Warming and CO_2 effects under oligotrophication on temperate phytoplankton communities

Marco J. Cabrerizo[1, 2, 3, *], M. Inmaculada Álvarez-Manzaneda[4], Elizabeth León-Palmero[4], Gerardo Guerrero-Jiménez[4], Lisette N. de Senerpont Domis[5, 6], Sven Teurlincx[5], Juan M. González-Olalla[3]

[1]Centro de Investigación Mariña da Universidade de Vigo (CIM-UVigo), Illa de Toralla s/n, 36331, Vigo (Spain)

[2]Department of Ecology and Animal Biology, Faculty of Marine Sciences, University of Vigo, Campus Lagoas Marcosende, 36310, Vigo (Spain)

[3]Department of Ecology, Faculty of Sciences, University of Granada, Campus Fuentenueva, s/n, 18071, Granada (Spain)

[4]Universitary Institute of Water Research, University of Granada, C/ Ramón y Cajal, P. O. 4, 18071, Granada (Spain)

[5]Netherlands Institute of Ecology (NIOO-KNAW), Droevendaalsesteeg, 10, 6708 PB Wageningen (The Netherlands)

[6]Aquatic Ecology and Water Quality Management group, Wageningen University, Wageningen (The Netherlands)

[*]Corresponding author: marcojc@uvigo.es

[1, 2] Present address

Emails: MJC (marcojc@uvigo.es), MIA-M (miams@ugr.es), EL-P (eleonpalmero@ugr.es), GG-J (ggj_2049@hotmail.com), LdSD

(L.deSenerpontDomis@nioo.knaw.nl), ST (S.Teurlincx@nioo.knaw.nl), JMG-O (jmolalla@ugr.es)

Submitted to: *Water Research*, October 2019

Revised version: submitted January 2020

Keywords: Eukaryotes, global change, photosynthesis, cyanobacteria, resource use efficiency, shallow lakes

Abstract

Eutrophication, global warming, and rising carbon dioxide (CO_2) levels are the three most prevalent pressures impacting the biosphere. Despite their individual effects are well-known, it remains untested how oligotrophication (i.e. nutrients reduction) can alter the planktonic community responses to warming and elevated CO_2 levels. Here, we performed an indoor mesocosm experiment to investigate the warming×CO_2 interaction under a nutrient reduction scenario (40%) mediated by an in-lake management strategy (i.e. addition of a commercial solid-phase phosphorus sorbent - Phoslock®) on a natural freshwater plankton community. Biomass production increased under warming×CO_2 relative to present-day conditions; however, a Phoslock®-mediated oligotrophication reduced such values by 30-70%. Conversely, the warming×CO_2×oligotrophication interaction stimulated the photosynthesis by 20% compared to ambient nutrient conditions, and matched with higher resource use efficiency (RUE) and nutrient demand. Surprisingly, at a group level, we found that the multi-stressors scenario increased the photosynthesis in eukaryotes by 25%, but greatly impaired in cyanobacteria (ca. -25%). This higher cyanobacterial sensitivity was coupled with a reduced light harvesting efficiency and compensation point. Since Phoslock®-induced oligotrophication unmasked a strong negative warming×CO_2 effect on cyanobacteria, it becomes crucial to understand how the interplay between climate change and nutrient abatement actions may alter the, ecosystems functioning. With an integrative understanding of these processes, policy makers will design more appropriate management strategies to improve the ecological status of aquatic ecosystems without compromising their ecological attributes and functioning.

1.- Introduction

Eutrophication constitutes the most pervasive global problem of the last decades affecting all types of aquatic ecosystems (Rabalais et al. 2009). Together global warming and rising atmospheric carbon dioxide (CO_2) levels, these pressures are altering life on Earth in unpredictable ways, with severe consequences for the goods and services that ecosystems provide for humanity (Steffen et al. 2015). In aquatic ecosystems, these stressors influence biogeochemical cycles, carbon-, oxygen- and nutrient-availability, as well as, species physiology and community dynamics (Basu & Mackey 2018). Moreover, freshwater ecosystems, which cover ~4% of the total Earth's, and are more active in terms of C-sequestration, processing and burial than terrestrial and marine ecosystems (Downing 2010; Tranvik et al. 2009), are particularly vulnerable to environmental changes as they are comparably smaller, and often undergo faster changes in ecosystem functions (Yvon-Durocher et al. 2017).

Despite the fact that warming and increasing CO_2 levels are intimately linked, with recent reports claiming the need to study their impacts jointly due to synergistic/antagonistic effects (Kim et al. 2013; Paul et al. 2015; Sett et al. 2018), most of available evidence come from studies: (1) performed in marine ecosystems and (2) with impacts assessed in isolation. On one hand, warming has been reported to exert stimulatory (O'Beirne et al. 2017) as well as an inhibitory (Boyce et al. 2010; Lassen et al. 2009; Osman et al. 2019) effect on the phytoplankton productivity. Also, warming can increase the biodiversity (Yvon-Durocher et al. 2015) and the C-use efficiency (Padfield et al. 2016), reduce species size and shift the community composition (Daufresne et al. 2009), alter the partitioning of the organic matter pools (Wohlers et al. 2009), and ultimately, weaken the CO_2-sink capacity of the ecosystems (Yvon-

89 Durocher et al. 2010; Yvon-Durocher et al. 2017). On the other hand, increased CO_2
90 concentrations have a fertilizing effect that stimulates primary production (Tortell et al.
91 2008), phytoplankton growth and biomass (Egge et al. 2009), and the efficiency of
92 phytoplankton in using limiting nutrients (Paul et al. 2015). Nevertheless, other reports
93 have also showed neither a stimulatory nor inhibitory effect of both stressors acting
94 separately (Li et al. 2019; Strecker et al. 2004; Verschoor et al. 2013) or in combination
95 (Keys et al. 2018) on different phytoplanktonic physiological and metabolic processes
96 (e.g. photosystem II efficiency, growth rates, carbon assimilation rates).

97 The above described changes exerted by temperature (T) and CO_2 on the biotic
98 environment are also affecting the abiotic environment of ecosystems i.e. nutrient
99 availability. Thus, an increased T changes the physiological requirements of
100 phytoplankton toward elevated carbon:nutrient content (De Senerpont Domis et al.
101 2014), and triggers a higher production of prokaryotic (i.e. cyanobacteria) vs. other
102 eukaryotic phytoplankton groups due to higher competitive advantage in nutrient
103 acquisition (Lürling et al. 2018) and resource use efficiency (RUE; Escalas et al. 2019;
104 Filstrup et al. 2014). High CO_2 concentrations favor the production of pico compared to
105 microphytoplankton (Hernández-Hernández et al. 2018; Ji et al. 2017), and do not seem
106 to alter the relative proportion of cyanobacteria (~25%) in phytoplankton communities
107 (Ullah et al. 2018). Surprisingly, most of these studies addressing the interaction
108 between warming (or high CO_2) focused on high nutrient conditions, revealing positive
109 synergistic effects (Schulhof et al. 2019; Villar-Argaiz et al. 2018; Zingel et al. 2018).
110 However, the net impact of the warming (or CO_2) × nutrients interaction may differ
111 when nutrient levels are naturally low (i.e. oligotrophic ecosystems), or when they are
112 reduced through bottom-up (i.e. biological activity) and / or top-down [i.e. management

process - (re)-oligotrophication] controls. In fact, a recent study by Verbeek et al. (2018a) reported that the interaction between warming and re-oligotrophication reduced biomass and diversity but increased RUE. By contrast, such negative effects on community structure, and positive on the RUE disappeared when nutrient concentrations were high.

Thus, as eutrophication constitutes a severe global economic and health problem (i.e. toxic cyanobacterial blooms, Glibert 2019; Jeppesen et al. 2007; Smith 2003), as well as one of the main pressures on water quality (Teurlincx et al. 2019), efforts for reducing the excessive nutrient loading in ecosystems have become a great challenge for water managers. In fact, it is well-known that the first step in reducing high nutrient concentrations is to tackle direct input of phosphorus (P) (Hilt et al. 2006). Based on this, the installment of the EU-Water Framework Directives (e.g. European Union WFD, Union 2000) with the aim to maintain and restore good water quality, may modify the magnitude and direction of the effects that the T×CO_2 interaction could have on ecosystems in the future.

