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ARGENTINE PAMPEAN SHALLOW LAKES

Long-term study of bloom-forming cyanobacteria in a highly fluctuating vegetated floodplain lake: a morpho-functional approach

**Inés O'Farrell · Alicia Vinocur ·
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Abstract This study portrays the frequency and intensity of bloom-forming cyanobacteria in relation to highly changing environmental conditions in a floodplain lake from the Chaco-Pampean region and identifies the factors promoting the growth of different species along a 15-year period marked by important drought and flood periods. Phytoplankton in Laguna Grande was increasingly dominated by cyanobacteria represented by eight bloom-forming species bearing different eco-physiological traits. The bloom development showed strong inter-annual variability in correspondence with the depth of the lake that was affected by local rainfall and the ENSO regional phenomenon. The water level of the lake strongly influenced the species composition, favoring non-heterocystous filaments in deep turbid waters and nitrogen fixing in very short well lit, nitrogen-depleted water columns. Species morphology, dimensions and occurrence of akinetes in the filaments also varied in relation to water level. We suggest that the akinete pool of the sediments played a key role on the occurrence of recurrent *Anabaenopsis* cf. *elenkinii* and *Sphaerospermopsis* cf. *aphanizonemoides* blooms.

Keywords Cyanobacteria · Blooms · Floodplain shallow lake · Morpho-functional traits · Pampean plain

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Introduction

Cyanobacterial bloom-forming species are increasingly getting established in shallow lakes over the world (Scheffer et al., 1997; Dokulil & Teubner, 2000; Downing et al., 2001; Chen et al., 2003; Nöges et al., 2008; Orihel et al., 2012). Though several separate causes are recognized to promote their massive development, there is increasing evidence of a synergistic interaction among an array of environmental drivers that promote cyanobacterial blooms. Paerl & Paul (2012) concluded that anthropogenic nutrient loading, rising temperatures, enhanced vertical stratification and increased atmospheric CO_2 favor cyanobacterial dominance in a wide range of aquatic ecosystems. This group has a shared reputation of ubiquity and rather generalized requirements, but in reality, individual species need quite specific conditions to establish

themselves (Reynolds, 1997). This explains why cyanobacteria may still dominate in a lake when conditions change, as revealed by replacement of species along the growing season or even over multi-year periods (Havens et al., 1998; Nixdorf et al., 2003; Ferber et al., 2004; Chu et al., 2007; Moustaka-Gouni et al., 2007; Yamamoto & Nakahara, 2009).

Several physiological characteristics act in concert to allow the dominance of different taxa under fluctuating conditions (Carey et al., 2012). These features are mostly related to nitrogen fixation in heterocytes, buoyancy regulation by means of aerotopes, enhanced ability to store phosphorus, high growth rates in warmer temperatures, efficient light capture over a wide range of wave lengths, survival during adverse conditions because of akinete development, and superior uptake kinetics for inorganic carbon (Dokulil & Teubner, 2000; Litchman et al., 2010). As the nature of resource limitation changes, one or more of these highly adaptable eco-physiological traits will place cyanobacteria in a highly competitive position.

The aim of this study was to portray the frequency and intensity of cyanobacterial blooms over a 15-year period in a vegetated floodplain lake located in the Chaco-Pampean region, in relation to changing environmental conditions. We aimed to identify which factors promoted the growth of different bloom-forming cyanobacteria species. In this lake, the availability of light and dissolved nitrogen, ultimately related to water level changes, was indicated as the main factors driving phytoplankton changes (Izaguirre et al., 2004; de Tezanos Pinto et al., 2007; O'Farrell et al., 2009, 2011). We hypothesize that in this highly fluctuating and enriched vegetated system, the changing nature of resource limitation (light and nitrogen) favors the bloom of different cyanobacterial species or of species bearing distinct eco-physiological traits. Also and in particular for Nostocales, we hypothesize that the akinete pool in the sediments of Laguna Grande plays a key role on the formation of blooms in the water column.

Materials and methods

Study area

Laguna Grande is the main water body of the Otamendi Natural Reserve, a Ramsar floodplain wetland that is delimited by the Paraná de las Palmas and Luján Rivers (34°10′−34°17′S, 58°48′−58°53′W, Buenos Aires Province, Argentina) in the Chaco-Pampean region. This shallow lake is included in the network project (PAMPA[2]) that encompasses several water bodies across a humidity gradient in the Pampean plain. This area is periodically flooded by rainfall and is affected by a high and fluctuating water table that is fed by the river pulse during high water periods, thus increasing the lake water level by underground connection (http://atlasdebuenosaires. gov.ar; Silva Busso & Santa Cruz, 2005). Laguna Grande is a eutrophic and vegetated shallow lake with an approximately 156 ha and a maximum depth of 1 m. The region has a temperate climate with hot summers and lacks a dry season sensu Koppen-Geiger (Peel & Huisman, 2009). Mean annual temperature varies between 16.7 and 18°C; rain occurs through all the year averaging 950 mm.

Field data set

This study comprises 83 sub-superficial water samples corresponding to surveys performed from spring 1998 through winter 2013 and collected at the limnetic area of the Laguna Grande, where no emergent vegetation develops. The National Meteorological Service (SMN) and the National Institute of Agriculture Technology (INTA Delta) provided the daily temperature and rainfall data. The Oceanic Niño Index (ONI) for each sampling occasion corresponded to the last month of the trimester as retrieved from http:// www.cpc.noaa.gov/products/analysis_monitoring/ ensostuff/ensoyears.shtml.

