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Summer phytoplankton composition and nitrogen limitation of the deep, naturally-acidic (pH~2.2) Lake Caviahue, Patagonia, Argentina

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Abstract

During the warm seasons of 1998–2004, the naturally-acidic (pH~2.2) Lake Caviahue was sampled for conductivity, temperature, oxygen, light, nutrients, and phytoplankton (density, biomass and chlorophyll *a*) with a view to studying the summer phytoplankton population changes with relation to environmental factors, as well as the significance of nitrogen limitation on the phytoplankton yield. Lake Caviahue is characterized by its low transparency, CO_2, and N concentration; significant P values; a distinctive vertical distribution of phytoplankton biomass with high values along the water column; and sometimes maximum meta-hypolimnion values. Biodiversity is very low as a result of extreme environmental conditions, Chlorophyceae being the prevailing algae group. Two types of bioassays were carried out to assess nitrogen limitation. For the first bioassay, a solution of ammonium–nitrogen chloride and/or wastewater (rich in ammonium and phosphorus) was used, while one of the lake's sediments was the source of nutrients for the second bioassay. Contrary to the case of acidic mining lakes, N-ammonium proved to be a significant supportive capacity limiting factor as to phytoplankton yield. The present paper provides for the first time information on phytoplankton nitrogen limitation in a naturally-acidic lake.

Keywords: Acidic lake; Acidophilic phytoplankton; Nitrogen limitation; Patagonia

Introduction

An understanding of the factors that restrict phytoplankton growth is essential towards the proper assessment of lake productivity. In many temperate freshwater lakes, phosphorus is plainly the limiting element (Horne & Goldman, 1994). Nevertheless, there are many instances of phytoplankton biomass in water bodies being constrained by nitrogen availability (Maberly, King, Dent, Jones, & Gibson, 2002; Pedrozo, Chillrud, Temporetti, & Diaz, 1993; Soto, Campos, Steffen, Parra, & Zuñiga, 1994). In contrast, the rate of primary production and biomass assembly has been found to be limited by the rate of released CO_2, especially within environments low in inorganic carbon, such as poorly buffered waters, even when the overall seasonal production or annual yield is restricted by factors such as other nutrients or water turbulence (Horne & Goldman, 1994). These situations could be found in acidic lakes, where pH is below 4 and the dissolved inorganic carbon is present only as molecular CO_2 and carbonic acid.

German mining lakes are an example of non-natural highly-acidic lakes, where the inorganic carbon is

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considered the potentially limiting factor for algal growth, together with phosphorus. This statement raises from the fact that these lakes possess low phosphorus (mostly $<10\,\mu g\,P\,L^{-1}$) and inorganic carbon concentrations (TIC = 0.5–6.2 $mg\,C\,L^{-1}$), as well as high nitrogen levels (mostly $>1\,mg\,N\,L^{-1}$) (Nixdorf, Mischke, & Lessmann, 1998). Olaveson and Nalewajko (1994) also quoted the inorganic carbon as the limiting nutrient in lakes that were acidified by the input of anthropogenic emissions via acid rain.

In contrast, naturally-acidic Lake Caviahue registered high phosphorus concentrations ($0.45\,mg\,P\,L^{-1}$), a very low N:P ratio (0.1–0.2) (Pedrozo et al., 2002), and a relatively-low concentration of inorganic carbon ($0.17\,mg\,C\,l^{-1}$) (Diaz and Maberly, unpubl.). In our previous work (Pedrozo et al., 2001), we observed a very low N:P ratio in both lake and river, while Baffico et al. (2004) suggested that the phototrophs in acidic tributary Agrio River would be nitrogen limited.

The following contribution is aimed at assessing the relevance of nitrogen limitation on phytoplankton yield in the naturally-acidic Lake Caviahue, as well as explaining phytoplankton population changes with relation to environmental factors.

Study area

The naturally-acidic Lake Caviahue (37°6753′S; 71°02′W – for a description of the area, see Pedrozo et al., 2001) is located in the depression of the Copahue Volcano – Caviahue caldera formed during the Pliocene–Holocene period, and later eroded by glaciers (Fig. 1). Lake Caviahue has a horseshoe shape, with North and South Arms. The North Arm has a Z_{max} of 95 m and a volume of $0.305\,km^3$, while the South Arm has a Z_{max} of 71 m and a volume of $0.169\,km^3$. The total volume of the lake is $0.474\,km^3$ (Pedrozo et al., 2001). Lake Caviahue receives two main inflow rivers, Dulce (pH = 6.0) and Upper Agrio (pH: 0.78–1.78 at inflow). The Upper Agrio River has its headwaters in the Copahue Volcano, and accounts for the lake's low pH and high salinity (Pedrozo et al., 2001) (Fig. 1). Lake Caviahue waters contain high concentrations of metallic iron ($18.4\,mg\,L^{-1}$) and sulphur ($130\,mg\,L^{-1}$), with low molar ratios of Fe:Al (0.22–0.63) (Pedrozo et al., 2002). The North arm of the lake receives sewage discharge from Caviahue Village (population: 500 inhabitants) (Fig. 1). The vigorous eruption of the Copahue Volcano, which occurred in July 2000, consisted of the emission of ashes, lapilli and bombs that covered the Caviahue Village. The ashes reached a maximum distance from the volcano of approximately 100 km.