To better understand these multiple pressures at play in aquatic systems, we have tested how the warming×CO_2 interaction under nutrient reduction modulates the responses of a natural plankton community from a highly productive shallow lake. To this end, we performed a controlled indoor mesocosm experiment comprising 9 so-called Limnotrons (Verschoor et al. 2013), in which we manipulated T (ambient or ambient + 3ºC), CO_2 (400 or 1000 ppm) and nutrient availability (ambient concentrations or reduced concentrations by 40% respect to ambient conditions) by adding Phoslock® in a cluster scenarios design (Valiñas et al. 2018; Xu et al. 2014). Throughout the experiment, we monitored the performance of the photosynthetic

apparatus of the phytoplankton, its photosynthetic activity, total biomass, and RUE to test the following questions:

(1) Will an oligotrophication process under a warming and high CO_2 scenario produce an earlier and stronger reduction in phytoplankton biomass by a lower RUE to sustain the algal growth?; (2) Will the warming and high CO_2 interaction produce higher (and maximum) photosynthetic rates during the bloom and under ambient nutrient concentrations by an increased RUE?; and (3): Will a future environmental scenario under oligotrophication unmask a higher sensitivity of prokaryotic (Prok, i.e. cyanobacteria) compared to eukaryotic (Euk) phytoplankton groups due to their higher nutritional requirements to sustain the growth and functioning of the photosynthetic apparatus?.

2. Material and Methods

Study area: This study was carried out with water collected at Lake Ertveldplas (mean depth 10.9 m) in s'Hertogenbosch (Hertogenbosch, The Netherlands, 51.71º N; 5.29º E). The study area (42.5 ha) is a turbid (mean light extinction, Kd_{PAR} = ~3.5 m^{-1}) and eutrophic ecosystem with recurrent phytoplankton blooms (Seelen et al. 2019). It is located close to an area of intense industrial activity and has a harbor used for commercial shipping from the Meuse River and the city of s'Hertogenbosch. Currently, almost all agricultural land surrounding the lake has been converted into a protected nature area (Maas 2014).

2.1. Experimental setup: Surface water (~10000 L, from the upper 1 m of the water column) was collected using a hydraulic pump mounted on a 20000L tank truck on the 17th of July 2018. Sediment (~150 first cm-depth of the sediment layer) samples

were collected in large opaque containers on July 16th, 2018 using an Ekman grab sampler at 10-15 m depth. Upon sampling, water and sediments were immediately transported to laboratory facilities of the Aquatic Ecology Department at the Dutch Institute of Ecology (NIOO-KNAW) and used to inoculate the Limnotrons.

Nine Limnotrons (Ø = 0.97 m; h = 1.35 m) were inoculated with natural sediment from the lake (~0.04 m^3 per Limnotron), and filled with Lake Erveldplas water (900 L per Limnotron), thus mimicking both the natural organismal composition,b, as the physical structure of shallow lake ecosystems. We homogenized both water and sediment prior to inoculating the Limnotrons, to ensure similar starting conditions. The biota was left to establish and acclimatize for fifteen days prior to the experiment (July 17th – August 1st, 2018).

The Limnotrons represented three clusters of environmental scenarios (in triplicate) as follows: (A) **Present**: Limnotrons filled with water maintained under similar conditions as prevailing in Lake Ertveldplas during time of sampling (Temperature = 23±0.7 ºC; carbon dioxide [CO_2] = 400 ppm; pH = 7.40), (B) **Future**: Limnotrons filled with water warmed 3ºC above ambient temperature (Supplementary information Fig. S1A-C), and aerated using a mix of CO_2-enriched air (1000 ppm; pH = 7.20); and (C) **Future + Phoslock®** ($Future_{Phos}$): Limnotrons filled with water that was warmed (Supplementary information Fig. S1A-C) and CO_2-enriched similar to the Future scenario, but here, Phoslock® was added, as a restoration tool, to lock the sedimentary mobile P (resulting in a dose of 1.02 g Phoslock® L^{-1} final concentration). The rationale for evaluating the combined effect of T and CO_2 underlies in the positive feedback found among both drivers over the past climate (Scheffer et al. 2006), and the fact that recent results suggest that much care must be taken in making inferences

184 related with the T and CO_2 effects to an ecosystem level from single-driver studies
185 (Harvey et al. 2013). Phoslock® is a lanthanum (La) modified clay, that has successfully
186 been applied to reduce the dissolved P concentrations in reservoirs, lakes and ponds,
187 and controlling eutrophication (Epe et al. 2017; Lürling and Van Oosterhout 2013).
188 Phoslock® contains 5% La by weight, and P is adsorbed by La at a molar ratio of 1:1
189 (Ross et al. 2008). For that, in dosage calculations, only the freely available P
190 components were considered (i.e. soluble reactive P in the water and mobile P in the
191 sediment; Meis et al. 2013). We chose this sorbent because it has the advantage of
192 stripping dissolved P from the water column, and blocking the P-release from the
193 sediment after settling in the lake bottom (Yuan et al. 2009). Using this chemical solid
194 phase sorbent, we try to simulate an oligotrophication process through a human-induced
195 management strategy (see below), decreasing the P-availability for plankton
196 communities under the above mentioned future global change conditions expected for
197 the end of the 21st Century (IPCC 2013).

198 Experimental warming was achieved by an external electronic element (AKO-
199 71025- 230V-600W) connected to a thermocouple with dual temperature forcing,
200 keeping the target temperature constant at ±0.5ºC (Supporting Information Fig. S1A).
201 Temperature at 0.5 and 1 m depth was continuously logged using the Farex SR mini
202 system with PT100 electrodes. CO_2 concentrations were achieved using a Witt-
203 Gasetechnik device (model KM60-2ME) that aerated both CO_2-enriched and ambient
204 into the Limnotrons at a constant flux rate of 0.5 L min^{-1}. In each Limnotron an impeller
205 was set at 0.54 rotations per min (rpm) to simulate comparable mixing conditions to
206 those that usually occur in the water column of shallow lakes. The illumination
207 (photosynthetically active radiation [PAR]) was provided by cool white-light tubes

208 (surface irradiances: 710.17±81.73 µmol photons m^{-2} s^{-1}; Agrilight AL2007 400;
209 Monster, The Netherlands), and had a 12h L:12h D cycle in order to mimic the daily
210 radiation doses received by communities in Dutch surface waters during the
211 experimental period. Phoslock® addition was implemented by creating a slurry with
212 897 g in a subsample (5 L of water coming from the corresponding Limnotron). Once
213 added, Phoslock® was allowed to settle for 24 h (i.e. August 2nd) prior to starting the
214 experiment. Based on the temporal evolution of the chlorophyll *a* (Chl *a*) over the
215 experimental period, we divided phytoplankton succession into three distinct phases; a
216 pre-bloom, bloom and a post-bloom period (see a description in results).

217 *2.2. Sampling and analysis:*

218 *2.2.1. Inorganic macronutrients:* 50 mL-samples for phosphate [PO_4^{3-}], nitrate +
219 nitrite [NO_3^- + NO_2^-] and ammonia [NH_4^+] were taken using a cylindrical sampler (Ø =
220 0.04 m; h = 1.20 m) that allows for depth-integrated samples throughout the water
221 column of the Limnotrons. Once taken, all samples were frozen and stored at -20ºC
222 until analysis. Nutrient concentrations were determined using a Seal QuAAtro39
223 continuous segmented flow analyzer (SEAL Analytical, Inc., USA).

224 *2.2.2. Dissolved organic and inorganic carbon (DOC):* 50 mL-samples were
225 pre-filtered through pre-combusted (2 h at 500ºC) glass-fiber filters (GF/F Whatman;
226 Whatman®, Sanford, ME, USA), and collected in combusted vials (3 h at 500ºC).
227 Samples were acidified with HCl 1N (2%), and measured by high-temperature catalytic
228 oxidation using a total organic carbon (TOC) analyzer (model TOC-V CSH/CSN;
229 Shimadzu, Kyoto, Japan).