Temperature, pH, conductivity and dissolved oxygen were measured with HANNA® and HORIBA® portable electronic meters. The dissolved fraction of nutrients and chlorophyll *a* concentrations were measured after sample filtration through fiberglass filters (Whatman® GF/F). Phosphate and nitrate were determined by ionic chromatography from 1998 to 2001; from 2003 onwards, phosphate and nitrate concentrations were analyzed using HACH® reagents by the stannous chloride and the cadmium reduction methods, respectively. Ammonium was evaluated by the phenate method (APHA, 2005), total phosphorus and nitrogen after alkaline digestion (Koroleff, 1983). Suspended solids were assessed after drying the nonfilterable residue until constant weight at 105°C

(APHA, 2005). Chlorophyll *a* concentrations were determined by spectrophotometry before and after acidification using hot ethanol (Nusch, 1980) and calculated with Lorenzen (1967) equations.

Akinetes were monthly monitored on five occasions throughout the growing season from November to March 2010–2011, from areas where cyanobacteria usually bloom. Sediments were sampled with a Lankford tube; the cores were sealed, maintained in cold and transported in upright position to the laboratory. The upper 2 cm were used for akinete quantification. A known amount of wet sediment was weighted, then diluted in a known volume of distilled water and fixed with lugol solution. Potentially viable akinetes (complete cell wall and with granular content) were quantified under light microscope in a Palmer Maloney chamber, based on their shape and dimensions.

Phytoplankton and cyanobacteria morphological analysis

The phytoplankton quantitative analysis was performed on samples fixed with acid Lugol iodine solution, and species were counted using an inverted microscope according to Utermöhl (1958). The bloom-forming cyanoprokaryota species were identified following Komarék & Anagnostidis (1999, 2005) and Kómarek (2013); their biovolumes were estimated following Hillebrand et al. (1999) and Jun & Dongyan (2003) on the basis of enumeration of (i) single cells for coiled filamentous species, (ii) on filaments for filamentous straight species and (iii) single cells of an area of the colony. For those species that produced several blooms along the study period, we analyzed its morphological traits using a light microscope (400x) with a digital camera and Lumenera Infinity 2 Analyze® package. From up to 7 samples that correspond to the most recent bloom events (since 2005), the following traits were measured for sixty filaments or colonies of each species: maximum linear dimension (MLD), diameter of the spiral (in coiled species), width and length of the vegetative cell, and number of akinetes. For the four most frequent blooming species, we ran Kruskall–Wallis's nonparametric test for independent samples—using the INFOSTAT package—to test whether there were temporal differences in the morphological traits.

Multivariate analysis

Pairwise Pearson's correlations were performed between the ONI index, depth of the lake, limnological features, total cyanobacteria and potentially toxic bloom-forming species abundance of the entire data set (81 samples over 15 years). Canonical correspondence analysis based on raw data was used to elucidate the relationships between the biovolume of the bloom-forming cyanobacterial species and environmental variables for the period sampled with a high frequency (a subset data from October 2008 to July 2013). Significance of the canonical axes was tested using Monte Carlo permutation of samples. A unimodal response model was selected by analyzing the length of gradient in a detrended correspondence analysis (DCA) (ter Braak & Smilauer, 2002).

Results

The minimum and maximum daily air temperature pattern shows distinct winter and summer seasons along the 15-year study period, with extreme values of −5 and 39°C on June 23, 2002 and December 26, 1999, respectively (Fig. 1a). All winters registered at least some days with temperatures below zero with the exception of the year 1998 in coincidence with the strongest Niño event registered, which is shown by the ONI index (Fig. 1a, b). The ONI index reveals the highest values at the beginning of the study period (1998) and also relatively strong Niño events by mid-summers of 2003 and 2010; opposite scenarios were observed during 1999–2000, 2008 and 2011, coinciding with scarce rainfall (Fig. 1b). Mean annual rainfall varied from 588 in 2008 to 1537 mm in 2001 (Fig. 1b). The lake depth and ONI index are directly related ($r = 0.42$, $n = 81$, $P < 0.000$) indicating deeper water columns during strong positive anomalies. When using the ONI values corresponding to 6 months previous to the sampling date, the correlation with lake depth scales up ($r = 0.47$, $n = 81$, $P < 0.000$).

