Responses of phytoplankton and related microbial communities to changes in the limnological conditions of shallow lakes: a short-term cross-transplant experiment

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ARGENTINE PAMPEAN SHALLOW LAKES

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Abstract We performed a short-term cross-transplant experiment (72 h) using dialysis bags in two shallow lakes with contrasting regimes (clear-vegetated and phytoplankton-turbid). We assessed the changes in the structure of the microbial planktonic communities in response to the variations in the limnological conditions. Important changes were observed in >2 μm-phytoplankton composition in the two transplanted communities (from clear-vegetated to turbid and vice versa). Cyanobacteria biomass (*Snowella* spp.) increased in the transplanted treatment from the clear to the turbid lake, whereas the contribution of *Ochromonas*-like sp. diminished. Colonial and filamentous Cyanobacteria species dominated the phytoplankton of the turbid lake throughout the experiment, both in non-transplanted and transplanted waters. Chrysophyceae showed an increasing trend at 72 h in the transplanted treatment (from turbid to clear waters). Heterotrophic bacteria biomass increased in the transplanted treatment from the clear to the turbid system, probably due to a higher availability of more labile sources of dissolved organic carbon (DOC). Our results evidenced changes in the microbial communities in response to important regulator factors (nutrients, light attenuation, DOC availability and top-down control at the microbial food web) in the two contrasting regimes.

Keywords Microbial plankton · Community structure · Shallow lakes · Turbid and clear regimes · Cross-transplant experiment

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Introduction

Different studies conducted in shallow lakes from the Pampa plain of Argentina have reported the contrasting features of clear-vegetated and turbid shallow lakes of the region (e.g. Quirós et al., 2002; Allende et al., 2009; Sánchez et al., 2010; Izaguirre et al., 2012). Due to the different human activities, nowadays, very few Pampean lakes still present a clear regime with abundant submerged macrophytes, whereas most lakes are in a turbid state. The majority of them have high phytoplankton biomass, and some

are inorganic turbid due to a high concentration of inorganic suspended material. Their optical properties were deeply analysed in Pérez et al. (2010), showing that clear-vegetated lakes have significantly lower mean vertical attenuation coefficient of photosynthetically active radiation (Kd_{PAR}) than turbid lakes. Later, Pérez et al. (2013) examined the differences among the Pampean turbid shallow lakes and found that although all have high light attenuation coefficients, in the inorganic-turbid lakes, much of the light energy is removed by unpigmented particulate absorption. Conversely, in the phytoplankton-turbid Pampean lakes, the light attenuation is closely related to changes in cell pigment content. Shallow lakes of this region also exhibit differences in their dissolved organic carbon (DOC) concentrations, with higher values in clear-vegetated lakes than in turbid systems (Pérez et al., 2010; Torremorell et al., 2014). Even though a considerable amount of DOC has probably allochthonous origin, the main source of autochthonous DOC probably differs among lakes: macrophytes are likely the main organic carbon sources in clear-vegetated systems, whereas DOC derived from high phytoplankton stocks would prevail in phytoplankton-turbid lakes. In inorganic-turbid systems, where both phytoplankton and macrophyte biomasses are low, terrestrial derived organic carbon likely dominates (Llames et al., 2013). DOC and suspended particles are very important in determining light transmission in lakes. Algae shift the absorption maximum to longer green wavelengths and dissolved humic substances to shorter yellow wavelengths (Lampert & Sommer, 2007).

Under the framework of the *Alternative Steady States* model (Scheffer et al., 1993), clear-vegetated and phytoplankton-turbid Pampean shallow lakes represent the two basic alternative states. Scheffer (1998) compiled field-documented examples of the passage from a clear-vegetated to a phytoplankton-turbid state in water bodies from different parts of the world. Nevertheless, although the empirical evidence obtained in hundred of lakes indicates bimodality (lakes are either clear or turbid, and rarely something in between), this is not a proof of alternative stable states, and the regime shifts might or might not correspond to catastrophic transitions (Scheffer, 2009).

Different studies focussed on the microbial plankton communities conducted both in marine and freshwater ecosystems have highlighted the importance of different factors (e.g. transparency, geographic region, DOC content and quality, grazing) in shaping their structure (Lindström et al., 2005; Šimek et al., 2005; Jezbera et al., 2006). Planktonic food web structure also changes according to the prevailing equilibrium regimes (Jeppesen et al., 1997). The effects of macrophytes on phytoplankton extend to changes in biomass, species size and composition (Søndergaard & Moss, 1998). Particularly, phytoplankton community structure in habitats with distinct ecological regimes shows a general tendency: higher importance of flagellates and predominance of picophytoplankton in clear-vegetated shallow lakes (Søndergaard & Moss, 1998; Mazzeo et al., 2003) with high representation of mixotrophic algae (Allende et al., 2009; Izaguirre et al., 2012). Contrarily, in phytoplankton-turbid shallow lakes, autotrophic phytoplankton is mainly represented by fast-growing algae such as certain chlorophytes, small diatoms and filamentous or colonial Cyanobacteria (Allende et al., 2009; Sánchez et al., 2010; Izaguirre et al., 2012). Studies performed on the picoplanktonic size fraction (Allende et al., 2009; Silvoso et al., 2011; Fermani et al., 2013, 2014; Torremorell et al., 2014) revealed that picocyanobacteria usually dominated photosynthetic picoplankton in the eutrophic Pampean shallow lakes.

The dialysis bag experiments constitute an excellent approach to investigate in situ the impact of environmental factors on microbial communities (Herndl et al., 1993; Gasol et al., 2002; Šimek et al., 2005; Grossart et al., 2008). We report here, a short-term experiment that consisted in the cross-transplant of water between a clear-vegetated and a phytoplankton-turbid lake using dialysis bags. We analysed the variations in the microbial plankton communities (composition, abundance and biomass) associated to changes in the limnological conditions. The experiments were conducted in two shallow lakes from the Pampa Plain (Argentina) that differ in their regimes. We aimed to detect the timing of the changes in the microbial assemblages, by sampling at short time-intervals considering the generational times of the plankton microbial components. We postulate the following hypotheses and predictions:

(a) The >2 μm-phytoplankton composition is affected by the different conditions in the lake

according to their regimes. We predict changes in the phytoplankton composition when the community is transplanted to a lake with a contrasting regime.

(b) In the autotrophic picoplankton, the proportion of picocyanobacteria (Pcy)/picoeukaryotes (Peuk) is affected by the turbidity of the system. According to some previously published evidences (see review by Callieri, 2007), we expect an increase of Peuk when the community is transplanted from a clear system to a turbid one.