2.2.3. Photosystem II (Φ_{PSII}) photochemical activity and total chlorophyll *a* (Chl *a*)*: In vivo* Chl *a* fluorescence was measured with a pulse amplitude modulated (PAM) fluorometer (Phyto-PAM, Walz, Effeltrich, Germany) equipped with an optical unit (ED101-US). For excitation of the Chl *a* fluorescence, the Phyto-PAM fluorometer applied an array of four different types of light emitting diodes (LED) with emission wavelengths peaking at 470, 520, 645, and 665 nm (Phyto-ML). We used blue, green and brown signals, respectively, as a proxy to differentiate the Chl *a* fluorescence of cyanobacteria (Prok), green algae and diatoms/dinoflagellates (Euk), but also to detect other eukaryotic phytoplankton in the water. For this end, we used an in-built reference spectra stored in the Phyto-PAM and routinely used by our group, which is constituted by three representative phytoplankton species of the above mentioned groups (i.e. *Synechococcus leopoliensis* [a cyanobacteria], *Chlorella vulgaris* [a green algae], and *Phaeodactylum tricornutum* [a diatom]) which allow us properly differentiate blue, green and brown in vivo Chl *a* fluorescence signals, respectively.

10-mL samples were taken from each Limnotron nine times (days 0, 3, 5, 7, 10, 13, 17, 21 and 24) over the experimental period. For each sampling day, we took samples at the beginning of the diel cycle, every 1.5-2 h during the light exposure period (12 h in total), every 30 min during the first 1.5-2 h of darkness and at the end of the darkness period (12 h in total) (10-12 measurements over each diel cycle). The samples were placed in a quartz cuvette and directly measured after sampling without any dark-adaptation. The effective photochemical quantum yield of photosystem II (Φ_{PSII}), a measurement of the cellular fitness, was measured following the equation of Maxwell & Johnson (2000):

$$\Phi_{PSII} = \Delta F / F'm = (F'm - Ft) / F'm \quad (1)$$

where F′m is the maximum fluorescence induced by a saturating light pulse (3832 μmol photons m^{-2} s^{-1} in 0.2 s) and Ft is the current steady-state fluorescence induced by a weak actinic light pulse (704 μmol photons m^{-2} s^{-1}) in light-adapted cells. All measurements were performed without any pre-concentration of the samples and at the same T and light intensity to which they were exposed in the Limnotrons.

The rate of electrons transported through the PSII (rETR, in μmol photons m^{-2} s^{-1}) i.e., an estimation of the photosynthetic rate, was calculated for each measurement over the experimental period from the values of Φ_{PSII} as:

$$rETR = \Phi_{PSII} \times E_{PAR} \times 0.5 \quad (2)$$

where Φ_{PSII} is the effective photochemical quantum yield, E_{PAR} is the PAR energy received by the phytoplankton cells (see above), and 0.5 is a correction factor as half of the absorbed light energy is diverted to the PSII (Kromkamp et al. 1998; Suggett et al. 2003). Daily integrated rETRs for each experimental condition were calculated integrating the rates measured each day vs. time.

Phyto-PAM was also used to measure other photosynthetic properties of the studied communities (Ralph and Gademann 2005). Rapid light curves (RLC) were measured at day 0 (hereafter, Initial) and the last experimental day. RLCs were run with actinic light intensities up to 1619 μmol photons m^{-2} s^{-1}, with each actinic light exposure lasting 10 s, in dark-adapted cells. Photosynthetic parameters such as the photoadaptive index (E_k, a measure of the light intensity for saturation of electron transport), maximum rates of electron transport ($rETR_{max}$), and light harvesting efficiency (α) were determined according to Ralph & Gademann (2005), and are related through the E_k equation (i.e. $rETR_{max}$ / α).

277 *2.2.4. Chl a:* 10 mL-samples were taken at the beginning of each sampling day
278 of the experiment and measured using the Phyto-PAM (Phyto-PAM, Walz, Effeltrich,
279 Germany). The total Chl *a* fluorescence-derived concentration was used as a proxy of
280 the total biomass in the community because recent results by Lürling et al. (2018)
281 evidenced a very high correlation between Chl *a* concentrations determined by classic
282 spectrophotometric techniques and by Phyto-PAM for natural communities from Dutch
283 lakes.

284 The growth rates (μ) in each environmental scenario were calculated based on
285 Chl *a* concentrations as:

286 $$\mu = \text{Ln} (N_{t+1} / N_t) / (t_{+1} - t) \quad (3)$$

287 where N_{t+1} and N_t represent Chl *a* concentrations at the end (t_{+1}) and at the initial
288 (t), respectively, of each phase of the phytoplankton succession.

289 *2.3. Data treatment and statistical analyses:*

290 As the biomass of the communities differed over the pre-bloom, bloom and post-
291 bloom periods, the effect of each environmental scenario on Euk and Prok was
292 compared using log response ratios (LRRs). LRRs are commonly used as effect size
293 metric in ecological research (Lajeunesse 2011), as well as, on the response of
294 phytoplankton communities to global change stressors (Galic et al. 2018; Lürling et al.
295 2018). Thus, the effect of each environment scenario on the response variable
296 considered was assessed as:

297 $$\text{LRR Future} = \text{Ln} (\text{Future} / \text{Present}) \quad (4)$$

298 $$\text{LRR Future}_{\text{Phos}} = \text{Ln} (\text{Future}_{\text{Phos}} / \text{Present}) \quad (5)$$

299 $$\text{LRR Phoslock}^{®} = \text{Ln}\,(\text{Future}_{\text{Phos}} / \text{Future}) \quad (6)$$

300 where LRR Future refers to the effect of warming and high CO_2, and LRR $\text{Future}_{\text{Phos}}$
301 represents the interactive effect of future conditions under oligotrophication (by
302 Phoslock® addition) respect to a present scenario. LRR Phoslock® represents the single
303 effect of oligotrophication by Phoslock® addition. Using LRR reveals the magnitude as
304 well as the direction of the responses relative to control conditions (i.e. Present
305 scenario).

306 RUE was used as a proxy for ecosystem functioning because it allows tracking
307 the functional change in relation or reaction to species change, and was defined as unit
308 biomass production in Chl *a* (µg L^{-1}) per unit total phosphorus (µg L^{-1}).

309 We used one-way analysis of the variance (ANOVA) to test significant
310 differences between environmental scenarios on T, macro-nutrients (PO_4^{3-}, NO_2^-+NO_3^-,
311 and NH_4^+), and DOC concentrations over the entire experimental period; and one-way
312 repeated measures (RM) ANOVA to test significant differences between environmental
313 scenarios over the different stages of the phytoplankton succession (i.e. pre-bloom,
314 bloom and post-bloom) on Chl *a*, RUE and growth rates. A two-way ANOVA was used
315 to test interactions between functional group (Euk and / or Prok) and scenario on the
316 performance of the photosynthetic apparatus (i.e. α, rETRmax and E_k), and on the LRR
317 of such properties. A two-way RM-ANOVA was used to test interactions between
318 functional group (Euk and / or Prok) and environmental scenario on rETR and LRR
319 rETR over the different stages of the phytoplankton succession. Prior to ANOVA
320 analysis, assumptions of Normality (by Q-Q plot residual analysis and Shapiro-Wilk's
321 test) homoscedasticity (by Levene's Equal Variance test) and sphericity (by Mauchly's
322 test) were checked. When the interaction was significant, differences between and

among groups were detected using a Least Significant Differences (LSD) *post hoc* test. All data were analyzed using R Core Team Environment (2014) and the *physcho*, and *car* packages.

