Huala Hue (13)	828	35	7	
	Trébol (14)	790	30	12	
	Los Patos (15)	1500	1.5	3	*Nothofagus pumilio*
	Sol (16)	1510	0.1	0.5	
	Verde (17)	1545	8	1.5	
High-altitude lakes	Tonchek (18)	1700	5	12	Rocks without vegetation
	Negra (19)	1620	11.6	10	
	Schmol (20)	1950	2.7	5	

et al. 2003). The absorbance coefficient at 350 nm (a_{350}) was considered as indicative of the lignin content in water (Fichot & Benner 2012), and the ratio of the absorbance at 250 nm to 365 nm (E_2:E_3) was calculated as an estimative of organic matter molecular size (De Haan & De Boer 1987). As the molecular size increases, the E_2:E_3 ratio decreases because of a strong light absorption by high-molecular-weight DOM at longer wavelengths.

The chlorophyll-*a* concentration (Chl-*a*) was determined by extraction with 90 % ethanol according to Nusch (Nusch 1980) using a fluorometer (Turner Designs, 10-AU), which was previously calibrated against spectrophotometric measurements. Bacterial samples (20 mL) were preserved adding filtered formaldehyde at a final concentration of 2 % v/v. Total bacterial abundance was determine by staining with the fluorocrome 4′,6′-diamidino-2-phenylindole (DAPI) at a final concentration of 0.2 % w/v according to Porter and Feig (Porter & Feig 1980). Counting was performed on 0.2-µm black polycarbonate filters (Poretics) at × 1,250 magnification in an epifluorescence microscope (Olympus BX50) using a UV light (U-MWU filter). Twenty fields per filter were counted.

Community-level physiological profiles

The CLPP was estimated with Biolog EcoPlates® (Biolog Inc., Hayward, CA) containing 31 different carbon sources (Table 2), representing five molecular types (Carbohydrates, Polymers, Aminoacids, Carboxilic acids and Amines) in triplicate wells (96 total wells), plus a tetrazolium salt, which is reduced to a coloured compound by active bacteria (Garland & Mills 1991) measurable colorimetrically. Detailed information on the EcoPlates is available at

http://www.biolog.com/pdf/milit/00A_012_EcoPlate_Sell_Sheet.pdf.

Each well was inoculated with a sample of 125 µL of unfiltered water, and incubated at 21 °C in darkness. Colour development was measured spectrophotometrically with a microplate reader (Chromate® Model 4300, Awareness Technology Inc.) at 595 nm at time zero, and followed daily until the maximum colour development was reached (≈ 7 days). The overall colour development of each plate is expressed as the average well-colour development (AWCD) 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. Negative values are considered as zero. The total plate-colour development was analysed by comparing the temporal dynamics, where the AWCD follows a sigmoid curve with time, and three kinetic parameters may be estimated by fitting a sigmoid function to the data (Lindstrom et al. 1998). The parameters a (maximum absorbance reached = $AWCD_{max}$), and x_0 (time when maximum colour development rate is achieved) were calculated using SigmaPlot 12.5.

Comparisons of substrate utilization were carried out at time x_0 (Garland 1996). After confirming a correlation between the AWCD and the bacterial abundance (Pearson correlation coefficient = 0.665, $p = 0.0014$), data were normalized by dividing the mean absorbance values of each triplicated substrate by the AWCD in order to minimize the influence of the incubation

Table 2. List of the 31 substrates in the Biologs EcoPlates grouped according to their chemical function. Abbreviations for the CCA and nMDS plots.