Table 1 shows the main limnological characteristics, which evidence strong fluctuations over the years at the open water area of Laguna Grande shallow lake. The lake depth, which varied from 4 to 113 cm (Table 1), negatively influenced conductivity ($r = -0.65$, $n = 81$, $P < 0.000$) and suspended solids
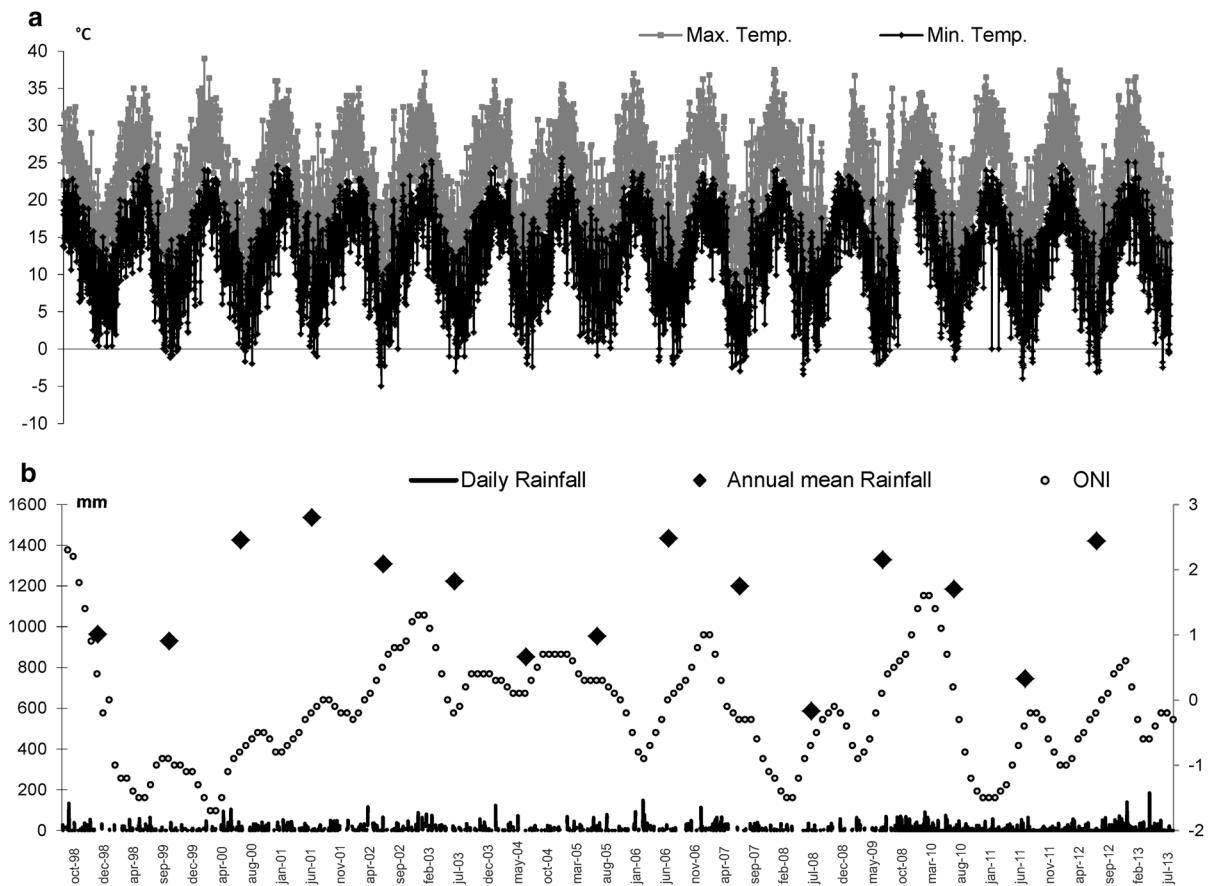
Fig. 1 Variation of meteorological characteristics in the study area from January 1998 to July 2013: **a** daily minimum and maximum air temperature, **b** monthly Oceanic Niño Index (ONI) and daily and mean annual rainfall (lower panel)

Table 1 Descriptive statistics of the main limnological features of the Laguna Grande corresponding to the 15-year period analyzed, 1998–2013

	Minimum	Maximum	Mean	Standard deviation	N
Lake depth (cm)	3.5	113	60.5	24.7	81
Temperature (°C)	5.8	31	20.5	6.5	83
Suspended solids (mg l^{-1})	0.6	266	45.4	49.5	61
Conductivity (µS cm^{-1})	362	7120	2041.9	1315.5	83
Dissolved oxygen (mg l^{-1})	0	19.4	7	4.3	80
pH	5	10	7.9	1.0	83
N-NH$_4$ (mg l^{-1})	0	1.9	0.15	0.3	76
N-NO$_3$ (mg l^{-1})	0	0.7	0.06	0.1	74
P-PO$_4$ (mg l^{-1})	0	2.2	0.43	0.4	81
Total N (mg l^{-1})	0.01	6.2	5.57	12.3	50
Total P (mg l^{-1})	0.01	4.8	1	1.0	55
Chlorophyll a (µg l^{-1})	0	>600	121.7	194.4	80

concentration ($r = -0.43$, $n = 59$, $P < 0.001$) and water color ($r = -0.46$, $n = 52$, $P < 0.000$). Dissolved nutrients ranged from non-detectable to very