3. Results

The changes observed in Chl *a* over the experimental period show the general evolution of phytoplankton blooms: an *initiation* period (or pre-bloom; days 0 – 10) in which biomass remains more or less stable, *succession* (or bloom; days 11 – 17) period in which biomass increases until reach the carrying capacity, and *decay* (or post-bloom; days 18 – 24) period from which the phytoplankton biomass begin to decay (Fig. 1A). Total Chl *a* exhibited a slight increase during the pre-bloom, from 10.9 to 14.1 $\mu g\ L^{-1}$ but without significant differences among environmental scenarios (LSD *post hoc* test, $p > 0.1$). During the bloom phase, Chl *a*, showed a strong increase, and peaked on day 14. Growth rates showed a similar increase with significant higher rates under a Future (255.20±21.30 $\mu g\ L^{-1}$ – 0.44±0.08 d^{-1}) than under a $Future_{Phos}$ (167.20±5.60 $\mu g\ L^{-1}$ – 0.32±0.03 d^{-1}) and Present (57.80±7.50 $\mu g\ L^{-1}$ – 0.20±0.01 d^{-1}) scenarios (Supplementary information Fig. S1D). In contrast, after the bloom phase, a consistent decline in Chl *a* occurred in all scenarios. The initial positive effect of a Future and $Future_{phos}$ scenario on Chl *a* production turned later into a negative effect, resulting in a reduction both in biomass as well as growth rates, which was reflected by a significant interactive Scenario×Time effect on both variables (Chl $a = F_{103.22}$, $p < 0.001$; $\mu = F_{18.01}$, $p < 0.001$) (Fig. 1A; Table S1). However, such reductions in growth were significantly higher in the Future than in the $Future_{Phos}$ scenario (-0.61 vs. -0.32 d^{-1}; Supplementary information Fig. S1D). Nevertheless, the response pattern found during the bloom

346 (Future > $Future_{Phos}$ > Present values) was maintained until the end of the experimental
347 period (Fig. 1A).

348 RUE significantly changed over time under the different environmental
349 scenarios tested (Scenario×Time = $F_{1392.15}$; $p < 0.001$; Table S1). During the pre-bloom,
350 RUE was significantly higher (LSD *post hoc* test, $p < 0.05$) under a $Future_{Phos}$ than
351 under Future and Present scenarios, where no significant differences were detected
352 (LSD *post hoc* test, $p > 0.05$). Maximum RUE values were reached during the bloom
353 phase (day 13 for $Future_{Phos}$ and Present scenarios and day 17 for Future scenario),
354 being significantly higher for $Future_{Phos}$ at the beginning of the phase, but decreasing
355 below the levels of Future scenario during the late bloom period. At the end of the
356 experimental period, maximum RUE was newly (and significantly) higher under a
357 $Future_{Phos}$ scenario (LSD *post hoc* test, $p < 0.001$).

358 Daily cycles of the rETR, a measurement of the photosynthetic activity, for each
359 group of the main components of the phytoplankton community are shown in figure 2.
360 In all scenarios and during the bloom and post-bloom, we observed an expected trough-
361 shaped pattern in which rETR decreased during the light exposure period, followed by a
362 slight recovery during darkness. However, during the pre-bloom the response pattern
363 found was opposite. Regarding the absolute rETR values, these were mostly higher at
364 the beginning of the exposure period of each individual day than at the end, excepting
365 during the post-bloom period where such rates were similar or higher at the end of the
366 diel cycle regardless of the scenario considered. For group-specific responses, our
367 findings show apparent similar (or slightly higher) rETR in Prok compared to Euk
368 during the pre-bloom period under the three environmental scenarios (Fig. 2A-C).

Noticeably, such rates were similar (i. e. bloom period) or even higher (e.g. $Future_{Phos}$) in Euk as the succession progressed.

Based on the previously shown daily responses, we calculated the daily integrated rETRs for each period of the phytoplankton succession (i.e. pre-bloom, bloom and post-bloom), and grouped them into Euk and Prok. Then, we used these values as a proxy of daily production in both compartments of the community (Fig. 3). In all scenarios, we observed that the maximum rates occurred during the bloom for Euk, and during the pre-bloom in Prok. In addition, whereas Euk generally has higher rETR during the succession, Prok showed a significant consistent decline over this period. The observed decrease during the experimental period on Prok was, however, most pronounced in the $Future_{Phos}$ scenario. It was reflected by a significant Scenario×Group (rETR = $F_{13.29}$, $p < 0.001$; Table S2) and a Scenario×Group×Time (rETR = $F_{5.31}$, $p < 0.01$; Table S2) interaction.

Overall, the future scenario exerted a greater stimulatory effect than the present scenario did (assessed as LRR) on daily production of Euk during pre-bloom and bloom, and a lower inhibitory effect during the post-bloom period than those found on Prok (Fig. 3B). This opposite effect on both groups was even stronger under the oligotrophication conditions derived from the Phoslock® addition. Thus, whereas the daily production was enhanced 12-25% in Euk, it was reduced 5-42% in Prok relative to the Present scenario. Oligotrophication derived from Phoslock® addition exerted a stimulatory effect in Euk over the phytoplankton succession, and negative on Prok (except during the bloom phase).

Finally, the reduction observed in the rETR mentioned above in Prok was also coupled with a significantly reduced light harvesting efficiency (α), rETRmax and light

compensation point (E_k; i.e. light intensity where photosynthesis and respiration rates are equal) (Fig. 4A-C and Table S3) over the experiment. In fact, when we quantified the net effect of the environmental scenarios tested, a $Future_{Phos}$ scenario reduced such parameters between -40 and -60% on Prok (Fig. 4D-F). Conversely, they were stimulated (e.g. α; 10-20%; Fig. 4D) or mildly impacted (~-15%; Fig. 4 E, F) in Euk, regardless of the scenario considered.

4. Discussion

This study constitutes the first empirical evidence showing that under a future warming and high CO_2 scenario, oligotrophication through an in-lake management strategy (i.e. Phoslock® application) may greatly impair cyanobacterial photosynthesis compared to other eukaryotic phytoplankton groups. The potential reductions in the carbon uptake were consequence of an impaired functioning of the photosynthetic apparatus due to a higher sensitivity of the cyanobacteria to the environmental drivers assayed. These findings are relevant for understanding how the ecosystem functioning could be impacted by reductions in nutrients availability in a warmer and more CO_2-enriched environment. Even though the duration of our experimental precluded communities from evolving under the prevailing conditions, we consider that our future warming and CO_2 conditions were quite realistic because: (1) the communities were gradually exposed to the future conditions, avoiding shock response which could exacerbate the susceptibility of them to the drivers tested. (2) Communities were exposed to realistic environmental scenarios as predicted by the IPCC (2019) for the end of this century; and (3) we quantified the phytoplankton responses at short- (i.e. diel cycles) and at generational time spans (i.e. weeks) which give us an idea about their acclimation capacity to the future expected conditions.

417 *4.1. Warming×CO_2 impacts on biomass and ecosystem functioning*

418 The faster increases in biomass reported under a future scenario agrees with
419 recent evidences reported in micro/mesocosm experiments investigating warming
420 (Yvon-Durocher et al. 2017; Wilken et al. 2018) and CO_2 (Low-Décarie et al. 2015;
421 Paquette and Beisner 2018) effects in isolation, and their combined impacts but only in
422 marine ecosystems (Sett et al. 2018; Sommer et al. 2015). This result suggests that the
423 patterns observed in marine systems may also apply to other aquatic biomes. This
424 stimulatory effect is likely due to enhanced cellular activities by warming, as stated by
425 the Metabolic Theory of Ecology (Brown et al. 2004), and high CO_2 concentrations
426 prompted a down-regulation of the CO_2-concentrating mechanisms, reducing the
427 cellular energetic expenditure (Raven et al. 2012).

428 After two weeks of incubation, a negative synergistic
429 warming×CO_2×oligotrophication interaction reduced between 30-70% the biomass
430 respect to observed under a future scenario. The observed trend is in support of recent
431 observational results by Verbeek et al. (2018b) showing a 71.5% decrease in
432 phytoplankton biomass over a 10-year period after reductions in the phosphorus
433 availability comparable to our experimental conditions. It is plausible that the
434 warming×CO_2 interaction boosted the nutritional requirements of phytoplankton to
435 grow, hence if such demands were not met by the nutrient availability, this would likely
436 lead to altered RUE and ultimately, to impaired biomass production. In fact, and despite
437 previous results have shown that global change stressors may decrease RUE when
438 nutrients availability does (De Senerpont Domis et al. 2014; Verbeek et al. 2018a), our
439 findings do not support neither reject our own expectations nor previous published
440 results, as maxima RUE was found under the future$_{Phos}$ scenario. This maximum RUE

441 evidenced could lead to potential stoichiometric trophic constraints (De Senerpont
442 Domis et al. 2014). Nevertheless, it is also shown that the RUE was variable as it
443 depended on the phase of the phytoplankton succession. Therefore, it could be plausible
444 that the different cellular status, loss of productive species or buffering of rare species
445 over this period have contributed to accentuate this decay process observed (Corcoran
446 and Boeing 2012) although high RUE values were found. Additionally, considering that
447 an isolated ecosystem, as assessed here, cannot gain species through immigration to
448 maintain the functional redundancy of the community, it is clear that the interaction
449 between several stressors could destabilize key functions in these ecosystems in the
450 future. Notwithstanding, based on our results, we can discard that the changes in
451 biomass reported were influenced by the zooplankton grazing, as we did not observed
452 differences neither in total abundances (~128.10±35.35 individuals L^{-1}) nor in the
453 relative abundance (18.10, 60.80 and 21.10% for cladocerans, copepopds, and rotifers,
454 respectively) of the main contributors to the zooplankton community between
455 environmental scenarios over the experiment (Álvarez-Manzaneda et al., unpub. data).

