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PHYTOPLANKTON

Comparison of morpho-functional phytoplankton classifications in human-impacted shallow lakes with different stable states

Irina Izaguirre · Luz Allende · Roberto Escaray · José Bustingorry · Gonzalo Pérez · Guillermo Tell

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Abstract The morpho-functional classifications of phytoplankton have been recently proposed as useful tools in the aquatic biomonitoring. In this study, we compared three different classifications in a range of different environmental conditions, a set of six shallow lakes with different stable states. The studied lakes are located in the Pampa Plain from Argentina, a region highly impacted as a consequence of the human activities. Among the selected lakes, three are in a turbid state, two of which have high phytoplankton abundances (phytoplankton-turbid), and one shows a high concentration of suspended inorganic matter (inorganic-turbid). Two lakes are clear and profusely colonized by submerged plants (clear-vegetated). Only one lake shows a typical alternative steady-state behavior, shifting turbid periods of high phytoplankton biomass with periods of more transparency and development of submerged macrophytes. We compared the three morpho-functional classifications applied by means of multivariate analyses in order to explore how much the variance of the biomass of the phytoplankton functional groups (for each functional classification) was explained by the environmental variables. The analyses performed showed a clear separation of the human-impacted turbid lakes from the clear-vegetated lakes. The advantages and disadvantages of the different morpho-functional classifications are discussed, concluding that the functional approach is adequate to analyze the phytoplankton communities in aquatic systems subjected to anthropogenic influence and for monitoring them.

Keywords Phytoplankton morpho-functional classifications · Alternative steady states · Shallow lakes · Human impact · Pampa Plain

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I. Izaguirre (✉) · L. Allende · G. Tell
Departamento de Ecología, Genética y Evolución, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, 1428 Buenos Aires, Argentina
e-mail: iri@ege.fcen.uba.ar

I. Izaguirre · L. Allende · G. Pérez · G. Tell
Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Buenos Aires, Argentina

R. Escaray · J. Bustingorry · G. Pérez
Instituto de Investigaciones Biotecnológicas, Instituto Tecnológico de Chascomús (IIB-INTECH), CONICET, Chascomús, Argentina

Introduction

Long time ago ecologists have tried to group organisms with similar structural and functional characteristics with the aim to obtain a better understanding of the functioning of the ecosystems (Salmaso & Padisák, 2007 and cites therein). In the phytosociological

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approach, the associations are defined as groups of species that are typically found together (e.g., Margalef, 1978; Reynolds, 1980, 1984), and the species of a particular association share common ecological attributes. This approach was successively refined and expanded by different phytoplanktologists. Reynolds (1997) proposed different associations following functional criteria based upon both morphological and ecological properties. Later on, Reynolds et al. (2002) considered the structure of the phytoplankton assemblages and proposed a general scheme with 31 functional groups, defining their distinctive ecological features (functional classification). Groups are often polyphyletic, recognizing commonly shared adaptive features. This classification has been frequently used by phytoplankton ecologists in different aquatic systems (e.g., Kruk et al., 2002; Tolotti et al., 2005; Devercelli, 2006; Sarmento & Descy, 2008). Recently, this classification was revised and updated by Padisák et al. (2009).

The concept of functional diversity (FD) coined by Weithoff (2003) highlights another aspect of the functional characterization of species and communities and provides a new understanding of phytoplankton ecology; it is related to the functional multiplicity within the community rather than the multiplicity of species. In this respect, functional traits were defined as a property of an organism that can be measured and that influences one or more essential functional processes such as reproduction, growth, etc. (Weithoff, 2003). Using multivariate analyses Salmaso & Padisák (2007) evaluated classifications based on the morphological and functional characteristics. More recently, Kruk et al. (2010) proposed a new morphologically based functional classification, where simple morphological traits were found to capture much of the variability in functional properties of phytoplankton; the classification was tested using phytoplankton information obtained in more than 200 lakes along a climatic gradient (tropical to subpolar).

In this investigation, we applied different phytoplankton functional classifications to capture much of the differences among different shallow lakes located in the Pampean region of Argentina. This region contains thousands of shallow lakes which are subjected to a progressive eutrophication due to a combination of human activities in their catchment such as land-use change, increase of agriculture, livestock, non-regulated urbanization, fish introduction, drainage, canalization, and damming (Quirós et al., 2006). Limno-regional studies conducted in this area allowed to recognize three main types of shallow lakes: clear with abundant submerged and emergent macrophytes, turbid with high phytoplankton biomass ("green" shallow lakes), and turbid with great concentrations of inorganic suspended material (Quirós et al., 2002). Scheffer et al. (1993) have described the mechanisms involved in the transition of the shallow lakes from a clear state with submerged macrophytes to a turbid one dominated by phytoplankton: at low nutrient concentrations the clear-water equilibrium is the only possible stable state, in a hypertrophic situation just the turbid equilibrium exists, and between these two extremes there is a range of nutrient levels over which two alternative equilibria can exist. In the Pampa Plain, clear-vegetated lakes and turbid lakes with high phytoplankton biomass represent the two basic alternative states described in the model proposed by Scheffer et al. (1993). The third type of lake would be the result of a direct human impact on their drainage basin and includes systems where primary production is severely light limited by inorganic turbidity (Quirós et al., 2002). Some previous papers have reported information on the phytoplankton structure of these different types of shallow lakes (e.g., Izaguirre & Vinocur, 1994; Cano et al., 2008; Allende et al., 2009; Silvoso et al., 2011).

We seasonally analyzed the structure of the phytoplankton assemblages of six selected shallow lakes from the Pampa Plain that are representative of the different scenarios that can be recognized in this region, which in turn are mainly the result of the human impact in the area: two shallow lakes are clear-vegetated, two are phytoplankton-turbid, one is inorganic-turbid, and one can alternate periodically between clear and turbid states. The aim was to explore the adequateness of the morpho-functional approach in detecting changes in the phytoplankton assemblages in human-impacted systems by comparing three different classifications: (a) Reynolds et al. (2002) revised by Padisák et al. (2009); (b) Salmaso & Padisák (2007); (c) Kruk et al. (2010). Using multivariate analyses, we estimated how much the variance of the biomass of the phytoplankton functional groups (for each functional classification) was explained by the environmental variables.

Study area

The Pampa Plain (35°32′–36°48′S; 57°47′–58°07′W) from Argentina is encompassed in a warm temperate region, where mean annual temperature is about 15.3°C and winds have a mean annual speed of 10.1 km h^{-1} (Torremorell et al., 2007). This region is characterized by a marked interannual variability between wet and dry periods with a mean annual precipitation of about 935 mm (Sierra et al., 1994). Due to the very low regional topographic gradient (<0.1%) of this plain, the networks of surface water and salt evacuation toward the ocean are poor and water excesses often translate into flooding and salt redistribution (Jobbágy et al., 2008).