high concentrations (1.9 and 2.2 mg l^{-1} of dissolved inorganic nitrogen –DIN– and P–PO$_4$, respectively) (Table 1), but there was no significant relationship
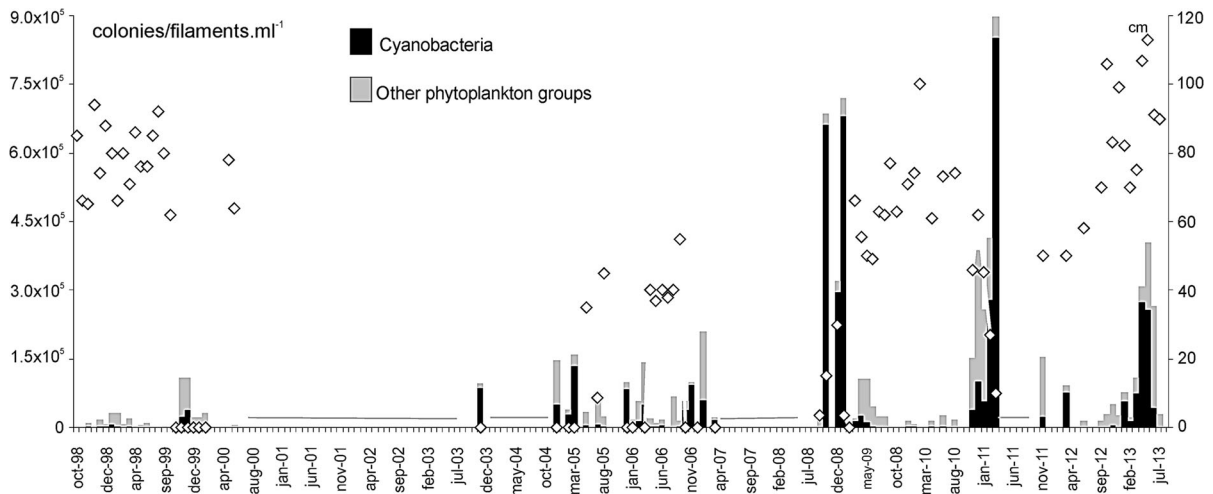
Fig. 2 Relative contribution of cyanobacteria to total phytoplankton density in the limnetic area of Laguna Grande from October 1998 to July 2013. *Gray straight lines* on the *Y*-axis indicate periods without samples. On the secondary axis, the depth of the lake is indicated for each sampling date

with lake depth. pH fluctuations (5–10) (Table 1) were mostly correlated with phytoplankton chlorophyll *a* ($r = 0.47$, $n = 81$, $P < 0.000$), and chlorophyll *a* was correlated with dissolved oxygen concentrations ($r = 0.35$, $n = 79$, $P < 0.001$). Phytoplankton chlorophyll ranged from undetectable values to very high concentrations (>600 μg l^{-1}) and was directly correlated with cyanobacterial abundance ($r = 0.74$, $n = 81$, $P < 0.00$), mostly due to the contribution of potentially toxic bloom-forming species ($r = 0.76$, $n = 81$, $P < 0.00$). There is a slight inverse relationship between lake depth and chlorophyll *a* ($r = -0.40$, $n = 79$, $P < 0.00$), and lake depth and abundance of cyanobacteria ($r = -0.38$, $n = 81$, $P < 0.00$).

During the first years of the study period (1998–1999), phytoplankton abundance was low (<3×10^4 colonies/filaments ml^{-1}) (Fig. 2), especially after the flood following the strong 1997–1998 El Niño ENSO event (Fig. 1b). In the summer 1999–2000, density was two or three times higher (ca. 10^5 colonies/filaments ml^{-1}), but the assemblages were mostly dominated by green algae. By spring 2003, similar phytoplankton abundance (Fig. 2) corresponded to dominance of filamentous non-heterocystous cyanobacteria: *Planktothrix agardhii* and *Raphidiopsis mediterranea* (Fig. 3a). The latter species still dominated through spring 2005 to autumn 2006 and was accompanied by *Dolichospermum* spp. and *Anabaenopsis* cf. *elenkinii* which had lower