456 *4.2. Warming×CO_2 impacts on photosynthesis over the phytoplankton succession*

457 Overall and partially confirming our initial expectations, the positive LRR-effect
458 reported under future conditions on photosynthesis was ~20% higher under
459 oligotrophication than ambient nutrients concentrations. This higher photosynthetic
460 activity found under these conditions could be supported by two processes: (1) the
461 higher RUE highlighted above; and (2) an increased nitrogen-demand by cells under
462 warming (Yu et al. 2018). Both processes would allow phytoplankton to invest extra
463 energy and resources in protein synthesis and assembly (Cotner et al. 2006). Although
464 we did not quantify neither nitrogen uptake nor denitrification (i.e. important ecological

processes that reduce nitrogen concentration in water bodies, Chen et al. 2012), we hypothesize that the decreased NO_2^-+NO_3^- (5-fold) coupled with the increased NH_4^+ (4-fold) concentrations only found under a $Future_{Phos}$ scenario would support an increased uptake and / or a dissimilatory nitrate reduction to ammonium (DNRA; Kamp et al. 2011). Thus, a higher N and P-cellular content joint to simultaneous increase in C derived from the biomass production already mentioned could trigger a boosting of the biological pump mediated by photosynthesis.

4.3. Sensitivity of cyanobacteria versus other eukaryotic phytoplankton groups to warming×CO_2 under oligotrophication

According to the results shown so far, climate change would be a single-edged sword, as it would result in large increases of community biomass, RUE and production (i.e. positive effect); however, when we zoomed into other organization levels, such as the community structure, the second edge appears (i.e. negative effects). Our results show an opposite impact of the scenarios tested on the main contributors of the phytoplankton community. In fact, the warming×CO_2 interaction exerted a consistent negative synergistic effect on Prok (i.e. cyanobacteria), and mostly positive on other Euk phytoplankton. Moreover, these negative and positive effects found on Prok and Euk, respectively, under *in situ* nutrient conditions were accentuated ~25% under oligotrophication.

This result constitute, to our knowledge, the first empirical evidence showing a higher sensitivity of Prok- compared to Eukaryotic phytoplankton to a warming and high CO_2 scenario. This higher sensitivity is consistent with the impaired photochemical performance i.e. a reduced light harvesting efficiency (ca. -65%), rETRmax (ca. -50%) and light compensation point (ca. -20%) found in this study. An impaired

cyanobacterial performance under such conditions contrasts with previous studies that shown a cyanobacterial dominance when N:P ratios in the water column decreased (Havens et al. 2003; Smith 1983). Thus, considering that: (1) nutrient concentrations (Rabalais et al. 2009; van Loosdrecht and Brdjanovic 2014), and (2) global climate change (Paerl and Huisman 2008;Yang et al. 2017) are the two main causes of the cyanobacterial blooms intensification, we propose that a displacement toward a higher competitive advantage of eukaryotic phytoplankton could potentially reduce the incidence of these blooms that increasingly threaten human and animal health, as many of them produce a suite of potent toxins (e.g. microcystin, Lürling et al. 2017). Additionally, a potential weakening of the cyanobacterial dominance could subsequently attenuate the strong disruption of the structure, and functioning of phytoplankton communities in conditions (i.e. high temperature or CO_2 and low N:P ratios) in which this group dominates, increasing their richness (Escalas et al. 2019; Visser et al. 2016).

5. Conclusion

This study revealed an unprecedented role of the warming×CO_2×oligotrophication interaction on production and functioning of a model freshwater ecosystem. As the positive effects of the warming×CO_2 became strongly negative on cyanobacterial (and positive on eukaryotes) photosynthesis when nutrient availability was lowered by an in-lake management strategy (i.e. Phoslock® addition), it is necessary to know how the interplay between climate change-derived problems and current management strategies alter the equilibrium and the dominance in phytoplankton communities. With a robust knowledge of these processes, policy makers could design more appropriate future management strategies to improve the ecological

513 status of the aquatic ecosystems (i.e. reduced cyanobacterial blooms, good water
514 quality) without compromising neither their ecological attributes (i.e. high total primary
515 production) nor functioning.

516 **6. Acknowledgements**

517 We are deeply acknowledged to Dr. Miquel Lurling for fruitful discussions at the
518 Netherlands Institute of Ecology (NIOO-KNAW). Dr. A. I. Funes and I. L. Lozano are
519 acknowledged by their help during the development of the proposal and experimental
520 setup, and Dr. Nico Helmsing, and Dr. Iris Chardon by carried out macro- and
521 micronutrients, and DOC analyses, respectively. Lora Strack van Schijndel, Eric
522 Reichmann and Cleo Stratmann are acknowledged by the help during the setting-up of
523 the Limnotron experiment. Comments and suggestions by the Editor Dr. Mark van
524 Loosdrecht and two anonymous reviewers on a previous version of the manuscript are
525 deeply acknowledged. This study was partly funded by a Transnational Access granted
526 to MJC through AQUACOSM project, and by the European Comision EU H2020-
527 INFRAIA-project (No. 731065), and by NIOO's own funding. MJC was supported by
528 a Juan de la Cierva-Formación contract (FJCI-2017-32318) funded by Ministerio de
529 Ciencia, Innovación y Universidades. MIAM was funded by a Ministerio de Economía
530 y Competitividad PhD contract (MINECO, CTM2013-46951-R), ELP and JMGO were
531 supported by a "Formación de Profesorado Universitario" contract from the Ministerio
532 de Educación, Cultura y Deporte (FPU14/02917 and FPU14/00977, respectively), and
533 GGJ by a PhD fellowship from the National Council of Science and Techonology of
534 Mexico (CONACyT). Authors declare no conflict of interest.

535

7. Author's contribution

Conceived the idea: MJC, MIA-M, EL-P, GG-J, LNdSD, ST, JMG-O. Developed and performed the research: MJC, MIA-M, EL-P, GG-J, LNdSD, ST, JMG-O. Analyzed the data, and drafted the manuscript: MJC. Discussed the presentation of the results and approved the final version of the manuscript: MJC, MIA-M, EL-P, GG-J, LNdSD, ST, JMG-O.

8. References

Boyce, D.G., Lewis, M.R. and Worm, B. (2010) Global phytoplankton decline over the past century. Nature 466, 591-596.

Brown, J.P., Gillooly, F., Allen, A.P., Savage, V.M. and West, G.B. (2004) Toward a metabolic theory of ecology. Ecology 85, 1771-1789.

Corcoran, A.A. and Boeing, W.J. (2012) Biodiversity increases the productivity and stability of phytoplankton communities. PloS One 7, e49297.

Cotner, J.B., Makino, W. and Biddanda, B.A. (2006) Temperature affects stoichiometry and biochemical composition of *Escherichia coli*. Microbial Ecology 52, 26-33.

Chen, X., Yang, L.H., Xiao, L., Miao, A. and Xi, B. (2012) Nitrogen removal by denitrification during cyanobacterial bloom in Lake Taihu. Journal of Freshwater Ecology 27, 243-258.

Daufresne, M., Lengfellner, K. and Sommer, U. (2009) Global warming benefits the small in aquatic ecosystems. Proceedings of the National Academy of Sciences USA 106, 12788-12793.

De Senerpont Domis, L., Van de Waal, D.B., Helmsing, N.R., Van Donk, E. and Mooij, W.M. (2014) Community stoichiometry in a changing world: Combined effects of warming and eutrophication on phytoplankton dynamics. Ecology 95, 1485-1495.