(c) The quality of DOC influences on the biomass of the heterotrophic bacteria (HB). An increase in HB is expected when the community is transplanted into a lake with more availability of DOC.

Materials and methods

Study site

The geomorphology of the Great Plains from Argentina is characterized by very gentle slopes (Quirós & Drago, 1999) that together with the succession of humid and dry climates led to the development of wetlands composed by many shallow lakes (Neiff et al., 1994). The Pampa Plain is a sub-region of the Great Plains, with more than 10,500 permanent water bodies (Dangavs, 2005). The area has suffered strong changes during the last century, many of them related to agriculturization (Quirós et al., 2006). This type of land use has been the main factor in the alteration of the grassland and wetland environments. Probably, the pristine condition of the Pampean wetlands was characterized by clear-vegetated shallow lakes (Diovisalvi et al., 2010). However, during recent years, the intense land use is rapidly modifying the shallow lakes, as they are becoming phytoplankton-turbid hypertrophic ecosystems and most of them lack submerged macrophytes.

The experiment was carried out in two typical shallow lakes from the Pampa Plain (Argentina) (El Triunfo: 35°51′06.29″S; 57°52′22.49″W and El Burro: 35°40′20.60″S; 57°55′32.02″W), which are located in the Great Plains, in the Warm Temperate Region. The selected water bodies belong to the Salado River Basin and show contrasting submerged plant development and optical properties. El Triunfo (area: 1.5 km^2, $z_{max} = \sim 2$ m), showed in the time of

our experiment a clear-vegetated regime, with large stands of macrophytes (mainly *Ceratophyllum demersum*). El Burro (area: 10.2 km^2, $z_{max} = \sim 2$ m) is a turbid shallow lake with high phytoplankton biomass (Allende et al., 2009; Sánchez et al., 2010). The distance between these two shallow lakes is about 25 km. Pérez et al. (2010) indicated strong differences between El Triunfo and El Burro as regards mean values of Secchi depth (1.05 and 0.17 m, respectively), nephelometric turbidity (4.0 and 27.3 NTU, respectively) and Kd_{PAR} (4.8 and 13.9, respectively). Both water bodies have high conductivities, high DOC concentrations and high phosphorus contents, whereas dissolved nitrogen concentrations can be limiting for phytoplankton growth in the clear-vegetated shallow lakes (Allende et al., 2009; Sánchez et al., 2010).

Experimental setup

The study was performed in summer (February 25–28, 2008). Two short-term (72 h) transplant experiments were carried out using dialysis bags, which allow the diffusion of dissolved nutrients and organic carbon but prevent the passage of organisms (Herndl et al., 1993). Thus, planktonic components can be incubated within the bags without suffering limitation for these resources for periods longer than 24 h. In our experiment, the dialysis bags (Sigma^{TM}, USA; 8,000–10,000 Da pore-size, ~ 8 cm flat width) were cut to a length of 60 cm (~ 1 l capacity). The bags were washed in hot tap water for 3 h and overnight in cold tap water. Subsequently, they were submerged in distilled water and rinsed.

In the field, we collected 12 L of water from each of the two shallow lakes: clear-vegetated (ET) and phytoplankton-turbid (EB) with a 2-l Niskin bottle from the superficial layer (depth ~ 0.5 m) into a plastic container. For each shallow lake, half of the water was used to fill six 1-l dialysis bags that were closed and hanged subsuperficially and horizontally in a floating device in its own lake water (El Triunfo non-transplanted water = W-ET; El Burro non-transplanted water = W-EB). Figure 1 summarizes the treatment labelling and the experimental setup. The other half was immediately transported in dark and cold conditions to the other shallow lake where other six 1-l dialysis bags were filled and hanged in a floating device (transplanted water El Triunfo = TW-ET; transplanted water El Burro = TW-EB). In the two water bodies, we also hanged six dialysis bags
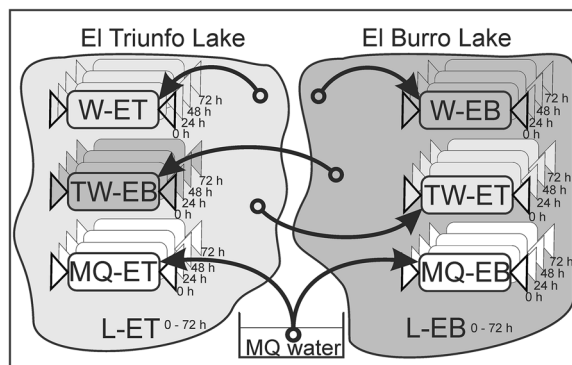
Fig. 1 Scheme of experimental design. Treatments corresponding to the transplant experiment from clear-vegetated El Triunfo (ET) to phytoplankton-turbid El Burro (EB) shallow lakes. *W-ET* non-transplanted water from ET incubated in ET, *TW-ET* transplanted water from ET incubated in EB, *W-EB* non-transplanted water from EB incubated in EB, *TW-EB* transplanted water from EB incubated in ET, *L-ET* ET natural lake water outside the microcosms, *L-EB* EB natural lake water outside the microcosms, *MQ-ET* MQ water incubated in ET, *MQ-EB* MQ water incubated in EB

containing MilliQ water in order to evaluate the diffusion of dissolved components inside the bags (MilliQ water in El Triunfo = MQ-ET; MilliQ water in El Burro = MQ-EB).

At all times and in both shallow lakes, the measurements of limnological variables were carried out both in the water from the dialysis bags (W-ET, W-EB, TW-ET, TW-EB, MQ-ET, MQ-EB) and in natural lake water (L-ET, L-EB). Temperature, pH and conductivity were measured with Hanna HI 8314 and HI 8033 portable electronic metres (HANNA, USA). Chlorophyll *a* (Chl *a*) was measured in situ using a Cyclops-7 Turner Designs (USA) portable fluorometer. For the analyses of dissolved nutrient concentrations—nitrites + nitrates (NO_2-N + NO_3-N), ammonium (NH_4-N), dissolved reactive phosphate (PO_4-P)—200 ml were collected and filtered (Whatman GF/F) at the beginning of the experiment (0 h) and at 24, 48 and 72 h. We used HACH DR/2010 (HACH Company, USA) spectrophotometer and the corresponding kits of reagents (detection limit for phosphates: 0.010 mg l^{-1}; nitrites + nitrates and ammonium: 0.001 mg l^{-1}). An aliquot (50 ml) of the filtered water was acidified and preserved in dark and cold conditions for the estimation of DOC concentrations. DOC was measured by a high-temperature Pt-catalyst oxidation method (Shimadzu TOC-5000) following the recommendations of Sharp et al. (1993).