abundances (one order of magnitude lower). These species did not occur in the summer 2007, and by late summer 2008, the lake was nearly dry. Following a slight water level recovery, the next warm period (2008–2009) was characterized by strong cyanobacterial blooms (ca. 1.4×10^5 colonies/filaments ml^{-1}) (Fig. 2) constituted by *A.* cf. *elenkinii* and *Sphaerospermopsis* cf. *aphanizonemoides* accompanied by *Microcystis aeruginosa* (*P. agardhii* and *R. mediterranea* were scarce) (Fig. 3a). In the following high water summer (2009–2010), overall phytoplankton densities were low with the absence of cyanobacterial blooms (Fig. 2). Cyanobacterial densities increased once again from spring 2010 to autumn 2011 (Fig. 2) and achieved the strongest blooms (same species as 2008–2009, Fig. 3a) registered for Laguna Grande (ca. 5×10^5 colonies/filaments ml^{-1}) in coincidence with extreme low water levels. By the end of the warm season 2012 (March), cyanobacterial abundance was one order of magnitude lower (4×10^4 colonies/filaments ml^{-1}) with dominance of *M. aeruginosa*. Finally, cyanobacteria grew steadily from January to April 2013 (2.4×10^5 colonies/filaments ml^{-1}) (Fig. 2) composed by several bloom-forming species: *Sphaerospermopsis torques-reginae*, *Dolichospermum* cf. *bituri*, *Cuspidotrix isaatschenkoi*, *S.* cf. *aphanizonemoides* and *R. mediterranea*. By mid-autumn, the latter species were accompanied by *M. aeruginosa* and *P. agardhii* (Fig. 3a) The general
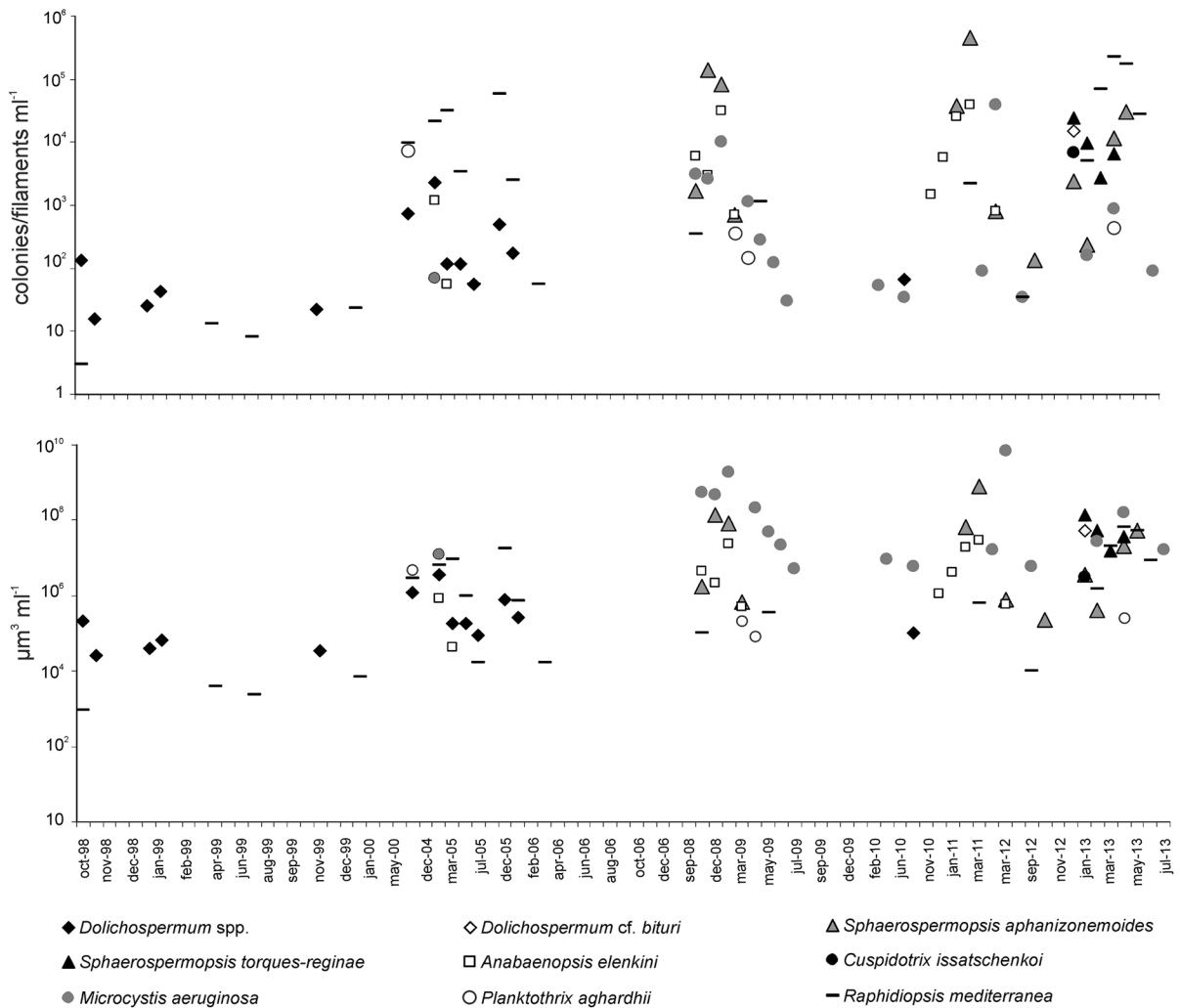
Fig. 3 Variation of bloom-forming cyanobacteria species in Laguna Grande in terms of **a** abundance and **b** biovolume during the study period corresponding to the sampling dates

species pattern match in terms of density (Fig. 3a) and biovolume (Fig. 3b), though the latter shows a marked increase of *M. aeruginosa* in their relative contribution to the bloom due to the big size of their colonies.

The eight bloom-forming species appeared with different frequencies and abundances along the study period (Table 2). *R. mediterranea* was the most frequent species as it occurred on 21 occasions showing a wide density variation. *S.* cf. *aphanizonemoides* was registered on 12 samples but with higher densities; *M. aeruginosa* and *A.* cf. *elenkinii* also appeared frequently but were less abundant than the former species. *C. issatschenkoi* only occurred once.

The first two axes of the redundancy detrended analysis (RDA) explained 80.7% of the biovolume of cyanobacterial bloom-forming species-environment variance (Fig. 4). The first axis explained 50.1% of the constrained total variance, influenced by lake water depth ($r = -0.64$), conductivity ($r = 0.32$), and dissolved inorganic nitrogen ($r = -0.12$), and the second axis by pH ($r = 0.48$) and water temperature ($r = 0.30$). The triplot (Fig. 4) shows that the species are separated along the first axis: The coiled *S. torques-reginae* and *A.* cf. *elenkinii* appear associated with shorter water columns, whereas the straight filaments of non-heterocystous cyanobacteria, *R. mediterranea* and *P. agardhii,* to deeper waters. At

