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PATAGONIAN LAKES

Global change effects on plankton community structure and trophic interactions in a Patagonian freshwater eutrophic system

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Abstract The short- and mid-term effects of a simulated global change scenario (i.e., Future) of increased nutrients, acidification, and solar radiation, in the presence or absence of grazers, were evaluated on a freshwater plankton community of Patagonia, Argentina. We used a cluster experimental design with microcosms incubated outdoors simulating the in situ (i.e., Present) and the Future conditions. Short-term changes in net productivity and respiration, together with mid-term changes in the community (abundance, biomass, and phytoplankton cell size) were measured. Phytoplankton had lower net productivity and higher respiration and zooplankton had, in general, higher respiration under the Future than that under the Present condition when organisms were exposed to UVR. The mid-term impacts of the Future condition were neither significant on zooplankton abundances, nor in phytoplankton abundances, biomass, and cell size. Nevertheless, the zooplankton–phytoplankton interaction strength was greater under the Future condition. Zooplankton exerted a strong top-down pressure, regardless of the experimental scenarios, grazing preferentially on small phytoplankton cells, thus decreasing their abundances and biomass. Overall, there were significant short-term impact of our Future global change scenario; however, its effects on mid-term time scales were not significant, and indeed, the zooplankton top-down pressure was the main driver that shaped the phytoplankton community.

Keywords Acidification · Nutrients · Phytoplankton · Solar radiation · Zooplankton

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Introduction

Global change is a complex process that might cause, among others, variations in the phenology and biodiversity patterns, reduction of water quality, modification of biogeochemical cycles, and diverse effects in the metabolism of organisms (Wrona et al., 2006; Winder & Sommer, 2012; Häder et al., 2014). Nowadays, one major scientific challenge involves the understanding of organisms' responses to global change to better predict its impacts on ecosystems'

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functioning and services. Freshwater ecosystems, such as lakes and lagoons, are among the most threatened by global change and growing human population (IPCC, 2013), and so, they have been considered as "sentinels" of change (Williamson et al., 2009). In these environments, the increased acidification (Findlay et al., 1999; Bleiwas & Stokes, 2011), temperature (Wojtal-Frankiewicz, 2012; Rasconi et al., 2015), and solar radiation levels (Sommaruga et al., 1997; Fischer et al., 2006b) as well as the increased nutrients inputs mostly due to human activities (e.g., growth of the cities, dumping of waste materials, agricultural use of the land, Smith et al., 1999; O'Neil et al., 2012), and the increased dissolved and particulate organic matter (DOM and POM) from terrestrial origin (Williamson et al., 2009; Häder et al., 2015; Kissman et al., 2017) have been reported as the main global change-related variables affecting the structure and dynamics of plankton communities. Some of these variables are interrelated and so, for example, increased rain may bring not only more DOM and POM to water bodies but also nutrients (Evans et al., 2006; Monteith et al., 2007). This higher content of organic matter in turn, interacts with solar radiation as it attenuates its penetration in the water column, somehow counteracting the predicted higher radiation levels as a consequence of shallower and more stable pycnoclines (Behrenfeld et al., 2006; Häder et al., 2015; Helbling et al., 2015). Thus, during the assessment of the impact of global change on aquatic communities, not only the direct but also the feedback effects should be considered (e.g., Helbling et al., 2015).

Previous studies on the global change impact have mainly focused on the responses of individual variables on aquatic communities (e.g., eutrophication: Smith et al., 1999, ultraviolet radiation: Williamson et al., 1999); more recently, however, several studies have highlighted the importance of considering multivariables approaches to better understand and predict how communities will respond to complex future environmental conditions (Garcia et al., 2013; Boyd et al., 2016). This is because in Nature, ecosystems are simultaneously subjected to the action of multiple variables which may act in a synergistic or antagonistic manner (Crain et al., 2008; Dunne, 2010); thus the observed responses do not necessarily represent the sum of the individual effects of each variable (Villafañe et al., 2015a). For example, previous studies showed that high nutrient availability reduces the negative effects of UVR by improving the phytoplankton's photosynthetic performance (Heraud et al., 2005; Marcoval et al., 2007; Cabrerizo et al., 2014). However, other studies evidenced that the interaction between UVR and phosphorous (P) enrichment negatively affected phytoplankton growth and primary production, possibly because the algae had been severely limited by P availability and thus, the UVR effects had been originally masked (Veen et al., 1997; Xenopoulos et al., 2002; Carrillo et al., 2008a). On the other hand, it had been observed that acidification and nutrient supply increased the richness of benthic diatoms in an additive way (i.e., the sum of the individual effects), while the same variables acted in a synergistic way reducing the richness of non-diatom taxa (Schneider et al., 2013). Hence, the wide variability in responses evidences the need to further investigate on this issue, as the impact of global change variables differs depending on the species/groups, and also on the type of interaction involved among the factors considered.

Organisms are linked one to another through different types of interactions, e.g., predation, mutualism, commensalism (Persson et al., 1992; Knight et al., 2005); thus, it would be logical to assume that any global change effect on one trophic level would potentially affect others. For instance, it was observed that water acidification drives alterations in the community structure (Hare et al., 2007; Biswas et al., 2011; Schulz et al., 2013), and in the biochemical and elemental compositions of primary producers (Torstensson et al., 2013) which indirectly constrains growth and reproduction of organisms of higher trophic levels (Rossoll et al., 2012). Other studies demonstrated that P enrichment mimicking Saharan dust inputs increased algal biomass (Carrillo et al., 2008b); these inputs, together with UVR exposure, reduced the seston carbon-to-phosphorus (C:P) ratio (Xenopoulos et al., 2002; Carrillo et al., 2008b; Hessen et al., 2008), enhancing consumers' growth by simultaneously improving both food quantity and quality (Villar-Argaiz et al., 2012). While bottom-up processes (i.e., nutrient availability) have been traditionally considered as the main drivers of the phytoplankton's community structure (Hairston et al., 1960; Tilman et al., 1982), several studies indicated that top-down processes (i.e., zooplankton predation pressure) can also strongly regulate it (Elser & Golman, 1991; Muylaert et al., 2006). For example, DOM has been

shown to increase the biomass of zooplankton, and through the subsequent intensified selective grazing of the zooplankton, it can stimulate shifts in the phytoplankton species' assemblage (Kissman et al., 2013). Other studies reported reductions in the grazing pressure of UVR-sensitive zooplankton species, resulting in the increase of primary producers' biomass under high UVR levels (Bothwell et al., 1994). Overall, and while there is an overwhelming number of studies that have focused on the responses of individuals from a specific trophic level (Walther et al., 2002), our understanding of the interactive effects of global change-related variables when two or more trophic levels are interacting is comparatively limited (Montoya & Raffaelli, 2010; Walther, 2010; but see Feuchtmayr et al., 2007; Katrina et al., 2012).