Plankton structure

The abundances of the different size-fractions of the plankton components were estimated within the dialysis bags (W-ET, W-EB, TW-ET, TW-EB) and in natural lake water (L-ET, L-EB) at 0 h and every 24 h. Water samples (40 ml) for quantitative analysis of heterotrophic flagellates (HF) were fixed with 10% ice-cold-glutaraldehyde (1% final concentration). For HF counts, 5 ml of sample stained with 50 μl of DAPI (4′,6-Diamidino-2-phenyindole, 0.5 mg ml^{-1}) following standard procedures (Porter & Feig, 1980) and filtered through a 0.8-μm pore-size black polycarbonate membrane (MSI). The filters were mounted on a microscope slide with a drop of immersion oil for fluorescence (Immersol 518 F) and stored at −20°C until counting.

Picoplankton (picocyanobacteria, Pcy; picoeukaryotic algae, Peuk; and heterotrophic bacterioplankton, HB) was analysed by flow cytometry. At each occasion, a 4 ml aliquot was fixed with 0.4 ml of ice-cold-glutaraldehyde (1% final concentration). All the samples were kept in cold and dark conditions, immediately transported to the laboratory, frozen in liquid nitrogen and then stored at −80°C. Two subsamples were taken for separate counts of HB and photosynthetic picoplankton (Pcy and Peuk). For HB determination, 4 μl of a DMSO-diluted SybrGreen I (Molecular Probes) stock (100:1) was added to 400 μl of sample (final concentration of 10^{-4} of the commercial solution), left for about 10 min in the dark to complete the nucleic-acid staining and run in the flow cytometer. We used a FACSAria II (Becton–Dickinson, USA) flow cytometer equipped with a 13 mW Argon-Ion blue laser (488 nm emission) and an 11 mW Helium–Neon red laser (633 nm emission). At least 60,000 events were acquired for each sample (usually 90,000 events). Fluorescent beads (1 μm, Yellow-Green Fluoresbrite carboxylate microspheres, Polysciences Inc., USA) were added at a known density as internal standards. The bead standard concentration was determined by epifluorescence microscopy. HB were detected by their signature in plots of side scatter light (SSC) versus green fluorescence of nucleic-acid bound stains (Gasol & del Giorgio, 2000). For photosynthetic picoplankton (Peuk and Pcy), we used the same procedure as for HB but without addition of stain. Small algae were easily identified in plots of SSC versus blue laser-

dependent red fluorescence (chlorophyll), and red laser-dependent far-red fluorescence (phycocyanin) versus chlorophyll (Olson et al., 1993). Data analysis was performed with the CellQuest (Becton–Dickinson) software.

The samples for >2 μm-phytoplankton quantification were fixed with acidified-lugol (1% final concentration) and identified to species level, whenever possible, under an inverted microscope (Utermöhl, 1958).

At the beginning of the experiment, zooplankton samples were obtained by filtering lake water through a 55-μm net and then fixed in situ with formalin 5%. In order to monitor the possible growth of the zooplankton components within the dialysis bags throughout the experiment (at 24, 48 and 72 h), zooplankton samples were obtained by filtering the content left in the dialysis bags, after the removal of samples for all the above-described analyses, and also fixed in situ with formalin 5%. Microzooplankton samples were analysed in a 1-ml Sedgwick-Rafter counting cell under a binocular microscope, and subsamples of dense samples were taken with a Hensen-Stempel pipette. Macrozooplankton samples were examined and enumerated in a 5-ml Bogorov chamber under a stereomicroscope, and large samples were subsampled with a Russell device. Naupliar stages were discriminated. The number of aliquots to be counted (at least three) was calculated in order to keep the estimation error below 10%.

Picoplankton biomass was estimated using the average single-cell HB, Pcy and Peuk biovolumes (0.053, 0.351 and 1.097 μm³, respectively) and the average width for filamentous HB (0.38 μm), previously measured by Kranewitter (2010) in other shallow lake from the same region. Bacterial cell carbon content (C_{bact}) was estimated according to Loferer-Krößbacher et al. (1998) as C_{bact} (fg C cell^{-1}) = 218 · V$^{0.86}$. Carbon content for the individual Pcy cells (C_{pcy}) was calculated using the conversion factor of 230 fg C μm^{-3} according to Worden et al. (2004). Peuk cell carbon content (C_{peuk}) was estimated following the C:V relationship proposed by Menden-Deuer & Lessard (2000) as: C_{peuk} (pg C cell^{-1}) = 0.216 V$^{0.939}$. For the HF biovolume determinations, we applied the sphere shape to each size-group category. The mean biovolume values were converted to carbon using the conversion factor of 0.22 pg C μm^{-3} proposed by Børsheim & Bratbak (1987).

To estimate the biovolume of >2 μm-phytoplankton, a minimum of 30 cells were measured. Linear measurements were converted to cell volume using geometric formulae according to Hillebrand et al. (1999) and were converted into biomass using carbon-volume regressions following Menden-Deuer & Lessard (2000).

Zooplankton lengths were measured under a microscope, and dry biomass was calculated using published length-weight regressions relationships (Culver et al., 1985; Malley et al., 1989). We converted dry weights of all zooplankton to carbon content with a conversion factor of 0.48 (Andersen & Hessen, 1991).

Data analyses

In order to analyse the statistical differences, ANOVA (hereinafter two-way ANOVA) were performed considering time and treatment of the different planktonic components (Pcy, Peuk, >2 μm-phytoplankton, HB, HF and zooplankton) as main factors (Underwood, 1997), using SPSS 15.0®. Posteriorly, Duncan's multiple comparison tests were carried out to identify the treatment(s) that showed significant differences; this test has rules for computing a minimum average risk least significant difference (Bliss, 1967).

A comparison of the phytoplankton composition encountered after the 72 h experiment in non-transplanted and transplanted waters for both shallow lakes was performed by means of the Stander's (1970) Similarity Index (SIMI) (Elber & Shanz, 1989). This index ranges between 0 and 1, where 1 represents the highest similarity between a pair of communities.

Results

The environmental conditions in each shallow lake during the 72 h experiment are presented in Table 1; the most marked differences between water bodies were observed in the Secchi depth, pH and Chl a.

The diffusion through the dialysis bags during the experiment worked properly, since at 24 h, the physical and chemical variables in MQ-ET and MQ-EB treatments did not differ significantly in comparison with the values obtained in the natural shallow lakes (ET and EB). The correct diffusion was also proved for the different treatments.