Materials and methods

Characterization of Lake Caviahue: sampling and lab analysis

The lake was sampled on seven occasions (November 1998, March 1999, January and April 2000, March 2001, March 2003 and March 2004), mainly during the warm season on account of severe climate conditions that made access to the area difficult throughout the rest

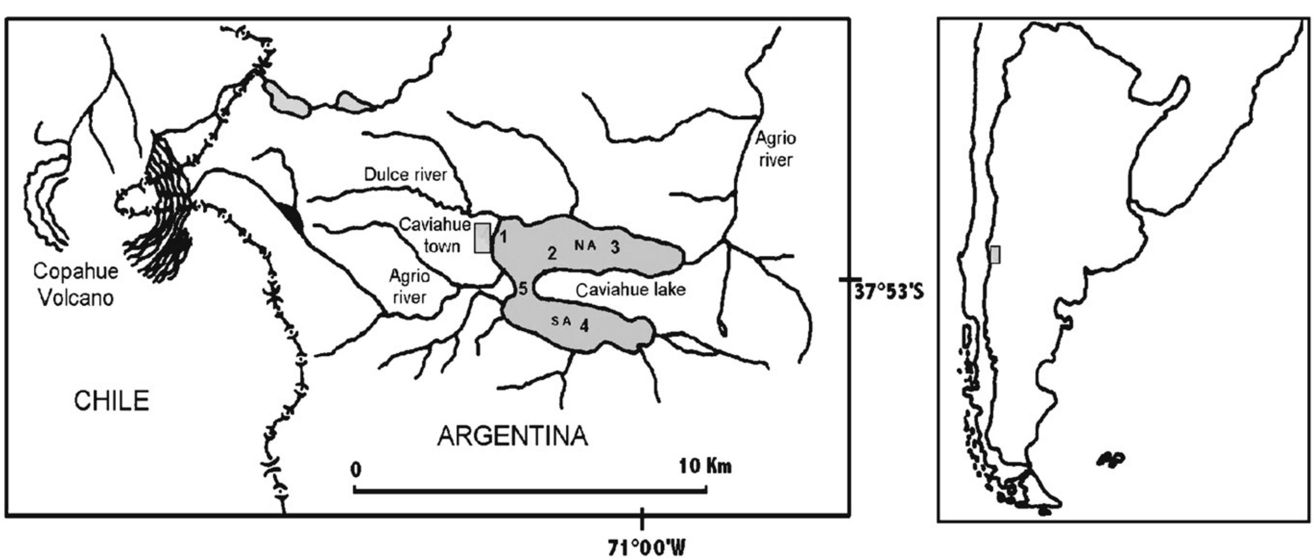

Fig. 1. Location map for Lake Caviahue, outflow and inflows. NA: North Arm, SA: South Arm. Sediment and water sampling sites: 1 wastewater inflow; 2 NA 20 m depth; 3 NA 83 m depth (deepest site); 4 SA 70 m depth (deepest site) and 5 C13 m depth (central part of the lake) (modified from Pedrozo et al., 2001).

of the year. Samples were taken in the North Arm (Fig. 1), which showed similar behaviour with respect to the South Arm (Pedrozo et al., 2001; Varekamp, 2003). Water samples for chemistry and phytoplankton counting were collected with a 5-litre Van Dorn bottle at depths of 0, 2, and 5 m, and every 10 m in 10-m-sections until reaching the bottom. We carried out depth profiles in the deepest site (90 m) of the limnetic area. Conductivity was measured with an Orion 135 probe corrected to standard temperature (25°) water temperature by means of a YSI thermistor, and pH with an Orion 265 probe previously calibrated using Orion buffer solutions at 1.00, 2.00, and 4.01. Dissolved oxygen was measured with an Orion 380 oximeter, and light was read using a LI-COR radiation meter equipped with a LI-192SB underwater quantum sensor. Transparency was measured using a 20-cm-diameter Secchi disc.

The following chemical and biological variables were analyzed according to APHA (1992): soluble reactive phosphorus (SRP) by the molybdate blue–ascorbic acid method; and total phosphorus (TP) by potassium persulphate oxidation with 1:4 sulphuric acid, and afterwards as SRP. With the aim to verify the importance of silica interference in the SRP analysis an exchange resin (Amberlite IR-120 H, 14–52 mesh, size of particule 0.30–1.18 mm) was used, as recommended by Durham University's Algal Research Laboratory, England. No significant differences were obtained between using and not using the resin ($R^2 = 0.998$). Three litres of lake water were passed through GFC Whatman filters for chlorophyll *a*, and further extracted with 90% acetone. At the laboratory, chlorophyll *a* concentrations were calculated at 665 and 750 nm from the absorbance readings. Dissolved inorganic nitrogen (DIN) chemical species were measured through automatic sequential flow photometry using the SFA 1000 Skalar (The Netherlands) continuous flow analyzer. DIN was considered as the sum of $NNO_3^- + NNO_2^- + NNH_4^+$. CO_2 was analyzed through gas chromatography (SRI 8160), the unit being equipped with a flame ionization detector. Dissolved organic carbon (DOC) was determined on a filtered sample (0.22 μm) via a Dima-TOC 100 (Dimatec, Germany) carbon analyzer. CO_2 and DOC concentrations were determined only for March 2000 and March 2004 samplings. Phytoplankton samples were fixed with Lugols' solution and counted at 400 × with a Hydrobios inverted microscope (Utermöhl, 1958). Cellular density was counted by fields until 100 cells were counted in each sample (maximum error: 20%) and further transformed to biomass as biovolume. With reference to algal cell volume, adequate dimensions were gathered for ten specimens of each species, and later expressed as fresh algal weight according to Wetzel and Likens (1991) and Goldman (1978). The species were taxonomically identified under a 1000 × Olympus BX30 optic microscope, the following being the literature consulted: Fott (1972), Huber-Pestalozzi (1961), Komárek and Fott (1983), and Tell and Conforti (1986).

Nutrient bioassays

After Pedrozo, Temporetti & Beamud (unpubl.) the wastewater (pH 8.02) has a large NNH_4^+ ($23\,mg\,N\,L^{-1}$) and TP ($3\,mg\,P\,L^{-1}$) content, and represents an external load of N and P to the Lake of 0.8 and 0.26 ton year^{-1}, respectively. Besides, Beamud, Temporetti, Friese, Mages, and Pedrozo (2002) found high values of P in the sediments of the both arms and in the central part of the lake. Based on these results wastewater and sediments were considered sources of N and P, assuming a possible nutrient liberation from the sediments toward the water column. According to the first wastewater bioassays results and with the aim to highlight the importance of the ammonium for the acidophilic algae, ammonium chloride solution ($1000\,\mu g\,L^{-1}$) was added as a new treatment.

