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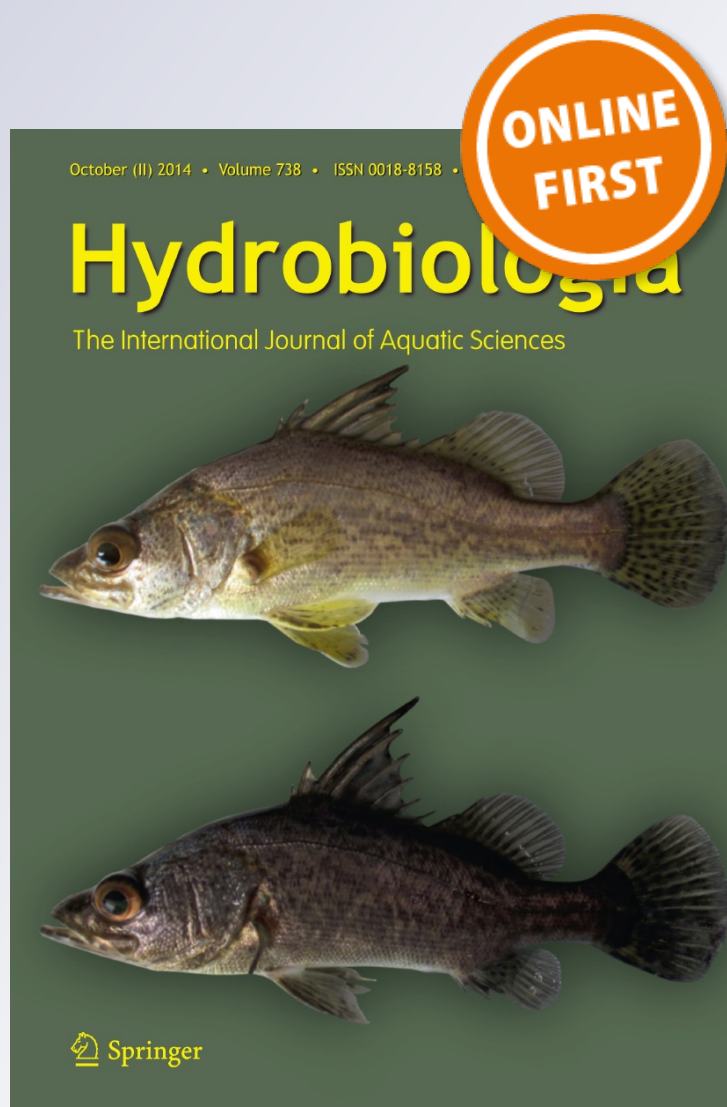
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ARGENTINE PAMPEAN SHALLOW LAKES

Microbial abundance patterns along a transparency gradient suggest a weak coupling between heterotrophic bacteria and flagellates in eutrophic shallow Pampean lakes

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Abstract The aim of this work was to study the components of the microbial food web and the degree of coupling between heterotrophic bacteria (HB) and heterotrophic flagellates (HF) in different shallow lakes of the Pampa Plain over a wide range of water transparency and nutrient levels. We hypothesized that not all microorganisms (i.e., HB, HF) equally increase with nutrient levels, thus, resulting in a weaker degree of coupling between HB and HF in more eutrophic waters. During the spring–summer 2009–2011, we sampled 40 Pampean lakes situated in different watershed of Buenos Aires Province. Abundances of most microbial components were very high, even higher than those reported in the literature. HB as well as picocyanobacteria (Pcy) increases with trophic state, while no clear relationship was found between protist (HF or ciliates) and Chl-*a* concentrations or nutrient levels. Photosynthetic picoplankton was generally dominated by phycocyanin-rich Pcy. Lakes with high HB and low HF were abundant, which suggest these components were weakly coupled. Based on the abundance of microorganisms, we do not accumulate enough evidence to segregate Pampean lakes into two discrete groups (clear vs turbid).

Keywords Microbial food web · Turbidity · Chlorophyll *a* · Nutrients · Pampean shallow lakes

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Introduction

Bacterial grazing by heterotrophic flagellates (HFs) is of fundamental importance in aquatic systems (Sherr & Sherr, 2002). It controls heterotrophic bacterial (HB) abundances in a wide range of ecosystem conditions, channels organic carbon to higher trophic levels (Pernthaler, 2005). Photosynthetic picoplankton

(PPP), represented by picocyanobacteria (Pcy) and picoeukaryotes (Peuk), is also currently considered as a major contributor to the carbon flow (Callieri, 2007; Stenuite et al., 2009). HFs are efficient predators of both HB and PPP (Boenigk & Arndt, 2002; Pernthaler, 2005; Izaguirre et al., 2012); in turn, they constitute the main food source for ciliates and crustaceans (Gasol et al., 1995; Jürgens & Jeppesen, 2000). Consequently, trophic interactions between microbial components determine total amount of carbon and nutrients available for zooplankton, and therefore for fish.

The function, structure, and dynamic of the microbial community and their response to nutrient levels in aquatic systems is still contradictory (Conty & Bécares, 2013). Several authors have observed that the carbon transfer through the microbial food web to higher trophic levels is significantly more important in oligotrophic than eutrophic systems (Porter et al., 1988; Weisse et al., 1990; Burns & Galbraith, 2007). However, the abundance and biomass of microorganisms increase with nutrients levels (Sommaruga & Robarts, 1997; Auer & Arndt, 2001) and, in high productive environments, they can also be of great importance (Riemann & Christoffersen, 1993; Kalinowska, 2004). Some studies showed that food source determines microbial abundance (Muylaert et al., 2002; Chróst et al., 2009; Roland et al., 2010). Nevertheless, due to the great amount of interactions between microbial components as well as with other planktonic organism of traditional food web in the more eutrophic systems, there are many works which showed different patters (Auer et al., 2004, Conty & Bécares, 2013; Burian et al., 2013). It is well documented that zooplankton composition could be of significant importance structuring the microbial trophic web (Jürgens & Matz, 2002; Pernthaler et al., 2004; Sommer & Sommer, 2006). For instance, some authors founded that picoplanktonic abundance could be strongly regulated by predation (top–down control), instead of its resource availability (bottom-up control) (Pernthaler et al., 1996; Hirose et al., 2003; Callieri, 2010). In addition, several authors have suggested that zooplankton exerts a higher control over HF abundances when the trophic status increases, resulting in a higher decoupling between HF and their preys (Gasol, 1994; Pernthaler, 2005).

The Pampa region in Argentina contains thousands of shallow lakes, which are naturally eutrophic (Canevari et al., 1999; Quirós et al., 2006). As many lakes of Northern Hemisphere and according to the alternative state theory developed by Scheffer et al. (1993), these waters bodies often present two contrasting states: a *clear state*, with macrophytes as the predominant autotrophic community, and a *turbid state* with phytoplankton as the predominant ones (Izaguirre & Vinocur, 1994; Allende et al., 2009). These lakes have been impacted by agriculture and livestock for more than a century, and nutrients levels have been increased (Quirós et al., 2002; Giraut et al., 2007). As a consequence, many lakes have shifted from a clear to a turbid state (Quirós et al., 2006). The alternative state theory was based primarily on the interaction between the phytoplankton and submerged vegetation; nevertheless, did not take into account the interaction between microorganisms. Despite a good knowledge of components of the traditional food web in Pampean shallow lakes (Izaguirre & Vinocur, 1994; Claps et al., 2004; Torremorell et al., 2009), little is known about the microbial components. Previous studies carried out in these lakes showed that phytoplankton-turbid waters bodies are characterized by high productivity and PPP abundances, mainly dominated by phycocyanin-rich Pcy (PC) (Allende et al., 2009; Torremorell et al., 2009). Moreover, in these water bodies, HB and Pcy numbers are usually very high (Silvoso et al., 2011; Fermani et al., 2013). However, Silvoso et al. (2011) found that Pcy abundances cannot be predicted considering only nutrient levels, light climate, and chlorophyll *a* (Chl-*a*) concentrations. Also, in a hypertrophic shallow lake of the Pampa Plain (Chascomús), we observed that the relationship between HB and HF was differentially affected by dominating zooplankton assemblages (Fermani et al., 2013).