Table 1 Ranges of the physical, chemical and biological variables measured in El Triunfo (clear-vegetated) and El Burro (phytoplankton-turbid) shallow lakes during the 72 h experiment

	El Triunfo (L-ET)	El Burro (L-EB)
Area (km^2)	1.5	10.2
Z_{max} (m)	~ 2	~ 2
Kd_{PAR} (m^{-1})	7.7	17.2
Secchi depth (cm)	Up to macrophyte bed (~ 70 cm)	19–22
Temperature (°C)	22.3–26.8	25.0–26.5
pH	7.80–8.13	8.90–9.60
Conductivity ($\mu S\ cm^{-1}$)	1,375–1,655	1,563–1,567
Ammonium-N (mg l^{-1})	0.025–0.050	0.005–0.080
Nitrate-N + nitrite-N (mg l^{-1})	nd–0.005	nd–0.020
Dissolved reactive phosphate (mg l^{-1})	nd–0.05	nd–0.14
Dissolved organic carbon (mg l^{-1})	41.1–47.3	31.9–40. 5
Chlorophyll a ($\mu g\ l^{-1}$)	7–14	34–57
>2 μm-phytoplankton (ind. ml^{-1})	2,750–4,155	23,405–38,972
Vegetation	Large stands of macrophytes (mainly *Ceratophyllum demersum*)	

nd no detected values, Z_{max} maximum depth, Kd_{PAR} vertical attenuation coefficient of photosynthetically active radiation

In ET, total >2 μm-phytoplankton biomass increased in transplanted waters (Fig. 2), with significant differences between 0 to 72 h ($P = 0.0003$, Table 2). These differences are given by a clear change in the species that are more abundant; in the TW-ET, the differences were mainly due to the increase of two *Snowella* species (*S. lacustris* and *S. septentrionalis*) with 176.4 ± 66.4 and 196.4 ± 65.0 μg C l^{-1}, respectively at 72 h. Followed by *Oocystis lacustris* (20.7 ± 7.0 μg C l^{-1}), *Scenedesmus* spp. (10.7 ± 5.2 μg C l^{-1}) and *Stephanodiscus hantzschii* (6.8 ± 2.1 μg C l^{-1}). On the other hand, biomass and abundance of *Ochromonas*-like sp. remained unchanged during the whole experiment in the lake and non-transplanted water of ET (L-ET, W-ET) with a biomass and density of about 4.5 ± 0.5 μg C l^{-1} and 517 ± 98 ind. ml^{-1}, respectively, being this group, the most important in terms of abundance. However, when the community of the clear lake was transplanted to the turbid lake (TW-ET), the relative importance of this group diminished after 48 h, decreasing their biomass and abundance to 2.2 ± 0.6 μg C l^{-1} (Fig. 2) and 241 ± 70 ind. ml^{-1} at 72 h, respectively. The high SIMI value obtained between non-transplanted W-ET and natural lake water from El Triunfo L-ET (SIMI = 0.94) evidenced that phytoplankton incubated in ET shallow lake was similar throughout the experiment when compared with the natural lake water.

However, W-ET showed strong differences in the final phytoplankton species composition in comparison with TW-ET (SIMI = 0.42).

The >2 μm-phytoplankton biomass in the turbid shallow lake EB was one order of magnitude higher than those of the clear lake ET (Fig. 2). The treatment TW-EB revealed a significant increase in this phytoplankton fraction throughout the experiment (0–72 h, $P < 0.001$, Table 2). In addition, the phytoplankton biomass of TW-EB (at 72 h) was significantly higher than L-EB and W-EB ($P < 0.01$, Table 2). Also in this case, no significant differences were found between the water of the natural lake (L-EB) and the non-transplanted treatment (W-EB), showing a similar pattern throughout the experiment. The most important species at 0 h EB were *Aphanocapsa delicatissima* (1562 ± 593 μg C l^{-1}) and the filamentous Cyanobacteria *Planktolyngbya limnetica* (93.6 ± 19.2 μg C l^{-1}), followed by *Planktolyngbya contorta* (60.1 ± 22.4 μg C l^{-1}). These three species were the most abundant in >2 μm-phytoplankton in all treatments along the incubation. At 72 h, phytoplankton biomass decreased in the lake (L-EB) and the non-transplanted treatment (W-EB) compared to 48 h. Contrarily, phytoplankton biomass increased in the transplanted treatment (TW-EB). Interestingly, the biomass of small-flagellated Chrysophyceae was low at 0 h
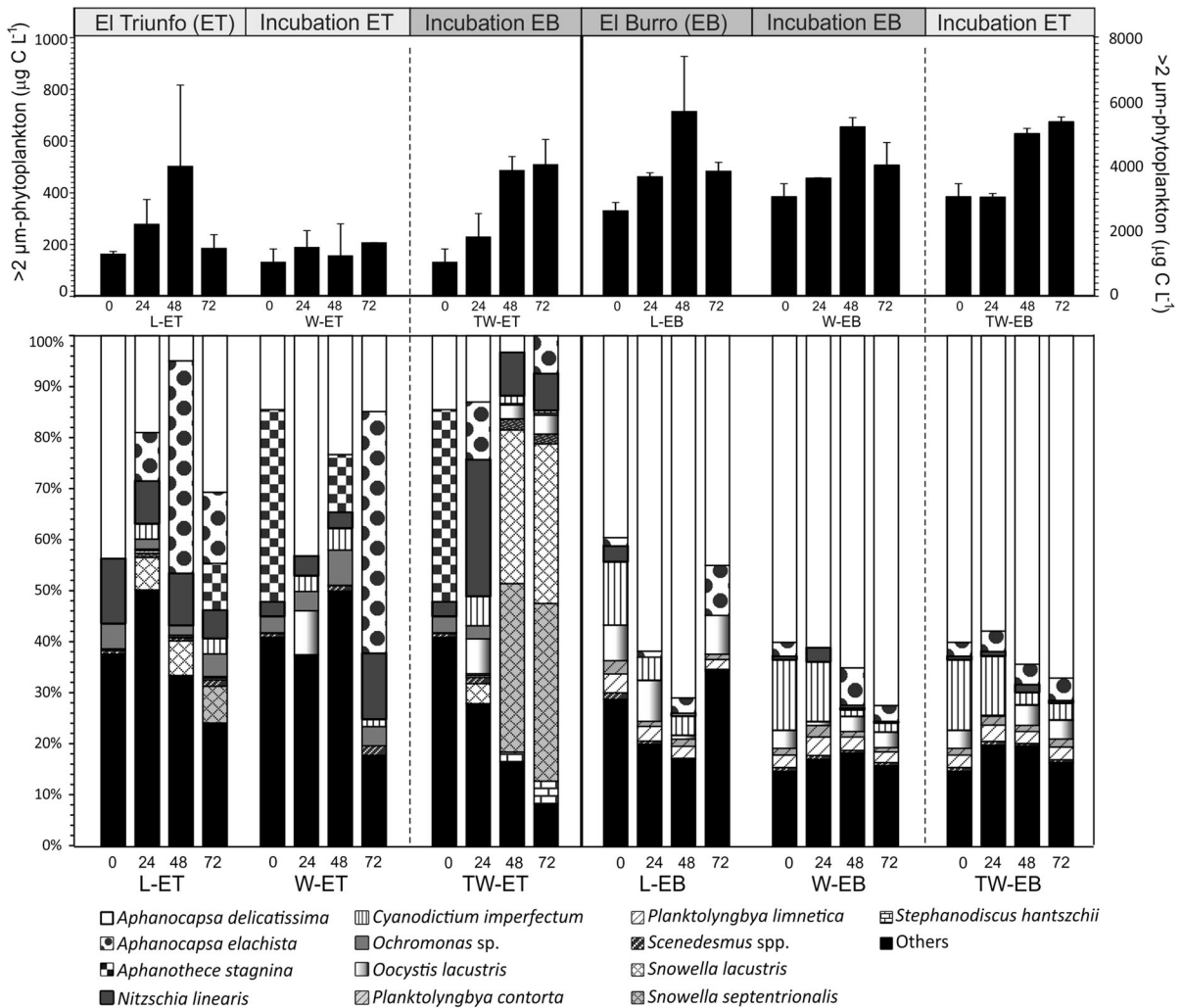
Fig. 2 *Upper panel* Total biomass of the >2 μm-phytoplankton during the 72 h-transplant experiment. *Lower panel* Relative proportion of the biomass for the main species corresponding to >2 μm-phytoplankton during the 72 h-transplant experiment. *L* natural lake water, *W* non-transplanted water, *TW* transplanted water