Bioassays design

The bioassays were carried out in 2000 and 2001. Tables 1 and 2 show the initial conditions. For the experiments, 60 ml Nunclon cell culture flasks with an angled neck adequate for inverted microscope counting were filled with filtered (Millipore, 0.22 μm) lake water, nutrient solution or sediments and the algal inoculum. The flask were incubated at the following controlled lab conditions according to the physical characteristics of the lake: 20 °C, 12/12 h light/dark cycle from cool white fluorescent tubes, ~100 μmol photon $m^{-2}\,s^{-1}$. The incubation period was of 21 days. Daily manual agitation was performed to prevent eventual cell sedimentation. Fresh weight biomass, SRP, and water pH were registered at the beginning and the end of the experiments. Nutrient yield limitation was estimated from the fresh weight biomass on day 21.

We performed separately the bioassays with the different nutrient sources, described above. First, wastewater and/or ammonium chloride solutions were used and the treatments were: $+NNH_4$ (NH_4Cl), +WW (wastewater), $+NNH_4$+WW, and Control (C), with none of either solution (Table 1). The addition of nutrients was adjusted to a target concentration of at least 10 times the initial concentration in lake water (Schelske, 1984). Second, when we used lake sediments, the treatments were established according to the places in the lake where the sediment samples collected, namely: NA20 m, NA83 m, SA70 m, and C13 m (Fig. 1; Table 2). All treatments had three replicates.

Table 1. Treatment composition in the two bioassays performed using wastewater and ammonium chloride solution as nutrient sources: +WW (wastewater), +N–NH_4^+ (NH_4Cl), +N–NH_4^+ +WW which has both solutions, and Control (C), which has none of either solution

Treatment	Initial nutrient concentration		Initial average algal density (±SD)
	N–NH_4^+ (μg N L^{-1})	P (μg P L^{-1})	(cells mL^{-1})
I			
C	31	499	220 (±40)
+WW	2566	756	220 (±46)
II			
C	31	499	500 (±55)
+ N-NH_4^+	510	499	500 (±40)
+WW	2566	756	500 (±49)
+ N-NH_4^+ +WW	3076	756	500 (±32)

±SD: Standard deviation.

Table 2. Treatments in the bioassays with sediments: NA20 m (North Arm, 20 m), NA83 m (North Arm, 83 m), SA70 m (South Arm, 70 m) and C13 m (Central, the place where both arms join)

	NA 20 m	NA 83 m	C 13 m	SA 70 m
Sediment texture	Clay	Clay	Silty–Clay	Sandy–Silty
Pore water N–NH_4^+ (μg L^{-1})	500	9620	7340	5930
Pore water SRP (μg L^{-1})	79	1090	720	110
Initial average algal density±SD (cells mL^{-1})	500 (±40)	500 (±47)	500 (±52)	500 (±55)
Initial SRP in water (μg L^{-1})	558	558	558	558
Sediments added (g)	20	20	20	20

Characterization of the sediments (texture) and the pore water (N–NH^{4+} and SRP concentrations) of Lake Caviahue. Initial conditions (algal density and SRP concentration in the water used) and grams of sediments added in each treatment.
±SD: Standard deviation.

Phytoplankton and sediment samplings and lab analysis

Phytoplankton samples were collected in the deepest area of the North Arm of the lake, and were concentrated with a 10-μm mesh net, inside of which a 63-μm net had been placed to remove zooplankton. Sediment samples were collected with an Ekman–Birge dredge. To verify the influence of the sewage water and the Agrio River, NA 20 m sample was taken in a point situated at 100 m (approximately) in between the input of the wastewater Treatment Plant and the Delta of the Agrio River (Fig. 1). The samples were preserved cold in the field and further dried in open air at the laboratory for use in the experiments. To evaluate the nutrient concentration released from the sediments more available to the algae, pore water was collected from a sediment subsample by centrifugation and subsequent filtration through membrane filters (0.45 μm). At the lab, once filtration NNH_4^+ and SRP were determined according to the methods indicated for water samples, the sediments' texture was determined according to Jackson (1970). Sewage or wastewater was collected directly from the Treatment Plant's outflow pipe before entering the lake.

For each bioassay, one-way univariate ANOVAs and *post hoc* analyses (Tukey test) with a 5% significance level were applied to test statistically-relevant differences between nutrient treatments, using STAT 6.1 statistical software.

Results

Vertical distribution of physical, chemical and biological variables

Fig. 2 provides an example of depth profiles of dissolved oxygen, phytoplankton biomass, temperature, chlorophyll *a*, conductivity, pH, DOC, and CO_2 performed in March 2004. For the rest of the sampling dates, the depth profiles remained similar to the profiles shown. Caviahue is a warm monomictic lake, thermally stratified during summer (December–April), with a