Freshwater ecosystems represent a small percentage of the Earth's surface; however, they are vital for populations, as they provide a wide range of essential services, such as drinking water and food, as well as transportation and recreation (Williamson & Saros, 2008). Freshwater bodies are particularly important in the eastern part of Patagonia, close to the Atlantic Ocean, as humans rely almost entirely on the relatively few of them found in this area (i.e., compared with the western part of Patagonia). These "eastern" water bodies are also distinct from their western counterparts as vegetation is limited in the former (Paruelo et al., 2007) and erosion due to wind stress bring particles and nutrients to them. With this background, the aim of the present study was to experimentally determine the impact of a simulated future global change scenario of increased nutrients, acidification and solar radiation, in the presence or absence of grazers, on a natural plankton community of an "eastern" Patagonian freshwater eutrophic system. To achieve this aim, we used a temperature-controlled outdoors "cluster-type" experiment (Quinn & Keough, 2002; Boyd et al., 2010; Villafañe et al., 2015b), and exposed plankton to two contrasting conditions: "Present," i.e., maintaining the in situ conditions and "Future,"i.e., increased nutrients, acidification, and solar radiation. For these two environmental clusters, we evaluated both the short-term (hours) metabolic responses (net phytoplankton productivity (NP) and respiration (R) of phytoplankton and zooplankton), and the mid-term (days) changes in the plankton community structure (abundance, taxonomic composition, and carbon allocation in terms of cell size), as well as the phytoplankton–zooplankton interaction strength. We hypothesized that Future conditions will benefit large phytoplankton cells, as, due to their low surface-to-volume ratios, the cells would take advantage of the increased nutrients and radiation conditions (Falkowski, 1981). The growth of large cells will be then translated into higher NP under the Future compared with the Present condition, thus changing the size structure of the community. In addition, we hypothesized that our simulated Future scenario will negatively affect zooplankton in a direct way, by reducing its abundance, and in an indirect way, by reducing the predation pressure on the phytoplankton community, as large phytoplankton cells are less edible. Thus, we expect that phytoplankton biomass would increase in the Future scenario.

Materials and methods

Experimental setup

The water samples and organisms used in our experiment were collected at the Cacique Chiquichano Lagoon, a shallow eutrophic freshwater body located on the eastern–central Patagonia (Chubut, Argentina, 43°14′S and 65°18′W) which was used as a model system to test the effects of global change on freshwater plankton communities. The samples were collected on October 1st, 2015 (i.e., the previous day to the start of the experimentation) with an acid-cleaned bucket (1N HCl), put in opaque containers, and immediately transported to the Estación de Fotobiología Playa Unión (EFPU; 20 min away from the sampling site). Experiments to evaluate the combined effects of increased solar radiation, nutrients, and acidification on natural plankton communities were carried out using outdoor microcosms. Traditionally, factorial experiments have been performed to study the combined effects of global change variables (e.g., Feng et al., 2008; Coello-Camba et al., 2014; Sobrino et al., 2014). However, the use of this type of approach, when a large number of variables are considered, is extremely difficult and sometimes is logistically impractical. Therefore, for our study, the responses of plankton communities were tested by comparing two contrasting environmental conditions, the Present and the Future, using a cluster design with

all factors (Morris, 1991; Quinn & Keough, 2002; Boyd et al., 2010) as follows:

(a) Present: Under this condition, the nutrients' concentration as well as the pH remained without modification as in the lagoon at the moment of sampling (i.e., $NO_3^- + NO_2^- = 60$ ($\pm$6.5) µM; $PO_4^{-3} = 22.4$ ($\pm$1.1) µM; and $SiO_3^{-2} = 192$ ($\pm$1.6) µM; and pH 8.2 ($\pm$0.02)). Solar radiation levels received by the cells in the microcosms under this condition were attenuated to 50%, using a layer of neutral density screen, in order to obtain a comparable irradiance level as within the epilimnion.

(b) Future: Under this condition, the nutrients' concentration was increased by $NO_3^- = 80$ µM, $PO_4^{-3} = 36$ µM, and $SiO_3^{-2} = 100$ µM. The acidification was increased by lowering the pH to 7.8, and this pH value was obtained with the additions of CO_3^{2-} (as Na_2CO_3), HCO_3^- (as $NaHCO_3$) and HCl (0.01N) to increase the pCO_2 and dissolved inorganic carbon (DIC) (Gattuso et al., 2010). Under this condition, the samples were left uncovered (receiving full solar radiation) thus simulating a scenario of increased solar radiation (i.e., the worst case), nutrients (ca. duplicating the in situ values), and acidification (decreasing ca. 0.4 U of pH). To the best of our knowledge, there are no predictions on how these variables would change in the future for the studied lagoon or similar water bodies nearby. Thus, our selection of the future conditions was mostly based on the general response patterns predicted for the eastern Patagonia (IPCC, 2013) and also on previous data collected by our group. In terms of solar radiation, we simulated the worst-case scenario of organisms receiving full solar radiation (both in quantity and quality). In the case of nutrients, by duplicating the in situ values, this would represent a conservative estimate due to increases in population, as well as the agricultural use of the land that almost duplicated in the last decade, together with the increased use of fertilizers (Antolini, 2012) resulting in higher inputs of nutrients into freshwater systems (Bermejo et al., unpublished). Furthermore, the aerosol input for the area increased over the last 20 years (Cabrerizo et al., unpublished) resulting in higher atmospheric deposition on waterbodies. In the case of acidification, we based our estimation of change on general ocean data predicted for this latitude (IPCC, 2013).

We evaluated the effects of the Present and Future conditions on the phytoplankton fraction as well as on the phytoplankton–zooplankton community; thus, we performed an additional treatment (hereafter called "grazer" treatment) as follows: (a) −zoo: Present and Future clusters where the zooplankton community was removed (only phytoplankton) and (b) +zoo: Present and Future clusters were both, the phytoplankton and zooplankton communities, were present. By contrasting the responses of phytoplankton in +zoo and −zoo treatments, we were able to obtain information about the predation pressure that zooplankton exerts on the phytoplankton community, as well as its changes as a function of the experimental scenario imposed (Present or Future).