$(0.9 \pm 0.5 \ \mu g \ C \ l^{-1})$ and showed an increasing trend at 72 h only in the transplanted water into the clear lake (TW-EB) $(9.5 \pm 3.6 \ \mu g \ C \ l^{-1})$, reaching relatively high abundance values at 72 h (from 144 ± 40 to 1767 ± 670 ind. ml^{-1}). As observed in ET, the SIMI values for the >2 μm-phytoplankton obtained between W-EB and L-EB at 72 h were high (SIMI = 0.97). In spite of the differences found between transplanted and non-transplanted waters from EB shallow lake, the SIMI index was high (TW-EB vs W-EB) at the end of the experiment (SIMI = 0.99). This result differs to what was observed for ET shallow lake.

In both lakes, the abundances of Pcy in the natural water (L-ET and L-EB) followed similar trends than

those of the non-transplanted treatments (W-ET and W-EB). Figure 3 shows the biomass of this pico-planktonic component within the dialysis bags during the experiment. Pcy were mostly represented by phycocyanin(PC)-rich cells (PC-rich Pcy) in both lakes, as confirmed by the FL4 signal in the flow cytometer. Even though no significant differences were found in all treatments, some tendencies were observed. The Pcy biomass in TW-ET and W-EB remained similar; in W-ET, the biomass was highest from 24 to 48 h and then decreased at 72 h, whereas in the TW-EB treatment, the Pcy biomass increased throughout the incubation (from $224.9 \pm 15.8 \ \mu g \ C \ l^{-1}$ at 0 h to $276.9 \pm 22.9 \ \mu g \ C \ l^{-1}$ at 72 h).

Table 2 Summary of the results of two-way analysis of variance (ANOVA) performed among the treatment and time for each lake (ET and EB). Only significant results are shown

	Significant results	Post hoc comparisons
>2 μm-phytoplankton	Lake ET: Treatment; *, $F(2,6) = 14.03$ Time; **, $F(1,6) = 38.03$	TW-ET$_{0h}$***, L-ET$_{72h}$**, W-ET$_{72h}$** < TW-ET$_{72h}$
	Lake EB: Treatment; *, $F(2,6) = 9.18$ Time; ***, $F(1,6) = 61.32$	TW-EB$_{0h}$**, L-EB$_{72h}$*, W-EB$_{72h}$* < TW-EB$_{72h}$ LEB$_{0h}$ < LEB$_{72h}$*, W-EB$_{0h}$ < W-EB$_{72h}$*
Peuk	Lake EB: Time; *, $F(1,6) = 11,73$	
HB	Lake ET: Treatment; *, $F(2,12) = 3.93$ Time; **, $F(3,12) = 10.39$	TW-ET$_{0h}$ < TW-ET$_{72h}$**
HF (<3 μm)	Lake ET: Treatment; *, $F(2,12) = 36.5$ Time; **, $F(3,12) = 18.39$ T × T; ***, $F(6,12) = 20.75$	L-ET$_{72h}$, W-ET$_{72h}$, <TW-ET$_{72h}$*** L-ET$_{0h}$, W-ET$_{0h}$, TW-ET$_{0h}$ < TW-ET$_{72h}$***

$T \times T$ interactions between treatment and time; *p-level* * $p < 0.05$; ** $p < 0.005$; *** $p < 0.0005$, F degree freedom

Initial Peuk biomass was 0.06 ± 0.02 and 1.96 ± 0.22 μg C l^{-1} (lakes ET and EB, respectively). The biomass of Peuk in ET remained low and fairly constant throughout the experiment in all the treatments, and no significant differences were found among them (Fig. 3). In EB, Peuk increased towards the end of the experiment in all treatments, resulting significant differences between the biomass at the beginning and the end of the experiment ($P = 0.025$, Table 2).

The HB biomasses in the natural lakes at 0 h were 21.7 ± 1.2 μg C l^{-1} for ET and 439.2 ± 11.1 μg C l^{-1} for EB (Fig. 3). An increase in the HB biomass was observed in ET for both treatments (W-ET and TW-ET) at 48 h; in TW-ET, this increment persisted until 72 h (59.8 ± 5.7 μg C l^{-1}), and significant differences were observed between 0 and 72 h ($P = 0.001$). Contrarily, a decrease at 72 h was observed in non-transplanted W-ET (36.3 ± 0.7 μg C l^{-1}). On the other hand, the turbid shallow lake EB showed a decrease in both treatments (W-EB and TW-EB) at the end of the experiment (445.2 ± 0.5 and 444.7 ± 22.6 μg C l^{-1}, respectively), whereas no significant differences were found among treatments and time.