Pampean shallow lakes are typically permanent, relatively homogeneous in depth (mean depth ~2 m), and eutrophic or hypertrophic (Quirós & Drago, 1999). The study was conducted in six selected shallow lakes (Fig. 1), which represent the different states that the shallow lakes of this region can exhibit. Two lakes (Kakel Huincul and El Triunfo) are clear and are profusely colonized by submerged plants (mainly *Myriophyllum* sp. and *Ceratophyllum demersum*) and emergent macrophytes (*Schoenoplectus californicus*); these water bodies show a limited development of pelagic algae (Allende et al., 2009). Other two lakes are characterized by low Secchi depth values and high phytoplankton abundances (San Jorge and Chascomús). One lake (La Limpia) presents a high concentration of suspended inorganic matter and low development of both submerged macrophytes and phytoplankton. Finally, the sixth shallow lake (Lacombe) may alternate between turbid periods of high phytoplankton development and periods of more transparency and development of submerged macrophytes (Cano et al., 2008). The main characteristics of the six shallow lakes are summarized in Table 1.

Materials and methods

Three sampling sites were established in the six selected shallow lakes above described. Each site was sampled for physical, chemical, and biological parameters. Samples were collected in spring, summer, autumn, and winter (from November 2005 to September 2006).

In each shallow lake, temperature, pH, dissolved oxygen (DO), and conductivity were measured with HI 8314 and HI 8033 Hanna portable instruments. The water transparency was estimated with Secchi disk. The vertical diffuse photosynthetic active radiation (PAR) attenuation coefficients (Kd_{PAR}) were studied in order to characterize the underwater light climate in each of the six shallow lakes and seasons. We used an underwater turbidimeter (SCUFA, Turner™) and a spectrum-submersible radiometer (USB2000, Ocean Optics), during

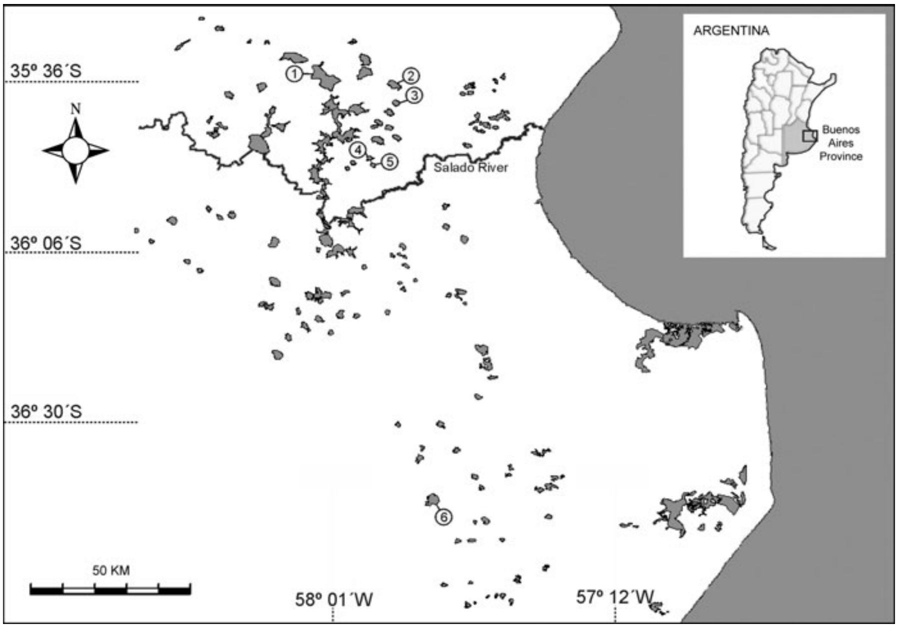

Fig. 1 Location of the studied shallow lakes in the Pampa Plain (Buenos Aires Province, Argentina). Chascomús (*1*); La Limpia (*2*); San Jorge (*3*); Lacombe (*4*); El Triunfo (*5*); Kakel Huincul (*6*)

Table 1 Geographic position, main morphometric features and states of the shallow lakes (data obtained from Silvoso et al. (2011)

	Kakel Huincul	El Triunfo	San Jorge	Chascomús	La Limpia	Lacombe
Geographic position	36°48′S; 57°47′W	35°51′S; 57°52′W	35°40′S; 57°47′W	35°36′S; 58°02′W	35°37′S; 57°48′W	35°49′S; 57°49′W
Surface area (km^2)	29.5	1.5	3.0	28.7	5.6	1.6
Max. depth (m)	4.0	nd	nd	1.9	2.3	2.0
Mean depth (m)	1.8	nd	nd	1.5	1.9	1.0
States	Clear-vegetated	Clear-vegetated	Phytoplankton-turbid	Phytoplankton-turbid	Inorganic-turbid	With alternative steady states

the annual period (2005–2006). The techniques used were described in detail in Pérez et al. (2010).

Samples for the determination of total suspended solids (TSS), percentage of organic matter in seston (%OM), total phosphorous (TP), and total nitrogen (TN) were collected subsuperficially (about 30 cm depth). All determinations were performed following the protocols given in APHA AWWA WEF (2005). Aliquots of filtered water (Whatman GF/F™) were acidified (pH 2) and stored at 4°C until analysis of dissolved organic carbon (DOC). DOC was determined using a high temperature Pt catalyst oxidation method (Shimadzu TOC-5000) following the recommendations of Sharp et al. (1993).

Phytoplankton samples were also collected subsuperficially at each of the three stations of the shallow lakes. Chlorophyll *a* (Chl *a*) concentration was estimated from triplicate samples filtered through glass-fiber filters (GF/F, Whatman™) by ion pairing reverse-phase HPLC, modified from Mantoura & Llewellyn (1983) and Hurley (1988), using an Äkta-basic chromatograph (Amersham™) controlled by the program Unicorn™ (C18 Phenomenex™; 5 µm particle size; 250 mm × 4.6 mm i.d.). The method applied has been described in detail by Laurion et al. (2002).

Quantitative phytoplankton samples were fixed with 1% acidified Lugol's iodine solution. Phytoplankton counts were performed using a Zeiss™ inverted microscope (Utermöhl, 1958) at ×400 magnification, and the counting error was estimated according to Venrick (1978). In all cases, we considered the individual algae as the unit (unicell, colony, coenobium, or filament), and we estimated cell numbers per colony or filament. Individual biovolumes were calculated using appropriate geometric formulae according to their shapes and the mean dimensions of the organisms in the samples (Hillebrand et al., 1999; Sun & Liu, 2003). For colonial organisms, calculations were made for the whole colony including mucilage. Biomass was estimated from biovolume, assuming unit specific gravity.