Different physicochemical and/or biotic factors in shallow Pampean lakes may set different scenarios for the development of microorganisms, being an excellent environmental frame to analyze the potential changes in microbial food web components at the extreme of trophic spectrum. In this regard, our work aims to study the microbial abundance patterns in 40 eutrophic shallow lakes of Pampa Plain along a gradient of water transparency and nutrient levels. We hypothesized that not all microorganisms (i.e., HB, HF) equally increase with increases in nutrient levels, thus, resulting in a weaker degree of coupling between HB and HF in more eutrophic waters.
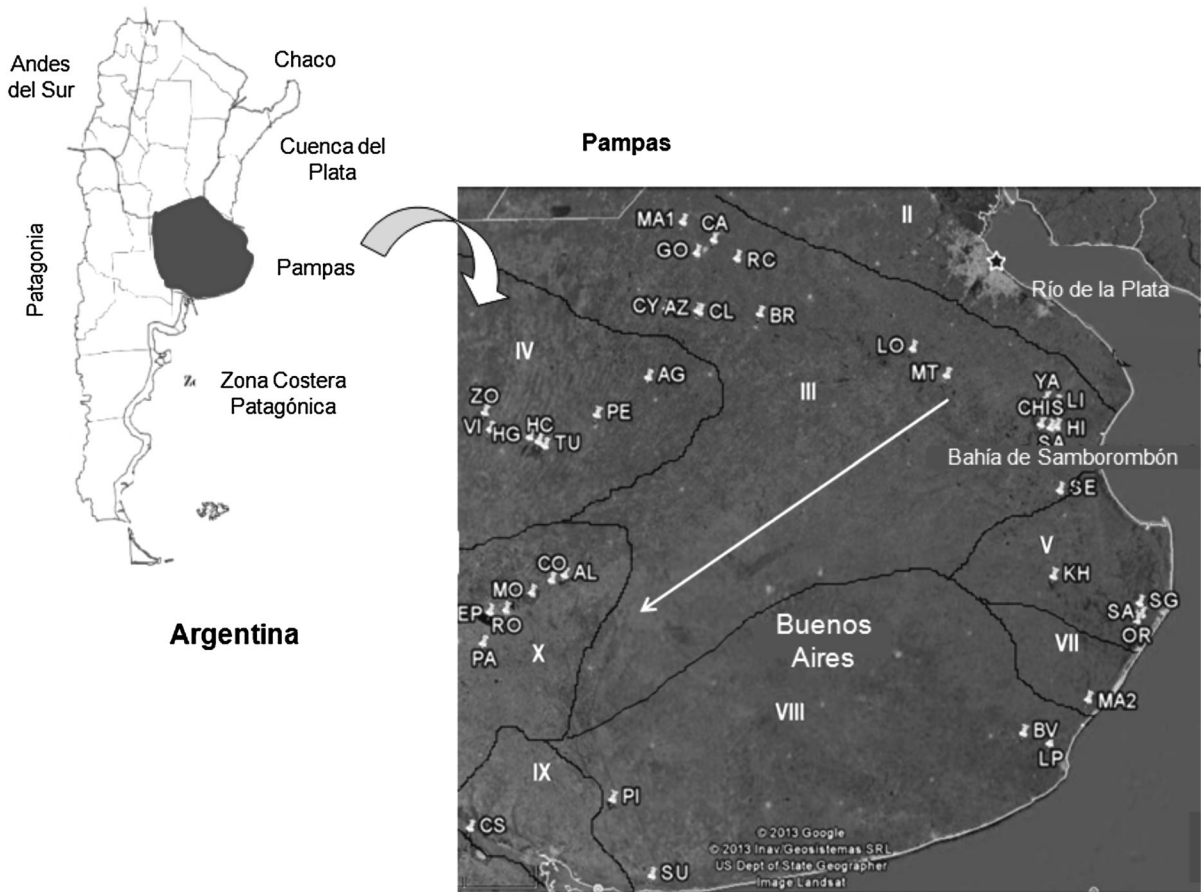
Fig. 1 Map of Argentina with their regions. *Right panel* showed Buenos Aires Province, representative of the Pampa region, with the sampled lakes (see references of lakes in Table 1). *White arrow* indicates a decreasing annual average precipitation gradient from 1000 mm in the NE to 600 mm in the SW. (Watershed Names: II: Noreste, III: Río Salado, IV: Noroeste, V: Atlántica Oriental, VII: Laguna Mar Chiquita, VIII: Atlántica Austral, IX: Bahía Blanca)

Methods

Area of study

Geographically, the Pampa region (Giraut et al., 2007) is an extensive plain of about 800.000 km^2 which covers the central-east of Argentina (33–39°S, 57–66°W) (Fig. 1). The region presents areas with different degrees of land use, ranging from places highly affected by agriculture, intensive livestock and effluent discharges untreated (flat Pampa and high areas of depressed Pampa), and sites with a smaller impact of farming activities (lower parts of depressed Pampa). Our study area encompassed 40 shallow Pampean lakes located between 34–39°S and 64–56°O situated in different hydrological basing of Buenos Aires Province (Fig. 1) (Giraut et al., 2007). The names of the lakes and their respective name codification are showed in Table 1. The region has a temperate climate, with a mean annual temperature and precipitation of about 15.3°C and 935 mm, respectively (Iriondo & Drago, 2004), but with a recurrent alternation of wet and dry periods (Sierra et al., 1994). The lakes were selected in order to cover a great range of morphometrical, physical, and chemical characteristics.

Sampling

Lakes were sampled once during the southern spring–summer (September–March) between 2009 and 2011. Measurements of temperature, pH (Orion pH-meter),

Table 1 Main geographical and morphometric characteristics of the 40 Pampean lakes, sorted by watershed according to Fig. 1

Lakes	Code	Geographic Position	Watershed	Surface area (km²)	Mean Depth (m)
Carpincho	CA	34°35′S; 60°54′W	III	4.4[d]	1.2[d]
Gómez	GO	34° 37′S; 61°07′W		36.6[c]	1.1[c]
Mar Chiquita 1	MA 1	34° 27′S; 61°11′W		60.0[b]	1.5[b]
Bragado	BR	34° 56′S; 60°27′W		3.5[b]	1.3[b]
Azotea	AZ	35° 03′S; 61°01′W		0.02[a]	0.3[e]
Coliqueo	CL	35° 04′S; 61°00′W		0.04[a]	0.7[e]
Chancay	CY	35° 03′S; 61°18′W		1.4[a]	0.3[e]
De Monte	MT	35° 27′S; 58°47′W		6.4[c]	1.4[c]
Lobos	LO	35° 20′S; 59°21′W		7.5[d]	1.2[d]
Rocha	RC	34° 41′S; 60°41′W		3.7[a]	2.5[a]
Yalca	YA	35° 35′S; 57°54' W		10.6e	0.7e
Burro	BU	35° 42′S; 57°55' W		12.0[b]	1.5[b]
Chis-Chis	CHIS	35° 46'S; 57°59' W		33.4[a]	1.5[e]
San Jorge	SJ	35° 40'S; 57°47' W		3.0[a]	1.5[e]
La Limpia	LI	35° 37' S; 57°48'W		0.5[a]	1.5[e]
Sevigné	SE	36° 12′S; 57°44' W		0.7[a]	0.9[e]
Algarrobos	AG	35° 31' S; 61°26' W	IV	3.7[a]	0.5[e]
Pehuajó	PE	35° 47' S; 61°54' W		6.3[a]	2.5[e]
Vidania	VI	35° 53' S; 62°52' W		0.9[a]	0.4[e]
Cuero de Zorro	ZO	35° 46' S; 62°54' W		10.9[a]	2.5[e]
Hinojo Grande	HG	35° 57' S; 62°25' W		100.0[a]	5.0[e]
Hinojo Chico	HC	35° 57' S; 62°31' W		11.0[a]	2.5[e]
Las Tunas del Medio	TU	35° 58' S; 62°21'W		15.0[a]	3.0[e]
Salada Grande	SG	36° 57′S; 57° 06' W	V	50.0[a]	1.8[e]
Saladita	SA	37° 01′S; 56°57' W		0.7[a]	0.4[b]
Los Horcones	OR	37° 03′S; 56°59' W		3.0[a]	1.5[e]
Kakel Huincul	KH	36° 48′S; 57°47' W		29.5[a]	1.8[e]
Mar Chiquita 2	MA 2	37° 40′S; 57°23' W	VII	45.0[a]	1.0[e]
De Los Padres	LP	37° 57′S; 57°44' W	VIII	3.8[a]	2.0[e]
La Brava	BV	37° 53′S; 57°58' W		2.7[a]	4.0[e]
Sauce Grande	SU	38° 56′S; 61°23' W		34.0[a]	1.6[b]
Paso de las Piedras	PI	38° 25′S; 61°44' W		40.0[a]	5.0[b]
Chasicó	CS	38° 37′S; 63°05' W	IX	120.0[a]	10.0[e]
Epecuén	EP	37° 08′S; 62°52′W	X	100.5[a]	4.0[b]
Alsina	AL	36° 51′S; 62°06′W		132.9[b]	1.1[b]
Cochicó	CO	36° 55′S; 62°18′W		66.7[b]	1.9[b]
Del Monte	MO	36° 59′S; 62°28′W		17.5[b]	5.2[b]
Del Venado	VE	37° 01′S; 62°39′W		100.2[b]	3.8[b]
Rolito	RO	37° 07′S; 62°43′W		3.0[a]	0.6[e]
Los Patos	PA	37° 20′S; 62°55′W		1.0[a]	1.0[e]

[a] Calculated with Google earth

[b] According to Wikipedia

[c] According to Quirós (1988)

[d] According to Quirós et al. (1988) and

[e] According to Quirós (2004)

conductivity (Hach conductimeter), dissolved oxygen concentration (DO)(YSI 5000 Meter), and turbidity (Hach turbidimeter 2100 P) were made *in situ*. Subsurface water samples were collected at a central point of the lake and transported to the laboratory in 10 l polypropylene containers for chemical and biological analysis.