Chemical group	Substrate	Abbreviation
Carbohydrates (CH)	D-Cellobiose	D-cel
	α-D-Lactose	α -Lac
	β-Methyl-D-Glucoside	β -glu
	D-xyloside	
	i-Erythritol	Ery
	D-Mannitol	D-Man
	N-Acetyl-D-Glucosamine	N-Ace
	Glucose-1-Phosphate	Glu-P
	D,L- α -Glycerol Phosphate	Gly-P
	D-Galactonic Acid γ-Lactone	
Carboxylic acids (CA)	D-Glucosaminic Acid	D-glu
	Pyruvic Acid Methyl Ester	
	D-galacturonic Acid	D-gal
	2-Hydroxy Benzoic Acid	
	4-Hydroxy Benzoic Acid	4-HBA
	γ-Hydroxybutyric Acid	γ-HdrB
	Itaconic Acid	Ita
	α-Ketobutyric Acid	α-Ket
	D-Malic Acid	
Polymers (Po)	Tween 40	T40
	Tween 80	T80
	α-Cyclodextrin	α-Cyc
	Glycogen	Gly
Aminoacids (AA)	L-Arginine	L-Arg
	L-Aparagine	L-Apa
	L-Phenylalanine	Phe
	L-Serine	
	L-Threonine	
	Glycyl-L-Glutamic Acid	Glut
Amines (Am)	Phenylethyl-amine	
	Putrescine	Put

time and the bacterial density (Garland & Mills 1991). We also compared the number of consumed substrate per lake, i.e. the number of positive wells (mean of triplicates) after subtracting the control average. The comparison of the use of the different chemical group of molecules (carbohydrates-CH, carboxylic acids-CA, polymers-Po, aminoacids-AA and amines-Am) was performed by dividing the sum of the absorbance of molecules of one type by the number of substrates of that type (CH = 10, CA = 8, Po = 4, AA = 6, Am = 2).

Experimental set-up

The experiment was performed in summer (February 2014) with samples from Lake Los Patos, a shallow lake located around 1500 m a.s.l. and surrounded by the deciduous *N. pumilio* (Table 1, Fig 1). The experiment consisted in the incubation of natural bacterial assemblage with and without the addition of different DOM sources: algal exudates and leaf leachates. Algal exudates were obtained by the incubation of algal mats of *Spyrogira* sp and *Mougeotia* sp collected on each sampling occasion and carried to the laboratory. Mats were carefully washed several times with MilliQ water to remove attached fauna and flora. Clean algae were put in MilliQ water in sterile 250-mL flasks and incubated at 20 °C and 90 µmol photon m^{-2} s^{-1} for 48 h. Leachates were obtained from *N. pumilio* senescent leaves collected from the trees just before excision to avoid soil contamination. Leaves were soaked in MilliQ water in sterile 250-mL flasks in a refrigerator for 48 h (Anesio et al. 2000). Both solutions were filtered through a pre-combusted GF/F filter and 0.2-µm cellulose acetate sterile filters before the experiment. We measured DOC concentration of these solutions to calculate the dilution needed to reach similar DOC concentrations in control and treatments.

The bacterial inoculum was obtained from filtered (2 µm) lake water, which was incubated with the different DOM sources: the control treatment consisted of filtered lake water (0.2 µm) without any DOM addition. The proportion of inoculum, lake water, and DOM solutions was 1:1:2 in treatments (algal exudates or leaf leachates). All treatments and control were run in 500-mL flasks in four replicates. All glassware was previously sterilized (autoclaved for 30 min). The flasks were incubated in a controlled temperature incubator at 20 °C in

Table 3. Environmental variables of the studied lakes: dissolved organic carbon (DOC), chlorophyll-*a* concentration (Chl-*a*), total dissolved phosphorus and total phosphorus concentration (TDP and TP), and spectrophotometric measurements (a_{440}, SUVA, a_{350}, E2:E3).