On the day of the collection (during the evening), water samples were dispensed into 12 UVR-transparent containers (20 cm × 20 cm × 25 cm, 10-l capacity; LDPE Cubitainers, Nalgene): in 6 of the containers, the water was pre-screened with a 200-µm mesh to remove large zooplankton (−zoo treatment), while in the other 6, the zooplankton were left (+zoo treatment). A preliminary analysis evidenced that the zooplankton community was entirely composed of macrozooplankton (0.5–5.0 mm) (see below); thus the use of a 200-µm mesh ensured that all the zooplankton individuals (including their larvae) were properly removed in the −zoo treatment. With this set up, triplicate microcosms with and without zooplankton (+zoo and −zoo, respectively) were exposed to the Present and to the Future conditions, as described above. The microcosms were placed outdoors at the EFPU, inside four 200-l water baths with running water to maintain the in situ temperature (12 ± 1°C, controlled every hour by using a handheld digital thermometer) and exposed to solar radiation for four days (October 2nd to 5th, 2015). The microcosms were manually shaken (i.e., hourly) during the daylight period, so that phytoplankton cells could not settle and thus they would receive homogeneous irradiances.

Incident solar radiation was continuously monitored using an European Light Dosimeter Network (ELDONET, Real Time Computers) broadband filter radiometer that measures UV-B (280–315 nm), UV-A (315–400 nm) and PAR (400–700 nm) every second, averages the data over a 1-min interval, and stores them in a computer. This instrument, permanently installed on the roof of the EFPU, is calibrated every year using a solar calibration procedure.

Sampling and determinations

Every day, early in the morning, samples were collected from the microcosms for the determination of chlorophyll *a* (chl-*a*) and pH. Additionally, to evaluate mid-term changes in the structure of the plankton community, samples for phytoplankton and zooplankton identification/counting were taken from each microcosms at the beginning and at the end of the experiment.

Chlorophyll a

Total chl-*a* content was measured by filtering between 150 and 400 ml of water sample from each microcosms onto MG/F glass fiber filters (25 mm, Munktell, Sweden) and extracting the photosynthetic pigments in 5 ml of absolute methanol (Holm-Hansen & Riemann, 1978). A scan between 250 and 750 nm was done using a spectrophotometer (Hewlett Packard, model HP 8453E, USA) and chl-*a* concentration was calculated using the equations of Porra (2002).

pH measurements

Measurements of the pH in the microcosms were done using a pH meter (Hanna, model HI-2211, USA); the pH was adjusted daily as required in the microcosms to keep a constant value of 7.8 (Future conditions) as described above.

Phytoplankton taxonomic analysis

Samples for the identification and enumeration of phytoplankton were placed in 125-ml brown glass bottles and fixed with buffered formalin (final concentration 0.4% of formaldehyde in the sample). Subsamples were allowed to settle for 24 h in 10-ml Utermöhl chambers (Hydro-Bios GmbH, Germany). Phytoplankton species were identified and enumerated using an inverted microscope (Leica, model DM IL, Germany) following the technique described by Utermöhl (1958). Cell dimensions were obtained from direct measurements under the inverted microscope, and the biovolume of each recognized plankton species was estimated according to Hillebrand et al. (1999). From these biovolumes, biomass (as autotrophic carbon concentration) was estimated using the equations of Menden-Deuer & Lessard (2000).

Zooplankton taxonomic analysis

Water samples for identification and counting of zooplankton were collected at the initial sampling time from the lagoon ($n = 3$), and at the end of the experiment from the microcosms in the +zoo treatments under the Present ($n = 3$) and Future conditions ($n = 3$). For the initial sampling time, a volume of 20 l of lagoon water was pre-screened with a 200-µm mesh for each replicate, whereas at the end of the experiment, the water of the entire microcosms (>8 l) was pre-screened; thus all zooplankton organisms were collected. The retained zooplankton were placed in 50-ml Falcon centrifuge tubes and fixed to a final concentration of 2% of formaldehyde. All organisms present in the samples were identified to the lowest possible taxonomic level and counted under a binocular stereoscope (Leica model L2, Germany). A preliminary analysis revealed that the zooplankton community at the time of sampling was completely dominated by the cladoceran *Daphnia* spp. (mainly *D. menucoensis*) and the cyclopoid copepod *Metacyclops mendocinus*. In the case of the *Daphnia* spp. there was high variability in the size of the individuals (30 cladocerans per sample were randomly selected and measured) thus two size classes' intervals were discriminated: 0.72–1.35, and 1.36–2.45 mm, total length; hereafter referred as "small" and "large" *Daphnia* spp., respectively.

Interaction strength

The interaction strength of the whole zooplankton community (i.e., the sum of copepods and large and small *Daphnia* spp. abundances) on their prey (total phytoplankton abundance) was calculated under the Present and Future conditions based on the dynamics indexes (DI; Wootton, 1997; Berlow et al., 1999). The DI calculations were based on data of the total abundance of both grazers and phytoplankton (once the experiment ended) using the following equation:

$$\mathrm{DI} = (\ln(N/D))/Yt$$

where N is the abundance of phytoplankton in the microcosms containing the grazers (i.e., +zoo treatment), D is the abundance of phytoplankton in the microcosms without grazers (i.e., −zoo treatment), Y is the total abundance of grazers (i.e., zooplankton individuals counted in the +zoo treatment at the end of

the experimental period), and t is the time interval in days over which the interaction was calculated (i.e., the duration of the experiment).