Chemical analyses

Lake water was filtered through Whatman GF/F filters. Organic nitrogen determinations (NK) were performed using the semi-micro-Kjeldahl method. Total phosphorus (TP) was determined from the unfiltered water, after an acid digestion with potassium persulfate, following the ascorbic acid method (APHA, 1998). Chlorophyll *a* concentrations (Chl-*a*) were determined spectrophotometrically after an extraction with methanol (Lopretto & Tell, 1995). Total suspended solid (TSS, also referred as seston) concentration was determined by weighing the dried residue (60°C) resulting from the filtration of a water sample through prerinsed and precombusted (530°C, 2 h) GF/F filters. Non-volatile particulate matter (also referred to as ash content) was estimated by reweighing the GF/F filters after combustion at 530°C for 3 h (APHA, 1998). The ash-free dry weight (AFDW) was estimated as the difference between seston and ash content. The trophic status of the water bodies was quantified by applying the Trophic State Index (TSI) developed by Carlson (1977).

Characterization of optical properties

Differences among lakes in water transparency were determined with the intrinsic estimator of light penetration; the vertical diffuse light attenuation coefficient (K_d PAR). Underwater vertical profiles of spectral (380-750 nm) downward irradiance [$E_d(\lambda)$] were performed using a calibrated USB2000 (Ocean Optics) spectroradiometer, which was attached to a fiber optic probe with a CC-3-UV-T cosine corrected diffuser yielding a 180° field of view. The attenuation coefficients were determined as the average values in the euphotic zone, which were derived from the slope of the linear regression of the natural logarithm of E_d (λ) versus depth (from the surface to the 1% light level) (Kirk, 1994a). Broadband K_d PAR was calculated in the same way by integrating E_d (λ) from 400 to

700 nm for each depth. The corresponding Kd for different colors: blue (Kd blue), green (Kd green), and red (Kdred) were calculated at 440, 550, and 675 nm, respectively (Kirk, 1994b). Differences in the spectral composition of the underwater light field among lakes were described as the relative changes of attenuation coefficients at reference wavelengths (i.e., Kd red/Kd green ratios) following Vörös et al. (1998).

Nephelometric turbidity, a common used proxy for light penetration, was measured with a bench-top 2100P turbidimeter (Hach) and calibrated against Formazin liquid standards (Hach).

Biological communities

Picoplankton and HF abundance

Within the picoplankton fraction (Sieburth et al., 1978), we distinguished heterotrophic bacterioplankton (HB), prokaryotic (picocyanobacteria: Pcy), and eukaryotic (picoeukariotic: Peuk) algae. Although heterotrophic picoflagellates (HPF) overlap the upper end of the picoplankton size range, they were processed slightly differently and their abundances were pooled with larger sized heterotrophic nanoflagellates (HNF). HPF and HNF are collectively referred to as HF. Samples for picoplankton and HF analysis were preserved in 45 ml falcon flask with ice-cold filtered glutaraldehyde 10% (final concentration 1%). When concentrations of suspended particulate matter in the lakes were very high, dilutions were performed prior to filtering. For picoplankton enumeration, original sample of about 0.01–0.5 ml was brought to 5 ml with distillate water, stained with 50 μl of DAPI 0.5 mg ml^{-1} (final concentration: 5 μg ml^{-1}) for 10 min (Porter & Feig, 1980), and filtered through a 0.22-μm pore-size black polycarbonate filter (MSI); while for HF quantification, original sample of about 0.05–3 ml was filtered through a 0.8-μm pore-size black polycarbonate filter (MSI).

All filters were mounted onto a microscope slide with a drop of immersion oil for fluorescence (Immersol 518 F) and stored at −20°C. The filters were examined at 1000× magnification using Nikon Eclipse 600 microscope equipped with HBO 50 W lamp, and a filter set for blue light excitation (BP 450–490 nm, FT 500 nm, LP 515 nm), green light excitation (BP 510–560 nm, FT 565 nm, LP 590 nm)

and UV excitation (BP 340–380 nm, FT 400 nm, BP 435–485). Single-cell HB was counted under UV light excitation. Pcy and Peuk were clearly recognizable under blue and green light excitation (Callieri & Pinolini, 1995). On some picoplankton samples, filamentous heterotrophic bacteria (FHB: >4 μm length) and Pcy microaggregates (Pcy-ag: 4–40 cells loosely attached within an inconspicuous mucilage) were recorded separately. On the other hand, HF were counted under blue and UV light excitation and sorted into four size categories: <2, 2–5, 5–10, and >10 μm. To obtain an error less than 20% in the density calculation, a minimum of 25 fields was inspected for picoplankton and 200 for HF.

Ciliates abundance

Unfiltered water samples were preserved in 100 ml PVC flasks with 1% acid Lugol's iodine solution. Although in general ciliates are customarily counted using the Utermöhl method (Jürgens & Jeppeseen, 2000; Küppers & Claps, 2012), the high density of particulate matter present in some lake water made this procedure unfeasible. To circumvent this problem, 6 ml of samples was bleached with thiosulphate and subsequently fixed with 2% formalin, 500 μl of this preparation were further diluted with MilliQ water (final volume: 5 ml), stained with 50 μl of DAPI (0.5 mg ml^{-1}), and gently filtered through a 2-μm pore-size black polycarbonate filter (Macek et al., 2008). Ciliates were enumerated by epifluorescence microscopy under blue and UV light excitation. In general, in more productive lakes, small bacterivorous ciliates prevails (Beaver & Crisman, 1989) and before counting cells, we inspect the entire field with a lower magnification of the microscope looking for large ciliates, in order to minimize underestimation of these ciliates that might be present in low abundance. Then, a minimum of 200 fields was counted at 1000× magnification and ciliates were sorted into three size categories: <10, 10–20, and >20 μm.

Empirical model

The relationship between HF and HB abundances was estimated according to a model developed by Gasol (1994). The model was used to estimate the main mode of regulation (bottom-up or top down) of HF abundances. This author computed a linear equation

that represents the HF maximum achievable abundance for a wide range of HB numbers (MAA, maximum attainable abundance), and proposed that points that lie along the line MAA indicate that all bacterial production is removed by HF, suggesting a bottom-up control exercised by the resource. On the other hand, based on a large data set from marine and freshwater systems he established the MRA (mean realized abundance) line which corresponds to the average of the periods of bottom-up and top–down regulation throughout the year. Points that lie below the line MRA means that HF abundance would be controlled by a top–down control exercised by zooplankton. The model assumes that HB are the only food source for HF and that HF responds in a proportional way to HB abundance regardless bacterial size.

Data analysis

To evaluate the possible relationships between abiotic and biotic parameters, the nonparametric Spearman rank correlation was applied for all data set using the statistical software Sigmaplot 12.0 (Systat inc.). All statistical analysis was accepted as significant at a probability level of $P < 0.05$.

To evaluate the spatial segregation of the lakes considering microbial components, we performed a nonparametric multidimensional scaling (NMDS: non-metric multidimensional scaling) analysis (Clarke & Ainsworth, 1993) with the program PAST 2.0 (Hammer et al., 2001) and it was plotted in 2-dimensional scale. This analysis allows to represent each object as a point in a low-dimensional space (in this case, 2 dimensions) whose management distances virtually reproduced the original multidimensional space. The degree of adjustment between the representation in a low-dimensional space and the original matrix was determined using the Kruskal stress index (formula 1) (Legendre & Legendre, 1998). Stress values vary from 0.2 (poor) to 0.0 (excellent) (Kruskal, 1964). The ordination obtained with the NMDS was used to investigate the relationship between environmental variables and lakes order based on plankton composition. For this purpose, we correlated each environmental variable with the scores of the sites on each of the two axes of arrangement using the nonparametric correlation coefficient of Spearman.

Results

Physical, chemical, and biological parameters

Tables 1 and 2 summarize the main geographical, morphometric, physical, and chemical characteristics of the 40 Pampean lakes studied. In particular, Table 2 shows the average values for all abiotic parameters while the values recorded in each lake are described in the online resource 1. There are some remarkable differences in lake area, water mean depth, physical, and chemical parameters among shallow lakes of Pampa Plain and many of these parameters were of a very big magnitude. Temperatures of water bodies were typical of spring–summer temperatures for this area, while DO values ranged from a minimum of 4.8 mg l^{-1} to an oversaturation maximum value of >20 mg l^{-1} in several lakes. Also, a wide range of conductivity measurements was observed. Likewise all lakes exhibited alkaline conditions, and according to the trophic state index most of them were eutrophic or hypertrophic. Indeed, general values of nutrients were extremely high; meanwhile Chl-*a* presented concentrations ranging between two and three orders of magnitude among lakes. Nephelometric turbidity records also varied widely from 4 to 1662 NTU and, for a better understanding of these results, lake values were sorted in increasing order (Fig. 2a). Lakes present in the various watersheds showed different levels of turbidity. This variable was highly correlated with all the analyzed light attenuation parameters (Kd PAR, blue, green, and red); as well as with seston, AFDW, Chl-*a*, and TP concentrations (Table 3; Fig. 2b). Moreover, studied lakes presented significant differences in the spectral composition of the underwater light field with Kd PAR values that changed 50-fold between lakes (Table 2). When we compare our results with those of Vöros et al. (1998), we observed primarily that our lakes presented higher Chl-*a* concentration than recorded by these authors and, secondly, unlike them, no clear relationship was observed between Kd red/green and Chl-*a* (Fig. 2c).