On the other hand, all the species recorded in the samples were classified into the functional groups proposed in the classifications of Reynolds et al. (2002)—reviewed by Padisák et al. (2009), Salmaso & Padisák (2007), and Kruk et al. (2010); the biomass corresponding to each functional group were also calculated for the numerical analyses.

Multivariate analyses

Redundancy analyses (RDA) were used to estimate how much variance of the biomass of the phytoplankton functional groups (Reynolds et al., 2002; Salmaso & Padisák, 2007; Kruk et al., 2010) was explained by the environmental variables. Previously, we preformed a detrended correspondence analyses (DCA), and as the data showed a linear response we applied RDA. Calculations were performed with the program CANOCO (ter Braak, 1988). The analysis was based on field data, on the biomass of the functional groups and on the environmental variables corresponding to each shallow lake and date. Species with a contribution of <5% of the total community biomass in any individual lake were excluded in this analysis. The statistical significance of the first axis and of all the axes was tested by a Monte Carlo permutation test. The importance of each variable was assessed using forward selection.

Results

Limnological variables

The contrasting features of the studied shallow lakes are indicated in Table 2. Clear-vegetated shallow lakes (El Triunfo and Kakel Huincul) showed the lowest Kd_{PAR} values (mean 5.2 m^{-1}) and TSS (mean 4.1 mg l^{-1}). The highest Kd_{PAR} and suspended solids concentrations were observed in the inorganic-turbid shallow lake La Limpia, with mean values of 36.9 m^{-1} and 256.5 mg l^{-1}, respectively. The phytoplankton-turbid shallow lakes (Chascomús and San Jorge) were also characterized by high Kd_{PAR} values (mean 23.2 m^{-1}) and high TSS concentrations (mean 126.4 mg l^{-1}). The shallow lake Lacombe, which as it was mentioned exhibits periodic alternative steady states (clear and turbid), presented intermediate values of Kd_{PAR} (mean 9.2 m^{-1}) and TSS (mean 48.2 mg l^{-1}); in relation to this shallow lake it is important to point out that during the year of our study the system was in a turbid state with high phytoplankton abundance.

All the studied shallow lakes are eutrophic, as it is expressed from the relatively high concentrations of TP and TN registered. Nevertheless, the lowest values were measured in the clear-vegetated lakes, whereas the highest ones in the turbid systems. For all studied lakes TP ranged from 0.05 to 0.87 mg l^{-1} and TN from 0.26 to 1.82 mg l^{-1}.

Due to the characteristics of the catchments, which contain sedimentary rocks rich in sodium and carbonates, the waters of the lakes in this region are alkaline (mean pH varied from 8.6 to 8.9), and conductivities are also relatively high (mean values ranged from 0.95 to 2.27 mS cm^{-1}). The concentrations of DOC were rather high in all the lakes, registering the maximum values in the clear-vegetated water bodies (mean 49.7 mg l^{-1}) and the minimum ones in the inorganic-turbid lake (mean 21.3 mg l^{-1}). All the shallow lakes exhibited well-oxygenated waters, with mean concentrations ranging between 8.3 and 9.3 mg l^{-1}.

The highest values of phytoplankton Chl *a* were recorded in the phytoplankton-turbid lakes (mean value 205.8 μg l^{-1}), and the lowest one in the clear-vegetated lakes (mean value 5.1 μg l^{-1}). Intermediate values were registered in the inorganic-turbid shallow lakes La Limpia and Lacombe (mean values 28.5 and 41.2 μg l^{-1}, respectively).

Phytoplankton community structure

The six water bodies showed strong differences in total phytoplankton biomass (Fig. 2). As expected, the

Table 2 Ranges of the main limnological features for the six shallow lakes studied in the Pampa Plain

	Kakel Huincul	El Triunfo	San Jorge	Chascomús	La Limpia	Lacombe
State	Clear-vegetated	Clear-vegetated	Phytoplankton-turbid	Phytoplankton-turbid	Inorganic-turbid	With alternative steady states
Kd_{PAR} (m^{-1})	3.4–3.7 (3.6; SD 0.13)	3.4–11 (6.9; SD 3.1)	11.5–23.4 (17.2; SD 4.9)	16.6–35.8 (29.2; SD 8.6)	21.1–49.8 (36.9; SD 12.9)	5.2–13.5 (9.2; SD 2.9)
Suspended solids (mg l^{-1})	1.6–12 (5.4; SD 4.7)	1.0–4.8 (2.2; SD 1.8)	55–98 (73.7; SD 18.7)	70–265 (165; SD 94.2)	129–351 (256.5; SD 101.9)	25.5–69 (48.2; SD 20.8)
Dissolved oxygen (mg l^{-1})	8.0–10.6 (9.4; SD 1.1)	7.6–11.9 (9.2; SD 1.9)	8.4–9.4 (8.9; SD 0.5)	8.2–11.0 (9.5; SD 1.2)	7.8–8.6 (8.3; SD 0.32)	7.8–9.8 (8.9; SD 0.88
pH	7.3–9.2 (8.4; SD 0.79)	8.4–9.4 (9.1; SD 0.5)	8.3–9.1 (8.8; SD 0.34)	8.4–9.1 (8.8; SD 0.30)	7.8–9.1 (8.6; SD 0.55)	8.3–9.2 (8.9; SD 0.36)
Conductivity (mS cm^{-1})	1.54–2.19 (1.8; SD 0.28)	1.32–1.87 (1.62; SD 0.23)	1.34–1.51 (1.41; SD 0.08)	1.58–1.80 (1.72; SD 0.09)	0.82–1.13 (0.95; SD 0.13)	2.14–2.5 (2.27; SD 0.14)
TN (mg l^{-1})	0.30–1.14 (0.62; SD 0.4)	0.23–0.69 (0.39; SD 0.21)	0.95–1.72 (1.43; SD 0.35)	0.30–1.82 (1.11; SD 0.75)	0.72–0.97 (0.82; SD 0.11)	0.26–1.05 (0.74; SD 0.30)
TP (mg l^{-1})	0.05–0.28 (0.13; SD 0.11	0.07–0.25 (0.17; SD 0.08)	0.23–0.34 (0.30; SD 0.05)	0.41–0.70 (0.60; SD 0.13)	0.62–0.87 (0.75; SD 0.11)	0.11–0.29 (0.21; SD 0.07)
DOC (mg l^{-1})	38.7–82.4 (58.5; SD 18.1)	37.3–49.8 (40.9; SD 5.9)	42.1–60 (48.9; SD 8.6)	17.6–47.2 (28.2; SD 13.2)	13.7–40.3 (21.3; SD 12.7)	31.0–67.4 (41.6; SD 15.0)
Chlorophyll *a* (μg l^{-1})	1.6–7.7 (3.9; SD 2.8)	2.7–5.5 (3.8; SD 1.3)	36–334.6 (207.7; SD 125.9)	50.2–135.4 (101.1; SD 38.2)	18.3–35.4 (28.5; SD 7.3)	20.6–63.2 (41.6; SD 21.3)

Mean values and standard deviations (SD) are indicated between brackets

Kd_{PAR} vertical diffuse photosynthetic active radiation attenuation coefficient, *TN* total nitrogen, *TP* total phosphorus, *DOC* dissolved organic carbon, *nd* no data highest biomasses were recorded in the two phytoplankton-turbid shallow lakes. Of these two lakes, San Jorge exhibited the highest values (217–1,730 mg l^{-1}), whereas in Chascomús they ranged between 146 and 232 mg l^{-1}. As the shallow lake Lacombe was in a turbid state during the year of this study, phytoplankton biomass was also very high, ranging from 56 to 1,620 mg l^{-1}.