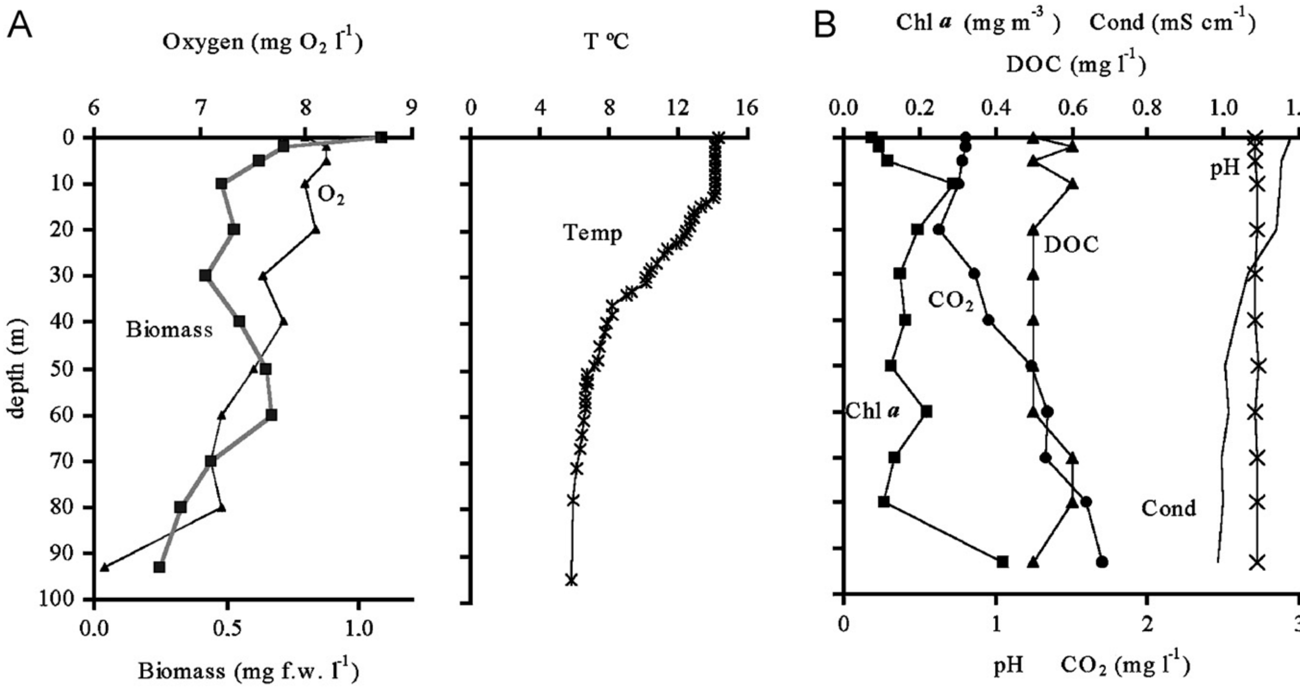

Fig. 2. Vertical distribution by March 2004 of (A) Phytoplankton biomass (grey line with squares), dissolved oxygen (small triangles), and temperature (crosses); (B) Phytoplankton chlorophyll *a* (squares), carbon dioxide (circles), dissolved organic carbon (triangles), conductivity (thin line), and pH (crosses).

thermocline between 20 and 40 m (Fig. 2A). Temperature ranged from 12 to 15 °C in the epilimnion, and 8 °C in the hypolimnion (Fig. 2A). The Secchi disc depth ranged from 3 to 6.5 m. The diffuse extinction coefficient (Kd PAR) was low, ranging from 0.32 to 0.26 m^{-1}. The lower limit of the euphotic zone (1% of surface PAR irradiance) was located at an approximate depth of 16 m, including only the epilimnion. The vertical distribution of dissolved oxygen concentrations showed concentrations of 8.1 $mg\,O_2\,L^{-1}$ (in average) for the epilimnion, and 7.0 $mg\,O_2\,L^{-1}$ for the hypolimnion (Fig. 2A). Phytoplankton biomass showed a maximum value at a 60-m depth (0.7 $mg\,fw\,L^{-1}$) (Fig. 2A). Chlorophyll *a* concentration was always very low and constant with depth (0.1–0.3 $mg\,Chl\,a\,m^{-3}$), with an extraordinary value of 0.4 $mg\,Chl\,a\,m^{-3}$ in the hypolimnion (Fig. 2B). Carbon dioxide concentration in the hypolimnion (0.7–1.7 $mg\,C\,L^{-1}$) was higher than in the epilimnion (0.6–0.8 $mg\,C\,L^{-1}$) (Fig. 2B). DOC concentrations in March 2004 ranged from 0.5 to 0.6 $mg\,C\,L^{-1}$ (Fig. 2B). Conductivity and pH values remained without variation with relation to depth (Fig. 2B).

N and P concentrations, conductivity and pH (1998–2004)

Conductivity changed from 1808 $\mu S\,cm^{-1}$ in November 1998 to 1157 $\mu S\,cm^{-1}$ in March 2004, reaching a maximum value of 3563 $\mu S\,cm^{-1}$ in March 1999 (Fig. 3A). pH increased from 1.9 (November 1998) to 2.7 (March 2004) (Fig. 3B).

Nutrients (N, P) were homogenously distributed along the water column. High concentrations of SRP were measured both in the epilimnion and hypolimnion of the lake. A strong variation was registered in P concentrations during the period under evaluation, decreasing from 600 to 200 $\mu g\,P\,L^{-1}$ (Fig. 3A). SRP was always the main fraction (50–90%) of the TP on account of acidity. DIN concentrations registered low values (on average): 80 $\mu g\,N\,L^{-1}$ in the epilimnion, and 113 $\mu g\,N\,L^{-1}$ in the hypolimnion (Fig. 3B).

Phytoplankton biomass and species composition (1998–2004)

The total biomass in the epilimnion was of some 0.7 $mg\,fw\,L^{-1}$ (November 1998), reaching values of up to 1.1 $mg\,fw\,L^{-1}$ during March 2003 (Fig. 3C). The lowest biomass value in the hypolimnion was 0.1 $mg\,fw\,L^{-1}$ in March 2001, the highest being 1.5 $mg\,fw\,L^{-1}$ in March 2003. We estimated averages of 0.7 $mg\,fw\,L^{-1}$ in epilimnion and 0.5 $mg\,fw\,L^{-1}$ in the hypolimnion. The exception were those values counted in the water column in March 2001 (less than 0.25 $mg\,L^{-1}$ fresh weight) after the eruption of the Copahue Volcano, which took place in July 2000. The