Abundances of microbial community

In Table 4, the microbial components measured in the 40 Pampean lakes are summarized; whereas in online resource 2, the microbial organisms densities of each lake are described. Most lakes showed very high HB abundances with some waters bodies which presented maximum values greater than 1×10^8 cells ml^{-1} (Fig. 3a, online resource 2). Moreover, the relative contribution of FHB to total bacteria was very small (6.2%). In contrast, PPP numbers were highly variable showing either not detectable values in many lakes or very high in the remaining ones (Fig. 3b, c, online resource 2); regardless the transparency of water bodies. Particularly, all lakes had single-cell Pcy (PC-rich) which varied between 1.1×10^3 and 7.7×10^7 - cells ml^{-1} and Pcy-ag represented an average of 23.7% of the total of Pcy abundances. Regarding Peuk abundances, many lakes presented undetectable values; while the maximum density was greater than 1.1×10^6 cells ml^{-1} (Fig. 3c). In general, bacterioplankton showed a more significant relative importance (>90%) with respect to PPP in almost all lakes analyzed (Fig. 4a). Taking into account only PPP relationships, Pcy dominated over Peuk in an average of 74%. However, there were some lakes (22% of the total) where higher percentages of Peuk (>60%) were registered, although no clear pattern was observed between the Pcy/PPP ratio and increasing Chl-*a* concentrations (Fig. 4b).

Despite high picoplankton densities were recorded in almost all studied lakes, not all of them showed large HF abundances. In fact, a wide range of values, between 150 and 3.9×10^5 cells ml^{-1}, was found (Fig. 5a, online resource 2). The size fraction between 2 and 5 μm was well represented in almost all water bodies with an average of 60.7%, whereas HF < 2 μm, HF 5–10 μm, and HF > 10 μm represented 17.4 , 20.1, and 1.8% of total HF numbers, respectively. Moreover, ciliates showed a random variation between lakes, with abundances ranging from undetectable values to the highest ones of 910 cells ml^{-1}; in which most individuals were >20 μm (Fig. 5b, online resource 2). On average, small (<10 μm), medium size (10–20 μm), and large ciliates (>20 μm) represented 29.1, 50.1, and 20.8% of total ciliates, respectively.

Relationships between communities and abiotic parameters

As shown in Table 3, HB abundances had a strong correlation with all transparency data as well as with optically active substance (OAS: AFDW and Chl-*a* concentrations) and TP concentrations. Regarding its relation to other microbial groups, HB was

Table 2 Average values (AVG), standard deviation (SD), and range (maximum and minimum values) of main physical, chemical, and biological parameters, measured in the 40 Pampean lakes

	AVG	SD	Maximum	Minimum
Temperature (°C)	23.2	4.1	31.0	14.0
pH	9.2	0.3	10.2	8.9
Conductivity (mS cm^{-1})	11.3	16.6	97.6	0.8
DO (mg l^{-1})	10.4	3.0	20.0	4.8
NK (μg l^{-1})	6635	4987	32626	1501
TP (μg l^{-1})	852	498	2220	174
Chl-a (μg l^{-1})	253	322	1550	2
AFDW (mg l^{-1})	107	112	567	9
Secchi Disk (cm)	16.4	17.0	78.0	2.0
Turbidity (NTU)	242.8	330.9	1662.0	4.0
Seston (mg l^{-1})	285.0	303.0	1188.0	16.0
Kd PAR (m^{-1})	18.9	15.9	65.2	1.3
Kd blue (m^{-1})	41.3	39.6	174.8	2.5
Kd green (m^{-1})	16.7	14.9	57.0	1.1
Kd red (m^{-1})	17.4	16.7	72.8	1.3
Kd red/green	1.1	0.3	2.2	0.6

DO dissolved oxygen, *NK* organic nitrogen, *TP* total phosphorous, *Chl-a* chlorophyll a, *AFDW* ash-free dry weight, *Kd* light attenuation coefficient, *PAR* photosynthetically active radiation

positively correlated with PPP (Pcy and Peuk) abundances. To a lesser extent, Pcy abundance was also correlated with all transparency data and OAS; whereas no relationship was found between HB or Pcy abundances and their possible predators (HF or ciliates densities). On the other hand, Peuk abundances did not correlate with abiotic parameters. In this manner, HB and Pcy tended to reach higher values with increasing trophic state; while Peuk abundances did not present any variations as Chl-*a* increased.

Protists did not exhibit significant changes with an increment in the trophic state. Total HF abundances did not show a clear relationship with the turbidity of water body and only a weak correlation with AFDW and seston concentrations was found (Table 3). On the other hand, total ciliate abundance was positively correlated with transparency data as well as with OAS.

For understanding the main mode of regulation of HF in Pampean lakes, we plotted HB and HF abundances in the framework proposed by Gasol (1994). In Fig. 6, we put data points registered in other

hypertrophic shallow lake of Pampa Plain (Chascomús) (Fermani et al., 2013) along with comprehensive Gasol's model dataset (Gasol, 1994) and six other values of eutrophic or hypertrophic lakes collected from the literature. The only values under the MRA correspond to samples from Wieltschnig et al. (2001) and some samples from Sommaruga (1995), Šimek et al. (1997), Nakano et al. (1998), Jürgens & Jeppesen (2000) and the second growing season of Chascomús lake (Fermani et al., 2013). However, as studies of this type of environment have been less frequent, we analyzed 40 Pampean lakes using Gasol's (1994) model. The results showed, firstly that most lakes presented roughly an order of magnitude higher than the upper limit of Gasol's (1994) dataset; and secondly, that most data is located below MRA line. Moreover, the average HB:HF ratio was 4.2×10^4. All these results suggest that a top–down control exerted by metazooplankton is the main mode of regulation of HF in most of Pampean lakes monitored.

Relationship between microbial component abundances and lakes

Figure 7 shows a two-dimensional array of 40 Pampas wetlands based on the microbial community structure of each lake. The Kruskal stress calculated was 0.027 which suggest an acceptable degree of order (Kruskal, 1964). The Spearmann test correlation carried out taking into account the abiotics parameters versus the scores of each axes of NMDS ordination indicated a high and positive correlation of axe 1 with nephelometric turbidity ($r = 0.70$, $P < 0.0001$) as well as with all transparency (Kd PAR: $r = 0.61, P < 0.0001$, Kd blue: $r = 0.71, P < 0.0001$, Kd green: $r = 0.67$, $P < 0.0001$, Kd red: $r = 0.69$, $P < 0.0001$), seston ($r = 0.67, P < 0.0001$), and AFDW values ($r = 0.65$, $P < 0.0001$) and to a lesser extent with Chl-*a* concentration ($r = 0.50$, $P < 0.05$). Besides, it showed a weakly negative correlation with depth mean (Z mean) of water body ($r = -0.36$, $P < 0.05$). On the other hand, axe 2 was negatively correlated with turbidity ($r = -0.35$, $P < 0.0001$) and all transparency data, seston ($r = -0.60$, $P < 0.0001$), AFDW ($r = -0.60$, $P < 0.0001$), and Chl-*a* ($r = -0.45$, $P < 0.05$), whereas it was weakly and positively correlated with area ($r = 0.34$, $P < 0.05$) and Z mean ($r = 0.35$, $P < 0.05$). Consequently, we observed a continuous distribution of lakes according to axes 1 and 2