In all four visits, the inorganic-turbid shallow lake (La Limpia) showed the lowest values of algal biomass, varying from 2.85 to 12.40 mg l^{-1}.

The two clear-vegetated lakes also presented low phytoplankton biomass. Values varied from 2.65 to 19.00 mg l^{-1} in the shallow lake El Triunfo. In Kakel Huincul, although the algal biomass was generally low, a peak was registered in summer; values in this lake varied from 0.59 to 273.00 mg l^{-1}.

The differences among the lakes were also reflected in the proportion of the algal groups (Fig. 3). In clear-vegetated lakes, total phytoplankton biomass was distributed in different groups, being the most representative Cryptophyceae, Chlorophyceae, Cyanobacteria, and Bacillariophyceae. In the phytoplankton-turbid shallow lakes, the dominant groups were Cyanobacteria, Chlorophyceae, and Bacillariophyceae, and the relative proportion of the algal groups was relatively stable in the different seasons. Cyanobacteria represented more than 90% of the total phytoplankton biomass in San Jorge. In the inorganic-turbid shallow lake, the dominant algal groups were Chlorophyceae and Bacillariophyceae. The shallow lake Lacombe was mainly dominated by Chlorophyceae and Cyanobacteria.

We identified a total of 184 phytoplankton species in the six shallow lakes. The main taxa recorded in each type of water body are listed in Table 3. In clear-vegetated lakes, different unicellular cryptophytes, chrysophytes, and chlorophytes were generally very abundant, together with small colonial cyanobacteria and colonial chlorophytes as accompanying species. In the turbid lake San Jorge, the phytoplankton community was almost completely dominated by the species cf. *Raphidiopsis mediterranea* in all sampling dates, which constituted a persistent algal bloom. In the other phytoplankton-turbid lake (Chascomús), the community was dominated by several unicellular and coenobial chlorophytes together with the diatom *Synedra berolinensis*; colonial cyanobacteria (mainly *Aphanocapsa delicatissima*) and colonial chlorophytes (mainly *Oocystis lacustris*) were less numerous but contributed considerably to the biomass. The inorganic-turbid lake La Limpia showed a higher contribution of filamentous diatoms (*Aulacoseira* spp.) and desmids (*Closterium aciculare*), and other taxa belonging to Chlorophyceae were also well represented in some seasons. In lake Lacombe, some small chlorophytes and filamentous cyanobacteria (mainly *Planktolyngbya limnetica*) were numerically abundant in certain seasons, whereas colonial cyanobacteria (*Cyanodictium imperfectum* and *A. delicatissima*) and several colonial chlorophytes were dominant in biomass in all sampling dates. The main morpho-functional groups according to the three classifications applied in this paper, are summarized in Table 4.

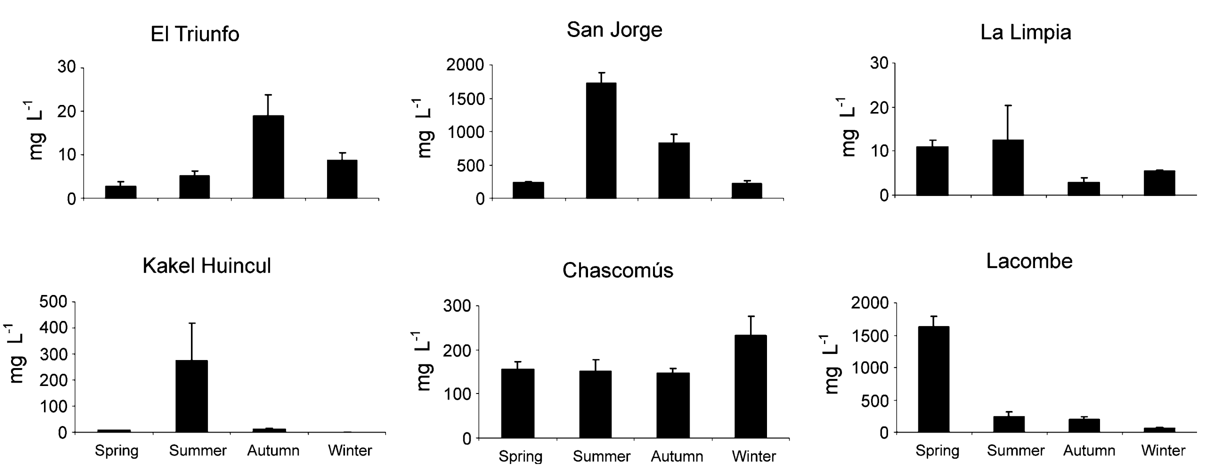

Fig. 2 Seasonal variation of total phytoplankton biomass in each shallow lake during the study period (*bars* standard deviation)