Lake		DOC (mg L^{-1})	Chl-*a* (µg L^{-1})	TDP (µg L^{-1})	TP (µg L^{-1})	TDN (µg L^{-1})	a_{440} (m^{-1})	*SUVA* (m^{-1} mg^{-1})	a_{350} (m^{-1})	E_2:E_3
Deep lakes	Correntoso	0.42	0.9	2.1	7.4	41.0	0.81	5.36	1.08	2.46
	Espejo	0.71	1.8	3.0	4.2	93.6	0.85	3.20	1.11	2.32
	Fonk	1.06	1.2	3.5	4.1	81.6	0.92	3.52	1.81	2.93
	Fonk chico	0.99	1.6	3.0	4.0	100.8	0.91	3.51	1.77	2.91
	Frias	0.73	0.4	12.6	18.6	92.1	1.22	13.48	3.18	3.78
	Guillelmo	0.64	1.2	3.4	4.8	69.4	1.01	4.95	1.40	2.54
	Gutierrez	0.49	1.5	3.3	4.7	58.1	0.55	3.30	0.74	2.55
	Mascardi	0.64	2.4	2.9	4.6	49.8	0.69	3.23	0.97	2.51
	Nahuel Huapi	0.50	2.5	2.2	4.2	45.0	0.87	4.95	1.17	2.46
	Roca	0.91	1.7	1.2	2.8	68.6	0.89	3.49	1.71	2.89
Shallow lakes	Escondido	4.89	1.7	4.3	4.9	205.1	1.31	4.68	5.23	5.68
	Pire	1.29	2.8	4.3	14.9	60.8	1.66	7.83	3.92	3.11
	Huala Hue	2.97	7.5	7.6	9.8	173.8	0.67	2.70	2.03	4.89
	Trébol	3.39	2.6	4.4	7.1	201.8	0.76	2.73	1.96	5.75
	Los Patos	3.25	2.55	6.7	11.8	71.4	2.48	3.06	4.65	2.54
	Sol	9.29	14.3	5.2	18.7	525.8	2.02	1.21	4.15	3.21
	Verde	4.33	10.6	12.5	10.3	208.2	3.31	7.14	9.21	4.18
High altitude lakes	Tonchek	0.41	0.7	3.4	5.7	31.5	0.60	3.70	1.17	2.84
	Negra	0.56	0.8	1.4	1.4	57.2	0.32	1.02	0.37	2.25
	Schmol	0.33	0.6	3.4	5.9	63.2	0.57	1.79	0.71	2.24

the dark for 96 h. Afterwards, we took samples to incubate the resulting bacterial communities from the control and the two treatments (exudates and leachates) on the Biolog plates.

Statistical analysis

A gradient analysis was performed of the substrate-utilization data from the 20 lakes, and related to the environmental variables (lake depth, altitude, DOC, Chl-*a* concentration, TDP, TP, TDN, a_{440}, $SUVA_{254}$, a_{350} and the E_2:E_3 ratio) by a Canonical Correspondence Analysis (CCA) with CANOCO for Windows 4.5 (Scientia Software) using the chi-squared (χ^2) distance. The Pearson correlation was used to analyze the correlation between the lake depth and altitude with the relative absorbance of the five groups of molecules (carbohydrates, carboxylic acids, polymers, aminoacids and amines). Differences in bacterial abundance, number of substrate utilization in the Biolog plates, and the kinetic parameters of the plate-colour development, were tested among the three lake types using one-way analysis of variance (ANOVA). Data of bacterial abundance were ln-transformed to achieve normality. When differences between lake types were found, an *a posteriori* all-pairwise multiple-comparison procedure (Tukey Test) was used to isolate that group or those groups different from the others.

In the experiment, the results of the biolog parameters were compared with one-way ANOVA. To analyze differences in substrate utilization in the different treatments of the experiment, we performed a similarity analysis instead of a gradient analysis. The matrix of substrate utilization from the Biolog plates was used to generate a similarity matrix using the Bray-Curtis index. A non-metric multidimensional scaling (nMDS) analysis was applied to visualize the ordination of sites according to these matrices. Vectors were plotted according to their correlation (Pearson correlation) with nMDS axes 1 and 2. Only substrates with a correlation > 0.7 were selected for visualization. Differences among experimental treatments were assessed by an analysis of similarities (ANOSIM). As the ANOSIM test revealed significant differences, we used the species contributions to similarity function (SIMPER) in PRIMER v.6 (Primer-E Ltd 2006, Plymouth, UK) to identify those substrates responsible for the observed differences.

Results

Field results

In the whole dataset of 20 lakes, we observed significant differences between the studied lakes (Table 3), with the DOC concentration ranging from 0.33 to 9.28 mg C L^{-1}, and negatively correlated with the lake depth (Pearson correlation coefficient DOC–ln(*Z*): −0.702, $p < 0.001$). The nutrient concentration and absorbance coefficients (a_{440} and a_{350}) showed the same trend (Table 3).

