Alternative states drive the patterns in the bacterioplankton composition in shallow Pampean lakes (Argentina)

Maria E. Llames,[1]* Paul A. del Giorgio,[2]
Horacio Zagarese,[1] Marcela Ferraro[1] and
Irina Izaguirre[3]

[1]Laboratorio de Ecología y Fotobiología Acuática, Instituto de Investigaciones Biotecnológicas, Instituto Tecnológico Chascomús (IIB-INTECH), CONICET, UNSAM, Camino de Circunvalación Laguna Km 8, Chascomús, pcia. de Buenos Aires, Argentina, CP (B 7130 IWA).
[2]Groupe de Recherche Interuniversitaire en Limnologie (GRIL), Département des sciences biologiques, Université du Québec à Montrèal, Montreal, Quebec, Canada.
[3]Departamento de Ecología, Genética y Evolución, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, CONICET, Buenos Aires, Argentina.

Summary

We assessed the influence of environmental factors in shaping the free-living bacterial community structure in a set of shallow lakes characterized by contrasting stable state patterns (clear-vegetated, inorganic-turbid and phytoplankton-turbid). Six temperate shallow lakes from the Pampa Plain (Argentina) were sampled over an annual cycle, and two fingerprinting techniques were applied: a 16S rDNA analysis was performed using denaturing gradient gel electrophoresis (DGGE) profiles, and a 16S–23S internally transcribed spacer region analysis was conducted by means of automated ribosomal intergenic spacer analysis (ARISA) profiles. Our results show that the steady state that characterized the different shallow lakes played a major role in structuring the community: the composition of free-living bacteria differed significantly between clear-vegetated, inorganic-turbid and phytoplankton-turbid shallow lakes. The state of the system was more important in determining these patterns than seasonality, geographical location or degree of hydrological connectivity. Moreover, this strong environmental control was particularly evident in the pattern observed in one of the lakes, which shifted from a clear to a turbid state over the course of the study. This lake showed a directional selection of species from a typical clear-like to a turbid-like community. The combined DGGE/ARISA approach revealed not only broad patterns among different alternative steady states, but also more subtle differences within different regimes.

Introduction

The early work by Scheffer and colleagues (1993) showed that shallow lakes could alternate between two distinctive regimes: a clear one, characterized by high water transparency, low phytoplankton biomass and the presence of macrophytes, and a turbid state, characterized by low transparency, often associated with phytoplankton blooms and the absence of rooted vegetation. The realization that ecosystems may shift back and forth between contrasting stable states (Beisner *et al.*, 2003) has, in turn, led to the development of concepts such as tipping points and environmental thresholds (Scheffer *et al.*, 2009; Briske *et al.*, 2010). Several other ecosystems have been reported to undergo sudden changes of state (e.g. Scheffer *et al.*, 2001; Aronson *et al.*, 2004; Jasinski and Payette, 2005; Baskett and Salomon, 2010), but it is probably fair to say that shallow lakes still stand as the most paradigmatic examples of ecosystems exhibiting regime shifts.

Regime shifts in shallow lakes are particularly apparent at landscape scales (i.e. presence or absence of macrophytes) and result in remarkable changes in phytoplankton community (Jeppesen *et al.*, 1997; Scheffer, 1998). Although most ecosystem components are likely to be involved or affected by lake state, many of them are still understudied. For example, bacterial communities are known to be highly responsive to changes in environmental conditions and resources (Ducklow, 2008; Poretsky *et al.*, 2010; Comte and del Giorgio, 2011), but only a handful studies have investigated the relationship between bacteria community structure and lake state. Studies carried out in freshwater shallow lakes in Belgium, differing in nutrient load and food-web structure, concluded that differences in bacterial community composition (BCC) among lakes were partly related to the

equilibrium state of the lake (Van der Gucht *et al.*, 2001; Muylaert *et al.*, 2002), and suggested that differential control mechanisms on BCC may operate in clear-water and phytoplankton-dominated systems. Haukka and colleagues (2006), on the other hand, used a field mesocosm experiment to show that BCC changes dramatically when the system shifts from a clear to a turbid state. Likewise, studies in tropical and subtropical shallow aquatic systems investigated intra-habitat differences in BCC between sites of the same lake representing alternating states and have suggested links between the alternative regime and bacterial community structure (Wu *et al.*, 2007; Ng *et al.*, 2010). Collectively, these studies suggest fundamental differences in BCC between turbid (algal-dominated) and clear (macrophyte-dominated) states.

The Pampa Plain of Argentina contains several thousands shallow lakes (Quirós and Drago, 1999), many of which have undergone progressive eutrophication because of increasing loads of nutrients from anthropogenic activities, such as cattle breeding, agriculture and urban discharges (Quirós *et al.*, 2002; 2006). Presently, the area comprises a mosaic of shallow lakes interconnected by fluvial networks, some of which are stabilized in either a turbid or a clear state, while others recurrently shift between different regimes (Quirós *et al.*, 2002; Allende *et al.*, 2009).

The work we present here builds upon previous limnological work conducted in this region (Quirós *et al.*, 2002; Allende *et al.*, 2009; Silvoso *et al.*, 2011; Izaguirre *et al.*, 2012) which identified three main types of shallow lakes: clear lakes with submerged as well as emergent macrophytes (clear-vegetated), turbid systems with high phytoplankton biomass (phytoplankton-turbid) and turbid lakes with elevated concentration of colloidal clays (inorganic-turbid). Clear-vegetated and phytoplankton-turbid lakes represent the two basic steady states described in the model proposed by Scheffer and colleagues (1993), while the third type (inorganic-turbid) results from human-induced changes in their drainage basins and subsequent sediment loadings (Quirós *et al.*, 2002).

The bacterial community of these highly interconnected systems may be assumed to belong to a regional metacommunity, which is expressed differently under the various regimes. Collectively, shallow Pampean lakes offer a unique opportunity to investigate microbial patterns across steady states. In this paper we explore the structure of the free-living bacterioplankton communities over an annual cycle of six selected shallow lakes from the Pampa Plain that are representative of the different regimes that can be recognized in this region. The sites selected comprised two clear-vegetated lakes, two phytoplankton-turbid systems, one inorganic-turbid and one that alternates periodically between clear and turbid

states. We assessed the patterns in BCC using an approach that combined two DNA fingerprinting methods that differ in the level of operational taxonomic units (OTUs) coverage: denaturing gradient gel electrophoresis (DGGE) profiles of 16S rDNA (Muyzer *et al.*, 1993; 1998) as a conservative estimate of the actual genetic diversity of the dominant components of the community, and automated ribosomal intergenic spacer analysis (ARISA) profiles (Fisher and Triplett, 1999), a method based on the size variability of the less conservative 16S–23S intergenic transcribed spacer region.