Downing, J. (2010) Emerging global role of small lakes and ponds: Little things mean a lot. Limnetica 29, 9-24.

Egge, J.K., Thingstad, F., Engel, A., Wohlers, J., Bellerby, R.G.J. and Riebesell, U. (2009) Primary production during nutrient-induced blooms at elevated CO_2 concentrations. Biogeosciences 6, 877-885.

Epe, T.S., Finsterle, K. and Yasseri, S. (2017) Nine years of phosphorus management with lanthanum modified bentonite (Phoslock) in a eutrophic, shallow swimming lake in Germany. Lake Reservoir and Management 33, 119-129.

Escalas, A., Catherine, A., Maloufi, S., Cellamare, M., Hamlaoui, S., Yéprémian, C., Louvard, C., Troussellier, M. and Bernard, C. (2019) Drivers and ecological consequences of dominance in periurban phytoplankton communities using networks approaches. Water Research 163, 114803.

Filstrup, C.T., Hillebrand, H., Heathcote, A.J., Harpole, A.J. and Downing, J.A. (2014) Cyanobacteria dominance influences resource use efficiency and community turnover in phytoplankton and zooplankton communities. Ecology Letters 17, 464-474.

Galic, N., Sullivan, L.L., Grimm, V. and Forbes, V.E. (2018) When things don't add up: Quantifying impacts of multiple stressors from individual metabolism to ecosystem processing. Ecology Letters 21, 568-577.
Glibert, P.M. (2019) Harmful algae at the complex nexus of eutrophication and climate change. Harmful Algae in press.
Harvey, B.P., Gwynn-Jones, D. and Moore, P.J. (2013) Meta-analysis reveals complex marine biological responses to the interactive effects of ocean acidification and warming. Ecology and Evolution 3, 1016-1030.
Havens, K.E., James, R.T., East, T.L. and Smith, V.H. (2003) N:P ratios, light limitation, and cyanobacterial dominance in a subtropical lake impacted by non-point source nutrient pollution. Environmental Pollution 122, 379-390.
Hernández-Hernández, N., Bach, L.T., Montero, M.F., Taucher, J., Baños, I., Guan, W., Espósito, M., Ludwig, A., Achterberg, E.P., Riebesell, U. and Arístegui, J. (2018) High CO_2 under nutrient fertilization increases primary production and biomass in subtropical phytoplankton communities: A mesocosm approach. Frontiers in Marine Science 5, 213.
Hilt, S., Gross, E.M., Hupfer, M., Morscheid, H., Mählmann, J., Melzer, A., Poltz, J., Sandrock, S., Scharf, E.-M., Schneider, S. and van de Weyer, K. (2006) Restoration of submerged vegetation in shallow eutrophic lakes – A guideline and state of the art in Germany. Limnologica 36, 155-171.
IPCC (2013) Climate Change. The Physical Science Basis, Cambridge University Press, New York, USA.
IPCC (2019) Global Warming of 1.5°C. An IPCC Special Report on the impacts of global warming of 1.5°C above pre-industrial levels and related global greenhouse gas emission pathways, in the context of strengthening the global response to the threat of climate change, sustainable development, and efforts to eradicate povert. Masson-Delmotte, V., Zhai, P., Pörtner, H.-O., Roberts, D., Skea, J., Shukla, P.R., Pirani, A., Moufouma-Okia, W., Péan, C., Pidcock, R., Connors, S., Matthews, J.B.R., Chen, Y., Zhou, X., Gomis, M.I., Lonnoy, E., Maycock, T., Tignor, M. and Waterfield, T. (eds), p. 630.
Jeppesen, E., Søndergaard, M., Meerhoff, M., Lauridsen, T. and Jensen, J.P. (2007) Shallow lake restoration by nutrient loading reduction—some recent findings and challenges ahead. Hydrobiologia 584, 239-252.
Ji, X., Verspagen, J.M.H., Stomp, M. and Huisman, J. (2017) Competition between cyanobacteria and green algae at low versus elevated CO_2: Who will win, and why? Journal of Experimental Botany 68, 3815-3828.
Kamp, A., de Ber, D., Nitsch, J.L., Lavik, G. and Stief, P. (2011) Diatoms respire nitrate to survive dark and anoxic conditions. Proceedings of the National Academy of Sciences 108, 5649-5654.
Keys, M., Tilstone, G., Findlay, H.S., Widdicombe, C.E. and Lawson, T. (2018) Effects of elevated CO_2 and temperature on phytoplankton community biomass, species composition and photosynthesis during an experimentally induced autumn bloom in the western English Channel. Biogeosciences 15, 3203-3222.
Kim, J.-H., Kim, K.Y., Kang, E.J., Lee, K., Kim, J.-M., Park, K.-T., Shin, K., Hyun, B. and Jeong, H.J. (2013) Enhanced of photosynthetic carbon assimilation efficiency by phytoplankton in the future coastal ocean. Biogeosciences 10, 7525-7535.
Kromkamp, J., Barranguet, C. and Peene, J. (1998) Determination of microphytobenthos PSII quantum efficiency and photosynthetic activity by means of variable chlorophyll fluorescence. Marine Ecology Progress Series 162, 45-55.

Lajeunesse, M.J. (2011) On the meta-analysis of response ratios for studies correlated and multi-group designs. Ecology 92, 2049-2055.
Lassen, M.K., Dewar Nielsen, K., Richardson, K., Garde, K. and Schlüter, L. (2009) The effects of temperature increases on a temperate phytoplankton community — A mesocosm climate change scenario. Journal of Experimental Marine Biology and Ecology 383, 79-88.
Li, W., Ding, J., Wang, T., Yang, Y., Li, Y., Campbell, D.A. and Gao, K. (2019) Functional responses of smaller and larger diatoms to gradual CO_2 rise. Science of the Total Environment 680, 79-90.
Low-Décarie, E., Bell, G. and Fussmann, G.F. (2015) CO_2 alters community composition and response to nutrient enrichment of freshwater phytoplankton. Oecologia 173, 875-884.
Lürling, M., Mendes e Mello, M., van Oosterhout, F., de Senerpont Domis, L. and Marinho, M.M. (2018) Responses of natural cyanobacteria and algae assemblages to a nutrient pulse and elevated temperature. Frontiers in Microbiology 9, 1851.
Lürling, M. and Van Oosterhout, F. (2013) Controlling eutrophication by combined bloom precipitation and sediment phosphorus inactivation. Water Research 47, 6527-6537.
Lürling, M., Van Oosterhout, F. and Faassen, E. (2017) Eutrophication and Warming Boost Cyanobacterial Biomass and Microcystins. Toxins 11, E64.
Maas, W.A.e. (2014) Projectplan: Natuurontwikkeling Ertveldpolder. Advies, B. (ed), p. 63, Hertogenbosch.
Maxwell, K. and Johnson, G.N. (2000) Chlorophyll fluorescence - a practical guide. Journal of Experimental Botany 51, 659-668.
Meis, S., Spears, B.M., Maberly, S.C. and Perkins, R.G. (2013) Assessing the mode of action of Phoslock® in the control of phosphorus release from the bed sediments in a shallow lake (Loch Flemington, UK). Water Research 47, 4460-4473.
O'Beirne, M.D., Werne, J.P., Hecky, R.E., Johnson, T.C., Katsev, S. and Reavie, E.D. (2017) Anthropogenic climate change has altered primary productivity in Lake Superior. Nature Communications 8, 15713.
Osman, M.B., Das, S.B., Trusel, L.D., Evans, M.J., Fischer, H., Grieman, M.M., Kipfstuhl, S., McConnell, J.R. and Saltzman, E.S. (2019) Industrial-era decline in subarctic Atlantic productivity. Nature 569, 551-555.
Padfield, D., Yvon-Durocher, G., Buckling, A., Jennings, S. and Yvon-Durocher, G. (2016) Rapid evolution of metabolic traits explains thermal adaptation in phytoplankton. Ecology Letters 19, 133-142.
Paerl, H.W. and Huisman, J. (2008) Blooms like it hot. Science 320, 57-58.
Paquette, C. and Beisner, B.E. (2018) Interaction effects of zooplankton and CO_2 on phytoplankton communities and the deep chlorophyll maximum. Freshwater Biology 63, 278-292.
Paul, C., Matthiessemn, B. and Sommer, U. (2015) Warming, but not enhanced CO_2 concentration, quantitatively and qualitatively affects phytoplankton biomass. Marine Ecology Progress Series 528, 39-51.
Rabalais, N.n., Turner, R.E., Díaz, R.J. and Justic, D. (2009) Global change and eutrophication of coastal waters. ICES Journal of Marine Science 66, 1528-1537.
Ralph, P.J. and Gademann, R. (2005) Rapid light curves: A powerful tool to assess photosynthetic activity. Aquatic Botany 82, 222-237.
Raven, J.A., Giordano, M., Beardall, J. and Maberly, S.C. (2012) Algal evolution in relation to atmospheric CO_2: carboxylases, carbon-concentrating mechanisms and