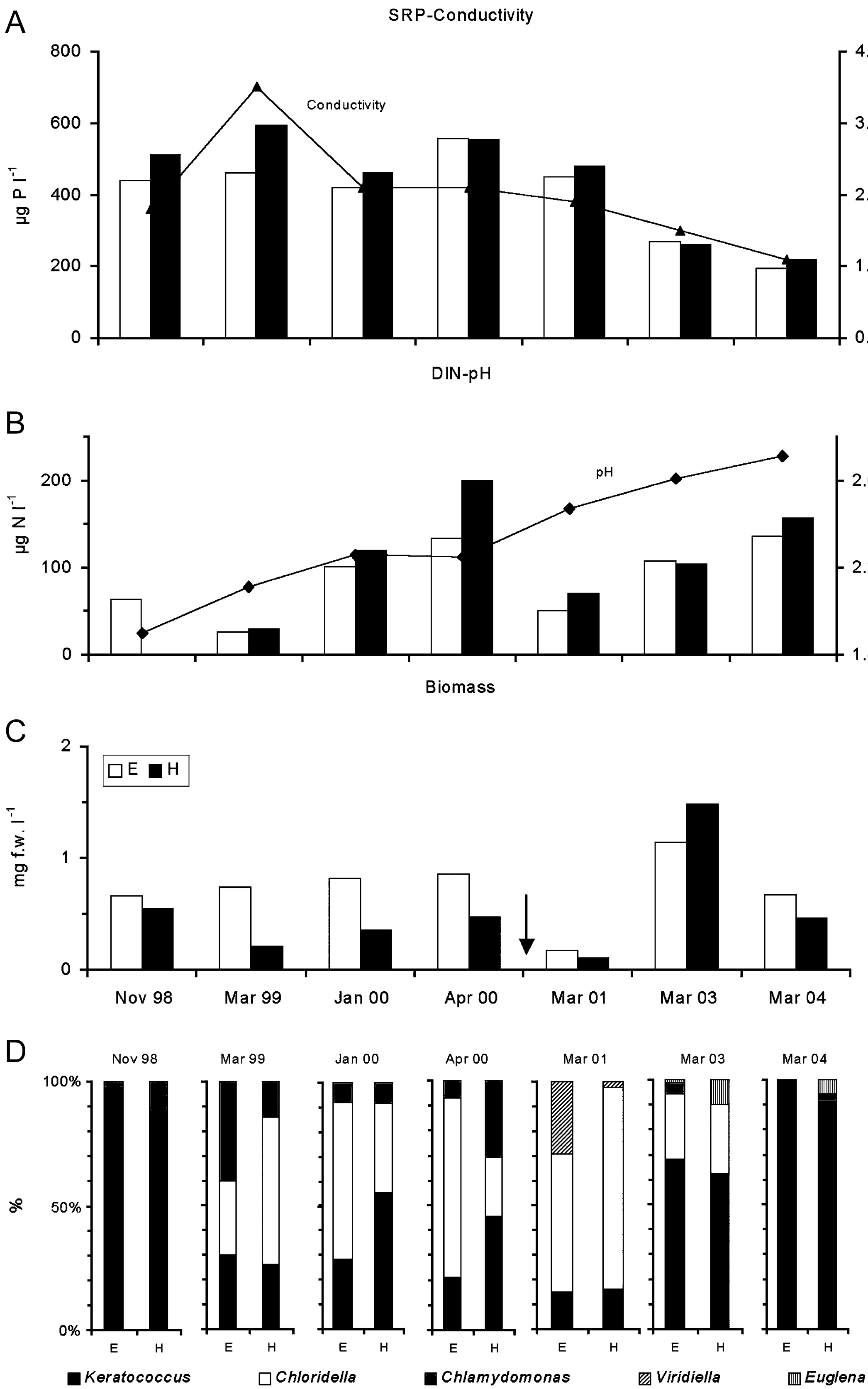

Fig. 3. Seasonal variation in the lake's epilimnion and hypolimnion of (A) soluble reactive phosphorus (SRP in $\mu g\,P\,l^{-1}$) and conductivity (in $\mu S\,cm^{-1}$), (B) dissolved inorganic nitrogen (DIN in $\mu g\,N\,l^{-1}$) and pH, (C) total phytoplankton biomass (mg f.w.l^{-1}), and (D) species contribution to the total phytoplankton biomass (in percentage), from 1998 to 2004. E: epilimnion; H: hypolimnion. The arrow indicates the volcanic eruption registered in July.

eruption brought an important shift in underwater light, with a decrease of the Secchi disc depth and an increase in suspended material due to the great amount of deposited volcanic ash. Algal biomass diminished as a result of this volcanic episode (Fig. 3C).

The phytoplankton of Lake Caviahue was characterized by a very low diversity of small size unicellular species. Five planktonic species have been found since 1998, although two dominant populations were present over the years: the small ($37\,\mu m^3$ of biovolume) *Keratococcus rhaphidioides* green algae, and the *Chloridella* sp. xanthophyte ($170\,\mu m^3$), both non-motile species that colonized the lake in all depths, and during the period under evaluation. Other species present were less quantitative, important, or rare: chlorophytes, motile *Chlamydomonas* sp. ($80\,\mu m^3$), non-motile *Viridiella* sp. ($25\,\mu m^3$), and one big euglenophyte: *Euglena mutabilis* ($4890\,\mu m^3$). Occasionally, we found certain species of diatoms and *Ulothrix* sp. coming from the stones on the shore of the lake or Inflow Rivers.

K. rhaphidioides occurred in very high abundance, representing from 15% to 100% of the total biomass.

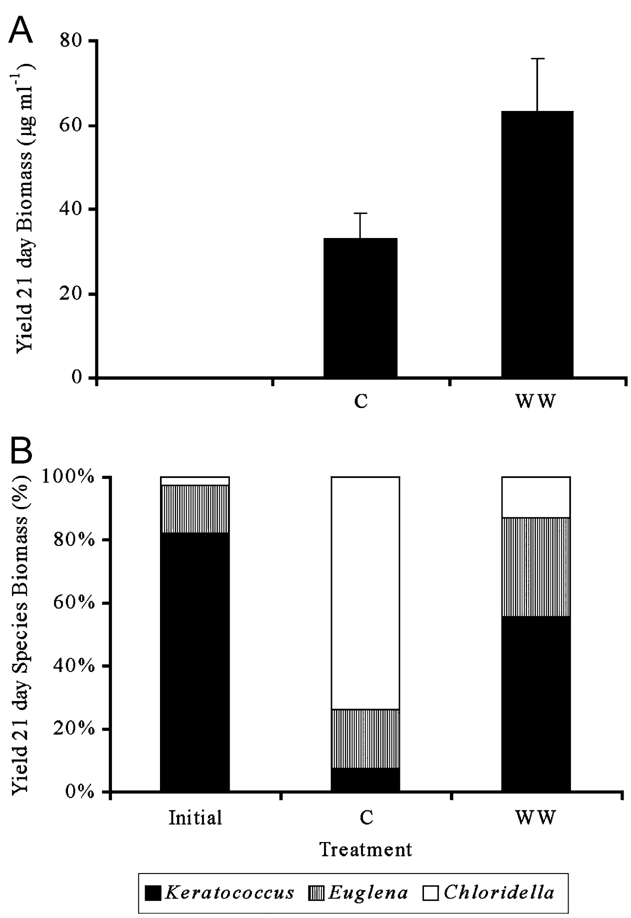

Fig. 4. Responses to C: control, and WW: plus wastewater treatments for phytoplankton yield (A), and species contribution to total biomass (in percentage) (B). Error bars represent one standard deviation.