Results and discussion

The studied lakes are located in Buenos Aires province (Argentina), in the warm temperate South American lowland region known as the Pampa Plain (35°32′–36°48′S; 57°47′–58°07′W). These wind-excavated lakes are typically polymictic and are located in basins characterized by nutrient-rich soils. Their trophic state varies from meso-eutrophic to hypereutrophic (Quirós and Drago, 1999; Quirós *et al.*, 2002; Quirós, 2005). Two of these lakes [Kakel Huincul (KH) and El Triunfo (TRI)] are in a clear-vegetated state, colonized by submersed plants (*Myriophyllum* sp. and *Ceratophyllum demersum*) and emergent macrophytes (*Schoenoplectus californicus*); two of these systems are in a turbid state [namely Chascomús (CH) and San Jorge (SJ) lakes] and are characterized by low Secchi depth values and high phytoplankton biomass (Allende *et al.*, 2009). One lake [Lacombe (LAC)] alternates between phytoplankton-turbid and the clear-vegetated states (Cano, 2008; Casco *et al.*, 2009). The sixth lake [La Limpia (LI)] corresponds to the third type described by Quirós and colleagues (2002) for this region. This system is also turbid, but turbidity is mainly due to suspended inorganic matter (colloidal clays); the system lacks vegetation and phytoplankton biomass is low (Allende *et al.*, 2009). All these lakes have a mean depth lower than 2 m. This study was conducted over a period of 13 months, from March 2008 to April 2009 and sampling was performed every 2 months from each lake.

Table 1 summarizes mean values ($\pm$ SD) of the main physical and chemical variables for each lake. Dissolved oxygen was generally close to saturation levels and no anoxic conditions were detected in the water column of these lakes. All lakes exhibited high pH, while conductivity and alkalinity varied within a range that is typical for the region. Clear-vegetated lakes TRI and KH were characterized by the lowest values of turbidity, seston concentration, chlorophyll-*a* and total phosphorous concentration. In contrast, much higher values in these parameters and different contributions of organic matter to seston were recorded in the phytoplankton-turbid systems of CH and SJ. The inorganic-turbid lake LI had

Table 1. Geographical position, alternative state, lake area (* data obtained from Silvoso *et al.*, 2011) and mean values (± SD) of water quality parameters of the studied lakes.

	Chascomús (CH)	La Limpia (LI)	San Jorge (SJ)	Lacombe (LAC)	El Triunfo (TRI)	Kakel Huincul (KH)
Location	35°36'S 58°02'W	35°37'S 57°48'W	35°40'S 57°47'W	35°49'S 57°49'W	35°51'S 57°52'W	36°48'S 57°47'W
Steady state	Turbid – high phytoplankton abundance	Turbid – high clay concentration	Turbid – high phytoplankton abundance	It alternates between phytoplankton-turbid and clear-vegetated	Clear-vegetated	Clear-vegetated
Surface area (km²)*	28.7	5.6	3.0	1.6	1.5	29.5
Temperature (°C)	18.4 (± 4.1)	18.2 (± 4.5)	19.2 (± 4.2)	20.8 (± 4.4)	18.1 (± 3.6)	17.9 (± 4.0)
Conductivity (μS cm^{-1})	2600 (± 400)	1400 (± 300)	1900 (± 300)	3300 (± 1100)	1590 (± 260)	1900 (± 600)
pH	9.1 (± 0.1)	9.1 (± 0.3)	9.1 (± 0.3)	9.0 (± 0.4)	9.3 (± 0.3)	8.6 (± 0.3)
Dissolved oxygen (mg l^{-1})	8.8 (± 0.8)	9.0 (± 1.3)	9.3 (± 1.1)	8.0 (± 1.7)	9.5 (± 1.8)	11.7 (± 3.8)
Alkalinity (μeq l^{-1})	10 665.2 (± 3305.3)	11 238.8 (± 3931.2)	12 038.1 (± 3518.3)	19 940.5 (± 9498.9)	9218.3 (± 3105.8)	7206.4 (± 2893.0)
Secchi (cm)	8.7 (± 2.4)	10 (± 1.1)	9.4 (± 4.1)	31.3 (± 12.3)	> 100	> 100
Turbidity (NTU)	162.0 (± 79.7)	199.1 (± 68.0)	190.1 (± 107.8)	19.3 (± 13.7)	3.2 (± 2.2)	13.6 (± 18.6)
K_d PAR (m^{-1})	20.2 (± 6.5)	18.8 (± 4.5)	20.8 (± 9.0)	7.1 (± 2.3)	4.6 (± 1.7)	4.8 (± 2.4)
Seston (mg l^{-1})	223.8 (± 107.0)	277.8 (± 113.6)	136.7 (± 84.4)	45.5 (± 24.0)	5.2 (± 3.7)	25.7 (± 37.7)
% Organic matter in seston	40.7 (± 5.6)	13.5 (± 4.6)	71.9 (± 16.7)	66.1 (± 25.9)	89.0 (± 11.8)	75.9 (± 7.2)
Total nitrogen (μg l^{-1})	3927.87 (± 1980.4)	3620.4 (± 2434.5)	6310.0 (± 4328.4)	3791.0 (± 2209.7)	3126.0 (± 1690.2)	3323.7 (± 2345.8)
Total phosphorous (μg l^{-1})	714.1 (± 209.2)	1036.6 (± 133.6)	448.7 (± 189.5)	289.1 (± 80.5)	94.7 (± 23.4)	128.2 (± 72.0)
CDOM absorption at 440 nm (m^{-1})	1.22 (± 0.27)	2.28 (± 0.94)	1.69 (± 0.34)	2.24 (± 0.47)	1.53 (± 0.33)	1.85 (± 0.50)
Ratio 250:365 nm	10.64 (± 0.51)	7.6 (± 3.10)	8.38 (± 2.11)	7.93 (± 2.72)	9.94 (± 0.49)	9.42 (± 2.75)
Chlorophyll-*a* (μg l^{-1})	322.0 (± 198.0)	79.5 (± 59.7)	783.1 (± 395.4)	91.8 (± 49.3)	19.5 (± 18.9)	46.4 (± 63.2)

K_d PAR, diffuse attenuation coefficient.

the highest turbidity, seston and total phosphorous but typically lowest chlorophyll-*a* and organic matter concentrations. Finally, lake LAC had intermediate values in all the parameters considered.

Mean diffuse attenuation coefficients of photosynthetically active radiation (K_d PAR) ranged between 4.6 and 20.8 m^{-1} for TRI and SJ respectively. These contrasting K_d PAR values indicated strong differences in the euphotic zones of these shallow lakes, which averaged 0.26 m in CH, SJ and LI, 0.71 m in LAC and 1.13–1.17 m in KH and TRI respectively. Also optical parameters related to dissolved organic matter (DOM) quality and quantity differed among systems. Mean chromophoric dissolved organic matter (CDOM) absorption coefficient at 440 nm ($\alpha_{(440)}$) ranged from 1.22 m^{-1} ($\pm$ 0.27 m^{-1}) in CH to 2.28 m^{-1} ($\pm$ 0.94 m^{-1}) in LI. On the other hand, the ratio 250:350 nm, which is an inverse index of DOM average molecular weight (De Haan *et al.*, 1982; Lindell *et al.*, 1995), showed the highest annual variation in LI (VC: variation coefficient = 41.2%) and LAC (VC = 34.4%), followed by KH (VC = 29.2%) and SJ (VC = 25.1%), while TRI and CH showed the least variation in this parameter (VC = 5.5% and 4.9% respectively). The study took place during an extended drought period, and as a result there was a general decrease in water level in all systems. This decrease in mean depth resulted in a general increase in turbidity in all systems (Fig. 1). In addition, during the study period, LAC shifted from a clear state at the beginning, to a predominantly turbid state towards the end of the study.