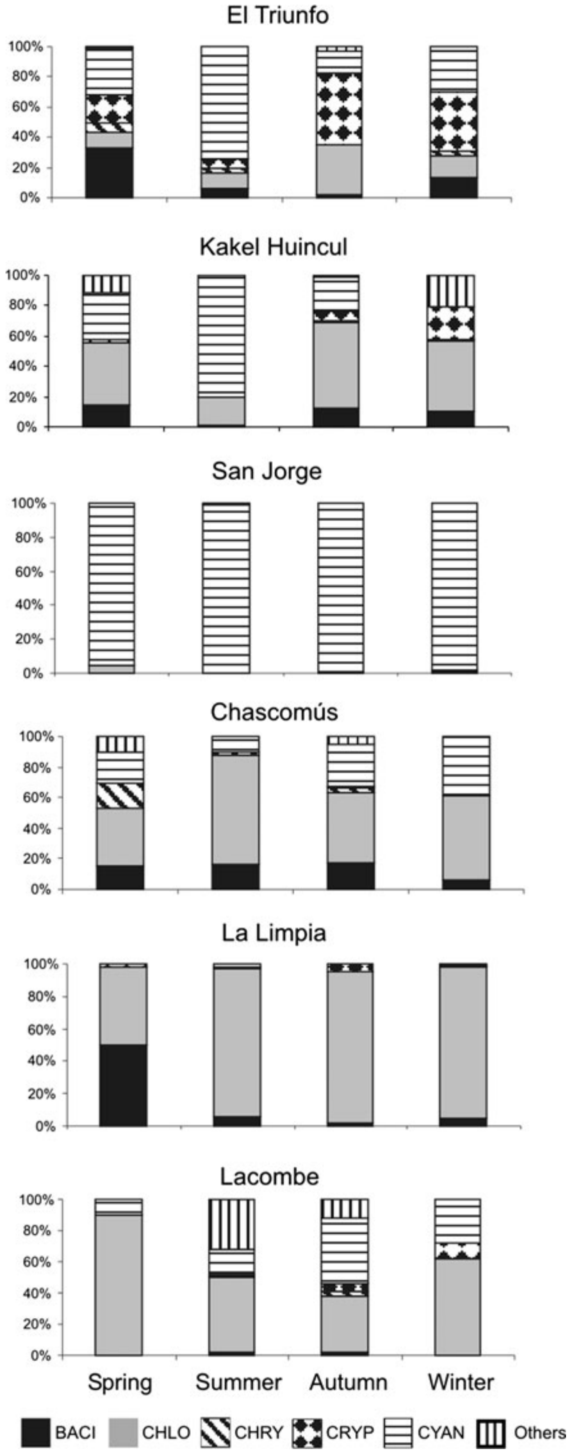

Fig. 3 Seasonal variation of the relative proportion of the different algal groups (in biomass) in the shallow lakes during the study period. Bacillariophyceae (*BACI*), Chlorophyceae (*CHLO*), Chrysophyceae (*CHRY*), Cryptophyceae (*CRYP*), Cyanobacteria (*CYAN*)

Multivariate analyses

In the redundancy analysis (RDA) based on the functional groups proposed by Reynolds et al. (2002), the first two axes accounted for 70.1% of the variance (axis 1: 39.9%; axis 2: 30.2%). The Monte Carlo test indicated that the environmental variables were significantly correlated with the first axis ($P = 0.012$) and the test of significance of all canonical axes was also significant ($P = 0.002$). The first axis was mainly correlated with Kd_{PAR}, TP, and DOC (intra-set correlation coefficients: 0.80, 0.71, and −0.62, respectively), and the second axis was mainly defined by conductivity (intra-set correlation coefficient: −0.61). Figure 4 shows the biplots (first two axes) of the shallow lakes (a) and the Reynold's groups (b) with respect to the environmental variables.

The results of the RDA based on the functional groups proposed by Salmaso & Padisák (2007) are shown in Fig. 5a, b. In this case, the total variance explained by the two first axes was 68.60% (axis 1: 40.90%; axis 2: 27.70%). The environmental variables were significantly correlated with the first axis ($P = 0.01$) and the test for all canonical axes was also significant ($P = 0.004$). In this analysis, the first axis was also mainly defined by a combination of TP, Kd_{PAR}, and DOC (intra-set correlation coefficients: 0.70, 0.67, and −0.57, respectively), and the second axis by conductivity (intra-set correlation coefficient: −0.66).

In the case of the RDA carried out with the morphologically based functional groups: MBFG (Kruk et al., 2010), the percentage of explained variance was 76% (axis 1: 46.60%; axis 2: 29.40%). The biplots corresponding to this analysis are shown in Fig. 6a, b. Monte Carlo test was significant for the first axis ($P = 0.012$) and for all canonical axes ($P = 0.006$). The first axis was correlated with conductivity and TN (intra-set correlation coefficients: −0.52 and 0.53, respectively), whereas the second axis was mainly defined by TP and Kd_{PAR} (intra-set correlation coefficients: 0.66 and 0.58, respectively).

The results of these analyses indicated that the biomass of the phytoplankton groups corresponding to the three classification approaches can be well predicted from the environmental variables. In all the ordinations, the turbid lakes are plotted separately from the clear-vegetated lakes. The samples of

Table 3 Dominant and frequent phytoplankton species in the studied shallow lakes from the Pampa Plain

Clear-vegetated shallow lakes	Phytoplankton-turbid shallow lakes	Inorganic-turbid shallow lakes	Shallow lake with alternative steady states
Cryptomonas marsonii	cf. *Raphidiopsis mediterranea*	*Aulacoseira granulata*	*Monoraphidium griffithii*
Cryptomonas erosa	*Aphanocapsa delicatissima*	*Aulacoseira granulata* var. *angustissima*	*Monoraphidium subclavatum*
Cryptomonas ovata	*Monoraphidium circinale*	*Closterium aciculare*	*Plagioselmis* sp.
Plagioselmis spp.	*Monoraphidium contortum*	*Coelastrum microporum*	*Aphanocapsa delicatissima*
Chlamydomonas spp.	*Monoraphidium griffithii*	*Pediastrum mustersii*	*Cyanodictium imperfectum*
Ochromonas spp.	*Monoraphidium minutum*	*Monoraphidium circinale*	*Bothryococcus braunii*
Monoraphidium circinale	Scenedesmus quadricauda	*Monoraphidium minutum*	*Oocystis marsonii*
Monoraphidium griffithii	*Tetrastrum staurogeniaeforme*	*Monoraphidium tortile*	*Oocystis nephrocystioides*
Monoraphidium komarkovae	*Oocystis lacustris*	*Monoraphidium obtusum*	*Planktolyngbya limnetica*
Aphanocapsa delicatissima	*Synedra berolinensis*	*Schroederia antillarum*	
Snowella lacustris		*Schroederia indica*	
Chroococcus minutus		*Schroederia setigera*	
Oocystis lacustris		*Cryptomonas marsonii*	
Bothryococcus braunii		*Plagioselmis* sp.	
Sphaerocystis schroeterii			

Table 4 Main morpho-functional groups according the three classifications used for to the different studied shallow lakes in the Pampa Plain

	Morpho-functional groups (Reynolds et al., 2002; Padisák et al., 2009)	Morphologically based functional groups (Kruk et al., 2010)	Morpho-functional groups (Salmaso & Padisák, 2007)
Clear-vegetated shallow lakes	X1, X2, Y, L0, K, F	V, IV, VII	2a, 2d, 5c, 5d, 11b
Phytoplankton-turbid shallow lakes	Sn, X1, K, F, D, J	III, IV, VI, VII	5c, 5d, 5e, 6b, 11a, 11b
Inorganic-turbid shallow lakes	P, X1, X2, Y, J	IV, V, VI	5c, 6b, 8a, 11a, 11b
Shallow lake with alternative steady states (in turbid state in this study)	X1, X2, S1, F, K	III, IV, V, VII	2a, 2d, 3a, 5c, 5d, 5e, 9b, 11b

Lacombe lake, that alternates between clear and turbid states, ordinated at a middle position, near the center of the graph or even closer to the samples of the clear-vegetated lakes. The optical conditions of the water bodies (expressed by the Kd_{PAR} values), together with conductivity and TP, seem to be the more important variables determining the algal groups. Nevertheless, the forward selection procedure showed that Kd_{PAR} was not significant in the analysis based on the MBFG of Kruk et al. (2010), whereas TP was not significant in the analysis based on the Reynold′s classification.