The results of the CCA ordination based on CLPPs show only 24.7 % of the variance in substrate utilization is accounted for by the first two ordination axes (35.8 % by the first four axes). The variance when environmental variables are added accounts for 50.5 % by the first two axes (73 % by the first four axes). The first ordination axis reflects a gradient positively related to the lake altitude, lignin content, dissolved nutrients and carbon and Chl-*a* concentrations, and negatively related to the lake depth (Fig. 2a). Deep

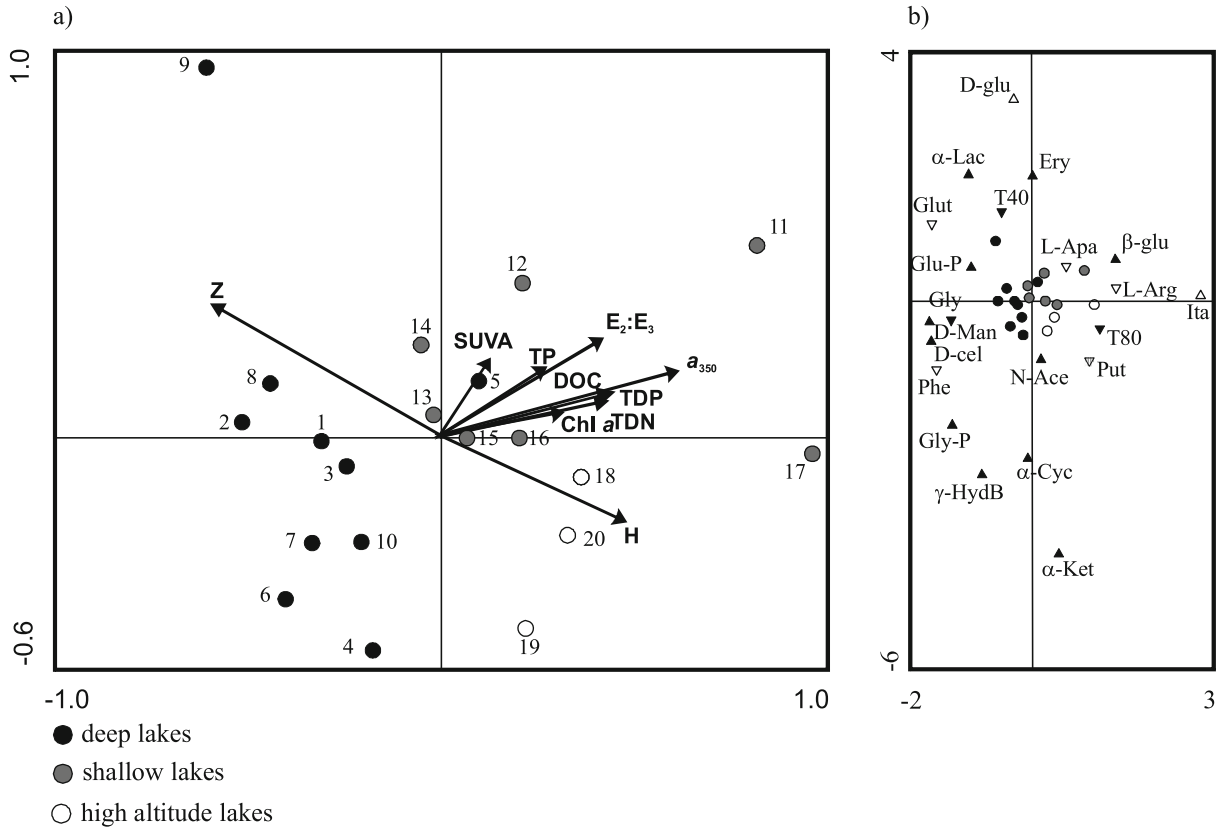

Fig. 2. Distribution of sampling sites based on the CCA. The environmental parameters used for this analyses are the lake depth (*Z*) and altitude (*H*), total and dissolved phosphorus (TP and TDP), dissolved nitrogen (TDN) and dissolved organic carbon (DOC), chlorophyll-*a* concentration (Chl-*a*), and specrophotometric variables (a_{440}, $SUVA_{254}$, a_{350} and the E_2:E_3 ratio). Site numeration as in Table 1. Substrate abbreviations are given in Table 2.