From a total of 41 biological samples obtained, 11 failed to be amplified and could not be included in this analysis. In particular, the ARISA amplification protocol did not work on LI samples. Nevertheless, as DGGE results did not change substantially when this lake was or was not taken into account, we decided to include it when results were analysed.

Based on DGGE profiles, a total of 58 different bands (OTUs) were detected. Only four OTUs were shared among all the lakes, and these shared OTUs represented, on average, between 8.7% and 81.4% of the total relative intensity in each lane (mean: 23.3%). Phytoplankton-turbid lakes (CH and SJ) shared four exclusive OTUs, while the clear-vegetated lakes (TRI and KH) shared only one OTU. There was one band exclusive to CH, and two bands that were only present in SJ. The remaining lakes (the inorganic-turbid LI and LAC) did not show any exclusive bands. On average, 18 ($\pm$ 8) OTUs were detected in CH, 13 ($\pm$ 5) in SJ, 9 ($\pm$ 5) in LI, 10 ($\pm$ 2) in LAC, 13 ($\pm$ 3) in KH and 13 ($\pm$ 5) in TRI. ARISA profiles yielded a total of 452 fragments (OTUs), two- to threefold higher than the number obtained using DGGE. Only four OTUs were present in all systems; 20 OTUs were shared exclusively by phytoplankton-turbid lakes (CH and SJ), while 10 OTUs

were only present in clear-vegetated lakes (TRI and KH). The mean number of fragments (OTUs) detected in each site was 38 ($\pm$ 26) in CH, 38 ($\pm$ 17) in TRI, 39 ($\pm$ 37) in SJ, 42 ($\pm$ 27) in LAC and 46 ($\pm$ 8) in KH.

Given that we failed to obtain the sequences of the bands obtained excised from DGGE, we could not rule out the presence of plastid and mitochondrial SSUrRNA derived from cyanobacteria and eukaryotes (e.g. Øvreås *et al.*, 1997; Boyer *et al.*, 2001). To overcome this limitation, we performed a pyrosequencing analysis (Ronaghi *et al.*, 1996; Ronaghi, 1998; 2001) on three randomly selected samples from each of three lakes differing in alternative state (CH, TRI and LAC) to assess the influence of non-bacterial amplicons in our analysis. The results form this analysis showed that only a minor proportion (~0.6%) of the sequences retrieved form pyrosequencing corresponded to plastids. We may therefore be confident that the results reported here represent an unbiased account of the patterns exhibited by the prokaryotic components.

As PCR amplification is known to introduce bias in the ratio of amplified products in mixed template reactions (Suzuki and Giovannoni, 1996; Wintzingerode *et al.*, 1997; Polz and Cavanaugh, 1998), and given that the difference in the number of operon copies represents a source of systematic error in community analysis (Klappenbach *et al.*, 2001; Crosby and Criddle, 2003), we used a conservative approach based on presence–absence data (as opposite to semi-quantitative data) obtained from DGGE and ARISA profiles to perform all multivariate analyses.

We carried out Mantel tests between presence–absence matrices generated from DGGE- and ARISA-based analyses, which yielded a significant correlation between both matrices ($r_S = 0.260$; $P < 0.0001$), suggesting an overall convergence and agreement in the BCC patterns based on these two independent techniques.