Initial HF biomass was 74.1 ± 17.9 and 371.0 ± 167.4 μg C l^{-1} for L-ET and L-EB, respectively (Fig. 4). No significant difference was found throughout the experiment in all the treatments. But in the transplanted water (TW-ET and TW-EB), a trend was observed where the biomass increased from 24 h to the end of the experiment in both lakes. At 72 h, the biomass proportion of smaller HF (cell sizes <3 μm) increased

significantly in TW-ET from 1.1 ± 0.8 μg C l^{-1} at 0 h to 6.0 ± 0.3 μg C l^{-1} at 72 h ($P < 0.0001$, Table 2) and also in relation to L-ET and W-ET at 72 h (both with $P < 0.00005$). In all treatments, the proportion of HF 3–5 and >5 μm remained almost constant, and no significant differences were found among treatments and time.

Total zooplankton biomass in the natural lakes were around 55.8 ± 14.5 μg C l^{-1} (L-ET) and 140.5 ± 81.9 μg C l^{-1} (L-EB) (Fig. 5), characterized by a high proportion of rotifers (78–92% in ET and 93–97% in EB). The biomass remained constant throughout the experiment in all treatments, and no significant differences were found among them, although in TW-ET, the biomass showed an increasing trend throughout the experiment (39.5 ± 24.7 μg C l^{-1} at 0 h to 71.0 ± 4.2 μg C l^{-1} at 72 h). In ET, the most abundant species were *Lecane* spp., *Keratella cochlearis* and some species of the class Bdelloidea, whereas in EB, the most abundant species were *Brachionus havanaensis*, *Keratella cochlearis*, *Hexarthra* sp., *Testudinella patina* and some species of the class Bdelloidea. The biomass of *B. havanaensis* remained unchanged during the whole experiment in the non-transplanted water (W-EB) with a biomass and abundance of about 65.15 ± 18.8 μg C l^{-1} and 1671 ± 482 ind. l^{-1}, respectively, being this group the most important in terms of abundance. However, when the community of the turbid lake was transplanted to the clear lake (TW-EB), the biomass and abundance of this species decreased at 72 h to 21.1 ± 10.1 μg C l^{-1} (Fig. 2) and 542 ± 257 ind. ml^{-1}, respectively.
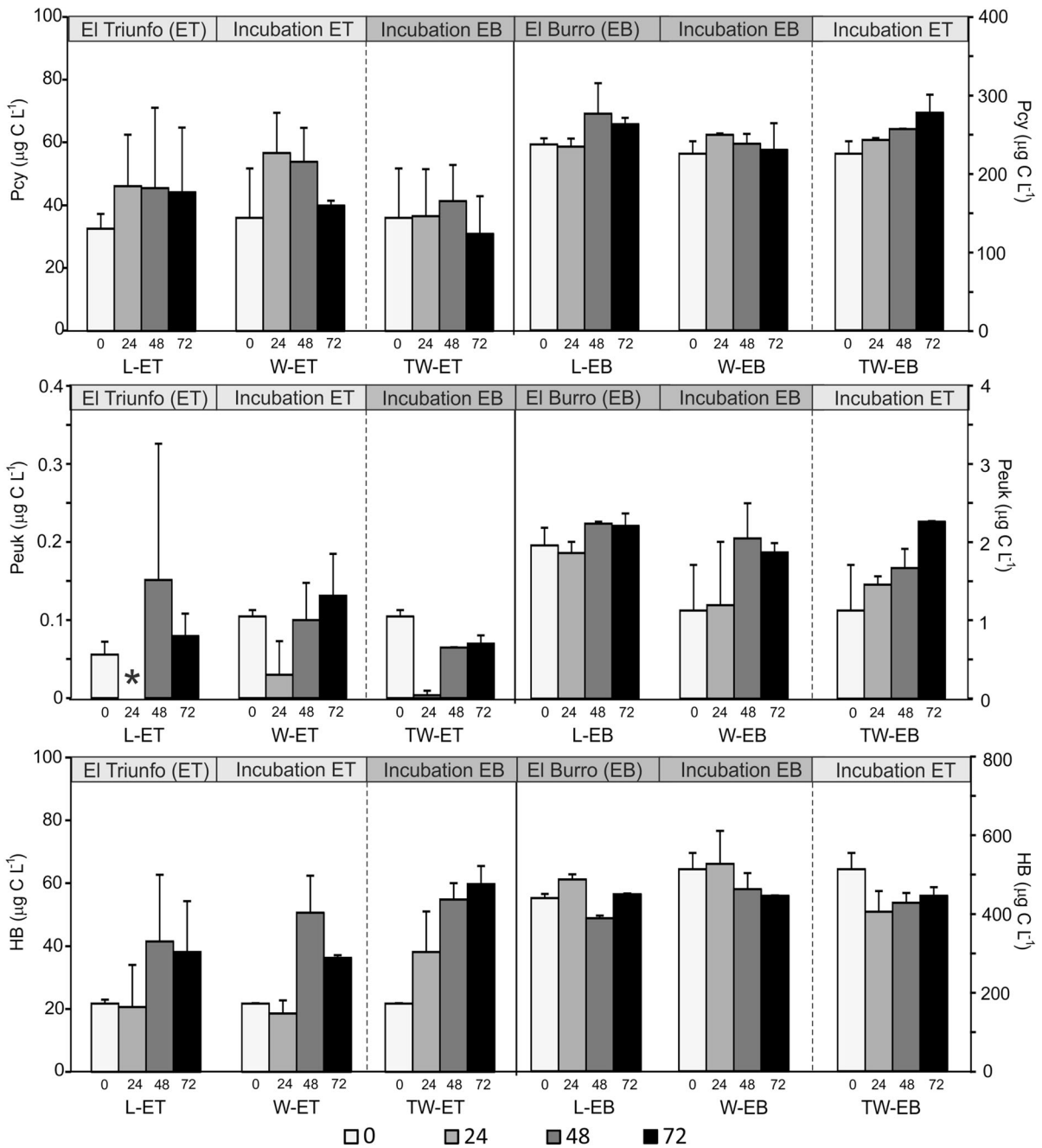
Fig. 3 Biomass of the picoplankton components during the 72 h-transplant experiment (0, 24, 48 and 72 h): biomass of picocyanobacteria (Pcy), picoeukaryotic algae (Peuk) and heterotrophic bacteria (HB). *Bars* represent total standard deviations. *L* natural lake water, *W* non-transplanted water, *TW* transplanted water. *Non-determined