lakes segregate in the negative values of the first axis, except Lake Frías, while almost all shallow and high-altitude lakes positively segregate with this axis. The second ordination axis is also correlated with the lake depth and with the aromatocity, TP value, E_2:E_3 ratio (positively) and altitude (negatively). In the positive values of axis 1, axis 2 segregates high-altitude lakes (in the negative values) and shallow piedmont lakes in the positive values (Fig. 2a).

The biplot of the sites and substrates (Fig. 2b) shows the substrates characterizing the deep and shallow lakes, with D-cellobiose, D-Manitol (CH) and glycogen (Po) associated with deep lakes, and β-methyl-D-glucoside (CH), L-Arginine (AA), L-Aparagine (AA) and Itaconic acid (CA) associated with shallow lakes, while Tween 80 (Po) and Putrescine (Am) are associated with high-altitude lakes.

Bacterial abundances are not correlated with lake depth, because they were in the same order of magnitude in the high-altitude lakes as in the deep lakes, despite their depth. However, when bacterial abundances are compared among lake types, they are higher in shallow (piedmont) lakes than in deep lakes (Table 4); Lake Frías is an exception, with bacterial abundance as high as in shallow lakes. Accordingly, the maximum plate absorbance reached ($AWCD_{max}$) is higher in shallow lakes, and the bacterial communities of high-altitude lakes react slower in the Biolog plates (higher x_0, Table 4). The number of consumed substrates varies from 8 to 26, but did not differ among lake types (Table 4). There were two substrates, 2-Hydroxy Benzoic Acid and L-Threonine, which were not used in any lake. However, the use of some groups of molecules differed according to lake depth: Carboxylic acids were more used by bacteria from deep lakes (Pearson correlation coefficient $=0.80$, $p<0.001$), while the use of amino-acids tended to be higher in communities from shallow lakes (Pearson correlation coefficient $= -0.43$, $p=0.059$). In contrast, the use of amines is correlated with the lake altitude (Pearson correlation coefficient $=0.615$, $p=0.004$). Carbohydrates and polymers were the most consumed substrates, without differences among lake types.

Table 4. Bacterial abundance, maximum average well-colour development (AWCD), time when maximum colour development rate is achieved (x_0), and number of total substrate utilization in the Biologs plates. Statistical results of the differences among lake types.

Lake		Bacteria (10^6 cells ml^{-1})	$AWCD_{max}$	x_0 (hours)	N^o substrates
Deep lakes	Correntoso	7.33	0.55	76	10
	Espejo	7.33	0.32	72	17
	Fonk	0.58	0.14	67	15
	Fonk chico	1.13	0.39	75	19
	Frias	12.25	0.68	61	21
	Guillelmo	0.93	0.16	50	11
	Gutierrez	0.98	0.16	116	8
	Mascardi	1.65	0.23	53	18
	Nahuel Huapi	6.58	0.25	135	11
	Roca	1.14	0.15	67	21
Shallow lakes	Escondido	8.00	0.40	75	11
	Pire	31.42	0.98	74	22
	Huala Hue	6.33	0.83	91	26
	Trébol	17.25	0.96	49	22
	Los Patos	10.16	0.98	65	23
	Sol	9.25	0.62	62	19
	Verde	9.18	0.44	74	11
High altitude lakes	Tonchek	3.17	0.59	98	15
	Negra	4.03	0.17	142	14
	Schmol	6.37	0.31	128	16
ANOVA results	F	6.610	9.011	6.038	1.524
	P	0.008	0.002	0.010	0.246