Cluster analyses based on the two fingerprint methods yielded a similar result, with groupings of lakes mainly corresponding to the prevailing regime (i.e. clear-vegetated, phytoplankton-turbid and inorganic-turbid) of the systems (Fig. 2). This pattern was evident when based on DGGE profiles (Fig. 2a). The cluster analysis based on ARISA profiles showed a somewhat more complex arrangement (Fig. 2b) than the pattern based on DGGE (Fig. 2a). With a few exceptions, clear-vegetated systems (i.e. TRI and KH) grouped separately from turbid systems (viz. CH, SJ and LI). Both techniques further evidenced a marked temporal pattern for LAC: at the beginning of the study BCC was more similar to that found in clear-vegetated lakes (namely, TRI and KH), whereas it became closer to that found in the turbid lakes by the end of the sampling period. This temporal pattern in BCC actually corresponds to a shift in state, from clear to
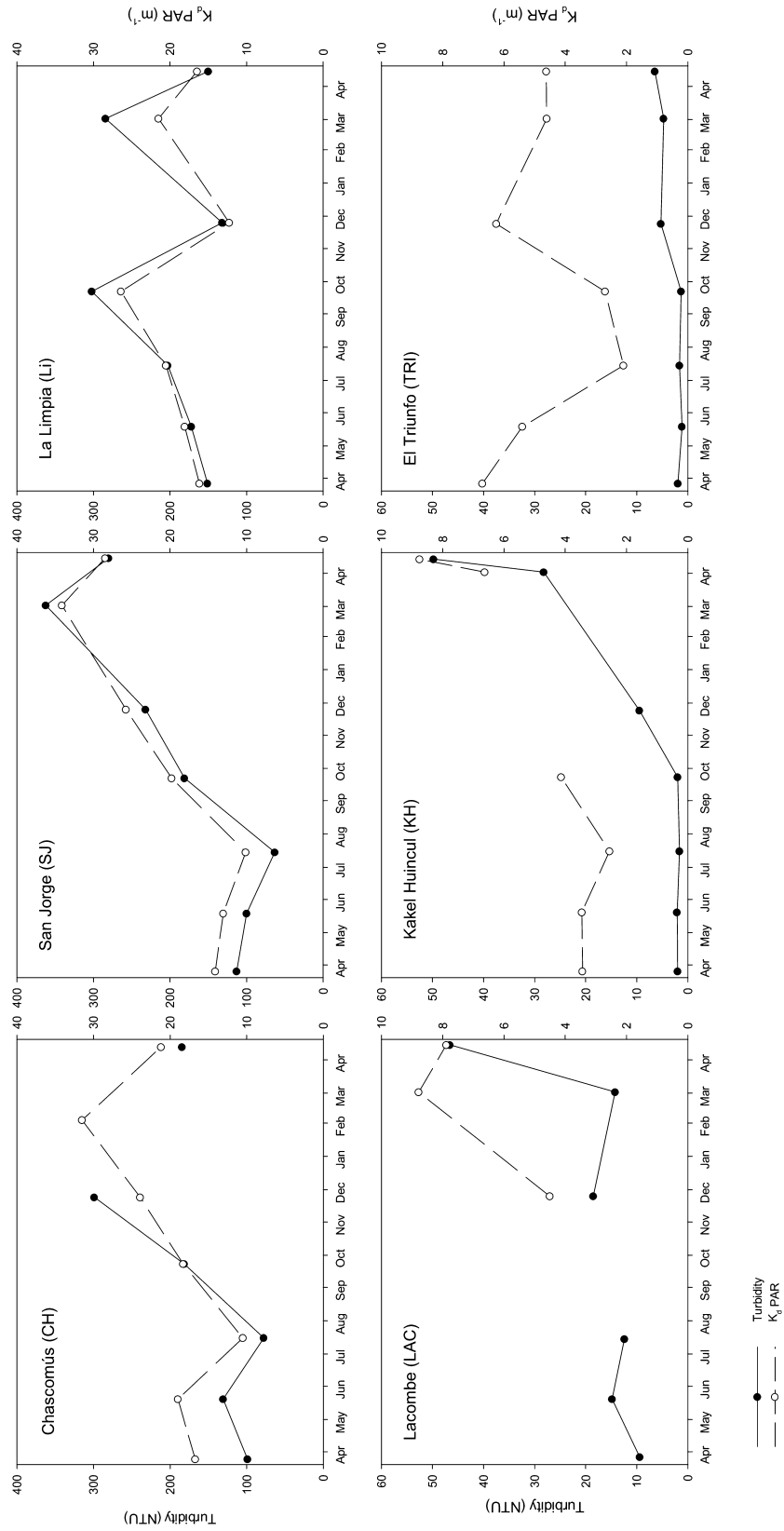
Fig. 1. Temporal variation of turbidity and diffuse attenuation coefficient (K_d PAR) in the sampled lakes. Upper panels correspond to turbid systems (higher turbidity and Kd); lower panels correspond to clear systems (lower values of turbidity and Kd).
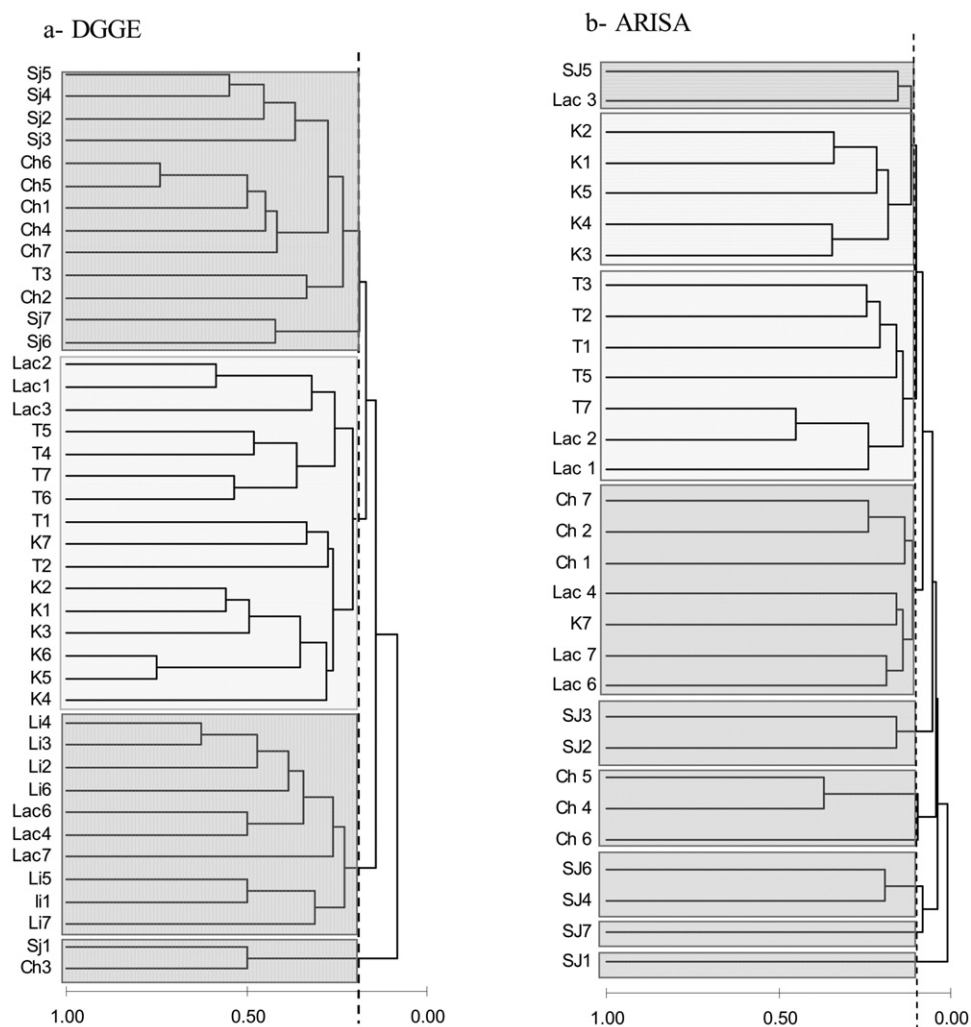
a- DGGE

b- ARISA

Fig. 2. Cluster analysis based on (a) DGGE and (b) ARISA profiles using Jaccard's similarity index and unweighted pair group method with arithmetic mean(UPGMA) linkage. Turbid systems are highlighted in dark grey, while light grey indicates clear-vegetated lakes. DNA extracted from LI failed to amplify for the ARISA protocol, thus this site could not be analysed with this technique.

turbid, which occurred in this lake during the sampling period.

Ordination of sites based on community composition [non-metric multidimensional scaling (MDS)] further confirmed the results from cluster analyses, with sites grouping on the basis of alternative state (Fig. 3). We tested the statistical significance of the groupings obtained by MDS using analyses of similarity (ANOSIM), and for both DGGE- and ARISA-based ordinations the ANOSIM test was statistically significant (ANOSIM $R_{DGGE} = 0.565$, ANOSIM $R_{ARISA} = 0.430$; $P < 0.0001$), such that the null hypothesis of 'no differences in BCC among sites' was rejected. Pairwise comparisons of sites based on their DGGE and ARISA patterns are shown in Table 2. The Bonferroni-corrected *P*-values indicated that for the DGGE-based composition there were three groups: LI, CH–SJ and LAC–TRI–KH, which differed significantly.

Pairwise comparisons based on ARISA profiles (Table 2) also distinguished three different groups based on BCC: the *CH*-like, the *SJ*-like and *TRI-LAC*-like communities. For KH, the within-group similarity did not differ significantly from among-group similarity, reflecting that BCC in KH did not show an exclusive pattern in bacterial composition that segregated this site from the other lakes. The OTUs recovered from these fingerprinting approaches, particularly DGGE, probably represent the dominant and best adapted taxa of each environment (Magurran and Henderson, 2003; Pedrós-Alió, 2006). In fact, our results show clear associations between alternative state and community composition, both based on DGGE and ARISA fingerprints.