carbon oxidation cycles. Philosophical Transactions of the Royal Society of London.Series B 367, 493–507.
Ross, G., Haghseresht, F. and Cloete, T.E. (2008) The effect of pH and anoxia on the performnace of Phoslock® a phosphorus binding clay. Harmful Algae 7, 545-550.
Scheffer, M., Brovkin, V. and Cox, P.M. (2006) Positive feedback between global warming and atmospheric CO_2 concentration inferred from past climate change. Geophysical Research Letters 33, L10702.
Schulhof, M.A., Shurin, J.B., Declerk, S.A.J. and Van de Waal, D.B. (2019) Phytoplankton growth and stoichiometric responses to warming, nutrient addition and grazing depend on lake productivity and cell size. Global Change Biology 25, 2751-2762.
Seelen, L.M.S., Flaim, G., Jennings, E. and De Senerpont Domis, L. (2019) Saving water for the future: Public awareness of water usage and water quality. JOurnal of Environmental Management 242, 246-257.
Sett, S., Schulz, K.G., Bach, L.T. and Riebesell, U. (2018) Shift towards larger diatoms in a natural phytoplankton assemblage under combined high-CO_2 and warming conditions. Journal of Plankton Research 40, 391-406.
Smith, V.H. (1983) Low nitrogen to phosphorus ratios favor dominance by blue-green algae in lake phytoplankton. Science 221, 669-671.
Smith, V.S. (2003) Eutrophication of freshwater and coastal marine ecosystems a global problem. Environmental Science & Pollution Research 10, 126-139.
Sommer, U., Paul, C. and Moustaka-Founi, M. (2015) Warming and ocean acidification effects on phytoplankton—From species shifts to size shifts within species in a mesocosm experiment. PloS One 10, e0125239.
Steffen, W., Richarson, K., Rockström, J., Cornell, S.E., Fetzer, I., Bennett, E.M., Biggs, R., Carpenter, S.R., de Vries, W., De Wit, C.A., Folke, C., Gerten, D., Heinke, J., Mace, G.M., Persson, L.M., Ramanathan, V., Reyers, B. and Sörlin, S. (2015) Planetary boundaries: Guiding human development on a changing planet. Science 347, 1259855.
Strecker, A.L., Cob, T.P. and Vinebrooke, R.D. (2004) Effects of experimental greenhouse warming on phytoplankton and zooplanktoncommunities in fishless alpine ponds. Limnology and Oceanography 49, 1182-1190.
Suggett, D.J., Oxborough, K., Baker, N.R., MacIntyre, H.L., Kana, T.M. and Geider, R.J. (2003) Fast repetition rate and pulse amplitude modulation chlorophyll *a* fluorescence measurements for assessment of photosynthetic electron transport in marine phytoplankton. European Journal of Phycology 38, 371-384.
Team, R.C. (2014) A language and environment for statistical computing. R foundation for statistical computing, Viena (Austria).
Teurlincx, S., Kuiper, J.J., Hoevenaar, E.C.M., Lürling, M., Brederveld, R.J., Veraart, A.J., Janssen, A.B.G., Mooij, W.M. and De Senerpont Domis, L. (2019) Towards restoring urban waters: Understanding the main pressures. Current Opinion in Environmental Sustainability 36, 49-58.
Tortell, P.D., Payne, C.D., Li, Y., Trimborn, S., Rost, B., Smith, W., Risselman, C., Dunbar, R.B., Sedwick, R.B. and DiTullio, G.R. (2008) CO_2 sensitivity of Southern Ocean phytoplankton. Gerophysical Research Letters 35, L04605.
Tranvik, L.J., Downing, J., Cotner, J.B., Loiselle, S., Striegl, R., Ballatore, T.J., Dillon, P., Finlay, K., Fortino, K., Knoll, L.B., Korelainen, P.L., Kutser, T., Larsen, S., Laurion, I., Leech, D.M., McCallister, S.L., McKnight, D., Melack, J., Overholt, E., Porter, J.A., Prairie, Y., Renwick, W., Roland, F., Sherman, B.S., Schindler,

723 D.E., Sobek, S., Tremblay, A., Vanni, M.J., Verschoor, A.M., Wachenfeldt, E.v.
724 and Weyhenmeyer, G.A. (2009) Lakes and reservoirs as regulators of carbon
725 cycling and climate. Limnology and Oceanography 54, 2298-2314.
726 Ullah, H., Nagelkerken, I., Goldenberg, S.U. and Fordham, D.A. (2018) Climate change
727 could drive marine food web collapse through altered trophic flows and
728 cyanobacterial proliferation. PloS Biology 16, e2003446.
729 Union, E. (2000) Directive 2000/60/EG of the European Parliament and of the Council
730 Establishing a Framework for the Community Action in the Field of Water Policy
731 of 23 October. PB L 327 of 22 December 2000.

732 Valiñas, M.S., Villafañe, V.E., Cabrerizo, M.J., Durán-Romero, C. and Helbling, E.W.
733 (2018) Global change effects on plankton community structure and trophic
734 interactions in a Patagonian freshwater eutrophic system. Hydrobiologia 816, 61-
735 77.
736 van Loosdrecht, M.C.M. and Brdjanovic, D. (2014) Anticipating the next century of
737 wastewater treatment. Science 344, 1452-1453.
738 Verbeek, L., Gall, A., Hillebrand, H. and Striebel, M. (2018a) Warming and
739 oligotrophication cause shifts in freshwater phytoplankton communities. Global
740 Change Biology 24, 4532-4543.
741 Verbeek, L., Vanhamel, M., van den Berg, E., Hanashiro, F.T.T., Gianuca, A.T.,
742 Striebel, M., Lemmens, P., Declerk, A.A.J., Hillebrand, H. and De Meester, L.
743 (2018b) Compositional and functional consequences of environmental change in
744 Belgian farmland ponds. Freshwater Biology 63, 581-596.
745 Verschoor, A., Van Dijk, M.A., Huisman, J. and Van Donk, E. (2013) Elevated CO_2
746 concentrations affect the elemental stoichiometry and species composition of an
747 experimental phytoplankton community. Freshwater Biology 58, 597-611.
748 Villar-Argaiz, M., Medina-Sánchez, J.M., Biddanda, B.A. and Carrillo, P. (2018)
749 Predominant non-additive effects of multiple stressors on autotroph C:N:P ratios
750 propagate in freshwater and marine food webs. Frontiers in Microbiology 9, 69.
751 Visser, P.M., Verspagen, J.M.H., Sandrini, G., Stal, L.J., Matthijs, H.C.P., Davis, T.W.,
752 Paerl, H.W. and Huisman, J. (2016) How rising CO_2 and global warming may
753 stimulate harmful cyanobacterial blooms. Harmful Algae 54, 145-159.
754 Wilken, S., Soares, M., Urrutia-Cordero, P., Ratcovich, J., Ekvall, M.K., Van Donk, E.
755 and Hansson, L.-A. (2018) Primary producers or consumers? Increasing
756 phytoplankton bacterivory along a gradient of lake warming and browning.
757 Limnology and Oceanography 63, S142-S155.
758 Wohlers, J., Engel, A., Zöllner, E., Breithaupt, P., Jürgensd, K., Hoppe, H.G., Sommer,
759 U. and Riebesell, U. (2009) Changes in biogenic carbon flow in response to sea
760 surface warming. Proceedings of the National Academy of Sciences USA 106,
761 7067-7072.
762 Xu, K., Fu, F.-X. and Hutchins, D. (2014) Comparative responses of two dominant
763 Antarctic phytoplankton taxa to interactions between ocean acidification,
764 warming, irradiance, and iron availability. Limnology and Oceanography 59,
765 1919-1931.
766 Yang, J.R., Lv, H., Isabwe, A., Liu, L., Yu, X., Chen, H. and Yang, J. (2017)
767 Disturbance-induced phytoplankton regime shifts and recovery of cyanobacteria
768 dominance in two subtropical reservoirs. Water Research 120, 52-63.