Experimental results

After the incubation on different dissolved organic sources, the maximum absorbance reached in the plates is similar for all treatments ($a = 0.94 \pm 0.03$, ANOVA, $p = 0.442$), but the time of reaction was lower in the control than in the enriched treatments (control $x_0 = 31.1 \pm 0.9$; exudates $x_0 = 47.8 \pm 0.9$; leachates $x_0 = 56.1 \pm 4.6$, ANOVA, $p = 0.002$). The number of substrates used ranged from 18 to 25 (ANOVA, $p = 0.518$), but there were differences in the use of the different molecular groups (Fig. 3). Bacteria from exudates consumed more carbohydrates than in the other treatments (ANOVA, $p = 0.042$), while amines were most consumed in the control followed by leachates and less consumed in exudates (ANOVA, $p = 0.006$). Also, the analysis of the CLPPs shows differences among resource treatments (ANOSIM, Global Rho = 0.811, $p = 0.004$) (Fig. 4). The SIMPER analysis indicates that the substrates that most contributed to the differences among treatments are β-methyl-D-Glucoside and N-Acetyl-D-Glucosamine, which were more consumed in the treatments exudates and leachates than in the control (Table 5). The carbohydrate D-cellobiose was more consumed by bacteria grown in the exudates treatment, and the polymer Glycogen was more consumed by bacteria incubated with leachates (Fig. 4).

Discussion

Differences in the environmental variables among the 20 studied North-Andean Patagonian lakes reveal that high-altitude lakes, though shallow, were more similar to deep lakes than to shallow piedmont ones (Fig. 2). Therefore, lake depth cannot be considered as a single factor affecting resource availability because the altitude also plays an important role. Another apparent discrepancy is Lake Frías, which is a deep lake more associated with shallow lakes because of its relative higher concentration of phosphorus. Lake Frías receives glacial clay inputs from the Frías Glacier of Mount Tronador, which is currently in drastic recession (Masiokas et al. 2008). As a consequence, Lake Frías has a decreasing transparency and increasing phosphorus content, as was observed in other aquatic environments of the area (Chillrud et al. 1994; Martyniuk et al. 2014).

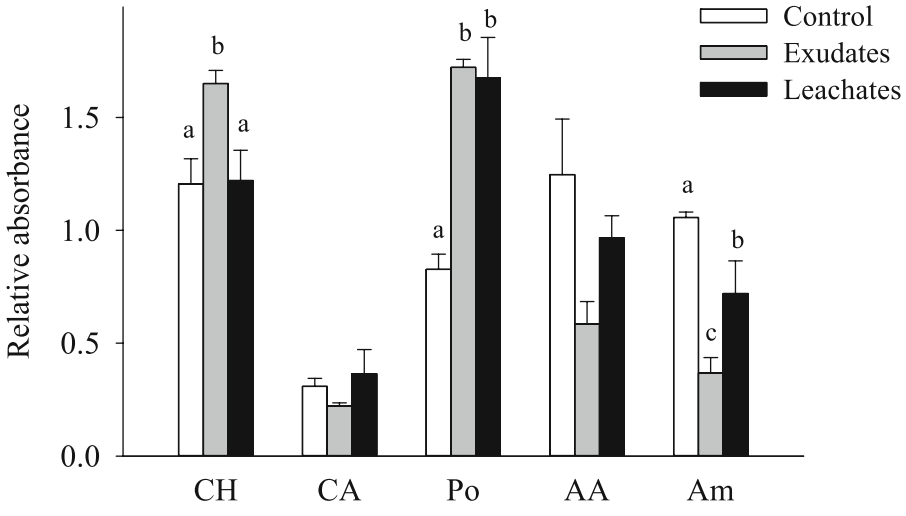

Fig. 3. Specific absorbance of the chemical groups of molecules (abbreviated as in Table 2) in the experiment. Error bars are the standard error. Differences among treatments (results of the *a posteriori* test) are marked with letters (a, b, c).

Table 5. Results of the SIMPER analysis. Average similarities among replicates in a treatment and dissimilarities between paired treatments; list of the organic substrates that most contributed to the similarity/dissimilarities up to ≈ 50 %, and the total contribution to the average similarity/dissimilarity.