Fig. 2 a Nephelometric turbidity (NTU) in the 40 Pampean lakes measured during the spring–summer seasons 2009–2011, **b** relationship between nephelometric turbidity and ash-free dry weight (AFDW), seston and Chlorophyll-*a* (Chl-*a*) concentration, and **c** underwater light climate expressed as Kd red/green ratio with increasing Chl-*a* concentration (see references of lakes in Table 1)
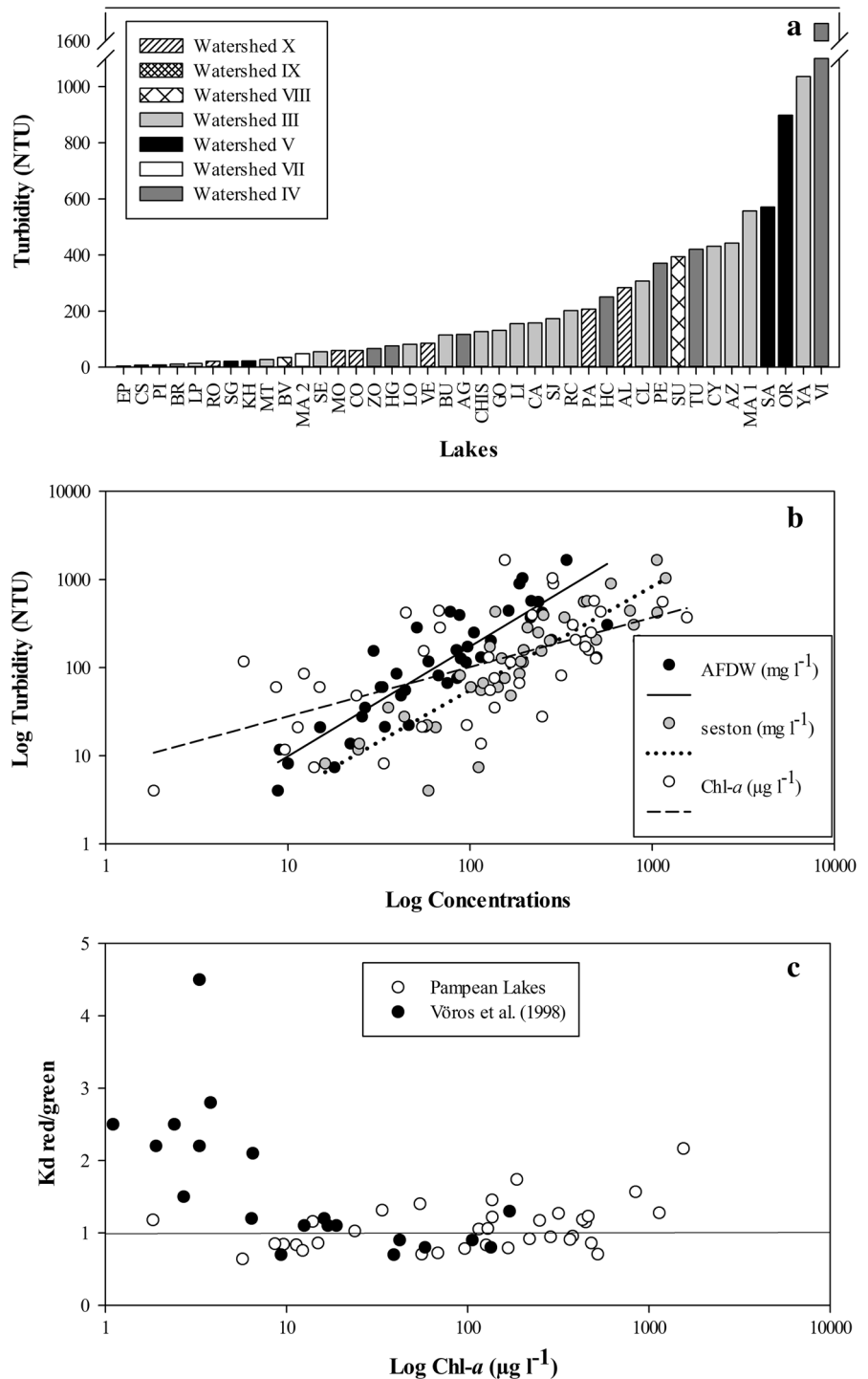
primarily due to the turbidity of water body. Under this ordination system, lakes with similar abundances communities are located close to each other, while the more different lakes have a distant location between them. In the upper left quadrant, the lakes with greater area, Z mean and more transparent water

Table 3 Spearman correlation coefficients between the abundances of different microbial groups and abiotics parameters. Only significant correlations are shown. Significant relationships were defined as: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

	Microbial community												Physics		Transparency						OAS		Nutrients	
	HB	Pcy	Peuk	HF < 2	HF 2–5	HF 5–10	HF > 10	tHF	Cil < 10	Cil 10-20	Cil > 20	t Cil	Cond.	DO	Turb.	Kd PAR	Kd blue	Kd green	Kd red	seston	AFDW	Chl-a	NK	TP
HB	–	0.41*	0.40**	–									–	–	0.79***	0.80***	0.76***	0.80***	0.74***	0.72***	0.71***	0.37 *	–	0.50 *
Pcy		–	–										–	–	0.45**	0.44**	0.48**	0.43*	0.40*	0.46**	0.51***	0.41**	–	–
Peuk			–	–																			–	–
HF < 2				–	0.69***	0.47**	–	0.74***	–											0.34*			–	–
HF 2–5					–	0.65***	–	0.95***	0.42**						0.36*					0.38**	0.47**		–	–
HF 5–10						–		0.78***	0.41**															
HF > 10							–	–	–															
tHF								–	0.40**											0.34*	0.37*	0.37*	–	–
Cil < 10									–	0.51***	0.75***	0.49**									0.33*			
Cil 10-20										–	0.88***	0.68**									0.32*		–	–
Cil > 20											–	0.76***												
t Cil												–				0.43**	0.50**	0.38*	0.46**		0.40*	0.43**	–	–
Cond.													–	–	–									0.33*
DO														–	–	0.37*	0.42*	0.35*	0.43*			0.54*	–	–
Turb.															–	0.97***	0.94***	0.96***	0.94***	0.88***	0.87***	0.61***		0.50**
Kd PAR																–	0.97***	0.99***	0.97***	0.80***	0.84***	0.73***		0.53**
Kd blue																	–	0.96 ***	0.95 ***	0.84 ***	0.87 ***	0.79 ***		0.55***
Kd green																		–	0.96 ***	0.80 ***	0.82 ***	0.67 ***		0.55 ***
Kd red																			–	0.76 ***	0.87 ***	0.81***		0.49 **
Seston																				–	0.87***	0.39**		0.54***
AFDW																					–	0.67***		0.42**
Chl-a																						–	–	–
NK																							–	–
TP																								–

OAS optically active substance, *HB* heterotrophic bacteria, *Pcy* picocyanobacteria, *Peuk* picoeukaryote, *HF* heterotrophic flagellate, *HF < 2* heterotrophic flagellates < 2 μm, *HF 2–5*: heterotrophic flagellates 2–5 μm, *HF 5–10* heterotrophic flagellates 5–10 μm, *HF > 10* heterotrophic flagellates > 10 μm, *tHF* total heterotrophic flagellates, *Cil < 10* ciliates < 10 μm, *Cil 10–20* ciliates 10–20 μm, *Cil > 20* ciliates > 20 μm, *t Cil* total ciliates, *Cond.* conductivity, *DO* dissolved oxygen, *Turb.* turbidity, *Kd* light attenuation coefficient, *PAR* photosynthetically active radiation, *AFDW* ash-free dry weight, *Chl-a* chlorophyll a, *NK* organic nitrogen, *TP* total phosphorous

Table 4 Average values (AVG), standard deviation (SD), and range (maximum and minimum values) of microbial communities registered in the 40 Pampean lakes

	AVG	SD	Maximum	Minimum
HB (cells ml^{-1})	6.4×10^7	8.5×10^7	4.2×10^8	1.4×10^6
FHB (fil ml^{-1})	3.9×10^6	1.2×10^7	6.1×10^7	2.2×10^4
Pcy (cells ml^{-1})	6.5×10^6	1.5×10^7	7.7×10^7	0.0
Pcy-ag (col ml^{-1})	1.2×10^6	1.7×10^6	6.8×10^6	2.2×10^4
Peuk (cells ml^{-1})	1.6×10^5	2.7×10^6	1.1×10^6	0.0
HF < 2 μm (cells ml^{-1})	10120	53482	339124	0
HF 2–5 μm (cells ml^{-1})	7393	12084	47919	18
HF 5–10 μm (cells ml^{-1})	1192	1616	7962	0
HF > 10 μm (cells ml^{-1})	69	175	737	0
t HF (cells ml^{-1})	18775	61566	388887	147
Ciliates < 10 μm (cells ml^{-1})	16	35	150	0
Ciliates 10–20 μm (cells ml^{-1})	25	46	255	0
Ciliates > 10 μm (cells ml^{-1})	30	133	835	0
t Ciliates (cells ml^{-1})	71	163	910	0

HB heterotrophic bacteria, *FHB* filamentous heterotrophic bacteria, *Pcy* picocyanobacteria, *Pcy-ag* picocyanobacteria aggregate, *HF* heterotrophic flagellates, *tHF* total HF, *t Ciliates* total ciliates

are predominated, whereas in the lower right quadrant, those lakes with higher turbidity records are grouped.

Discussion

### Characteristic of Pampean lakes

Surface waters generally reflect the type and intensity of human activities carried out in their drains. The effects of agriculture and livestock could be reflected in the levels of phosphorus and nitrogen of most Pampean lakes. Therefore, nutrient concentrations depend on both the basin to which each water body belongs and the land use in the surrounding area (Quirós et al., 2002, 2006; Carpenter et al., 2011).

According to TSI index, most Pampean lakes are eutrophic and hypertrophic. This index was developed to quantify the trophic status of most lakes through Europe and United States. Quirós et al. (2006) proposed new indexes to assess Pampa wetlands in which water bodies were classified according to total area, and large clear lakes could be classified as meso-eutrophic. In this work, however, we observed values of TP and Chl-*a* which are much higher than those previously reported by these authors. Although we have registered significant correlations between lake distributions and both area and mean depth they were very weak, which suggest that all lakes have a high trophic state, regardless of their sizes. Previously,

Quirós et al. (2002) had suggested the possibility that Pampean lakes were distributed along an environmental gradient from clear lakes dominated by macrophytes to phytoplankton-dominated hypertrophic and turbid ones. Our analysis by NMDS (Fig. 7) provides no evidence on which to discriminate the boundary between clear and turbid lakes. Moreover, our results show that lakes are aligned along a continuous gradient. In a study of South American lakes, Kosten et al. (2012) observed that the distribution of frequency of lakes based on either Chl-*a* concentration or percentages of lakes covered by macrophytes could fit into a bimodal distribution, suggesting the occurrence of alternative states, although it was not sufficient to proof the theory. In spite of this, the distribution of frequencies of water transparency observed by these authors did not confirm this pattern. Our results support the latter, since we do not observe a segregation of water bodies in groups, existing a continuous distribution along water turbidities.