Table 2 List of the bloom-forming species in Laguna Grande, indicating the number of occasions each one occurred, their maximum and mean densities ± standard deviation and the assigned eco-strategy

	Blooms (N)	Maximum density	Mean density	Eco-strategy
Order Nostocales				
Anabaenopsis cf. *elenkinii*	11	38,754	1,637 ± 6,626	NF, SF
Cuspidothrix issatschenkoi	1	6,890	104.3 ± 848.1	NF, SF
Dolichospermum cf. *bituri*	1	14,764	211 ± 1,764.6	NF, SF
Raphidiopsis mediterranea	21	224,409	7,593 ± 32,374.5	HD
Sphaerospermopsis cf. *aphanizonemoides*	12	447,824	10,804 ± 56,671.6	NF, SF
Sphaerospermopsis torques-reginae	4	24,212	614 ± 3,187.1	NF, SF
Order Oscillatoriales				
Planktothrix agardhii	4	8,103	129.2 ± 969.4	HD
Order Chroococcales				
Microcystis aeruginosa	16	39,370	834.4 ± 4,856	SF

NF nitrogen fixer, *SF* scum forming, *HD* homogeneously dispersed

Fig. 4 Triplot showing the results of the redundancy detrended analysis (RDA) based on the data set corresponding to the high-frequency sampling. Environmental selected data are indicated with *arrows*, *circles* correspond to sampling dates and diamonds to the bloom-forming species (biovolume)
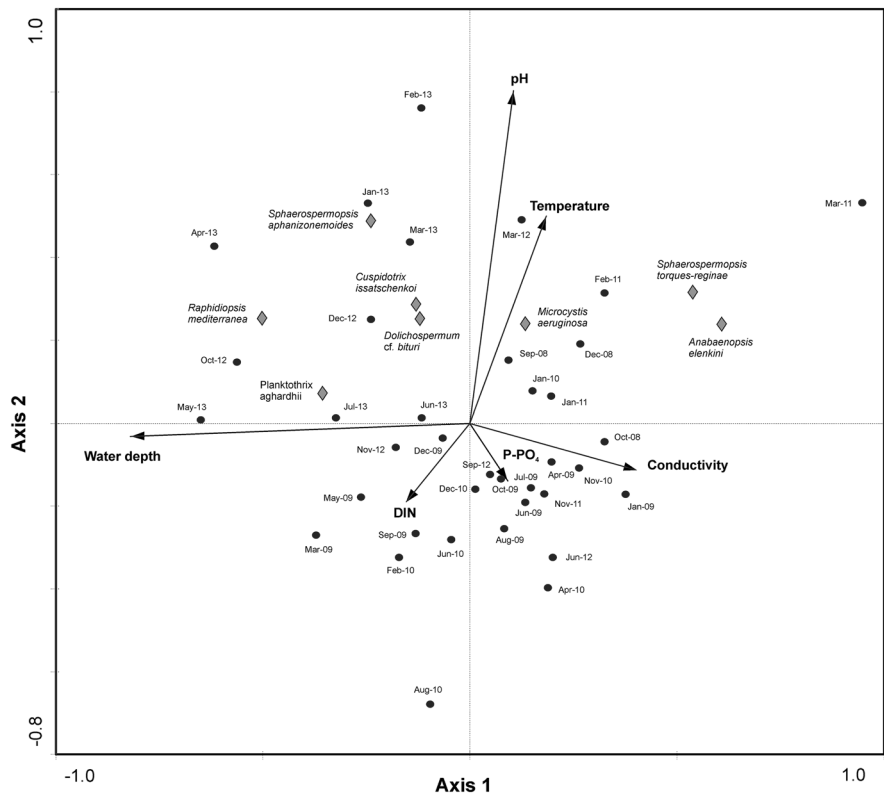
intermediate water levels, the colonies of *M. aeruginosa* appear close to coiled Nostocales. Filaments of the straight N-fixing *S. cf. aphanizonemoides, C. isaatschenkoi* and *D.* cf. *bituri,* were related to straight non-fixing cyanobacteria, at higher depths. The

ordination of species in relation to conductivity shows a very similar pattern, with a single inversion in the position of the gradient between *P. agardhii* and *S. cf. aphanizonemoides*. The inverse correlation of the first axis with dissolved inorganic nitrogen is weak; higher

concentrations are mostly related to non-N-fixing filaments. Regarding the second axis, the segregation of species is not so marked; *P. agardhii* is related to lower temperatures and pH values, whereas both *Sphaerospermopsis* species and *M. aeruginosa* appear related to higher values of these variables. The plot of the dates (Fig. 4) indicate that higher cyanobacterial abundance correspond to the summer samples mostly located in the upper section of the graph; coiled filaments and colonies developed profusely in 2008, 2010 and 2011, whereas straight filaments occurred mainly in 2012 and 2013. The significance value calculated from the Monte Carlo permutation test for all constrained eigenvalues is 0.036.

The morphological attributes of the blooming species also showed marked variations:

– *R. mediterranea*, the median of the maximum linear dimension (MLD), varied from 74.6 to 153 μm ($P < 0.00$) and its median width from 2.5 to 3.4 μm, in correspondence with extremely low (10/2008) and high waters (1/2006) periods. *R. mediterranea* akinetes were very scarce in the analyzed samples ($P > 0.99$) and were only observed on few filaments on March 2013 (high waters).