Control **Average similarity: 71.89 %**			**Exudates** **Average similarity: 89.96 %**			**Leachates** **Average similarity: 78.82 %**		
D-Mannitol	CH	13.0 %	N-Acetyl-D-Glucosamine	CH	13.4 %	Glycogen	Po	12.5 %
L-Aparagine	AA	11.6 %	D-Cellobiose	CH	13.0 %	N-Acetyl-D-Glucosamine	CH	10.0 %
L-Arginine	AA	10.5 %	β-Methyl-D-Glucoside	CH	11.7 %	L-Aparagine	AA	9.9 %
Putrescina	Am	9.8 %	D-galacturonic Acid	CA	11.3 %	β-Methyl-D-Glucoside	CH	9.2 %
Tween 80	Po	8.8 %				D-galacturonic Acid	CA	7.8 %
Total contribution to similarity		53.7 %			49.4 %			49.4 %
Control-Exudates **Average dissimilarity: 46.5 %**			**Control-Leachates** **Average dissimilarity: 39.7 %**			**Exudates-Leachates** **Average dissimilarity: 26.6 %**		
β-Methyl-D-Glucoside	CH	11.5 %	Glycogen	Po	12.6 %	D-Cellobiose	CH	12.4 %
L-Arginine	AA	9.5 %	β-Methyl-D-Glucoside	CH	10.8 %	D-galactonic Acid γ-Lactone	CH	11.0 %
D-Cellobiose	CH	9.5 %	L-Arginine	AA	8.8 %	L-Aparagine	AA	9.7 %
L-Aparagine	AA	8.4 %	D-glucosaminic Acid	CA	7.9 %	Glycogen	Po	8.5 %
N-Acetyl-D-Glucosamine	CH	8.4 %	N-Acetyl-D-Glucosamine	CH	7.0 %	D-galacturonic Acid	CA	6.4 %
Total contribution to dissimilarity		47.3 %			47.1 %			48.1 %

The maximum plate-colour development of Biologs may be related with bacterial activity (Freixa & Romaní 2014). In our study, the $AWCD_{max}$ value was significantly higher in shallow lakes, which is probably related with the higher DOC and nutrient concentrations observed in these lakes (Table 2). In contrast, in the ultraoligotrophic deep Andean lakes, the bacterial production is strongly P-limited (Bertoni et al. 2008), thus the low bacterial activity observed in this type of lake could be associated with the low nutrient concentration. Accordingly, the value of x_0 was higher in the high-altitude lakes, which are environments with very low nutrient and DOC concentrations, and particularly low coloured DOM (a_{440}) and lignin (a_{350}) values (Table 2). The bacterial communities of these transparent environments are exposed to stressful conditions of high UV irradiance (Corno et al. 2009), which may cause negative effects on the bacterial production (Chatila et al. 2001; Buma et al. 2003) and on the biogeochemical C cycle, specifically on the