There is a controversy over the use of the theory of alternative states in different climate regions (temperate vs subtropical shallow lakes) (Meerhoff et al., 2007a, b). Firstly, how effective might be the aquatic vegetation as a refuge depends on the composition and plant architecture (submerged, floating and emerged plant) (Meerhoff et al., 2003); as well as on the density of patches and predators that these plants host (Jeppesen et al., 1998). The theory was developed in

Fig. 3 a Abundances of single-cells heterotrophic bacteria (HB) and filamentous heterotrophic bacteria (FHB), **b** picocyanobacteria (Pcy) and picocyanobacteria aggregate (Pcy-ag), and **c** picoeukaryote (Peuk); in the 40 Pampean lakes measured during the spring–summer seasons 2009–2011 and ordered by increasing nephelometric turbidity (see references of lakes in Table 1)
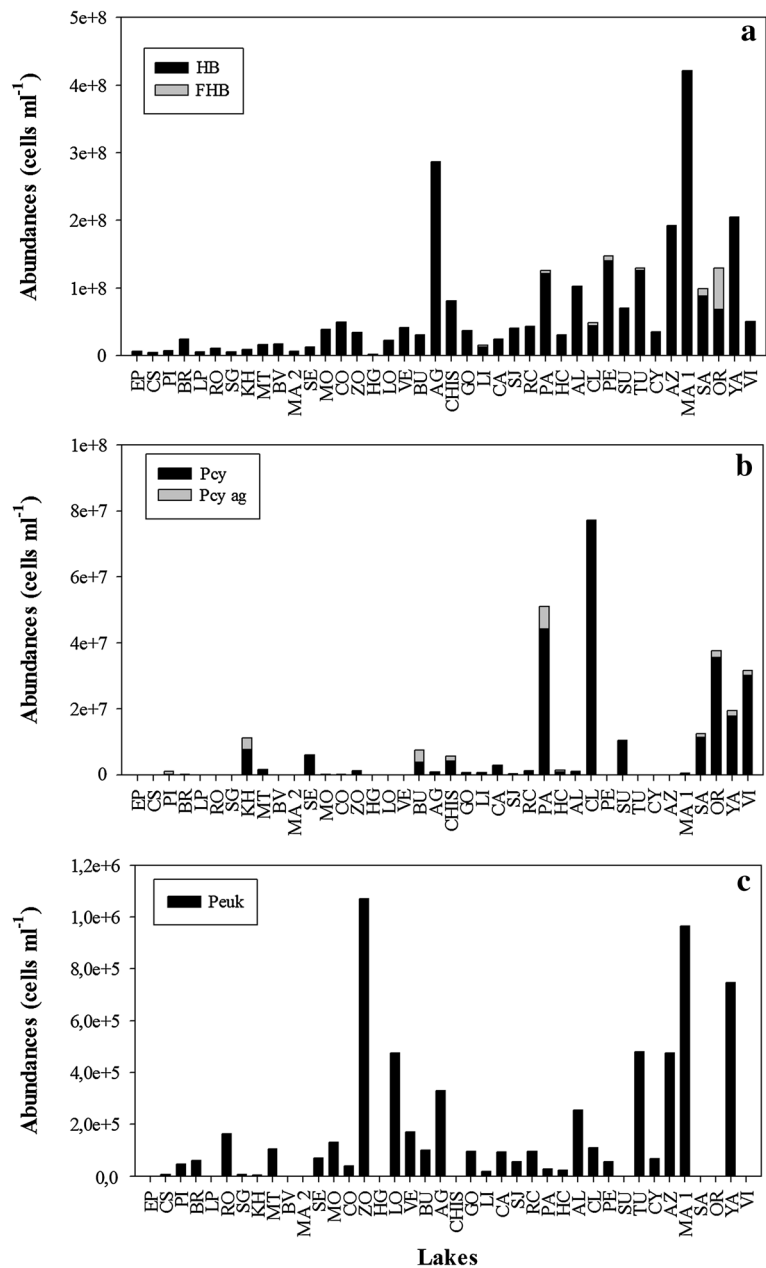
the pelagic zones without taking into consideration the litoral areas in which macrophytes are abundant. In the temperate European lakes, aquatic vegetation serves as a shelter for zooplankton against fish predation. However, in the South American lakes a different pattern of zooplankton migration has been observed and, in these systems, submerged vegetation hosts many fish predators as well as macroinvertebrates (Meerhoof et al., 2006). The latter are effective predators of cladocerans and copepods (Meerhoff et al., 2007a, b; González Sagrario et al., 2009). As a result, trophic chain could be affected allowing for the development of phytoplankton and, consequently, increasing the turbidity of water bodies. Secondly, in shallow lakes of Pampa plain there are much higher nutrients levels than those formerly used to develop

Fig. 4 **a** Relative abundance of heterotrophic bacteria (HB), picocyanobacteria (Pcy), and picoeukaryotes (Peuk) in the 40 Pampean lakes measured during the spring–summer seasons 2009–2011 and ordered by increasing nephelometric turbidity (see references of lakes in Table 1), **b** relative contribution of Pcy to total picophytoplankton (PPP) abundances with increasing tropic state
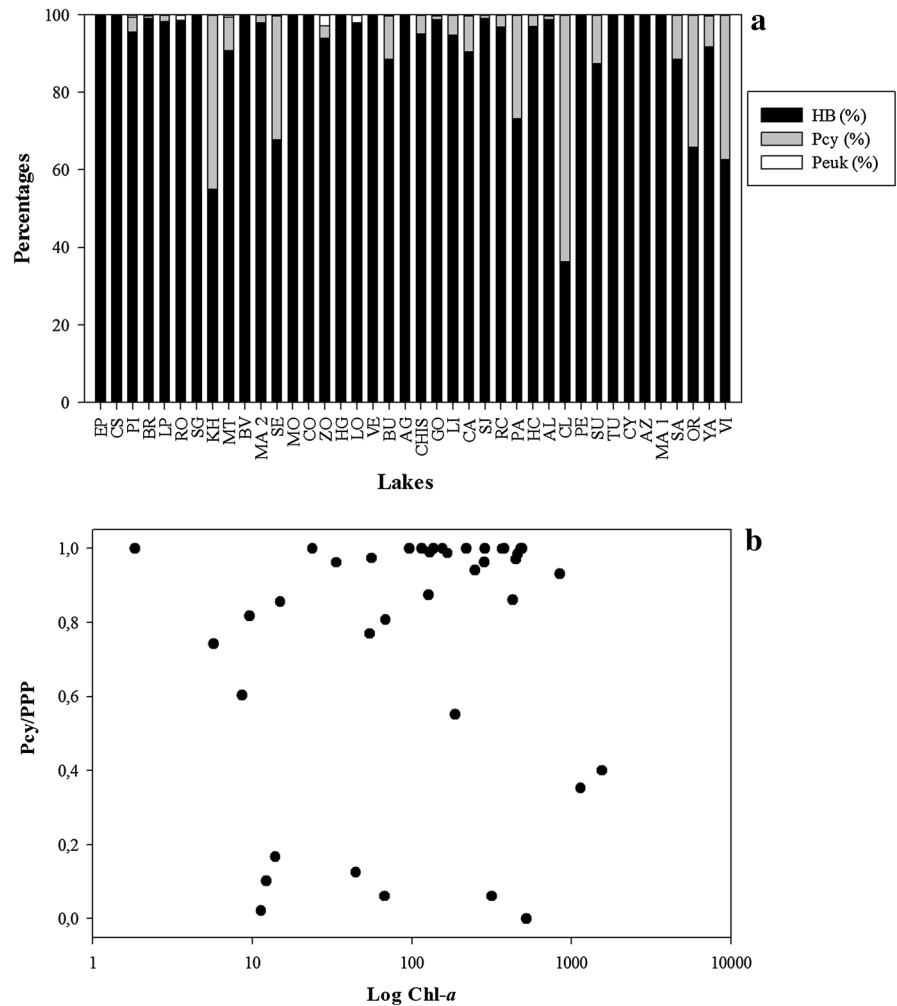
the theory (25–100 μg TP l^{-1}) (Jeppesen et al., 1990). Besides, lake size, depth, and climate are key factors affecting the nutrient levels (Scheffer & van Ness, 2007). Most of shallow Pampean lakes cover a small area (Kosten et al., 2012) and are connected to rivers, thereby receiving water from direct precipitations, groundwaters, and streams (Soriano, 1992). During dry periods, turbidity increases (Quirós et al., 2002). In addition, there are some differences in the structure of food chain between regions (Iglesias, 2010). While the temperate lakes of Europe are dominated by great daphnids, in the southern lakes, daphnids are scarce and rotifers are usually dominant (Sommaruga, 1995; Fermani et al., 2013). These differences in composition impact differentially in the structure of the microbial food web (Jürgens &Matz, 2002; Sommer

& Sommer, 2006; Fermani et al., 2013). As a whole, these features cause nutrient concentrations and turbidity values to be higher than those expected for temperate lakes and rendering not possible to separate them in two alternative states. Additionally, Pampean lakes exhibit highly variable salinity, depending on the drought-flood cycles characteristic of the region (Quirós, 2005); and most of them could be classified like oligo to mesohaline (Ringuelet, 1962). Despite the conductivity values recorded in this study were similar to those recorder in soda lakes (Zinabu & Taylor, 1997; Zinabu et al., 2002), Pampean shallow lakes cannot be considered within this category, due to the presence of not only sodium but also a high concentration of calcium (Fernández-Cirelli & Miretzky, 2004; Quirós, 2005).