These results reinforce previous reports of a strong connection between system state and microbial community composition in shallow lakes elsewhere (Van der
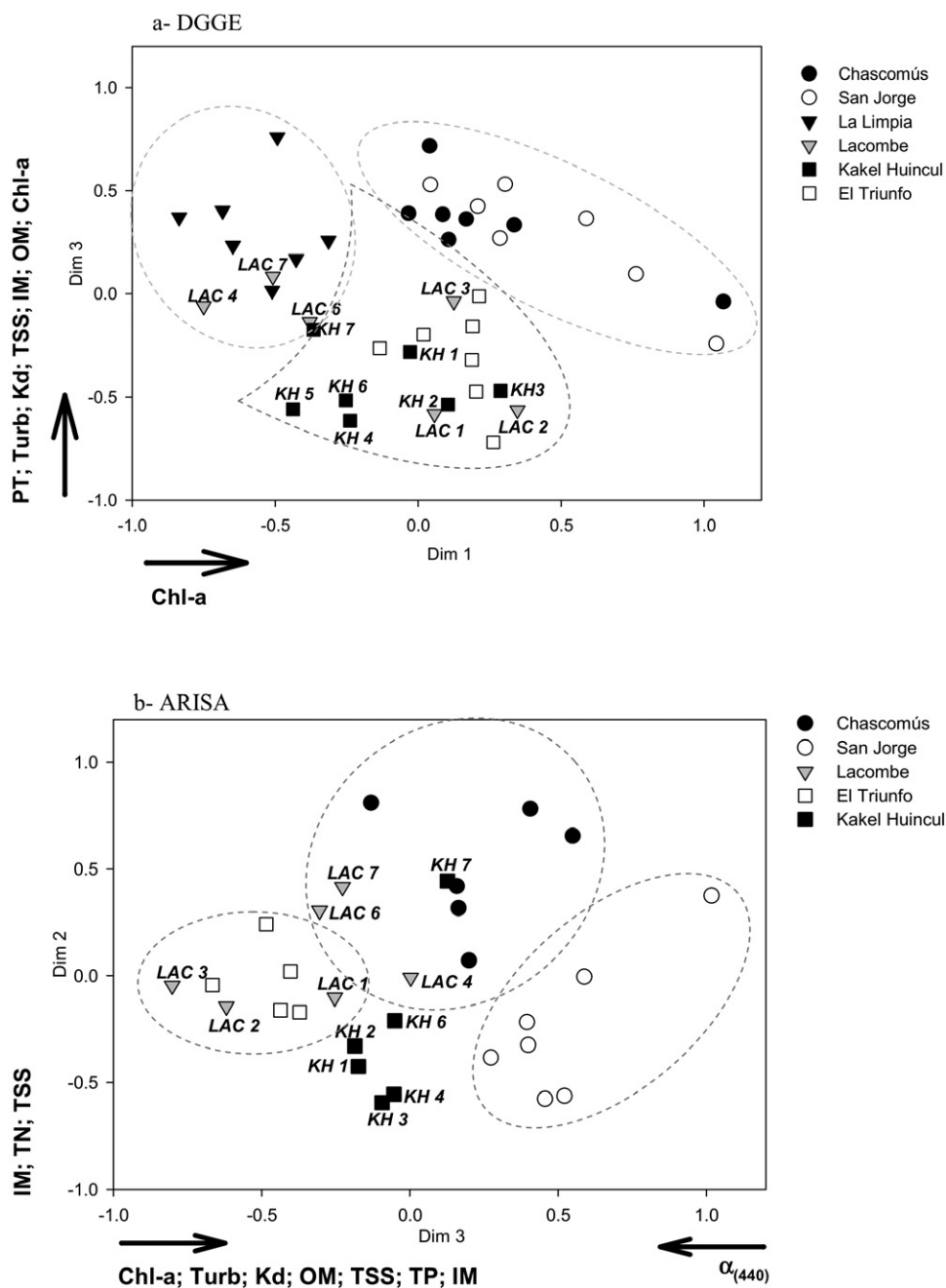
Fig. 3. a. Dim 1 versus Dim 3 of the three-dimensional non-metric MDS plot for DGGE profiles (stress = 0.17). Arrows indicate the direction of significant correlation of the axes with environmental variables. TP, total phosphorous concentration; Turb, turbidity; Kd, diffuse attenuation coefficient; TSS, total suspended solids; IM, inorganic matter content; OM, organic matter content; Chl-a, chlorophyll-*a* content.
b. Dim 3 versus Dim 2 of the three-dimensional non-metric MDS plot for ARISA profiles (stress = 0.16). Arrows indicate the direction of significant correlation of the axes with environmental variables. TP, total phosphorous concentration; TN, total nitrogen concentration; Turb, turbidity; Kd, diffuse attenuation coefficient; TSS, total suspended solids; IM, inorganic matter content; OM, organic matter content; Chl-a, chlorophyll-*a* content; $\alpha_{(440)}$, CDOM absorption coefficient at 440 nm. Temporal trajectories for LAC and KH are detailed: 1 – March 2008; 2 – May 2008; 3 – July 2008; 4 – September 2008; 5 – November 2008; 6 – February 2009; 7 – April 2009. Regions in the panels were delineated according to ANOSIM results.

Gucht *et al.*, 2001; Muylaert *et al.*, 2002; Wu *et al.*, 2007; Ng *et al.*, 2010). In addition, our conclusions regarding the links between ecosystem state and BCC are consistent with previous reports of systematic patterns in other features of these same Pampean shallow lakes, including on physical–chemical characteristics, macrozooplankton and fish communities (Quirós *et al.*, 2002), phytoplankton community composition (Allende *et al.*, 2009; Izaguirre

Table 2. ANOSIM statistics for global as well as pairwise comparisons based on both DGGE and ARISA profiles.

a – DGGE	R	P-value
Global test	0.565	< 0.0001
CH versus SJ	0.257	0.1305
CH versus LI	0.806	0.0075
CH versus LAC	0.685	0.0195
CH versus KH	0.769	0.0120
CH versus TRI	0.450	0.0195
SJ versus LI	0.661	0.0075
SJ versus LAC	0.534	0.0135
SJ versus KH	0.710	0.0030
SJ versus TRI	0.391	0.0135
LI versus LAC	0.409	0.0210
LI versus KH	0.800	0.0075
LI versus TRI	0.789	0.0030
LAC versus KH	0.589	0.0075
LAC versus TRI	0.347	0.1380
KH versus TRI	0.196	0.7155

b – ARISA	R	P-value
Global test	0.430	< 0.0001
CH versus SJ	0.470	0.0280
CH versus LAC	0.546	0.0189
CH versus KH	0.631	0.0640
CH versus TRI	0.851	0.0251
SJ versus LAC	0.542	0.0123
SJ versus KH	0.248	0.2390
SJ versus TRI	0.580	0.0165
LAC versus KH	0.237	0.4650
LAC versus TRI	0.155	1.0000
KH versus TRI	0.521	0.0460

Results in grey highlight no significant differences among sites.

et al., 2012), as well as the structure of picoplankton communities (Silvoso *et al.*, 2011).