Discussion

As a consequence of the human activities, most shallow lakes located in the more impacted areas of the Pampa Plain have become turbid systems (Quirós et al., 2002, 2006). Once in a turbid state, the lake morphometry, the flatness of the terrain, and the persistence and strength of winds favor mixing and prevent stratification, and probably act as stabilizing factors of the turbid state (Torremorell et al., 2007). In turbid systems, the high light attenuation in the water

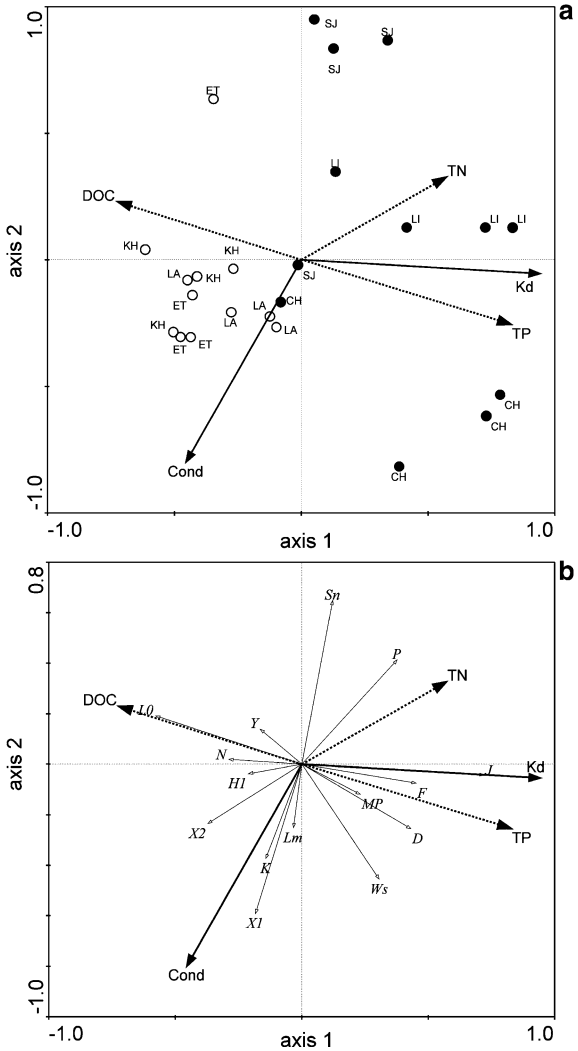

Fig. 4 Biplots of the RDA based on the biomass of the morpho-functional groups proposed by Reynolds et al. (2002). **a** Ordination of the samples and environmental variables (*CH* Chascomús, *SJ* San Jorge, *LI* La Limpia, *LA* Lacombe, *ET* El Triunfo, *KH* Kakel Huincul). **b** Functional groups and environmental variables. Turbid shallow lakes (*black circles*); clear-vegetated shallow lakes and lake with alternative steady states (*white circles*). Significant environmental variables ($P < 0.05$) are indicated with *solid arrows*, while *dotted arrows* are not significant. Conductivity (*Cond*), dissolved organic carbon (*DOC*), vertical attenuation coefficient (*Kd*), total nitrogen (*TN*), total phosphorus (*TP*)

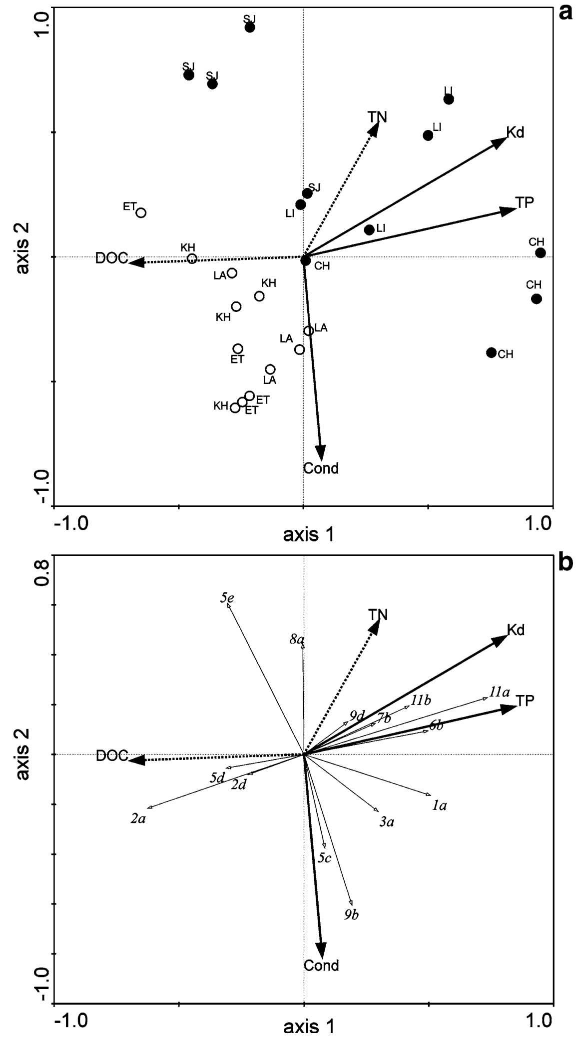

Fig. 5 Biplots of the RDA based on the biomass of the morpho-functional groups proposed by Salmaso & Padisák (2007). **a** Ordination of the samples and environmental variables (*CH* Chascomús, *SJ* San Jorge, *LI* La Limpia, *LA* Lacombe, *ET* El Triunfo, *KH* Kakel Huincul). **b** Functional groups and environmental variables. Turbid shallow lakes (*black circles*); clear-vegetated shallow lakes and lake with alternative steady states (*white circles*). Significant environmental variables ($P < 0.05$) are indicated with *solid arrows*, while *dotted arrows* are not significant. Conductivity (*Cond*), dissolved organic carbon (*DOC*), vertical attenuation coefficient (*Kd*), total nitrogen (*TN*), total phosphorus (*TP*)

column is one of the ecological key factors, and exerts profound effects on phytoplankton. Light availability affects algal competition (Huisman et al., 1999; Reynolds, 2006) and phytoplankton diversity (Reynolds, 1998; Stomp et al., 2004). Light-limiting conditions prevail in Pampean turbid lakes (Llames et al., 2009), and previous investigations have shown that the underwater light climate strongly affects the phytoplankton structure and the primary production in the lakes of this region (Allende et al., 2009). In the