Fig. 5 a Abundances of total heterotrophic flagellates (HF) and its different size fractions (HF < 2 μm, HF 2-5 μm, HF 5–10 μm, HF > 10 μm) and **b** abundances of total ciliates together with its different size fractions (ciliates < 10 μm, ciliates 10–20 μm, ciliates > 10 μm); in the 40 Pampean lakes measured during the spring–summer seasons 2009–2011 and ordered by increasing nephelometric turbidity (see references of lakes in Table 1)
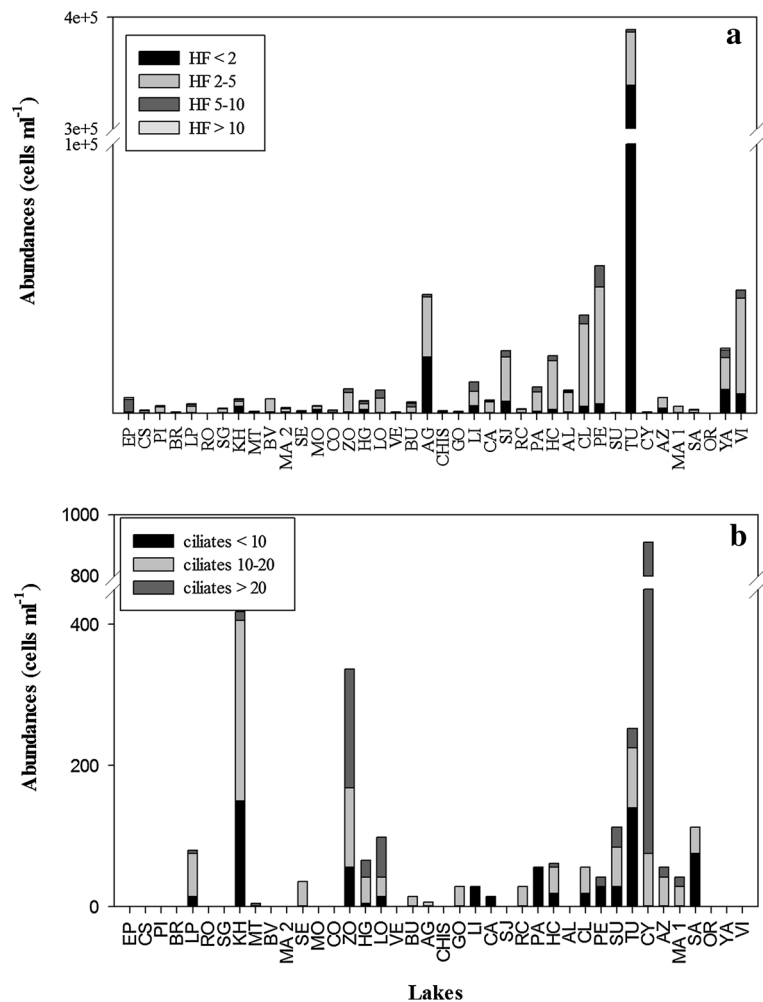
Regarding to above mentioned, in Pampean lakes, we can distinguish water bodies with presence of vegetation, lakes without vegetation, and lakes with salinities highly variable. Therefore, we found lakes with transparent water not only in the Pampa Deprimida region as Quirós et al. (2002) described previously but also throughout the Pampa region depending on particular characteristics of each lake.

Microorganisms in Pampean lakes

Data of microorganisms in hypertrophic lakes have been comparatively less reported (Sarmento, 2012). Regression models used by Bird & Kalff (1984) and Cole et al. (1988) in temperate systems registering positive correlations between HB abundance and Chl-*a* concentrations in a wide range of environments;

although did not take into account environments with Chl-*a* concentrations greater than 200 μg l^{-1}. On the other hand, Sommaruga (1995) suggests that in highly trophic state HB levels decrease with extremely high Chl-*a*. In a more recent study carried out in more than 1000 subtropical lakes, Roland et al. (2010) reported high concentration of Chl-*a* (reaching 437 μg l^{-1} in costal lakes) and a weak but significant positive relationship between Chl-*a* and bacterioplankton. Additionally, stronger relationship was observed when they analyzed each particular system.

In this work, according to Roland et al. (2010), Chl-*a* concentrations were very high and were correlated with HB abundances. Zinabu & Taylor (1997) showed similar values and relationships of these parameters in Ethiopian soda lakes. In particular, these authors demonstrated that the relationship between bacterial
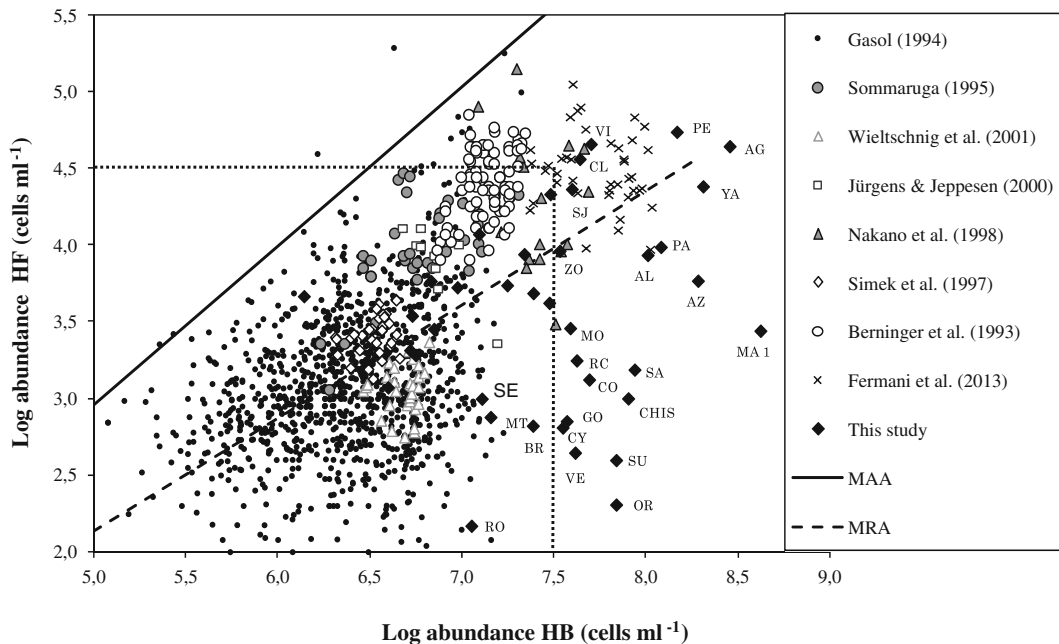
Fig. 6 Logarithms of total bacterial (HB) and heterotrophic flagellates (HF) abundances of the 40 Pampean lakes studied in this work, together with the data set from Gasol (1994) and other simultaneous estimations of HB and HF abundances of different eutrophic to hypertrophic waters bodies collected from the literature. Data of eutrophic and hypertrophic shallow lakes were taken from tables or read from graphs with an image analyzer. A total of six articles were found to be included in this plot. The *upper boundary line* is the empirically derived maximum attainable abundance (MAA) for HF, and the dashed line is the mean realized abundance (MRA), according to Gasol (1994) (*dotted lines* forming a *box* indicate the limits of the model; see references of lakes in Table 1)

abundance and chlorophyll is different in saline and freshwater lakes; and they attributed this difference to a weaker grazer control in soda lakes, leaving bacterial growing with increasing phytoplankton biomass. In this sense, in many Pampeans lakes we registered HB abundances higher than 1×10^8 cells ml^{-1} which are greater than those reported in most hypertrophic lakes worldwide (Sommaruga, 1995; Tijdens et al., 2008; Chen et al., 2010) and similar to those corresponding to a Brazilian reservoir by Roland et al. (2010) and many soda lakes (Kilham, 1981; Zinabu & Taylor, 1997). Therefore, we observed that in these highly eutrophic environments the HB abundances follow the same pattern observed by Cole et al. (1988) for environments with Chl-*a* < 200 µg l^{-1}. Our results also agree with data reported by several authors who suggest that abundances of microbial components increase with the trophic status (Beaver & Crismann, 1989; Berninger et al., 1991). In addition, the strong correlation between HB and TP along with all transparency data and the lack of a relationship with

theirs predators (HF and ciliates) suggest that the bacterioplankton of most lakes might be regulated by resource availability.