We further explored the environmental links that may underlie the patterns in community composition across the different alternative states, by carrying out Mantel tests between the matrix of environmental variables and the matrices of BCC. There was a significant correlation between the ensemble of environmental variables and BCC derived from both approaches, and this correlation was much stronger based on ARISA profiles (r_{DGGE} = 0.109, P = 0.002; r_{ARISA} = 0.338, P < 0.0001). These results suggest that the type of stable state selects a common set of dominant species, as likely reflected in the DGGE profiles, and that local environmental differences within a given type of state further modulate composition at a finer scale, as reflected in the profile yielded by ARISA. The latter pattern was clearly evident for turbid lakes: lake CH was dominated by a mixed phytoplankton community, whereas lake SJ had an almost continuous bloom of the filamentous cyanobacteria *Raphidiopsis mediterranea*. These drastically different algal communities probably resulted in differences in organic matter and nutrient dynamics between the two, which in turn led to selection of subdominant taxa, yet the actual state

(phytoplankton-turbid), and not the prevailing algal community, appeared to have an overriding effect in determining the dominant bacterial species in these lakes.

We further investigated the influence of individual environmental variables on BCC, by correlating the scores derived from the ordination of sites shown in Fig. 3 with the 16 environmental variables considered here. We only used the combination of axes that best separated sites, and significant correlations of environmental variables with these ordination scores are shown in Table 3. Our analysis suggests that the patterns in bacterioplankton composition were related to a combination of phytoplankton biomass (Chl-a), water transparency (Turb, TSS, Kd) and resource availability (OM, TP, TN, $\alpha_{(440)}$ and the ratio 250:365 nm), while temperature and solar irradiance (parameters related to seasonality) did not show a significant correlation. Altogether, the variables that best explained the pattern observed were those connected to the optical properties of these systems and also to the characteristic of carbon sources in these lakes.

In this regard, shallow lakes in this Pampa Plain region markedly differ in their optical characteristics because of the nature of main absorbing components (Pérez *et al.*, 2010). In clear-vegetated lakes, CDOM is the most important absorbing fraction, while in inorganic-turbid lakes colloidal clays contribute significantly to light attenuation. On the other hand, in spite of CH and SJ being both phytoplankton-turbid systems, in CH a significant fraction of light absorption (*c.* 46%) is due to non-chlorophyllous particles, while in SJ absorption is predominantly due to algae. These differences in optical signatures are probably also related to differences in the main organic matter pools and thus in the dominant substrates for bacteria in each type of regime.

It has now been well established that the source, concentration and composition of organic substrate are among the factors shaping the composition of the bacterial communities in lakes (Kritzberg *et al.*, 2006) and it has been shown that different groups of bacteria exhibit different capabilities for utilizing qualitatively different substrates (e.g. Hutalle-Schmelzer *et al.*, 2010; Šimek *et al.*, 2011). Thus, we suggest that in these shallow lakes, which are all characterized by relatively high nutrient concentrations, patterns in BCC are probably mainly driven by differences in the nature of the predominant organic matter sources and pools: among the six selected lakes, clear-vegetated lakes are characterized by low chlorophyll concentration and macrophytes are likely the main organic carbon source for bacteria, through the release of particulate and dissolved organic compounds during active photosynthesis and also during senescence and early stages of decomposition (Wetzel, 2001; Demarty and Prairie, 2009). In the case of phytoplankton-turbid systems, organic matter produced by the high standing

Table 3. Spearman rank order correlation coefficients of the MDS axes with environmental variables.

	a – DGGE			b – ARISA		
	Axis 1	Axis 2	Axis 3	Axis 1	Axis 2	Axis 3
Chlorophyll-*a* (Chl-a)						
r	0.390		0.579			0.738
P-value	0.0292		< 0.0001			< 0.0001
n	31		39			20
Total phosphorous (TP)						
r			0.735			0.528
P-value			< 0.0001			0.0005
n			32			26
Total nitrogen (TN)						
r					0.443	
P-value					0.030	
n					24	
Turbidity (Turb)						
r			0.726			0.731
P-value			< 0.0001			< 0.0001
n			39			28
Diffuse attenuation coefficient (Kd)						
r			0.726	−0.413		0.659
P-value			< 0.0001	0.0486		< 0.0001
n			35	24		24
Total suspended solids (TSS)						
r			0.673		0.424	0.623
P-value			< 0.0001		0.0275	< 0.0001
n			38		27	27
Inorganic matter content (IM)						
r			0.610		0.457	0.485
P-value			< 0.0001		0.0167	0.0105
n			38		27	27
Organic matter content (OM)						
r			0.606			0.639
P-value			< 0.0001			0.0003
n			38			27
CDOM absorption at 440 nm ($\alpha_{(440)}$)						
r		−0.480				−0.412
P-value		0.005				0.045
n		33				24
Ratio 250:365 nm						
r		0.384				
P-value		0.028				
n		33				

A total of 16 environmental variables were tested: temperature, incident irradiance, conductivity, alkalinity, pH, dissolved oxygen concentration, chlorophyll-*a* concentration, total nitrogen and total phosphorous concentration, total suspended solids, organic and inorganic matter concentration, turbidity, diffuse attenuation coefficient, CDOM absorption coefficient at 440 nm and the ratio 235:365 nm. Only significant correlations are shown.

stocks of phytoplankton probably dominates the carbon pools, and it is possibly that the nature of this dissolved organic carbon (DOC) differs from the macrophyte-derived DOC (Guillemette and del Giorgio, 2011). In contrast, in inorganic-turbid systems where both phytoplankton and macrophyte biomass is low, terrestrially derived organic carbon likely dominates the organic matter pool. In addition, the particulates are composed mostly of suspended clays, which provide surfaces for the selective adsorption of DOC, altering its availability to bacteria (Lind and Dávalos-Lind, 1991 and references therein).

These assumptions remain as hypotheses to be tested, and further work is needed in this regard, as other factors such as top-down controls or differences among system types in regard to the quality and availability of structures suitable for attachment were not taken into account in this analysis. For example, suspended material, such as suspended clays, can interference with the feeding and growth of microbial components (Pfandl and Boegnick, 2006), and also promote alternative pathways for the microbial loop by providing surfaces for the adsorption of DOC, which becomes more readily available to bacteria and, subsequently, to higher trophic levels (Lind and Dávalos-Lind, 1991 and references therein).

That the alternative state plays a key role in determining BCC is further evidenced by the pattern observed in LAC, which shifted from a clear to a turbid state over the course of the study (Fig. 3). This lake clearly aligned with the two

non-turbid lakes in terms of BCC in the early stages, and then clustered with the turbid lakes in the later stages of the study. These shifts in BCC are not due to seasonality; rather, the evidence supports a strong influence of the environment on BCC, and also suggests a strong directional selection of species out of the metacommunity pool, since upon the regime shift, BCC in this lake did not simply diverge to a different configuration, but rather tended to converge to that found in the other turbid lakes.

The progression observed in another of the lakes (KH) provides further evidence for the above conclusion. As we pointed out above, the study period had much lower than average precipitations, and this led to a decline in water level in all the lakes, and an overall increase in turbidity. The lake that was most affected by this gradual increase in turbidity was KH, which while remaining in its basic vegetated-clear water state (as opposed to LAC, which completely shifted in state as discussed above), become significantly more turbid by the end of the study period. Interestingly, we note that there was a gradual shift in the positioning of BCC of this lake, progressively shifting towards that of the turbid lakes (Fig. 3). This suggests a pattern of environmentally driven species sorting from a common regional metacommunity pool, particularly by the type of alternate regime, with the possibility of finding intermediate configurations between the extreme alternative states.