Impact of global change variables on plankton metabolic responses

Net productivity (NP) and respiration (R) of phytoplankton

We assessed the impact of UVR under the Present and Future environmental conditions, by performing oxygen measurements to determine NP and R of phytoplankton at the beginning (October 2nd) and at the end (October 5th) of the experimental period. In this way, we had a measure of the sensitivity of the plankton community after being exposed to both environmental conditions. For these measurements, subsamples from each microcosms were taken and placed into 30-ml UV-transparent Teflon FEP narrow-mouth bottles (Nalgene). In the +zoo treatments, zooplankton were removed from the water samples using a 200-µm mesh, just before being placed in the Teflon bottles so that only the responses of phytoplankton were determined. Samples from both, Present and Future conditions, were exposed to two radiation treatments (triplicate samples for each treatment): (1) PAB treatment: samples receiving PAR + UV-A + UV-B (>280 nm, unwrapped Teflon bottles), and (2) P treatment: samples receiving only PAR (>400 nm), Teflon bottles wrapped with Ultraphan (UV 395 Opak Digefra) film. The Teflon bottles were placed in an illuminated culture chamber (Sanyo MLR-350, Japan) at the in situ temperature, and the radiation conditions were provided by 10 Philips daylight fluorescent tubes for PAR and 5 tubes Q-Panel UVA-340 for UVR. The samples were exposed during 8 h to irradiances of 164.1, 42.8, and 0.7 W m^{-2} for PAR, UV-A and UV-B, respectively, which are comparable to the mean daily values during the month of October (Helbling et al., 2005) so that the doses received by the samples during this time period were of 4726, 1232, and 20 kJ m^{-2} of PAR, UV-A and UV-B, respectively. Oxygen measurements were done every hour during the exposure period; then, the samples were maintained inside the chamber for another 3 h in darkness to measure the phytoplankton oxygen consumption, via respiration, every hour. This latter time period was chosen based on preliminary studies (Cabrerizo et al., 2014). Net productivity and respiration rates of phytoplankton were calculated as the slope of the regression line of increases or decreases in the carbon-specific oxygen concentration (i.e., normalized by the phytoplankton carbon content) versus time; thus oxygen rates were expressed as µg O_2 µg C^{-1} h^{-1}.

Oxygen concentration was measured using an Optode Presens system (Mini 10 - PreSens GmbH, Germany) connected to a computer equipped with the Oxyview 6.02 software to register the data. The system was calibrated by a two-point calibration, together with data of atmospheric pressure and temperature, before each set of measurements, following the manufacturer's recommendations.

Zooplankton respiration

Respiration of copepods, small *Daphnia* spp. and large *Daphnia* spp. were determined independently, via oxygen measurements, at the beginning (October 2nd) and at the end of the experimental period (October 5th). At the initial time, 5 large and 10 small *Daphnia* spp., and 10 copepods directly collected from the lagoon were put separately into Teflon bottles filled with autoclaved freshwater from the lagoon. The number of individuals used at the end of the experiment was doubled in order to enhance the signal response of zooplankton. The Teflon bottles -unwrapped or wrapped with Ultraphan film; PAB and P treatments respectively- were placed in the same culture chamber as described above for phytoplankton, so that the individuals received either PAR only or PAR + UVR for 8 h. After this period, oxygen concentration was measured every hour (as described above for phytoplankton) in darkness for 6 h. Respiration rates were obtained from the slope of the regression lines of the individual-specific oxygen concentration versus time, and expressed as mg O_2 l^{-1} $individual^{-1}$ h^{-1}.

UVR effects

The UVR effects on NP (for phytoplankton) and R (for both phytoplankton and zooplankton) during the short-term incubations (i.e., samples under PAB and P radiation treatments) at the beginning and at the end of the experimental period were calculated as:

$$\text{UVR effect} = \left([O_2]_P - [O_2]_{PAB}\right) / [O_2]_P$$

where $[O_2]_P$ is the oxygen concentration rates in the P-treatment, and $[O_2]_{PAB}$ is the oxygen concentration rates in the PAB-treatment. Values obtained for UVR effects on R were multiplied by -1, so that positive values indicate higher R rates under the PAB radiation treatment, while negative values indicated higher R rates under the P radiation treatment.

Data analysis

The relative changes in the abundance of phytoplankton and zooplankton were calculated as the difference between the values at the end and those at the beginning of the experiment. The same calculation was done for changes of the biomass and carbon content per phytoplankton cell—this latter to evaluate changes in the size structure of the phytoplankton community. Thus, for these calculations, positive values indicate an increase, while negative numbers indicate a decrease in the abundance, biomass, or carbon content per cell, with respect to the initial time. Similarly, relative changes in the UVR impact on zooplankton R, and on NP and R of phytoplankton, were calculated as the difference of the impact at the end minus that at the beginning of the experiment for all the conditions. In the case of the relative change in the UVR effects, positive values indicate an inhibition of the NP and an increase in R when previously exposed to UVR, while negative values indicate the opposite.

To determine significant differences in the relative change in the abundance of the different zooplankton groups, *t*-tests were separately performed for copepods, and small and large *Daphnia* spp. Also, differences in the *per capita* interaction strength between Present and Future conditions were evaluated using a *t*-test (Zar, 1999). Additionally, and in the case of phytoplankton, two-way analysis of variance (ANOVA; Zar, 1999) was used to test differences in the relative change in the abundance, biomass, and carbon content per cell, of each phytoplankton group (i.e., Chrysophyceae, Bacillariophyceae, Chlorophyceae, etc.) with environmental conditions (Present/Future) and grazers (±zoo) as factors. When multiple comparisons were done, *P*-values were adjusted using the sequential Bonferroni correction (Rice, 1989) based on the number of *t*-tests (for zooplankton abundances) or ANOVAs conducted (for phytoplankton's relative changes in cell abundance, biomass, and cell size).

Differences in the relative change in the UVR effects on NP and R rates of phytoplankton, with environmental conditions (Present/Future) and grazers (±zoo) as factors, were evaluated with two-way ANOVAs. To determine significant differences in the relative change in the UVR effects on R of the different zooplankton groups, *t*-tests, followed by Bonferroni sequential adjustment, were separately performed for copepods, small and large *Daphnia* spp.

For all statistical analyses, normality (by the Kolmogorov–Smirnov test) and homoscedasticity (by the Levene test) of the datasets were checked before ANOVAs analyses. When necessary, data were transformed to fit parametric assumptions. When significant differences were detected in the ANOVAs, a post hoc Tukey test was performed (Zar, 1999).

Results

Solar radiation

Surface PAR and UVR levels (Fig. 1) were rather similar during the first two days of the experiment, with mostly sunny conditions, whereas variable cloud cover was observed during the last two days (October 4th and 5th). The mean (±SD) daily irradiances (i.e., sunrise to sunset) received by the samples during the whole experiment were 152 ± 37, 19 ± 4, and 0.45 ± 0.09 W m^{-2} for PAR, UV-A, and UV-B, respectively. Daily radiation doses were similar along the experiment (mean ± SD: 7300 ± 311, 930 ± 38, and 21 ± 1.3 kJ m^{-2} for PAR, UV-A, and UV-B, respectively) with the exception of the third day, when the doses were lower, i.e., 4200, 572, and 13 kJ m^{-2} for PAR, UV-A, and UV-B, respectively.