Abundances of photosynthetic picoplankton could be highly variable (Sommaruga & Robarts, 1997). In our work, we observe intermediate picophototrophs abundances in the most transparent lakes, while very elevated or undetectable values were registered in the most turbid ones. However, as phytoplankton biomass increases an extremely high abundance of picophytoplankton was registered. Our results are similar to those observed by Vöros et al. (1998) who analyzed 32 water bodies in a trophic gradient, and to those observed in smaller group of Pampean lakes (Allende et al., 2009; Silvoso et al., 2011). We found a positive correlation between Pcy and trophic status as well as with optical properties. Several studies have shown that underwater light quality is a key factor determining the distribution patterns of Pcy types on natural waters (Vöros et al., 1998; Stomp et al., 2007). Changes in the underwater light climate have been
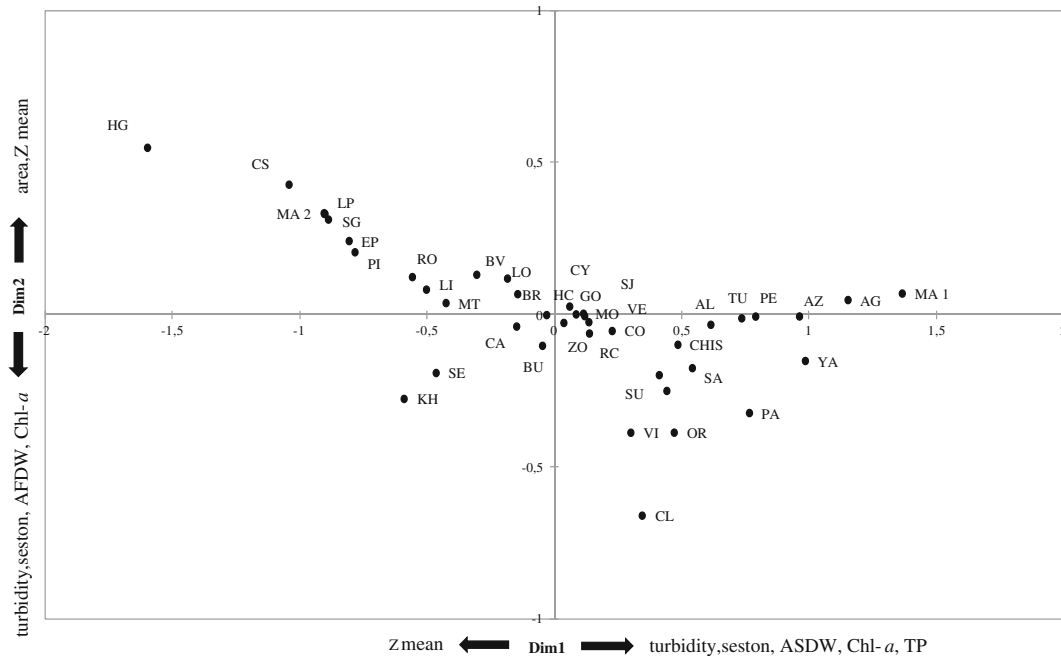
Fig. 7 Arrangement by NMDS of 40 Pampean lakes based on the microbial abundances (see references of lakes in Table 1, *Z* depth, *NK* organic nitrogen, *TP* total phosphorus, *ASDW* ash-free dry weight, *Chl-a* chlorophyll-*a, Dim 1* axis 1, *Dim 2* axis 2). *Arrows* indicate the direction of correlation of physical and chemical variables

related with different trophic states of aquatic environments. Vöros et al. (1998) showed that in lakes with low chlorophyll concentration (<10 μg l^{-1}) the ratio Kd red/green was higher than 1 and green light dominate the euphotic zone, whereas red light prevails in more eutrophic lakes. These authors found an increase in the PE-rich cells percentage of total Pcy with the increment of Kd red/green ratio. Differences in Pcy accessory pigmentation and therefore distinctive selective absorption bands allow better alternative exploitation of green or red light with direct implications on their growth rates. The result of this is a gradual transition from predominance of PE-rich cells in clear waters to PC-rich cells in eutrophic ones. Interestingly, our findings do not show a clear pattern between Kd red/green ratio and Chl-*a* concentration. Most Kd red/green values were around 1, while higher values were observed at extremely high Chl-*a* concentrations (ca. 1000 μg l^{-1}). The explanation for these results is related to the optical characteristics of some Pampean lakes. In highly turbid lakes with high phytoplanktonic biomass, light attenuation coefficients were determined mainly by the phytoplankton light absorption and scattering (Pérez et al., 2011,

2013). In these lakes, red light is more rapidly attenuated than green light due to the second absorption band of Chl-*a* and therefore green light penetrates deeper. In this sense, the observed dominance of PC-rich cells in several Pampean lakes by Allende et al. (2009), and in this work, could not be explained only by the spectral composition of the underwater light field. In turn, other factors such as low-light adaptation and predation could also shape niches of Pcy types. On the other hand, in this study, Peuk abundances were also highly variable but unlike those reported in other temperate and eutrophic shallow lakes (SØndergaard, 1991; Mózes et al., 2006; Callieri, 2007), we observe that the relationship with Pcy abundances in general is low and in most water bodies Pcy prevails over Peuk. Only, 9 out of 40 lakes presented Peuk abundance greater than 60% of PPP; and in one lake (Cy) Peuk accouted for 100% of PPP. Factors that regulate the number and distribution of Peuk are different than those controlling Pcy. The availability of nutrients and light intensity in the water column may favor the Peuk development (Callieri & Stockner, 2002), but the long-term behavior is difficult to predict since Peuk easily adapt to local conditions (Callieri, 2007).

According to Silvoso et al. (2011), we observe a higher relative importance of Pcy with respect to Peuk in almost all lakes studied. All these results also are comparable with others highly eutrophic turbid soda lakes of Central Europe (Felföldi et al., 2009) who found PPP abundances extremely high and the contribution of Peuk to total PPP ranged from 0 to 100%.

In different pelagic environments, HF abundances vary between 20 and more than 2×10^4 cells ml^{-1} (Boenigk & Arndt, 2002). However, in this study this variation was observed only in the eutrophic-hypertrophic range, and we recorded the highest abundances ever reported in the literature (Hirose et al., 2003; Chen et al., 2010; Fermani et al., 2013). Several studies showed that HF abundances increased along with trophic gradient (Auer & Arndt, 2001; Auer et al., 2004) and that hypertrophic water bodies were dominated by HF > 10 μm. In contrast to this observations, the most HF in Pampean lakes were smaller than 5 μm, which are one of the main bacterivores in aquatic ecosystems (Sherr & Sherr, 2002). However, no correlation was found between HB and HF; neither with others indicators of thophic state, indicating that other factors, like predation, could be responsible for the control of HF abundances. On the other hand, ciliates numbers were also difficult to predict, since the values recorded varied widely among lakes without any clear pattern. Many of these lakes have even higher values than those reported in previous studies for eutrophic systems (Sommaruga, 1995; Wichelen et al., 2013); and about half of them are <30 μm, as expected for this type of lakes (Beaver & Crisman, 1989). Some authors suggest that in eutrophic systems ciliates are usually more affected due to predation by metazooplankton rather than food availability (Jürgens et al., 1999). Despite in our study, we found significant correlation between ciliates abundance and light availability, AFDW and Chl-*a* concentrations, they were very weak and it is possibly that ciliates numbers also were regulated by predation.

Relationship between HB and HF

Several authors have suggested that the top–down control on the HF abundance should prevail when the trophic status of the water body increases (Gasol, 1994; Gasol et al., 2002; Pernthaler, 2005). The available evidence in previous studies (see Fig. 6), however, is not entirely consistent with this prediction.

Nevertheless, since studies in this type of environment were less frequent we analyzed 40 Pampean lakes using Gasol's (1994) model. The low number of HF obtained in relation to the amount of HB was probably due to a top–down effect on HF by metazooplankton, which is an important factor that influences the structure of the microbial food web (Jürgens & Jeppesen, 2000). In this sense, experiments of grazing by Burian et al. (2013) support the hypothesis of Zinabu & Taylor (1997), who proposed that in soda lakes HF abundances would be preyed upon by zooplankton.

For a better understanding, the results we estimated the Bacterial Production (BP) using the empirical model proposed by White et al. (1991) for freshwater systems (Eq. 5) and the Bacterial grazing by HF (GR) following Vaqué et al. (1994, Eq. 1). Our results showed that in average PB (852 ± 1200 μgC l^{-1} day^{-1}) is greater than G_{HF} (330 ± 1030 μg C l^{-1} day^{-1}), and most lakes presented a PB/GR $\gg 1$, probably suggesting that not the entire PB is consumed by HF. Most waters bodies in our study were located below the MRA line in Gasol's model, which suggest that a top–down effect could regulate HF population. On the other hand, only 20% of total lakes presented PB/GR ≤ 1 which were plotted above MRA line. Furthermore, it is well established that HF predation may induce the development of resistance morphologies on picoplankton, such as filaments and aggregates (Jürgens & Matz, 2002; Callieri, 2010). However, there was no obvious relationship between HF and FHB and Pcy-ag, indicating a weak coupling degree between those microbial components. All these evidences could lead to the conclusion that in most Pampean lakes, a weak coupling between HF and picoplankton could be due to a top–down effect on the HF exerted by metazooplankton.

Conclusions

In general, most of picoplankton community increased with trophic state; while no clear relationship was found between heterotrophic protist and Chl-*a* concentrations. In particular, several lakes showed HB and HF values which exceed the limits of the original Gasol (1994) model and they are comparable, or even higher, than the highest records reported in the literature. Our data also allows to describe a set of

lakes with high bacterial and low HF abundances. Comparison of HB and HF with the framework of Gasol's model suggested that these two components are generally not coupled in Pampean lakes, possibly due to high metazooplankton abundances. Additionally, the most saline water bodies seem to behave as soda lakes. From the point of view of the theory of alternative states, we do not accumulate enough evidence to segregate Pampean shallow lakes into two discrete groups based on microbial communities abundance. In other words, we found that Pampean lakes are distributed according to a turbidity gradient that does not explain the microbial abundance pattern observed in the studied systems.

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