Previous studies have shown that mass effects may be the dominant process in determining community composition in lakes with relative short hydrological retention times (< 6 months, Lindström *et al.*, 2006). In this regard, water residence time in these shallow Pampean lakes varies greatly, but is generally short, usually in the order of 3–5 months (Quirós and Drago, 1999). It could thus be expected that dispersal might homogenize BCC across these lakes, especially in lakes within the same drainage basin, but this clearly was not the case. Moreover, of the six lakes that we targeted in this study, five do not have any river inlet and are fed only by groundwater and rainfall, whereas the remainder, lake CH, has a major river inlet, yet the latter had a very similar BCC as the other phytoplankton-turbid lake (LAC) that had no river connection. The evidence thus confirms the importance of environmental selection of species over dispersion and mass effects in determining BCC in these lakes. The convergence in BCC for a given alternative state would in turn suggest that either this regional metacommunity pool is limited, in terms of the range of phylotypes available, or that even if this pool is very broad, the environmental selection is very stringent and results in essentially the same configuration of phylotypes selected under a particular set of conditions; this latter scenario is the most likely in our opinion. Our results thus suggest a hierarchy in the environmental sorting, wherein the steady state selects the dominant phylotypes, and variations within each state, such as differences in the phytoplankton or macrophyte community composition, further select taxa from these dominant players.

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References

Allende, L., Tell, G., Zagarese, H., Torremorell, A., Pérez, G., Bustingorry, J., *et al.* (2009) Phytoplankton and primary production in clear-vegetated, inorganic-turbid, and algal-turbid shallow lakes from the pampa plain (Argentina). *Hydrobiologia* **624:** 45–60.

Aronson, R.B., Macintyre, I.G., Wapnick, C.M., and O'Neill, M.W. (2004) Phase shifts, alternative states, and the unprecedented convergence of two reef systems. *Ecology* **85:** 1876–1891.

Baskett, M.L., and Salomon, A.K. (2010) Recruitment facilitation can drive alternative states on temperate reefs. *Ecology* **91:** 1763–1773.

Beisner, B.E., Haydon, D.T., and Cuddington, K. (2003) Alternative stable states in ecology. *Front Ecol Environ* **1:** 376–382.

Boyer, S.L., Flechtner, V.R., and Johansen, J.R. (2001) Is the 16S–23S rRNA internal transcribed spacer region a good tool for use in molecular systematics and population genetics? A case study in cyanobacteria. *Mol Biol Evol* **18:** 1057–1069.

Briske, D.D., Washington-Allen, R.A., Johnson, C.R., and Lockwood, J.A. (2010) Catastrophic thresholds: a synthesis of concepts, perspectives and applications. *Ecol Soc* **15**. [WWW document]. URL http://www.ecologyandsociety.org/vol15/iss3/art37/

Cano, M.G. (2008) Fitoperifiton de un lago somero y su relación con los estados de biequilibrio. PhD Thesis. La Plata, Argentina: Universidad Nacional de La Plata.

Casco, M.A., Mac Donagh, M.E., Cano, M.G., Solari, L.C., Claps, M.C., and Gabellone, N.A. (2009) Phytoplankton and Epipelon responses to clear and turbid phases in a seepage lake (Buenos Aires, Argentina). *Int Rev Hydrobiol* **94:** 153–168.

Comte, J., and del Giorgio, P.A. (2011) Composition influences the pathway but not the outcome of the metabolic response of bacterioplankton to resource shifts. *PLoS ONE* **6:** e25266. doi: 10.1371/journal.pone.0025266.

Crosby, L.D., and Criddle, C.S. (2003) Understanding bias in microbial community analysis techniques due to rrn operon copy number heterogeneity. *Biotechniques* **34:** 2–9.

De Haan, H., De Boer, T., Kramer, H.A., and Voerman, J.

(1982) Applicability of light absorbance as a measure of organic carbon in humic lake water. *Water Res* **16:** 1047–1050.

Demarty, M., and Prairie, Y.T. (2009) *In situ* dissolved organic carbon (DOC) release by submerged macrophyte–epiphyte communities in southern Quebec lakes. *Can J Fish Aquat Sci* **66:** 1522–1531.

Ducklow, H. (2008) Microbial services: challenges for microbial ecologists in a changing world. *Aquat Microb Ecol* **53:** 13–19.

Fisher, M.M., and Triplett, E.W. (1999) Automated approach for ribososmal intergenic spacer analysis of microbial diversity and its implication for freshwater bacterial communities. *Appl Environ Microbiol* **65:** 4630–4636.

Guillemette, F., and del Giorgio, P.A. (2011) Reconstructing the various facets of DOC bioavailability in freshwater ecosystems. *Limnol Oceanogr* **56:** 734–748.

Haukka, K., Kolmonen, E., Hyder, R., Hietala, J., Kirsi, V., Kairesalo, T., *et al.* (2006) Effect of nutrient loading on bacterioplankton community composition in lake mesocosms. *Microb Ecol* **51:** 137–146.

Hutalle-Schmelzer, K.M.L., Zwirnmann, E., Krüger, A., and Grossart, H.P. (2010) Enrichment and cultivation of pelagic bacteria from a humic lake using phenol and humic matter additions. *FEMS Microbiol Ecol* **72:** 58–73.

Izaguirre, I., Allende, L., Escaray, R., Bustingorry, P., Pérez, G., and Tell, G. (2012) Comparison of morpho functional phytoplankton classifications in human impacted shallow lakes with different stable states. *Hydrobiologia* **698:** 203–216.

Jasinski, J., and Payette, S. (2005) The creation of alternative stable states in the southern boreal forest, Québec, Canada. *Ecol Monogr* **75:** 561–583.

Jeppesen, E., Jensen, J.P., Søndergaard, M., Lauridsen, T., Pedersen, L.J., and Jensen, L. (1997) Top-down control in freshwater lakes: the role of nutrient state, submerged macrophytes and water depth. *Hydrobiologia* **342/343:** 151–164.

Klappenbach, J.A., Saxman, P.R., Cole, J.R., and Schmidt, T.M. (2001) rrndb: the Ribosomal RNA Operon Copy Number Database. *Nucleic Acids Res* **29:** 181–184.

Kritzberg, E.S., Langenheder, S., and Lindström, E.S. (2006) Influence of dissolved organic matter source on lake bacterioplankton structure and function – implications for seasonal dynamics of community composition. *FEMS Microbiol Ecol* **56:** 406–417.

Lind, O., and Dávalos-Lind, L. (1991) Association of turbidity and organic carbon with bacterial abundance and cell size in a large, turbid, tropical lake. *Limnol Oceanogr* **36:** 1200–1208.

Lindell, M.J., Granéli, W., and Tranvik, L.J. (1995) Enhanced bacterial growth in response to photochemical transformation of dissolved organic matter. *Limnol Oceanogr* **40:** 195–199.