Plankton community structure and interaction strength

The phytoplankton community at the initial time was co-dominated by Chlorophyceae and Bacillariophyceae (Table 1) not only in terms of abundances (i.e., 81% of the total) but also of biomass (i.e., 88% of the total). The initial zooplankton samples were dominated by small *Daphnia* spp. (size class 0.72–1.35 mm) with a mean value of 138 ind l^{-1}

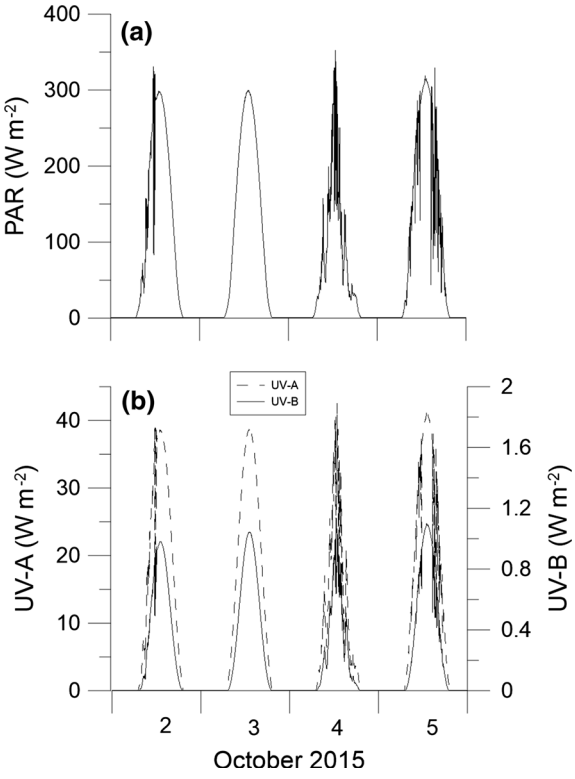

Fig. 1 Incident surface solar radiation for **a** photosynthetic active radiation, PAR (400–700 nm), **b** ultraviolet-A radiation, UV-A (315–400 nm), and ultraviolet-B radiation, UV-B (280–315 nm) over the study area during the experimental period (October 2–5, 2015)

(±5.6), followed by copepods and large *Daphnia* spp. (size class 1.36–2.45 mm) with abundances of 57 (±6.9) and 28 ind l^{-1} (±0.1), respectively. The zooplankton community compositions at the end of the experiment, both under the Present and Future conditions, were similar to that at the initial time, with small *Daphnia* spp. being the most abundant, followed by copepods, and large *Daphnia* spp., respectively. There were no differences (*t*-tests $P > 0.05$) in the relative changes of the abundances of copepods and *Daphnia* spp. (for both size classes) between the Present and Future conditions.

The grazing pressure exerted by the zooplankton throughout the experiment, estimated via the dynamic index (DI) (Fig. 2) revealed that the interaction strength was significantly greater (i.e., more than double) under the Future compared with the Present condition (*t* test $P < 0.05$). The zooplankton grazing pressure on the phytoplankton community throughout the experiment was also reflected in the relative changes in the abundances and biomass of some of the phytoplankton groups that had significantly lower values in the +zoo compared with the −zoo treatment (Fig. 3; Table 2). These lower values in the relative change in the phytoplankton abundances in the +zoo compared with the −zoo treatment were only significant for Chrysophyceae, Bacillariophyceae, and Chlorophyceae (Fig. 3a; Table 2). In spite of the fact that cell abundances decreased due to the zooplankton predation pressure, the Chlorophyceae was still the dominant group at the end of the experiment in the +zoo treatments. A similar situation was observed in the phytoplankton biomass (Fig. 3b) with a general decreasing trend in the relative changes of carbon content in the +zoo (~ -5.8 and ~ -6.5 µC l^{-1} under the Present and Future conditions, respectively) compared with the −zoo treatment where the relative changes of the carbon content increased (~ 2.9 and ~ 3.6 µC l^{-1} under the Present and Future conditions, respectively). Significant differences in the relative change in the biomass between the +zoo and −zoo treatments were only found in the Chrysophyceae and Bacillariophyceae groups (Fig. 3b; Table 2). No significant differences between the Present and Future conditions when comparing the relative change in the abundance/biomass were found for any of the phytoplankton groups (Table 2).

Table 1 Mean (± SD, $n = 3$) cell abundances (in cell ml^{-1}), autotrophic biomass (in µg C l^{-1}) and cell size (in ng C $cell^{-1}$) for the main taxonomic phytoplankton groups at the initial sampling time

	Cell abundance	Autotrophic biomass	Cell size
Chlorophyceae	611 (±131)	4.8 (±0.55)	7.9 (±0.8)
Cryptophyceae	40 (±1.7)	0.33 (±0.01)	8.2 (±0.06)
Chrysophyceae	80 (±25.1)	0.80 (±0.2)	10.1 (±0.6)
Bacillariophyceae	348 (±170.5)	6.12 (±2.1)	18.3 (±2.8)
Cyanophyceae	98 (±109.7)	0.33 (±0.4)	3.05 (±0.6)

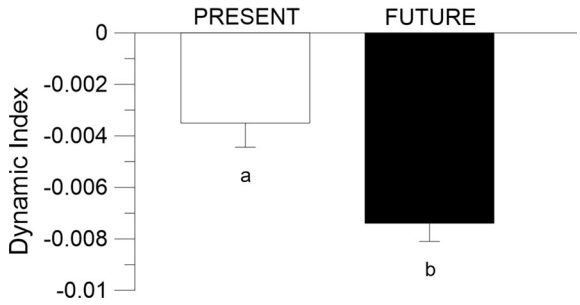

Fig. 2 Dynamics index (DI) as an estimate of the interaction strength between the zooplankton and the phytoplankton community under the Present and Future conditions. The *bars* represent the mean ($n = 3$) and the vertical lines the standard deviation. The greater the modulus of the *bar*, the greater the interaction strength. The *different letters* indicate significant differences between Present and Future conditions