Table 3. ANOVAs for the different treatments in the bioassays using I: wastewater; II: wastewater and ammonium chloride solution; III: sediments

Bioassay	dF	*F*
I		
pH	7	37.5*
Biomass	7	93.3*
II		
pH	15	33.2*
Biomass	15	32.0*
III		
pH	7	63.2*
SRP	7	19.6*
Biomass	7	18.8*

*$p = 0.05$.

The following were the minimum and maximum contributions of the other species to total biomass: *Chloridella* sp. 26–82%, *Chlamydomonas* sp. 4–40%, *Viridiella* sp. 2–30%, and *Euglena mutabilis* 1–10% (Fig. 3D).

Phytoplankton nutrient limitation

The final biomass in the treatments was significantly different to the control one ($p = 0.05$) (Fig. 4A and Table 3), a small increase in pH being registered during the treatments ($C = 2.15$, $+\mathrm{WW} = 2.23$, $p < 0.005$, Table 3). At the end of the incubation period, *K. rhaphidioides* density reached values of 5% and 55% for C and WW treatments, respectively. *Chloridella* and *Euglena* were the prevailing species in the C treatment (Fig. 4B).

In the bioassays using ammonium chloride solution and wastewater, the final biomass differed strongly from the control one ($p = 0.05$) (Fig. 5A and Table 3). The results showed a small increase in pH during the treatments ($C = 2.46$, $+\mathrm{NNH4} = 2.46$, $+\mathrm{WW} = 2.49$, $+\mathrm{NNH4}+\mathrm{WW} = 2.5$; $p < 0.005$, Table 3). The algae biomass had a sharp increase when ammonium was added alone or with the wastewater, being higher during the +NNH4+WW treatment (Fig. 5A). Phytoplankton composition registered no changes as to species proportion during the C treatment. *E. mutabilis* was the only species favoured by the addition of ammonium and wastewater (Fig. 5B).

In the bioassays using sediments, all the treatments final biomass differed widely from the control ($p = 0.05$) (Fig. 6A and Table 3). The results showed an increase in pH during the treatments (NA 20 m = 2.37, NA 83 m = 2.30, C 13 m = 2.54, SA 70 m = 2.33; $p = 0.05$, Table 3). The results yielded by these bioassays are related to those shown above: the greatest phytoplankton growth was found in the treatments with highest ammonium concentration in the pore water (Table 2, Fig. 6A). Fig. 6A represents the SRP uptake in relation with the SRP available at the beginning of the

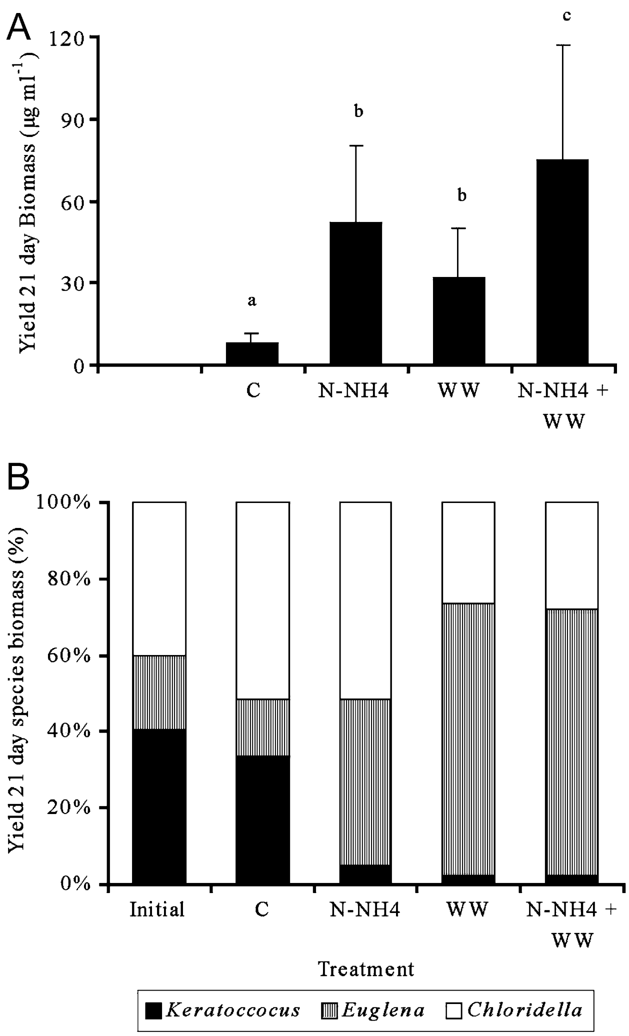

Fig. 5. Responses to C: control, N–NH_4: plus ammonium–nitrogen solution, WW: plus wastewater, and N–NH_4 + WW: plus ammonium–nitrogen solution and wastewater treatments for phytoplankton yield (A), and species contribution to total biomass (in percentage) (B). Error bars represent one standard deviation. The letters indicate homogeneous groups between treatments ($p = 0.05$).

experience. The greatest drop in SRP was found during the SA 70 m treatment, followed by N 20 and N 83 m. At the end of incubation *E. mutabilis* and the diatoms diminished their biomass. *K. rhaphidioides* increased during all treatments, although *Ulothrix* sp. proved to be a better competitor during the treatment with sediments coming from the Central site (Fig. 6B).

Discussion

Lake characterization

This study presents Lake Caviahue as a relatively low transparent lake with a low CO_2 concentration, a distinctive vertical distribution, as well as high depth values of phytoplankton biomass and chlorophyll *a* in the meta and hypolimnion. Such high values could be explained if the algal populations would be adapted to growing well at low light intensities with high available inorganic nutrients or if they would be able to uptake organic nutrients, dissolved or particulate. This last capability of the algae is called mixotrophy, and it could be advantageous in ecosystems where light and inorganic carbon are quite low. Tittel et al. (2003) reported that mixotrophy was responsible for deep algal accumulations in the acidic mining lake 111.