Lindström, E.S., Forslund, M., Algesten, G., and Bergström, A.-K. (2006) External control of bacterial community structure in lakes. *Limnol Oceanogr* **51:** 339–342.

Magurran, A.E., and Henderson, P.A. (2003) Explaining the excess of rare species in natural species abundance distributions. *Nature* **422:** 714–716.

Muylaert, A.E., Van Der Gucht, K., Vloemans, N., De Meester, L., Gillis, M., and Vyverman, W. (2002) Relationships between bacterial community composition and bottom-up versus top-down variables in four eutrophic shallow lakes. *Appl Environ Microbiol* **68:** 4740–4750.

Muyzer, G., Waal, E.C., and Uitierlinden, A.G. (1993) Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA. *Appl Environ Microbiol* **59:** 695–700.

Muyzer, G., Brinkhoff, T., Nübel, U., Santegoeds, C., Schäfer, H., and Wawer, C. (1998) Denaturing gradient gel electrophoresis (DGGE) in Microb. Ecol. In *Molecular Microbial Ecology, Manual*. Akkermans, A.D.L., van Elsas, J.D., and de Bruijn, F.J. (eds). Dordrecht, the Netherlands: Kluwer, pp. 1–27.

Ng, H.-T., Motta Marques, D., Jeppesen, E., and Søndergaard, M. (2010) Bacterioplankton in the littoral and pelagic zones of subtropical shallow lakes. *Hydrobiologia* **646:** 311–326.

Øvreås, L., Forney, L., Daae, F.L., and Torsvik, V. (1997) Distribution of bacterioplankton in meromictic Lake Saelenvannet, as determined by denaturing gradient gel electrophoresis of PCR-amplified gene fragments coding for 16S rRNA. *Appl Environ Microbiol* **63:** 3367–3373.

Pedrós-Alió, C. (2006) Marine microbial diversity: can it be determined? *Trends Microbiol* **14:** 257–263.

Pérez, G., Torremorell, A., Bustingorry, J., Escaray, R., Pérez, P., Diéguez, M., and Zagarese, H. (2010) Optical characteristics of shallow lakes from the Pampa and Patagonia regions of Argentina. *Limnologica* **40:** 30–39.

Pfandl, K., and Boegnick, J. (2006) Stuck in the mud: suspended sediments as a key issue for survival of chrysomonad flagellates. *Aquat Microb Ecol* **45:** 89–99.

Polz, M.F., and Cavanaugh, C.M. (1998) Bias in template-to-product ratios in multitemplate PCR. *Appl Environ Microbiol* **64:** 3724–3730.

Poretsky, R.S., Sun, S., Mou, X., and Moran, M.A. (2010) Transporter genes expressed by coastal bacterioplankton in response to dissolved organic carbon. *Environ Microbiol* **12:** 616–627.

Quirós, R. (2005) La ecología de las lagunas de las Pampas. Investigación y Ciencia 1–13. [WWW document]. URL http://www.agro.uba.ar/users/quiros

Quirós, R., and Drago, E. (1999) The environmental state of Argentinean lakes: an overview. *Lakes Reserv Res Manag* **4:** 55–64.

Quirós, R., Renella, A.M., Sosnovsky, A., and Boveri, M.B. (2002) Factores que afectan la estructura y el funcionamiento de las lagunas pampeanas. *Ecol Austral* **12:** 175–185.

Quirós, R., Boveri, M.B., Petracci, C.A., Renella, A.M., Rosso, J.J., Sosnovsky, A., and von Bernard, H. (2006) Los efectos de la agriculturación del humedal pampeano sobre la eutrofización de sus lagunas. In *Eutrofização na América do Sul: Causas, conseqüências e tecnologias de gerenciamento e controle*. Tundisi, J.G., Matsumura-Tundisi, T., and Galli, C.S. (eds). São Carlos, Brazil: Instituto Internacional de Ecologia, Instituto Internacional de Ecologia e Gerenciamento Ambiental, Academia Brasileira de Ciências, ConselhoNacional de Desenvolvimento Científico e Tecnológico, InterAcademy Panel on Interna-

tional Issues, InterAmerican Network of Academies of Sciences, pp. 1–16.

Ronaghi, M. (1998) Pyrosequencing: a tool for sequence-based DNA analysis. Doctoral Thesis. Stockholm, Sweden: The Royal Institute of Technology.

Ronaghi, M. (2001) Pyrosequencing sheds light on DNA sequencing. *Genome Res* **11:** 3–11.

Ronaghi, M., Karamohamed, S., Pettersson, B., Uhlen, M., and Nyren, P. (1996) Real-time DNA sequencing using detection of pyrophosphate release. *Anal Biochem* **242:** 84–89.

Scheffer, M. (1998) *Ecology of Shallow Lakes*. London, UK: Chapman & Hall.

Scheffer, M., Hosper, S.H., Meijer, M.-L., Moss, B., and Jeppesen, E. (1993) Alternative equilibria in shallow lakes. *Trends Ecol Evol* **8:** 275–279.

Scheffer, M., Carpenter, S.R., Foley, J.A., Folke, C., and Walkerk, B. (2001) Catastrophic shifts in ecosystems. *Nature* **413:** 591–596.

Scheffer, M., Bascompte, J., Brock, W.A., Carpenter, S.R., Dakos, V., Held, H., *et al.* (2009) Early-warning signals for critical transitions. *Nature* **461:** 53–59.

Silvoso, J., Izaguirre, I., and Allende, L. (2011) Picoplankton structure in clear and turbid eutrophic shallow lakes: a seasonal study. *Limnologica* **41:** 181–190.

Šimek, K., Kasalický, V., Zapomělová, E., and Horňák, K. (2011) Algal-derived substrates select for distinct betaproteobacterial lineages and contribute to niche separation in *Limnohabitans* strains. *Appl Environ Microbiol* **77:** 7307–7315. doi: 10.1128/AEM.05107-11.

Suzuki, M.T., and Giovannoni, S.J. (1996) Bias caused by template annealing in the amplification of mixtures of 16S rRNA genes by PCR. *Appl Environ Microbiol* **62:** 625–630.

Van Der Gucht, K., Sabbe, K., De Meester, L., Vloemans, N., Zwart, G., Gillis, M., and Vyverman, W. (2001) Contrasting bacterioplankton community composition and seasonal dynamics in two neighbouring hypertrophic freshwater lakes. *Environ Microbiol* **3:** 680–690.

Wetzel, R. (2001) *Limnology, Lake and River Ecosystems*. London, UK: Academic Press.

Wintzingerode, F., Göbel, U.B., and Stackebrandt, E. (1997) Determination of microbial diversity in environmental samples: pitfalls of PCR-based rRNA analysis. *FEMS Microbiol Rev* **21:** 213–229.

Wu, X., Xi, W., Ye, W., and Yang, H. (2007) Bacterial community composition of a shallow hypertrophic freshwater lake in China, revealed by 16S rRNA gene sequences. *FEMS Microbiol Ecol* **61:** 85–96.

Supporting information

Additional Supporting Information may be found in the online version of this article:

Experimental procedures.