– *S. cf. aphanizonemoides* median MLD of ranged between 72.7 and 156 μm ($P < 0.05$), also at low (1/2009) and high waters (1/2013), respectively. Median width (3.38–4.93 μm) and length (3.89–4.57 μm) of vegetative cells differed among samples, but unrelated to water level. Akinetes were more abundant in the filaments at high waters ($P < 0.00$) (January and April 2013), whereas on October 2008, during very low waters, akinetes were completely absent.

– *Anabaenopsis* cf. *elenkini* morphology varied ($P < 0.001$) in terms of the median diameter of the spiral coil (14.67–34.54 μm), and median width (3.29–5.60 μm) and length (6.40–7.46 μm) of vegetative cells; no pattern could be associated with water level. Akinetes occurred in the filaments more frequently in January 2009 when waters were extremely low and were less frequent in December 2010 when the depth of the lake was high. Mean diameter and length of the akinetes ranged from 5.74 × 9.3 μm in March 2012 to 8.44 × 12.7 μm in March 2012 and differed between periods of high and low waters, respectively ($P < 0.012$).

– *Microcystis* spp. The median MLD of the colonies ranged between 29.98 and 57.98 μm in March 2012 and October 2008, respectively. The smallest colonies were registered at high waters (March 2012).

Regarding akinetes in the sediments, in the summer of 2010–2011, the density of potentially viable akinetes ranged between 2020 and 5040 per ml of wet sediment. These were oval shaped, with a mean width and length of 8.65 and 11.04 μm, respectively. Cylindrical akinetes were also present, but were either empty or had the cell wall broken (e.g., because of germination or degradation). The density of akinetes, (oval plus cylindrical shapes) which were empty or had broken walls (e.g., because of germination or degradation) ranged between 2100 and 126,000 per ml of wet sediment.

Discussion

Shallow lakes are highly vulnerable to increasing external impacts such as changes in water level, arrival or introduction of exotic species, increase in nutrient loading and agrochemicals (Meerhoff et al., 2010). The over-enrichment of waters with nutrients has traditionally been indicated as the primary cause of cyanobacterial blooms (Schindler et al., 2008). However, O'Farrell et al. (2011) found that despite a slow eutrophication of Laguna Grande, water level changes accounted for the shift from a floating macrophyte community to phytoplankton dominance. The data set here presented indicates that phytoplankton abundance showed an increasing tendency and was progressively dominated by bloom-forming cyanobacterial species throughout the 15-year study period, which was marked by both important drought and flood periods. Despite the increasing trend, there is a strong inter-annual variability in correspondence with the influence exerted by water level (Fig. 2); for example, strong blooms were observed in the warm season of 2008–2009 in coincidence with low ONI values and local rainfalls, and no blooms occurred in the growing period of 2009–2010 with opposite characteristics. Global warming and associated hydrological changes strongly affect the physico-chemical environment and biological processes, most notably metabolism, growth rates and bloom formation (Paerl

& Paul, 2012). The high hydrological variability influenced phytoplankton growth and seemed to favor the establishment of cyanobacteria, as whenever floods demised, one or two species evidenced ecological fitness on behalf of different morpho-functional traits (e.g., aerotopes, heterocytes, akinetes, accessory pigments) and could thrive from the existing propagules. The sequence of nutrients mobilization from land to receiving waters after intense rainfall and flushing subsidence followed by protracted periods of drought explains massive cyanobacterial blooms in estuaries, reservoirs and lakes; in this particular case, sediments rich in organic matter also act as a nutrient pool periodically replenished by decomposition of floating and rooted aquatic plants. Phlips et al. (2007) related the frequency and intensity of algal blooms to their sensitivity to rainfall patterns associated with El Niño events and La Niña periods, indicating a potential relationship with multidecadal cycles and global warming effects. O'Farrell et al. (2011) suggested a link between the enhanced cyanobacteria development with the ENSO events, and in this sense, the ONI may provide a tool for the prediction of strong blooms several months prior to the onset of their explosive growth.

The high-frequency analysis performed in this shallow Pampean floodplain lake during 2008–2013 indicates that water level also affected the composition of cyanobacterial blooms. The eight species concerned are relatively large celled, filamentous or colonial, contain aerotopes for buoyancy regulation, and some may develop heterocytes for N fixation in well-lit waters and akinetes for surviving during adverse periods. In this way, different ecological traits may succeed to promote a rapid growth leading to extremely high biomass despite the changing scenarios characterizing the lake. Literature describes assemblages with different ecological strategies related to both light and nutrient acquisition as well as to their persistence over adverse circumstances: For example, N-fixing cyanobacteria in well-lit water columns, scum formers in not too turbulent waters and homogeneously dispersed organisms in turbid water columns (Reynolds, 1997; Chorus & Bartram, 1999). The RDA shows a clear differentiation of the species along a depth gradient in apparent accordance with species morphology: straight filaments, colonies and coiled filaments were associated with water columns ranging from deep to very short. The straight filaments