Nitrogen concentrations are quite low in Lake Caviahue, ammonium (70 $\mu g\,N\,L^{-1}$) being the main form of inorganic nitrogen, mainly of volcanic origin (Pedrozo et al., 2001). The values of total organic nitrogen (TON) in the lake, estimated as TN-DIN by Pedrozo et al. (unpubl.) varied between 32 and 91 $\mu g\,N\,L^{-1}$. These values are very low compared with those found by Maberly et al. (2002) for freshwater lakes in United Kingdom and Nixdorf, Wollmann and Deneke (1998) for acidic mining lakes in Germany. The N:P and the C:N molar ratios averaged 0.3 and 8.9, respectively, which is symptomatic of nitrogen deficiency. These N:P ratios are lower than those quoted for mining acidic lakes in Germany (Nixdorf, Mischke et al., 1998).

Conductivity in March 1999 was the highest during the period under evaluation, while the rise in dissolved ions in the water could be accountable for the change observed in phytoplankton species composition, although not in the total algal biomass. After this date, *Chloridella* and *Chlamydomonas* together represented over 50% of the total biomass during two consecutive summers, until the eruption of 2000, when *Chlamydomonas* was replaced by *Viridiella*, and the lowest biomass value was registered. Since March 2003, it was possible to witness the recovery of *K. rhaphidioides* and the increase of the biomass. No correlations were found between nutrients and biomass. In spite of the drop in phosphorus concentration three times during the period under analysis, and the oscillations in nitrogen concentration, the total biomass was constant except when the eruption covered the lake with tons of ashes.

In comparison with other acidic water bodies, phytoplankton biomass values and their vertical distinctive distribution in Lake Caviahue are similar to the mining lakes of eastern Germany, although these acidic lakes have been formed at the end of the 19th century and are shallow, with extremely high iron contents which causes the red colouration of the water, a high potential primary production and the plankton community is very diverse (Kamjunke, Gaedke, Tittel, Weithoff, & Bell, 2004; Nixdorf, Mischke et al., 1998; Nixdorf, Wollmann et al., 1998) in contrast with Lake Caviahue. Because of these differences, besides the low

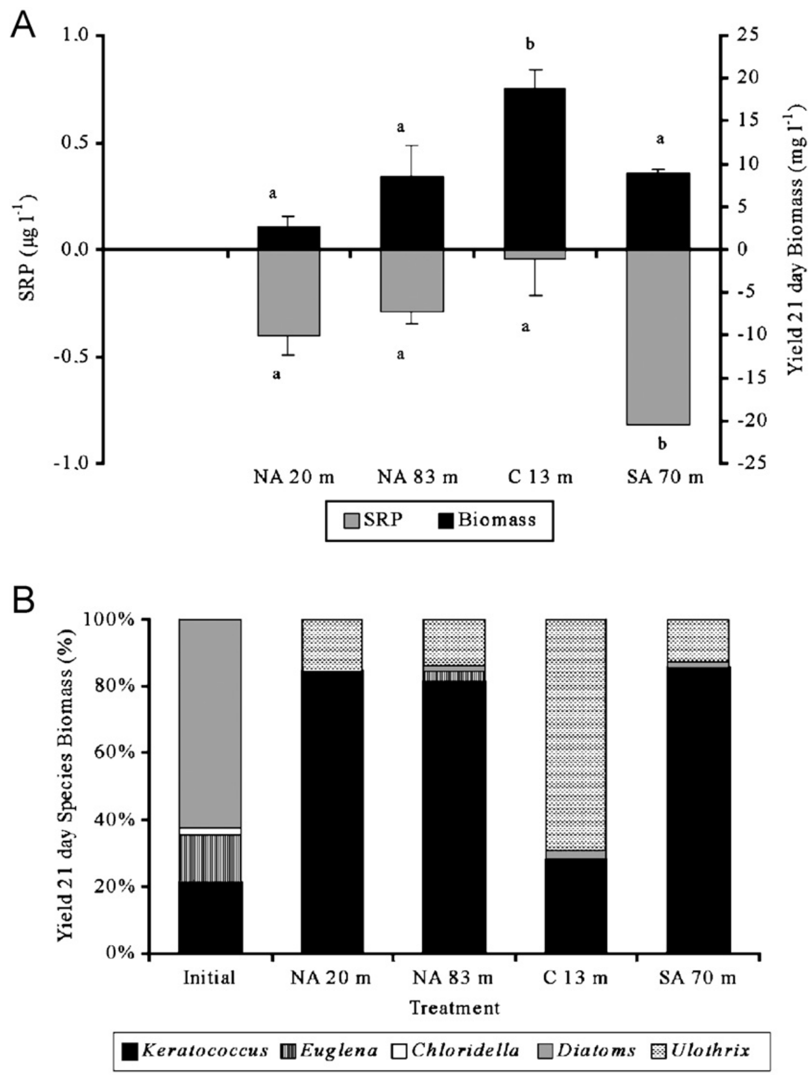

Fig. 6. Mean values (±Standard Deviation) of (A) algal biomass yield and net increment in SRP concentration ($\mu g\,l^{-1}$). This is the difference in SRP values at the beginning and at the end of the experience. (B) Species contribution to total biomass (in percentage) of the different treatments in bioassays with sediments as a nutrient source. NA 20 m: North Arm at 20 m depth; NA 83 m: North Arm at 83 m depth; C 13 m: Centre of the lake at 13 m depth; SA 70 m: South Arm at 70 m depth. The letters indicate homogeneous groups between treatments ($p = 0.05$).

pH, phytoplankton growth conditions in Lake Caviahue could differ widely from those prevailing in anthropogenically-acidified lakes, when natural phytoplankton populations have been living in acidic waters for 10,000 years.