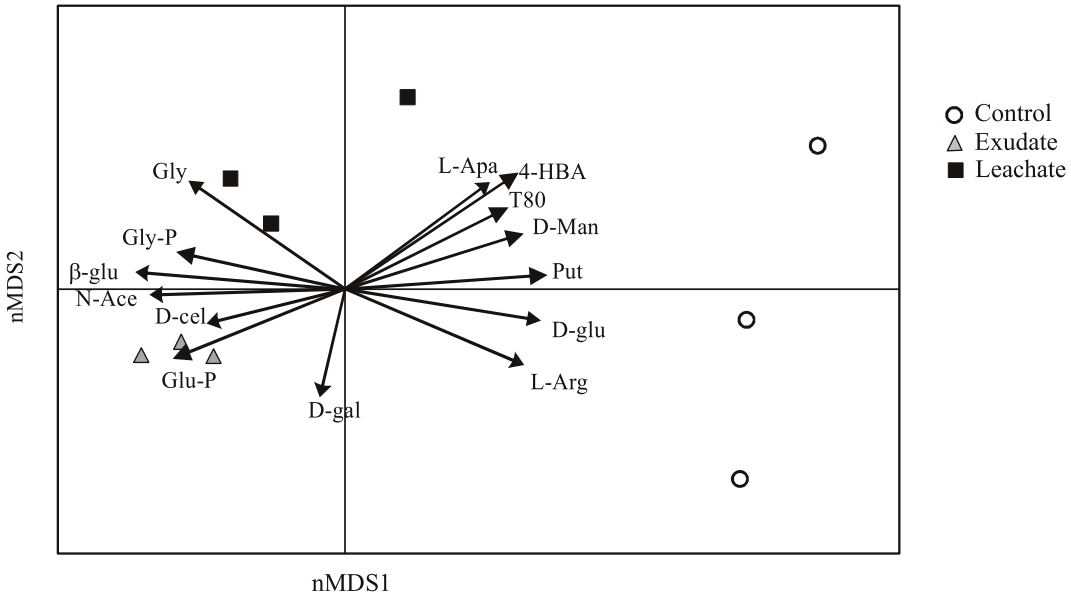

Fig. 4. Non–metric multidimensional scaling (nMDS) distribution of treatments (control, exudates and leachate) based on CLPP. Substrate abbreviations are given in Table 2.

efficiency in which DOM is transformed into bacterial biomass (Hörtnagl et al. 2011). In our study, all the incubations in the Biolog plates were carried out with bacterial communities coming from subsurface samples, thus negatively affected by short wavelengths (Corno 2009; Modenutti et al. 2010). This stressful condition could also potentially account for the low bacterial activity, i.e. longer time to react to the different substrata, in the Biolog incubation.

Our results also confirmed the hypothesis that bacterial communities from deep and shallow lakes have different abilities to consume organic substrates offered on the Biolog plates. Changes in terrestrial input and the importance of cDOM in the C pool are important factors in functional bacteria divergence (Ruiz-González et al. 2015). The use of a specific carbon substrate from the EcoPlates does not mean that this substrate was available in the environment, though the appearance of specific metabolic capabilities may suggest that similar substrata may exist at the collecting site (Sala et al. 2006; Tiquia 2010; Freixa & Romaní 2014). In general, carbohydrates and polymers were the most consumed compounds. However, in deep lakes, carboxylic acids were used in higher proportion than in the other studied lakes. This pattern was also found in oligotrophic coastal waters compared with more eutrophic waters from a harbour site (Sala et al. 2006). Carboxylic acids are considered to be part of the labile pool of DOM and a common product of photochemical processes (Bertilsson & Tranvik 1998). As photobleaching of DOM by UVR is a common process in the highly transparent, deep North-Patagonian Andean lakes (Osburn et al 2001), carboxylic acids can be expected in these environments, and, therefore, bacteria may be adapted to make use of this substrate. Among the carbohydrates, D-cellobiose is one of the most related to deep lakes, and was also found to be one of the most consumed substrates by marine bacterioplankton (Sala et al. 2010). Since D-cellobiose derives from phytoplankton cellulose degradation (Overbeck & Chróst 2012), a higher consumption of this compound indicates that cellobiose would be an available resource when the DOM is predominantly of a benthic algae or phytoplankton origin. This is confirmed in the experiment carried out with the bacterial community from Los Patos incubated in different DOM sources (algal exudates and leaf leachates), in which the nMDS analysis shows that bacteria growing in the algal exudates treatment is characterized by the consumption of D-cellobiose, like the bacterial communities from deep lakes. In contrast, in shallow lakes characterized by higher DOC and nutrient concentrations, there is a trend to higher aminoacid consumption, in particular L-Aparagine and L-Arginine. Aminoacids are a high-quality resource, and may be related to the higher Chl-*a* concentration in shallow lakes (Jørgensen 1982).

Summarizing, here we showed that the bacterial functional traits of 20 shallow and deep North-Andean Patagonian lakes are linked with differences in resource availability. Although most of the offered substrata were consumed, we observed significant

differences in the time to reach the maximum colour development (x_0) and in the specific consumption of some compounds. In particular, the low activity and the higher consumption of carboxylic acids of the bacterial communities from deep lakes can be related with the low nutrient concentration and the high UVR exposure in these lakes. Higher DOC and nutrient concentration with a concomitant increase in the importance of the terrestrial influence was observed for shallow piedmont lakes, in which bacterial CLPP was related with sources of external origin.

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List of authors' contributions

VDV designed the experiment, analysed data, and wrote the paper, MBN performed the laboratory analysis and carried out the experiment, BM analysed data and wrote the paper.

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