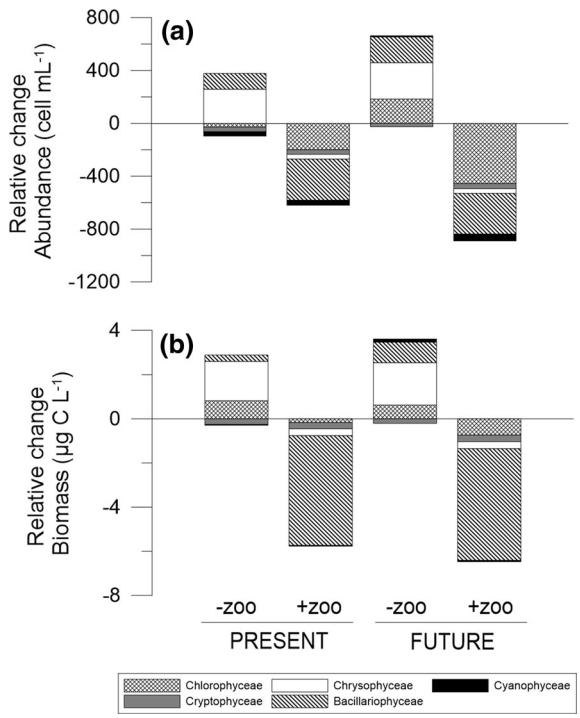

Fig. 3 Relative changes, with respect to the initial sample (t_0), in the main phytoplankton taxonomic groups, Chlorophyceae, Cryptophyceae, Chrysophyceae, Bacillariophyceae, and Cyanophyceae, of **a** abundance, and **b** biomass under the Present and Future conditions, with (+zoo) and without (−zoo) zooplankton. *Positive and negative values* indicate an increase and decrease, respectively, in the abundance and biomass with respect to the initial values

There were no significant changes in the relative cell sizes, when comparing the relative carbon contents per cell between the Future and Present conditions (Fig. 4; Table 2). However, there were significant increases in the cell sizes of Chlorophyceae, Chrysophyceae, and Bacillariophyceae in the +zoo compared with the −zoo treatment, for both the Present and Future conditions (Fig. 4; Table 2) which indicated a highly selective predation pressure on small phytoplankton cells.

UVR effects on NP and R

UVR had a significant impact on R on both, copepods and *Daphnia* spp., with samples previously receiving PAR + UVR having higher R than samples that received only PAR (i.e., positive values of relative change in the UVR effect, Fig. 5). This was especially evident for both size classes of *Daphnia* spp. under the Present, and for copepods and large *Daphnia* spp., under the Future conditions. There were also significant differences between the Present and Future conditions in all groups (*t*-tests $P < 0.05$), with the relative change in the UVR effect on R in the latter being higher (for copepods and large *Daphnia* spp.) and lower (for small *Daphnia* spp.) than the respective value under the Present condition.

There were significant changes in the UVR impacts on NP and R of phytoplankton between Present and Future conditions (Fig. 6). Under the Present condition, the UVR impact on NP was negative indicating a better performance of the cells under UVR exposure, while under the Future condition, the change in NP was rather small. Under both, Present and Future conditions, cells had higher R rates in samples previously exposed to PAR + UVR (positive values) with the UVR effects under the Present condition being significantly lower than that under the Future condition.

Discussion

The main outcomes of our study can be summarized as follows: (a) there was a significant short-term impact of the Future conditions on the plankton communities due to UVR that resulted in higher inhibition of NP and higher R of both phytoplankton (Fig. 6) and zooplankton (with the exception of small *Daphnia* spp.; Figure 5); (b) there was no significant mid-term impact of the Future conditions on zooplankton abundances, nor in phytoplankton

Table 2 Results of the two-way ANOVAs for the effects of Present and Future conditions and grazing (+zoo and −zoo treatments) on the relative changes in abundance, biomass, and cell size of the main phytoplankton taxonomic groups: Chlorophyceae, Chrysophyceae, Cyanophyceae, Cryptophyceae, and Bacillariophyceae

Class	Effect	Abundance		Biomass		Size	
		F	*P*	*F*	*P*	*F*	*P*
Chlorophyceae	Pres/Fut	0.07	0.796	0.71	0.423	2.47	0.160
	+zoo/−zoo	22.45	**0.001**	0.12	0.737	12	**0.010**
	Interaction	7.46	0.026	0.48	0.506	4.86	0.063
Cryptophyceae	Pres/Fut	0.27	0.617	0.03	0.875	1.6	0.247
	+zoo/−zoo	0.64	0.446	1.05	0.336	0.13	0.725
	Interaction	0.86	0.381	0.27	0.617	0.69	0.435
Chrysophyceae	Pres/Fut	0.016	0.901	0.02	0.893	0.01	0.915
	+zoo/−zoo	16.34	**0.004**	19.87	**0.002**	24.46	**0.001**
	Interaction	0.01	0.915	0.02	0.890	0.15	0.712
Bacillariophyceae	Pres/Fut	0.21	0.656	0.03	0.859	2.12	0.184
	+zoo/−zoo	31.20	**0.001**	13.70	**0.006**	42.5	**0.0001**
	Interaction	0.18	0.682	0.06	0.817	0.85	0.384
Cyanophyceae	Pres/Fut	0.04	0.837	0.065	0.806	0.24	0.635
	+zoo/−zoo	0.23	0.647	0.103	0.756	6.82	0.031
	Interaction	0.17	0.691	0.110	0.748	0.18	0.679

The *P* values in bold indicate significant differences after the Bonferroni sequential correction. The degree of freedom for all comparisons was 1

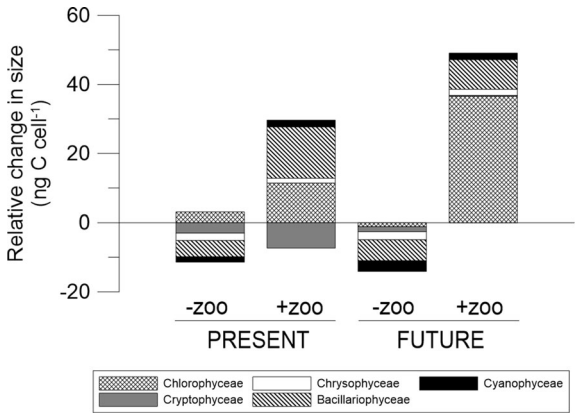

Fig. 4 Relative changes, with respect to the initial sample (t_0), in the carbon content per cell of the main taxonomic phytoplankton groups, Chlorophyceae, Cryptophyceae, Chrysophyceae, Bacillariophyceae, and Cyanophyceae for the Present and Future conditions, with (+zoo) and without (−zoo) zooplankton. *Positive and negative values* indicate larger and smaller cells, respectively, with respect to the initial values

abundance, biomass (Fig. 3; Table 2) and carbon content per cell (Fig. 4; Table 2); and (c) There was a significant mid-term impact of zooplankton grazing upon the phytoplankton community that was evidenced in a significant decrease in cell abundances and carbon biomass of some phytoplankton groups (Fig. 3; Table 2), together with a dominance of large cells in the +zoo treatments at the end of the experimental period (Fig. 4; Table 2). Additionally, DI indices calculated for Present and Future conditions revealed that the phytoplankton–zooplankton interaction strength was greater under this latter condition (Fig. 2). In the following paragraphs, we will discuss in detail each of these results obtained.