of *R. mediterranea* and *P. agardhii* appeared associated with longer water columns, lower temperatures (better homogenization) and higher dissolved inorganic nitrogen concentrations. Higher dissolved inorganic nitrogen levels in Laguna Grande are mostly related to ammonium concentrations, and when the lake gets depleted from nitrate during the growing season, the inflow of dissolved inorganic nitrogen from the anoxic nutrient-rich sediments (O'Farrell et al., 2009) would favor these non-N-fixing cyanobacteria. As the water level subsides, straight filaments of Nostocales appear related to shorter columns and better illuminated waters, which will provide if necessary the required energy for the high cost involved in N fixation (Hense & Beckmann, 2006). Contrasting underwater photosynthetically available radiation (PAR) and DIN concentrations were associated with different species in this lake: 0.1–1.2 μmol photon m^{-2} s^{-1} and 0.7 mg l^{-1} of DIN with *P. agardhii* and *R. mediterranea* under dense free-floating plants (ca. 50 cm depth) (de Tezanos Pinto et al., 2007), and 300 μmol photon m^{-2} s^{-1} and <0.04 mg l^{-1} of DIN with *S. aphanizomenoides* and *A. elenkini* bearing heterocytes (less than 30 cm depth) (unpublished data). Moustaka-Gouni et al. (2010) found that *C. issatschenkoi* and *R. mediterranea* preferred different water depths, with the latter dominating the deep water layer, suggesting that it was positively affected by low light conditions and also by high water temperature. Even shallower waters are associated with colonies of *Microcystis* and further on, to coiled N-fixing filaments. The water level drop in Laguna Grande also implied increasing conductivity, which generally evidences a species replacement toward saline tolerance (Moisander et al., 2002; Tonk et al., 2007), and a short and highly illuminated water column that ensures the high energetic cost of fixation. An example of the investment on heterocyte formation was observed in the warm season of 2008–2009 when dissolved inorganic nitrogen concentration decreased from 220 to 10 μg l^{-1} and 97% of the filaments had heterocytes (pers. observation). These cells are a good indicator of depletion of ammonium and reliance on atmospheric N, as Nostocales with access to ammonium produce few of these specialized cells (Ferber et al., 2004). Which species dominates depends, among other factors, on the source of N more easily reached with low cost. From our findings, it appears that straight

filaments and colonies are more efficient than coiled forms to regulate buoyancy in order to reach benthic ammonium. As asserted by Ferber et al. (2004), we do not reject the hypothesis that cyanobacteria dominate in eutrophic lakes because of their ability to fix N when N:P supply ratio is low and N a limiting resource (Downing et al., 2001), but coincide with their assertion that the situation is more complex due to the interplay between benthic sources of ammonium and the competitive abilities provided by buoyancy to access to nutrient sources.

Another interesting feature observed in this highly fluctuating environment is related to the morphological variation observed in the dimensions of the filaments and colonies. There were clear trends for *R. mediterranea* and *S.* cf. *aphanizonemoides* showing shorter filaments when water level was very low and viceversa; our results may have reflected either nutrient limitation or the difficulty for long filaments to be entrained in short water columns. There are still some inconsistencies for the other species, especially coiled filaments and colonies, probably as the data set did not encompass very high water levels. Several of the species here represented have morphotypes with large differences in cell and trichome size: *Raphidiopsis mediterranea* (Moustaka-Gouni et al., 2009), *Anabaenopsis elenkinii* (Ballot et al., 2008; Santos et al., 2011), *S. torques-reginae* (Werner et al., 2012), *S.* cf. *aphanizonemoides* (Zapomelová et al., 2009). The species that recurrently bloomed in Laguna Grande are widely distributed (Komárek & Anagnostidis, 1999, 2005; Komárek, 2013) and as said before, show a broad morphological plasticity that provides a competitive advantage in fluctuating environments. Finally, akinete-forming cyanobacteria can potentially persist if water bodies that alternate between drought and wet conditions (Paerl et al., 2011). The densities of viable akinetes in the sediments of Laguna Grande were lower than those encountered in systems with dense blooms (ca. 10^4 ml^{-1}) (Faithfull & Burns, 2006). However, perturbations caused by wind and animals in this shallow lake may easily expose akinetes to environmental conditions that favor germination. The high pool of unviable akinetes, (where many were germinated), added to those potentially viable is comparable to the figures described by Faithfull & Burns (op.cit.). Based on shape and size, the akinetes found in the sediments probably correspond to *A.* cf. *elenkeni* and *S.* cf. *aphanizonemoides,*

species that recurrently bloom in the lake and whose filaments frequently carried these resting cells. The environmental conditions that seem to trigger their development corresponded to different water levels of the lake. This suggests that there is a pool that potentially favors the persistence and re-colonization of these nostocaleans in the water column with inocula sufficient to produce blooms. Also, the lack of akinetes of *R. mediterranea* may be related to their low frequency in the filaments of the planktonic populations.

Final remarks

The frequency and intensity of cyanobacterial blooms increased in Laguna Grande during the last 15 years, which were characterized by marked water level changes. We propose that the high inter-annual variation of cyanobacteria abundance was related to the effect of water depth, mainly on the acquisition of light and nitrogen. The fluctuating environmental conditions promoted the massive growth of eight different cyanobacterial species with distinct ecophysiological traits that provided competitive advantage when the nature of the limiting resource or condition changed. The akinete pool in the sediments of the floodplain lake would enable the reappearance of blooms in the water column of two of the most abundant cyanobacterial species, after periods lacking these events in coincidence with high water levels.

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