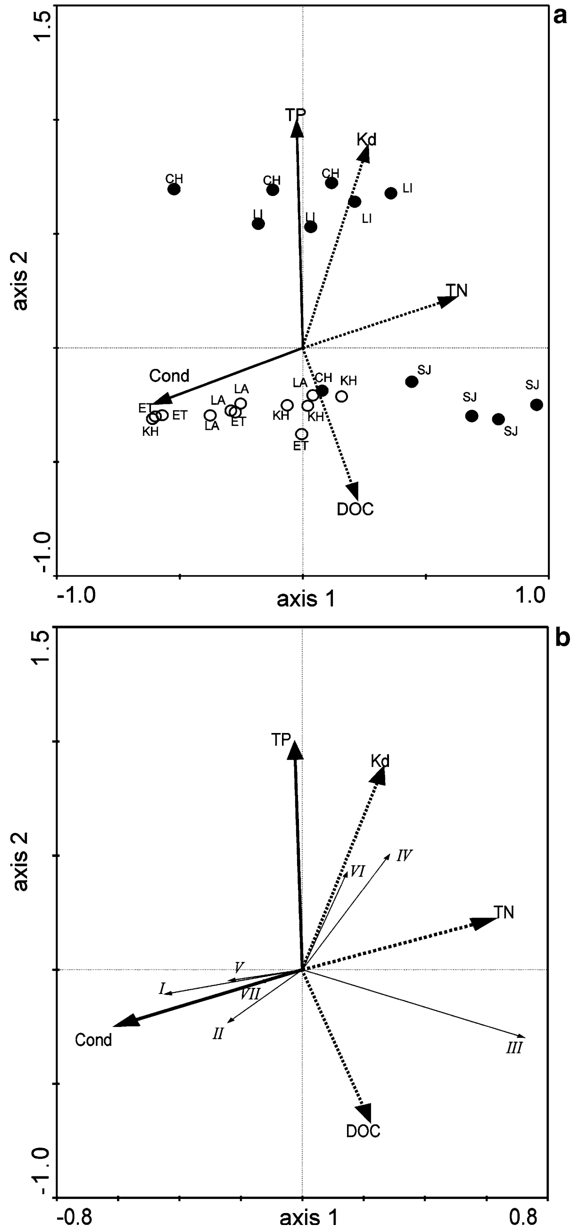

Fig. 6 Biplots of the RDA based on the biomass of the morphological-based functional groups proposed by Kruk et al. (2010). **a** Ordination of the samples and environmental variables (*CH* Chascomús, *SJ* San Jorge, *LI* La Limpia, *LA* Lacombe, *ET* El Triunfo, *KH* Kakel Huincul). **b** Functional groups and environmental variables. Turbid shallow lakes (*black circles*); clear-vegetated shallow lakes and lake with alternative steady states (*white circles*). Significant environmental variables ($P < 0.05$) are indicated with *solid arrows*, while *dotted arrows* are not significant. Conductivity (*Cond*), dissolved organic carbon (*DOC*), vertical attenuation coefficient (*Kd*), total nitrogen (*TN*), total phosphorus (*TP*)

passage of the shallow lakes from a clear-water to a turbid state, changes in phytoplankton community not only entail an increment in the pelagic algal biomass but also changes in algal composition. For example, a study conducted in the Pampa plain in the 1990s (Izaguirre & Vinocur, 1994) reported that almost 50% of the shallow lakes surveyed were in a clear state and these systems were characterized by low phytoplankton abundances, relatively high species richness, and a high proportion of nanoplanktonic species. Our current investigations in the region show that nowadays most lakes are characterized by high phytoplankton biomass, with dominance of one or few species, generally belonging to Cyanobacteria, Bacillariophyceae, and Chlorophyceae.

The Gleasonian line of reasoning assumes that individual species respond independently to the environment (Gleason, 1926), and the community composition reflects the response of individual species to the environmental conditions. As the environmental conditions select groups of species that share similar adaptive characteristics, there is a broad consensus in that communities are more reliable indicators of habitat conditions that are the presence or absence of component species (Naselli-Flores & Barone, 2011 and cites therein), and the morpho-functional phytoplankton approaches tends to simplify the taxonomical approach in environmental biomonitoring. In this sense, the aim of our investigation was to evaluate the strength of different classifications based on morphological and functional characteristics of phytoplankton to discriminate among the different types of shallow lakes.

Our results showed that the biomass of the phytoplankton groups corresponding to the three classification approaches used can be well predicted from the environmental variables. All the analyses showed the separation of the turbid lakes from the clear-vegetated ones.

The RDA performed using the Reynold′s functional classification showed a noticeable separation of the samples in relation to the turbidity of the lakes. The functional groups related with higher Kd_{PAR} values and nutrient concentrations were J, MP, D, and F. Codon J is commonly associated to shallow, mixed, highly enriched systems; codon MP includes meroplanktonic species (mostly diatoms) that are drifted to the plankton living in turbid shallow lakes; codon D includes diatoms that live in shallow turbid waters (Padisák et al., 2009). Codon F is also typical of deeply mixed meso-eutrophic lakes, and although it was described living in clear epilimnia of the lakes, is tolerant to high turbidity (Reynolds et al., 2002). The codon Sn (dominant in the lake San Jorge) is typical of warm mixed environments; it comprises Cyanobacteria tolerant to low light conditions and as they usually have heterocysts, can also tolerate nitrogen deficiency. In the lake San Jorge, this functional group was almost exclusively represented by cf. *Raphidiopsis mediterranea*; it is important to mention that during the period of our studies we have never observed heterocysts in this species, but the system is not N-limited, and as it was discussed by Litchman et al. (2010), the development of heterocysts (the specialized cells where fixation occurs) indicates low nitrogen availability. Thus, we cannot discard that this population may belong to the genus *Cylindrospermopsis*. In the clear-vegetated lakes, the most representative functional groups were: Y mainly represented by cryptomonads; L0, which was described for mesotrophic systems and that seems to be sensitive to prolonged or deep mixing (Reynolds et al., 2002), and that in our lakes was represented by small colonial cyanobacteria; K that includes some small non-gas-vacuolated cyanobacteria typical of shallow, nutrient-rich water columns, and also sensitivity to deep mixing; X2 that comprises some unicellular flagellated species typical of shallow meso-eutrophic environments; X1, a codon described for shallow, eu-hypertrophic environments, mainly represented by small chlorococcaleans (Reynolds et al., 2002; Padisák et al., 2009). The functional groups proposed in the Reynold′s classification provided an appropriate characterization of the shallow lakes of the region, and the main groups observed in each type of lake reflect the main features of the phytoplankton assemblages previously described using complete lists of species (Allende et al., 2009). Particularly, the classification was able to detect the importance of mixotrophic flagellates (Chrysophyceae and Cryptophyceae) and small colonial cyanobacteria in the clear-vegetated lakes that can be explained by their lower sedimentation rates as compared with large non-buoyant cells (Søndergaard & Moss, 1998). One of the disadvantages of this classification is that the criteria for assigning species to groups are not formalized, and it is necessary a deeper knowledge of the autoecology of the species.