Short-term global change impact on plankton metabolic responses: Previous studies have shown that a short-term UVR exposure can inhibit photosynthesis (Sobrino et al., 2008; Cabrerizo et al., 2014) or increase respiration in both phytoplankton and zooplankton (Fischer et al., 2006a; Hamilton, 2011). In our experiment, however, phytoplankton under the Present condition were benefited when exposed to UVR (negative values in Fig. 6) suggesting PAR limitation, as was previously shown in other studies (Barbieri et al., 2002; Gao et al., 2007). However, there were no changes in the relative UVR effects on

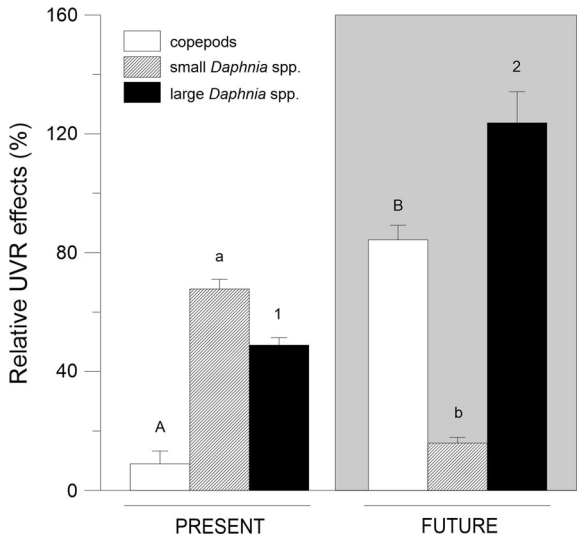

Fig. 5 Relative changes, with respect to the initial sample (t_0), of the UVR effects (%) on respiration (R) of the main zooplankton groups present in the samples, copepods, small *Daphnia* spp., and large *Daphnia* spp. under the Present and Future conditions. *Positive values* indicate higher R rates when previously exposed to UVR. The *bars* represent the mean ($n = 3$) and the vertical lines the standard deviation. Significant differences among treatments are denoted by *capital letters*, *lowercase letters* and *numbers* for copepods, small and large *Daphnia* spp., respectively

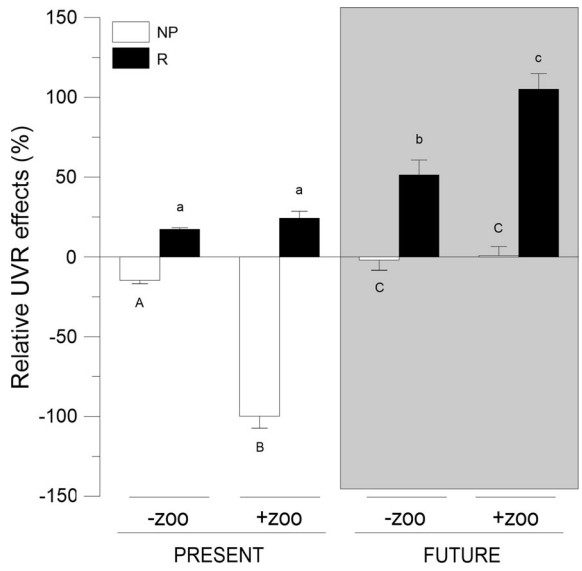

Fig. 6 Relative changes, with respect to the initial sample (t_0), of the UVR effects (%) on net productivity (NP), and respiration (R) of phytoplankton under the Present and Future conditions, with (+zoo) and without (−zoo) zooplankton. For NP, *positive values* indicate an inhibition of oxygen production rates due to UVR, while *negative values* indicate an increase of them when samples were exposed to UVR. In the case of R, *positive values* indicate higher respiration rates when previously exposed to UVR, while *negative values* indicated lower ones when previously exposed to UVR. The *bars* represent the mean ($n = 3$) and the vertical lines the standard deviation. Significant differences of UVR effects on NP among treatments are denoted by *capital letters*, while differences on R are denoted by *lowercase letters*

NP for the Future condition. These contrasts most probably reflect a differential acclimation of the phytoplankton communities under the Present and Future conditions that received different irradiances and thus organisms might need different time lapses to acclimate to the new conditions (Van de Poll & Buma, 2009).

The enhanced respiration rates due to UVR, found in both phytoplankton and zooplankton communities, could reflect in part, the higher energetic costs for repairing cellular components e.g., via DNA and nucleotide excision repair (Sancar, 1996; Sinha & Häder, 2002), or synthesis of protective antioxidant enzymes (Borgeraas & Hessen, 2002) which are all ATP-dependent processes. Previous study demonstrated that both *D. menucoensis* and *M. mendocinus* had a high efficiency for photorepairing UV-B-induced damage to the DNA molecule (Gonçalves et al., 2002). On the other hand, under acidified conditions the copepod *Centropages tenuiremis* increased its food acquisition to compensate the extra energy demand via enhancement of respiration (Li & Gao, 2012). Although food ingestion was not estimated, the DI indices revealed a significantly greater impact of zooplankton upon the phytoplankton community under the Future condition. This suggests that the predation pressure and consequently the *per capita* food consumption were greater under this latter condition and could partly explain the higher respiration rates observed under the Future condition (Fig. 5). While our findings indicate a change in respiration responses from lower values under the Present condition for copepods and large *Daphnia* spp. compared with the Future condition, the opposite was observed for small *Daphnia*. In this latter case, the effect of UVR on respiration decreased significantly under the Future conditions (Fig. 5), suggesting a size-related tolerance to UVR of this cladoceran species. This agrees with previous studies (Grad et al., 2003) that found that juveniles of the rotifer *Asplanchna girodi* were more tolerant to UVR than older individuals; however, some other studies also found the opposite

results (Karanas et al., 1979; Leech & Williamson, 2000; Lacuna & Uye, 2001).