Lake Caviahue posses a very low diversity of phytoplankton species and a prevalence of green algae. This situation is quite common in extreme environments (Blouin, 1989). The reason for the preponderance of certain algae groups in these extremely acidic environments lies in their fitting capacity, their ecophysiological specialization, and the ecological advantages that can be offered (Nixdorf, Wollmann et al., 1998). Acidophiles are able to grow in acidic environments, mainly because they can maintain their internal pH at external high H^+ concentrations, as is the case of *E. mutabilis*, which can keep it above pH 6 (Lane & Burris, 1981), and *Chlamydomonas* sp., which can grow at pH 2 with a mean cytosolic pH of 6.6 (Messerli et al., 2005). Chlorococcals algae (*K. rhaphidioides*) could be present in light of the group's ample P assimilation capacity (Reynolds, 1997; Tilman, Kiesling, Sterner, Kilham, & Jhonson, 1986). Of the five phytoplanktonic species living in Lake Caviahue, only *Chlamydomonas* sp., *Viridiella* sp., and *E. mutabilis*, have been registered in other acidic environments and quoted as highly tolerant to metals (Volcanic areas of Italy: Albertano, Pollio, & Taddei, 1991; Canada: Olaveson & Nalewajko, 2000; Mining lakes Germany: Beulker, Lessmann, & Nixdorf, 2003; Rio Tinto Spain: Amaral-Zettler et al., 2002). *Chlamydomonas* sp. and *E. mutabilis* have been referred to as capable of growing heterotrophically in acidic conditions (Bissinger, Jander, & Tittel, 2000; Olaveson & Stokes, 1989). In the dark deeper layers of Lake Caviahue, below a 20-m depth, phytoplankton could be using dissolved organic carbon as a source of carbon. Although mixotrophy was not proved in any of the species living in this acidic lake, Vincent and Goldman (1980) provided evidence of mixotrophy in *Monoraphidium contortum* in freshwater Lake Tahoe, species that belong to the same family of *K. rhaphidioides* that grows in a very high density in Lake Caviahue.

Nutrient limitation

The CO_2 concentration estimated in the experiments ($0.14\,mg\,C\,L^{-1}$, laboratory altitude: 760 m; water temperature: 20 °C) was lower than that measured ($0.4–1.7\,mg\,C\,L^{-1}$) and the calculated air-equilibrium concentrations for the lake ($0.3\,mg\,C\,L^{-1}$, altitude: 1600 m; water temperature: 10 °C). Nevertheless, the adding of nitrogen yielded the maximum growth in the experiments under conditions of lower carbon concentrations than in the lake, which suggests that CO_2 availability is sufficient to meet the carbon requirements of phytoplankton in the lake, and that the experiments and algae growth should not be limited by carbon. The adding to the lake water of N as ammonium in the presence of high levels of available P had a synergistic effect and resulted in an increase in phytoplankton biomass. It is worth noting that the rise in algae growth in response to N additions was registered at high P concentrations, where P may no longer be considered a limiting factor. This is similar to the results obtained by Diaz and Pedrozo (1996) in a series of non-acidic Andean lakes. Several studies indicate that N could be limiting primary production as a secondary nutrient, having a synergistic effect with P (Canfield, 1983; Goldman, 1978). In certain lakes, the sole addition of N produces an increase in the algal growth rate, as observed in Lake Tahoe (Goldman, 1981). Nevertheless, algal growth experiments under nutrient enrichment conditions show that there is higher response when N and P are added together than when each is added separately.

The relevance of ammonium as key nutrient in Lake Caviahue can be expected as the acid production mechanism (i.e., the oxidation of ammonium into nitrate) should be inhibited at a low pH (Bock, Koops, & Harms, 1986; Schindler, Turner, & Hesslein, 1985). Hence, ammonium would be the only inorganic nitrogen species of autochthonous origin. Concerning the nitrogen organic fraction, the TON values presented by Pedrozo et al. (unpubl.) were low. Besides, only a small proportion (urea, amino acids) of the dissolved fraction could be exploited by algae (McCarthy, Pratum, Hedges, & Benner, 1997) and as a result, probably very low amount available for the growth. On the other hand, applying the equations given by Reynolds (1992) to estimate chlorophyll *a* from available SRP and DIN, and Quiros (1988) to estimate chlorophyll *a* from TON, Pedrozo et al. (unpubl.) calculated values of $2.34\,mg\,Chl\,a\,m^{-3}$ using SRP, $0.01\,mg\,Chl\,a\,m^{-3}$ using DIN and $0.23\,mg\,Chl\,a\,m^{-3}$ using TON. The estimated Chl *a* value from DIN and TON matches the value of chlorophyll *a* observed in the lake, which indicates that nitrogen is a better predictor of algal Chl *a* in this lake.

Ammonium concentration in the pore water was quite high in the Centre and South Arms, the sediments being silty-clay to sandy, and thus less efficient to bind nutrients than those with a high clay proportion (as the North Arm sediments). The low ammonium content in the North Arm (at a 20-m depth) sediments could explain the low algal biomass by the end of the experiment. But, this situation is not the expected one, due to the sediments being influenced by the waste water coming form the near Treatment Plant and from the Agrio River. As nitrification is not registered under low pH conditions, the high ammonium concentration in comparison with the water column can be explained by the accumulation of the nutrient in the pore water. The adding of nutrient brought changes in the N:P ratios and consequently in the algal biomass and in the structure of the phytoplankton community. This situation was expected according with the Resource-ratio Theory developed by Tilman (1977) for phytoplankton, which anticipates that changes in the environmental ratios of two nutrients will cause changes in the community structure. In general, *E. mutabilis* and *Ulothrix* replaced the other two species prevailing in the lake, namely *Chloridella* sp. and *K. rhaphidioides*. In the experiments with sediments, *K. rhaphidioides* was limited by phosphorus and nitrogen, and was able to use labile P and ammonium, while *Ulothrix* was limited only by nitrogen, and used available ammonium released from the sediments.

The changes observed under experiment condition in the algae composition due to the increase in the N:P ratios, and the small increase in water pH (probably as a consequence of a higher photosynthesis), allows to expect that the continued discharge of wastewater in the lake could alter the trophic state of this naturally acidic environment.

Conclusions

Biodiversity is quite low as a result of extreme environmental conditions, Chlorophyceae being the prevailing algae group. We found a distinctive vertical distribution of phytoplankton biomass and chlorophyll *a* with high values along the water column, and maxima below the metalimnion, this particular phytoplankton distribution was also found by Tittel et al. (2003) in an acidic mining lake. This biomass distribution along the water column could be explained by heterotrophic or mixotrophic nutrition of the algae, although this behaviour was not tested in the species of Lake Caviahue. In spite of the low pH in Lake Caviahue, nitrogen (as ammonium) is the limiting factor, which is in contrast with the situation in lakes acidified by mine discharge, where high anthropogenic N (ammonium and nitrate) concentrations release the algae from N limitation, the system thus becoming P and C limited.

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