Discussion

In this cross-transplant experiment, we assessed the changes triggered in the structure of the microbial planktonic communities in response to the variations in the limnological conditions from a clear-vegetated to a phytoplankton-turbid shallow lake and vice versa. We observed evident changes in >2 µm-phytoplankton
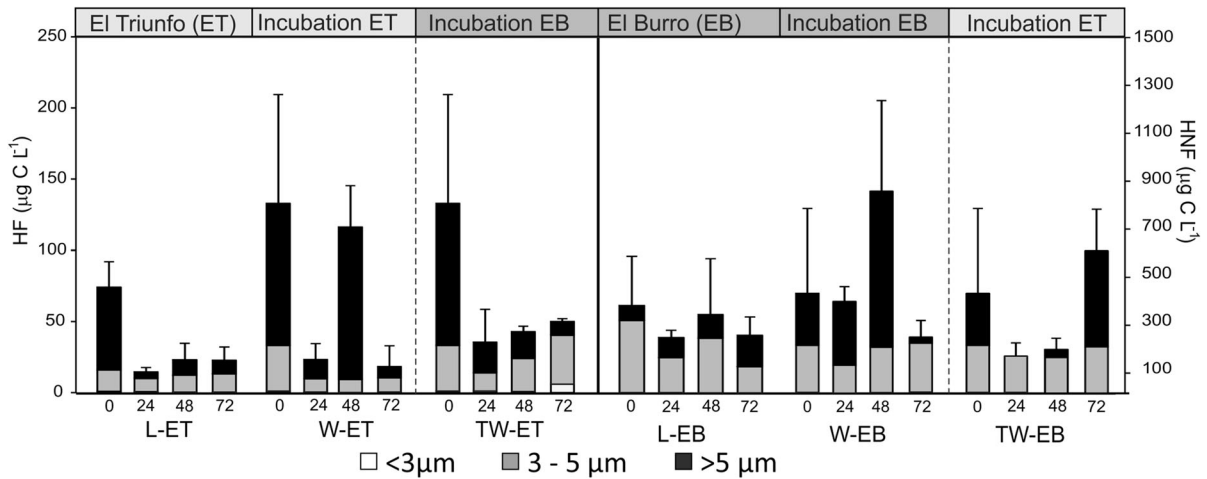
Fig. 4 Biomass fluctuations registered during the 72 h-transplant experiment for heterotrophic flagellates (biomass of cell sizes: <3, 3–5 and >5 μm). *Bars* represent total standard deviations (three cell sizes). *L* natural lake water, *W* non-transplanted water, *TW* transplanted water
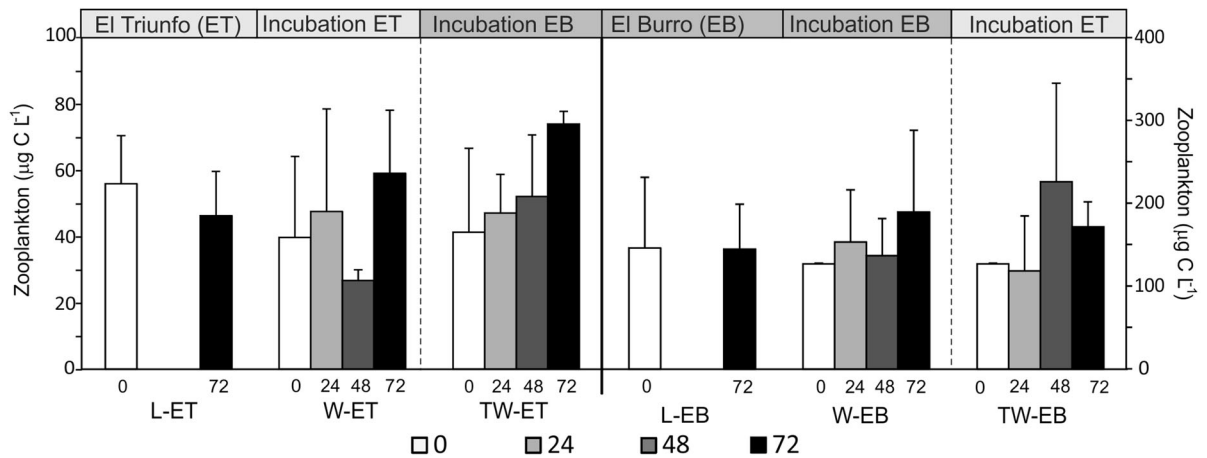

Fig. 5 Biomass of total zooplankton registered during the 72 h-transplant experiment. *W* non-transplanted water, *TW* transplanted water

composition in the two transplanted communities (TW-ET and TW-EB), thus, confirming the first hypothesis of our study (Hyp. a).

The changes in >2 μm-phytoplankton observed when the community was transplanted from the clear-vegetated lake into the turbid lake (TW-ET), can be explained by the shift in the optical conditions and nutrient contents. Particularly, the increase in Cyanobacteria species (*Snowella* spp.) in this treatment could be explained since this group is well adapted to low light conditions as observed in eutrophic turbid lakes (Tilzer, 1987; Scheffer et al., 1997). Although total nutrients (N and P) concentrations in the studied lakes

are typically high (Allende et al., 2009; Sánchez et al., 2010; Silvoso et al., 2011), inorganic forms (nitrites + nitrates; ammonium; phosphates) were particularly low throughout the experiment, especially nitrogen in the clear-vegetated lake ET. In this shallow lake, the nitrogen concentration can be limiting for algal growth according to the ranges reported by Reynolds (2006), mainly due to the uptake of this nutrient by the abundant submerged macrophytes (Villar et al., 1998). The natural phytoplankton of clear-vegetated lake was dominated in numbers by mixotrophic (i.e. phagotrophic) chrysophyceans, which decreased both in abundance and biomass when

water was transplanted to the turbid lake EB. Several experiments support the idea that the mixotrophic mode of nutrition is an adaptive strategy for growing under resource (i.e. nutrient) limiting conditions (Nygaard & Tobiesen, 1993; Rothhhaupt, 1996; Samuelsson et al., 2002; Unrein et al., 2007). Katechakis & Stibor (2006) experimentally demonstrated that the mixotrophic chrysophyte *Ochromonas tuberculata* has a competitive advantage over specialist phototrophs and specialist phagotrophs when nutrients are limiting. Moreover, Olrik (1998) found that high abundances of mixotrophic chrysophytes mostly occur at inorganic nitrogen concentrations below 0.5 mg l^{-1}, and their abundance was higher close to the detection limit of this nutrient. Our findings fit this trend as the abundance and biomass of mixotrophic algae decreased with the increase of nitrogen in the transplanted community. Besides the higher nutrient content, another possible cause of the decrease of chrysophytes in the transplanted water of the clear lake (TW-ET) can be the CO_2 availability; as it was demonstrated by Maberly et al. (2009), high pH waters (like lake EB) do not contain free CO_2, and chrysophytes do not have the ability to make use of bicarbonate as an alternative source of inorganic carbon.