The morpho-functional classification proposed by Salmaso & Padisák (2007) constitutes another interesting approach where the criteria adopted to discriminate the groups include the traits proposed by Weithoff (2003) that are valuable for characterizing functional aspects of phytoplankton: motility, the potential capacity to obtain carbon and nutrients by mixotrophy, specific nutrient requirements, size and shape and presence of envelopes. The results of our multivariate analyses showed that three functional groups were prevalent in clear-vegetated lakes with higher DOC concentrations: 2a, 2d, and 5d; the two first include potentially mixotrophic nanoflagellates (Chrysophytes and Cryptophytes, respectively), and the third one comprises small colonial cyanobacteria belonging to Chroococcales. These results were similar to those obtained with the Reynold′s classification, but the dominant components in the assemblages of the clear-vegetated lakes were even more clearly reflected. In the turbid shallow lakes, the morpho-functional groups more related with high Kd_{PAR} values were 6b and 7b (large and small pennate diatoms, respectively); 11a and 11b (naked and gelatinous colonial Chlorococcales, respectively). Other groups, such as 1a (large Chrysophytes) and 3a (unicellular Phytomonadina) were also related with high turbidity. The groups 5e (Nostocales) and 8a (large Conjugatophytes), also abundant in phytoplankton-turbid lakes of the region, were associated to high TN levels.

An obvious requisite to use this classification, as well as that proposed by Reynolds et al. (2002), is that a deeper knowledge of the taxonomy and the functional aspects of phytoplankton are necessary.

The third classification used (Kruk et al., 2010) based on purely morphological traits, offers several advantages. Among them, its objectivity, its independence from taxonomic affiliations and the relative ease of its application to the majority of species for which physiological traits are unknown and are not readily determined. These features make this classification very useful for monitoring of ecosystems. The disadvantages with respect to the other two classifications may be the relatively low sensitivity to detect certain phytoplankton functional aspects that may be relevant in the system. For example, some mixotrophic taxa like

Cryptomonas spp., well represented in clear-vegetated lakes, were included in the same group (V) that other flagellate species that are strictly autotrophic, although these taxa are well represented in the opposite alternative steady state. Kruk et al. (2011) argued that the species in any particular group are basically interchangeable and ecologically equivalent, but this would not be the case in the mentioned example. Nevertheless, in our study, the multivariate analyses based on the MBFG proposed by Kruk et al. (2010) also showed a clear separation of the clear-lakes from the turbid ones. The percentage of explained variance obtained using this classification was higher than for the other two classifications, but this is because with a lower number of groups the total variation in the data set (total inertia) decreases. Contrarily to that observed with the other two classifications, in this analysis, the forward selection procedure indicated that Kd_{PAR} was not significant in the ordination obtained, whereas TP had more importance. The samples of the two turbid shallow lakes (Chascomús and La Limpia) were placed very close to each other, toward higher values of TP. The more representative MBFG in these turbid systems were IV (organisms of medium size lacking specialized traits) and VI (non-flagellated organisms with siliceous exoskeletons = diatoms). In the clear-vegetated lakes, the prevalent groups were I (small organisms with high S/V), II (small flagellates with siliceous exoeskeletal structures), V (represented in the studied systems by unicellular flagellates of medium size), and VII (mucilaginous colonies). The group III (large filamentous with aerotopes) was related with higher values of TN.

With all the classifications used, the results of the RDA showed that the samples of the phytoplankton-turbid lake Chascomús plotted closer to the samples of La Limpia (inorganic-turbid) than to the samples of the other phytoplankton-turbid lake (San Jorge). These results evidence the great importance of the light attenuation on the phytoplankton assemblages, as these two lakes are the most turbid among the studied systems. Investigations conducted in Chascomús, perhaps the most extensively studied Pampean shallow lake, indicate that the system has stabilized in a turbid state (Llames et al., 2009), and the results obtained by Torremorell et al. (2009) are consistent with a theoretical expectations based on a light limitation scenario.

Interestingly, in the two algal-turbid lakes, the dominant algal classes and the main functional groups were relatively constant throughout the study period. Other studies carried out in Chascomús have reported similar results. In particular, Torremorell et al. (2009) observed that the phytoplankton assemblage did not displayed significant variability in species composition during an annual period, and suggested that the seasonal trend in photosynthesis photoinhibition could have resulted from physiological adaptations of the cells. These observations seem to indicate that the phytoplankton assemblages of these turbid shallow lakes are in a steady state, and probably the stress due to a high light constraint would be the key factor in the selection of species well adapted to such conditions. Different investigations have shown that steady-state conditions occur regularly in shallow lakes and the stress factors may be one of the causes involved (Naselli-Flores et al., 2003 and cites therein).

Our results showed that in general the morpho-functional classifications constitute a good approach to analyze the differences among the shallow lakes of the Pampa plain. Although taxonomy cannot be replaced by a morpho-functional classification since the unequivocal link between any species and its traits is the basis for a correct inclusion of species into functional groups (Padisák et al., 2009), this approach can be very valuable in the environmental biomonitoring. In particular, the morphological-based classification proposed by Kruk et al. (2010) may be useful for long-term monitoring of aquatic systems or in the comparison of a great number of lakes, due to its relatively simplicity and objectivity. Nevertheless, in our study, the analysis based on this classification underestimated the importance of the light climate, one of the main regulator factors of the phytoplankton communities in the shallow lakes of this region, and it was not very sensitive to detect the importance of certain species in the clear-vegetated lakes (particularly some mixotrophic taxa). The functional classification proposed by Reynolds et al. (2002) provides more information, and allows a more detailed description of the algal assemblages. However, although the revision performed by Padisák et al. (2009) clarified many misplacements and modified some of the original habitat templates facilitating its application, this classification requires a deeper knowledge of the autoecology of single species or species groups.

The classification of Salmaso & Padisák (2007) reflected the differences in the algal assemblages among the systems, is also relatively simple, and in our study highlighted the importance of the variables that define the different steady states in the region. Thus, in this regional context, this classification seemed to be particularly sensitive and appropriate for monitoring the aquatic systems of the region.

All the classifications applied resulted adequate to separate the more deteriorated systems (turbid lakes) from the clear-vegetated ones, which are comparatively less impacted, concluding that the functional approach is an adequate tool to analyze the phytoplankton assemblages in human-impacted lakes.

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