Mid-term global change impact on the plankton community structure: The structure of both phytoplankton and zooplankton communities in terms of abundance, biomass, or carbon content per cell (these two latter for phytoplankton) was not affected by the Future conditions imposed in our study (Figs. 3, 4; Table 2). These findings contrast with a recent work done by Villafañe et al. (2015b) which showed that a scenario of global change of increased nutrients and acidification increased the abundance and the specific growth rates of marine phytoplankton, and also produced changes in the taxonomic composition of the community. On the other hand, and similar to what we observed in the current study, a remarkable resilience of the plankton communities to acidification was reported by Suffrian et al. (2008) who did not find significant effects of the increased CO_2 levels neither on phytoplankton nor in zooplankton. There is a wide range of responses in plankton communities to acidification, and there is consensus that this variability is most probably explained by differences in the organisms' physiology (Hurd et al., 2009). For example, Verschoor et al. (2013) found that elevated CO_2 levels did not have a significant impact on the biomass of algal species, but they shifted their elemental composition toward higher carbon-to-nitrogen and phosphorus ratios. In a study performed by Cripps et al. (2016), it was found that elevated pCO_2 caused biochemical changes to phytoplankton, and this adversely affected the copepod population structure and recruitment (i.e., bottom-up effect). Our results show that the structure of the zooplankton community was not affected by the Future condition, highlighting the lack of direct and indirect mid-term effects of a global change scenario on the abundance of the grazers community. Even though we did not perform food-quality determinations, we cannot rule out at least two possibilities to explain why the global change scenario imposed in our experiment did not indirectly impact on the structure of the zooplankton community: (i) The Future condition did not affect the quality of the phytoplankton cells and, (ii) Changes in the food quality could occur under the Future condition, and they might affect the zooplankton community, but the duration of the experiment was too short to detect such changes in the consumers. A hint toward this latter possibility is the greater DI index (Fig. 2) under the Future compared with the Present condition, which indicates, as we previously discussed, that the *per capita* food consumption was greater under the Future condition. This suggests that zooplankton were ingesting more cells of potentially lower food quality to compensate its energetic needs, a strategy commonly displayed among invertebrates (Cruz-Rivera & Hay, 2003; Prince et al., 2006; Siuda & Dam, 2010). Thus, it is obvious that there is the need to perform experiments in longer-term scales, including the analysis of the food quality to fill this gap of knowledge.

Mid-term impact of zooplankton on phytoplankton community: Even though the DI index for zooplankton–phytoplankton interaction strength was greater under the Future as compared with the Present condition (Fig. 2) this did not translate into differences in the structure and composition of the phytoplankton community (Figs. 3, 4; Table 2). The top-down pressure exerted by zooplankton strongly decreased the abundance of phytoplankton (by ca. 67 and 87% under the Present and the Future conditions, respectively) and hence their biomass (Fig. 3; Table 2) but not in equal magnitude for all the phytoplankton groups. Indeed, a selective predation was observed on Chlorophyceae, Chrysophyceae, and Bacillariophyceae, evidenced by comparing the phytoplankton community structure under the +zoo and −zoo treatments (Fig. 3; Table 2). These results agree with a large amount of evidence that showed strong zooplankton top-down impact on phytoplankton biomass in enclosure experiments (e.g., Elser & Golman, 1991; Vanni & Layne, 1997; Bertolo et al., 2000). Moreover, zooplankton showed a higher predation pressure on small than on large phytoplankton cells (Fig. 4). Several studies found that *Daphnia* species feed preferentially on small cell sizes (Fussmann, 1996; Sommer & Sommer, 2006) while copepods tend to feed on larger ones when they are able to choose among different types (Katechakis et al., 2004; Sommer & Sommer, 2006). Nevertheless, other characteristics than cell size are important in the food selection by zooplankton: While in *Daphnia* spp. the prey size is the main factor determining feeding preferences (Sommer et al., 2003), the selection of food in copepods is also determined by the motility (Tiselius & Jonsson, 1990) as well as the chemical quality, i.e., ''taste'' of the prey (DeMott, 1988). Thus, the top-down pressure effects observed in the present study might be the result of the presence of zooplankton groups (i.e.,

copepods, small and large *Daphnia* spp.) with different foraging strategies allowing grazing on cells of different sizes or characteristics. Nevertheless, considering that both, copepods and *Daphnia* spp. were incubated together in the experimental containers, we cannot discern if a specific phytoplankton group was preferentially affected by a zooplankton species in particular but, at least, we can confirm that overall, they preferentially grazed on small cells. This key role of zooplankton in shaping the phytoplankton community has a particular significance during the late Spring and early Summer when zooplankton density reaches its maximum as a consequence of their high predation pressure upon phytoplankton. During these periods, and due to the zooplankton predation, the water column has a significant increase in transparency thus enhancing the exposure of the organisms to high levels of UVR (Williamson et al., 2007; Gonçalves et al., 2011).

In conclusion, we rejected our hypothesis that large phytoplankton cells will dominate under the Future conditions, as this only occurred when zooplankton were present, due to the strong predation pressure on small cells. We also rejected the hypothesis that the Future conditions will negatively affect the zooplankton community in both direct and indirect ways, as (a) there were no differences in the abundance of the different zooplankton groups between Present and Future conditions, and (b) contrary to what we proposed, the predation pressure was higher under the Future condition, as the interaction strength showed greater values under this scenario. Finally, we also rejected the hypothesis that phytoplankton biomass will increase under the Future scenario as no differences were detected between this condition and the Present one. Plankton communities from eutrophic freshwater environments in eastern Patagonia seem to be sensitive to increased levels of nutrients, acidification, and solar radiation, when short-term effects on their physiology are determined. However, they showed resilience on mid-term scales, and thus, the short-term impacts on NP or R were not scaled-up on changes in abundances and biomass. Moreover, the top-down pressure exerted by zooplankton, and not the Future global change conditions imposed during our experiments, was responsible for shaping the phytoplankton community structure. In our study, we addressed the impacts of some variables related to global change at different time scales; however, interactions among organisms (as shown here) and among other variables, relevant to other environments, should be also taken into account, to gain a greater understanding of the impact of global change on freshwater ecosystems.

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