Interestingly, in our experiment, in the transplant from the turbid lake to the clear-vegetated one, an increase in the autotrophic phytoplankton was observed. This result seems to be contrary to the expected diminishing in the phytoplankton biomass that usually is associated to the shift from a turbid to a clear regime. This difference could be related to the relatively short time of our experiment. In eutrophic turbid systems, the poor light conditions may be limiting for phytoplankton growth, and even species well adapted to turbid waters may be favoured under better light conditions. Thus, the improvement of light quality and quantity may have stimulated the increase in the autotrophic fraction. In relation to phytoplankton composition, the most important change was the increasing trend of chrysophytes when the water of the turbid lake was transplanted into the clear-vegetated lake.

In relation to the picoplanktonic fractions (autotrophic and heterotrophic), the different responses observed in our experiment are probably related to the following factors: light quality, dominant DOC fractions and grazing pressure of picoplanktivorous protists. As it was reported by Pérez et al. (2010), significant differences in Secchi depth, nephelometric turbidity and Kd_{PAR} were found between ET and EB shallow lakes, showing the contrasting regimes of both lakes. The increase in Pcy biomass when the community was transplanted from the turbid lake (EB) into the clear-vegetated lake (ET)—together with a relatively constant pattern observed in transplant from ET to EB—might be explained by differences in the underwater light climate. We hypothesize that better light conditions in the clear lake would have favoured Pcy growth. Different studies have shown that light is a very important factor in niche differentiation for Pcy (Callieri, 2010 and cites therein). The pigment composition of Pcy defines individual strains, but closely related strains can have different pigment composition (Everroad & Wood, 2006). On the other hand, although some previous studies have reported that the Peuk contribution to the total picoplankton biomass would increase at higher Kd values (e.g. Pick & Agbeti, 1991; Vörös et al., 2009), in our study, the contribution of Peuk was relatively low in both lakes, and no clear trend was observed in the cross-transplant. From these results, the second hypothesis postulated in our experiment (Hyp. b) has to be rejected.

Even though the recognition of the importance of DOC in lakes is not new, there is a burgeoning interest in it lately, and it is becoming more clear that this component plays a crucial role in many processes and in particular in those that involve anthropogenic impact (Williamson et al., 1999). In our study, ET and EB shallow lakes presented similar high DOC concentrations (about 40 mg l^{-1}), which are not limiting for heterotrophic bacterial growth sensu del Giorgio & Peters (1993). However, the study, carried out by Pérez et al. (2010) regarding the main light absorbing components in these two water bodies, evidenced a difference in the prevailing origin of their DOC. An interesting finding derived from the study of the absorption coefficients at 440 nm revealed that whereas in ET, coloured dissolved organic matter (CDOM) was the fraction with the highest absorption of surface quanta (representing 80% of the total light absorption), phytoplankton absorption was the most relevant fraction absorbing at the red spectral segment (43%) in EB shallow lake. Thus, in the turbid lake EB, where algal biomass was very high algal-derived DOC would prevail over other autochthonous sources (i.e. macrophyte-derived DOC). Contrarily, in the clear-vegetated lake ET, autochthonous DOC would mostly

derive from the degradation of macrophytes, resulting in a more humic and aromatic CDOM than phytoplankton-dominated lakes (Torremorell et al. 2014, this special issue). Differences in DOC origin would imply differences in DOC quality, which would affect bacterial metabolic rates. Recently, a comparison of microbial pelagic metabolism between a phytoplankton-dominated and a macrophyte-dominated shallow lake suggests a higher importance of phytoplankton-derived DOC related to other sources of DOC (either from macrophytes or terrestrial) shaping bacterial production and, a dependence of bacteria on phytoplankton for a supply of labile DOC in the macrophyte-dominated lake (Torremorell et al., op cit.). It is now well known that the availability of DOC to bacterioplankton is mainly related to the lability of the molecule, its origin and age, and other factors such as the accessibility to phosphorus and nitrogen and sunlight, which induces photochemical transformations of recalcitrant compounds into simple DOC molecules (Farjalla et al., 2002). The higher availability of simple carbon molecules in the phytoplankton-turbid lake could have promoted a steeper response in the HB development as the one encountered in the transplanted community from the clear lake to the turbid one towards the end of the experiment (72 h), which seems to confirm our third hypothesis (Hyp. c). It is important to note that this increase in HB occurred in spite of the increment of HF < 5 µm. On the other hand, in our study, a decrease in HB was observed when the community of the turbid lake was transplanted into the clear-vegetated lake (TW-EB), probably due to the lower concentration of labile DOC availability. Our findings are in line with the results obtained by Horňák et al. (2005) in a transplant experiment between two sites with different nutrient concentrations and nature/quality of organic C sources. In this experiment, bacterial abundance was efficiently controlled by their predators after 48 h, following a relatively fast initial growth. The consumers decreased bacterial abundance in treatments transplanted to a nutrient-limited area back to their initial numbers, while in parallel, nutrient-rich incubated treatments remained approximately the same or decreased only slightly despite the much higher bacterivory.

In relation to zooplankton, the most important change in the experiment was the decline in *B. havanaensis* when the community was transplanted from the turbid lake to the clear lake. This species was reported as typical of turbid eutrophic systems (Frutos et al., 2009).

The results obtained in our cross-transplant experiment reflect some of the most important changes that may take place in the microbial communities when the lakes experience a regime shift from a clear-vegetated to a phytoplankton-turbid and vice versa. Although the design of our experiment did not include all the variables involved in the regime shifts of shallow lakes (e.g. fish, waves, macrophyte decay), it reproduced well the variations in the main factors that regulate the microbial communities (nutrients, light attenuation, DOC availability and top-down control at the microbial food web) in the two scenarios (clear and turbid).

The nutrient enhancement and subsequent eutrophication of lakes are likely to be a continuing problem as human populations and development expand, and as stated earlier, the agriculturization of plain regions induces the shift of shallow lakes from a clear-vegetated to a phytoplankton-turbid regime. Under this scenario, our results provide the first evidence of the rapid changes that occur in the microbial components when the community is exposed to a different lake regime, with consequences in the aquatic trophic food web.

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