769 Yu, C., Li, C., Wang, T., Zhang, M. and Xu, J. (2018) Combined effects of
770 experimental warming and eutrophication on phytoplankton dynamics and
771 nitrogen uptake. Water 10, 1057.
772 Yuan, X.-Z., Pan, G., Chen, H. and Tian, B.H. (2009) Phosphorus fixation in lake
773 sediments using $LaCl_3$-modified clays. Ecological Engineering 35, 1599-1602.
774 Yvon-Durocher, G., Allen, A.P., Cellamare, M., Dossena, M., Dossena, M., Gaston,
775 K.J., Leitao, M., Montoya, J.M., Reuman, D.C., Woodward, G. and Trimmer, M.
776 (2015) Five Years of Experimental Warming Increases the Biodiversity and
777 Productivity of Phytoplankton. PloS Biology 13, e1002324.
778 Yvon-Durocher, G., Hulatt, C., Woodward, G. and Trimmer, M. (2017) Long-term
779 warming amplifies shifts in the carbon cycle of experimental ponds. Nature
780 Climate Change 7, 209-213.
781 Yvon-Durocher, G., Jones, J.I., Trimmer, M., Woodward, G. and Montoya, J.M. (2010)
782 Warming alters the metabolic balance of ecosystems. Philosophical Transactions
783 of the Royal Society B: Biological Sciences 365, 2117-2126.
784 Zingel, P., Cremona, F., Noges, T., Cao, Y., Neif, E.M., Coppens, J., Iskin, U.,
785 Lauridsen, T.L., Davidson, T.A., Sondergaard, M., Beklioglu, M. and Jeppesen,
786 E. (2018) Effects of warming and nutrients on the microbial food web in shallow
787 lake mesocosms European Journal of Protistology 64, 1-12.

788 **Figure captions**

789 Figure 1.- Mean (±SD) (A) Total chlorophyll *a* (Chl *a*) concentrations and (B) resource
790 use efficiency (RUE) of the phytoplankton community exposed to three environmental
791 scenarios (Present, Future and Future + Phoslock®) during the experiment.

792

793 Figure 2.- Mean (±SD) diel relative electron transport rates (rETR) of eukaryotes (i.e.
794 green algae, diatoms and dinoflagellates) and prokaryotes (i.e. cyanobacteria) exposed
795 to three environmental scenarios (Present, Future and Future + Phoslock®) during the
796 experiment.

797 Figure 3.- Mean (±SD) (A) Integrated relative electron transport rates (rETR) in
798 eukaryotes (Euk, green algae, diatoms and dinoflagellates) and prokaryotes (Prok,
799 cyanobacteria) exposed to three environmental scenarios (Present, Future and Future +
800 Phoslock®) during the experiment (pre-bloom, bloom and post-bloom phases). (B) Log-
801 response ratios (LRR) of the rETR in Euk and Prok under the above mentioned
802 scenarios. Letters on the top of bars represent represent significant differences by the

803 Least Significant Differences (LSD) *post hoc* test. Positive and negative values of LRR
804 indicate a stimulatory and an inhibitory effect, respectively.

805 Figure 4.- Mean (±SD) (A) Light harvesting efficiency (α), (B) relative maxima electron
806 transport rates ($rETR_{max}$), and (C) light compensation point (E_k) in eukaryotes (Euk,
807 green algae, diatoms and dinoflagellates) and prokaryotes (Prok, cyanobacteria)
808 exposed to three environmental scenarios (Present, Future and Future + Phoslock®) at
809 the initial and at the end of the experiment (pre-bloom, bloom and post-bloom phases).
810 Log-response ratios (LRR) of α (D), rETRmax (E) and E_k (F) in Euk and Prok under the
811 above mentioned scenarios. Letters on the top of bars represent significant differences
812 by the Least Significant Differences (LSD) *post hoc* test. Positive and negative values
813 of LRR indicate a stimulatory and an inhibitory effect, respectively.

814

815

816

817

818

819

820

821

822

823

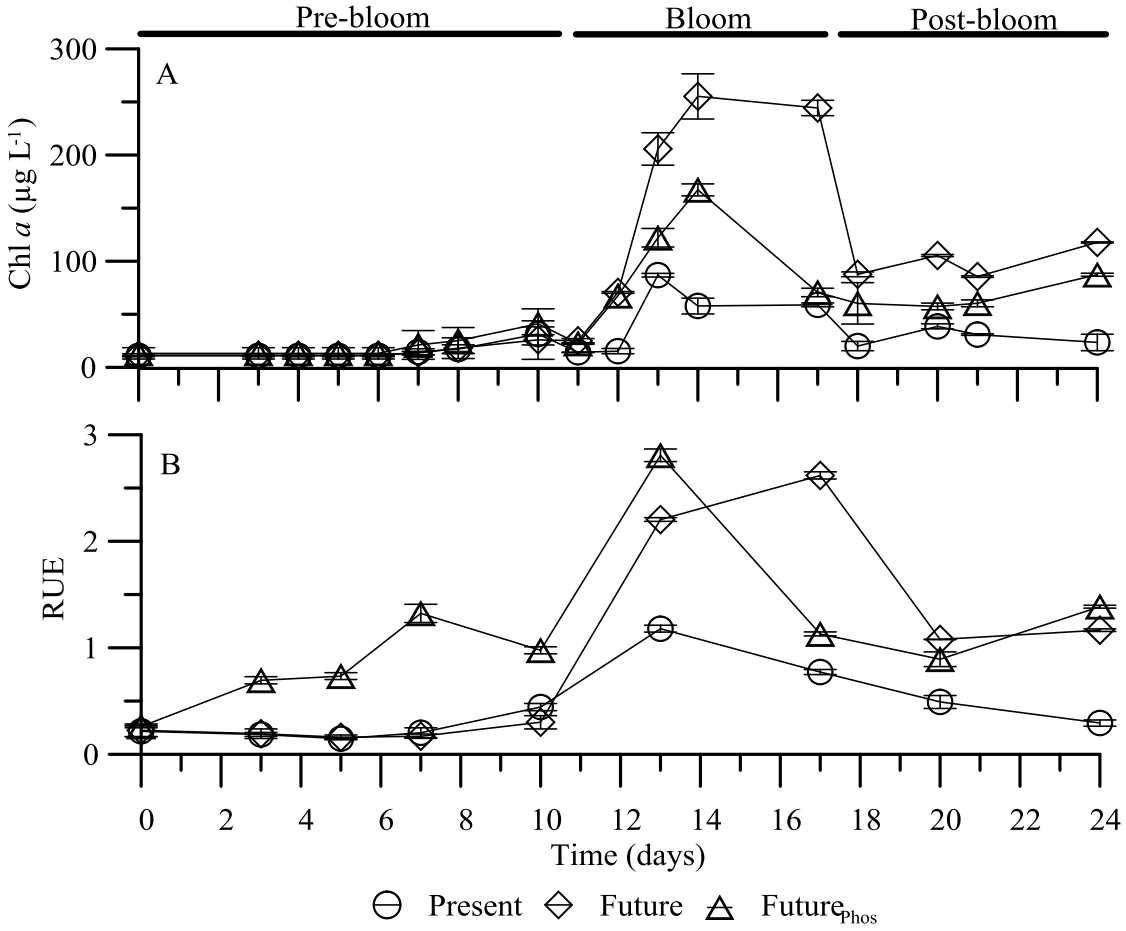

Pre-bloom
Bloom
Post-bloom
A
Chl a (µg L-1)
300
200
100
0
B
RUE
3
2
1
0
0
2
4
6
8
10
12
14
16
18
20
22
24
Time (days)
Present
Future
Future Phos

Figure 1

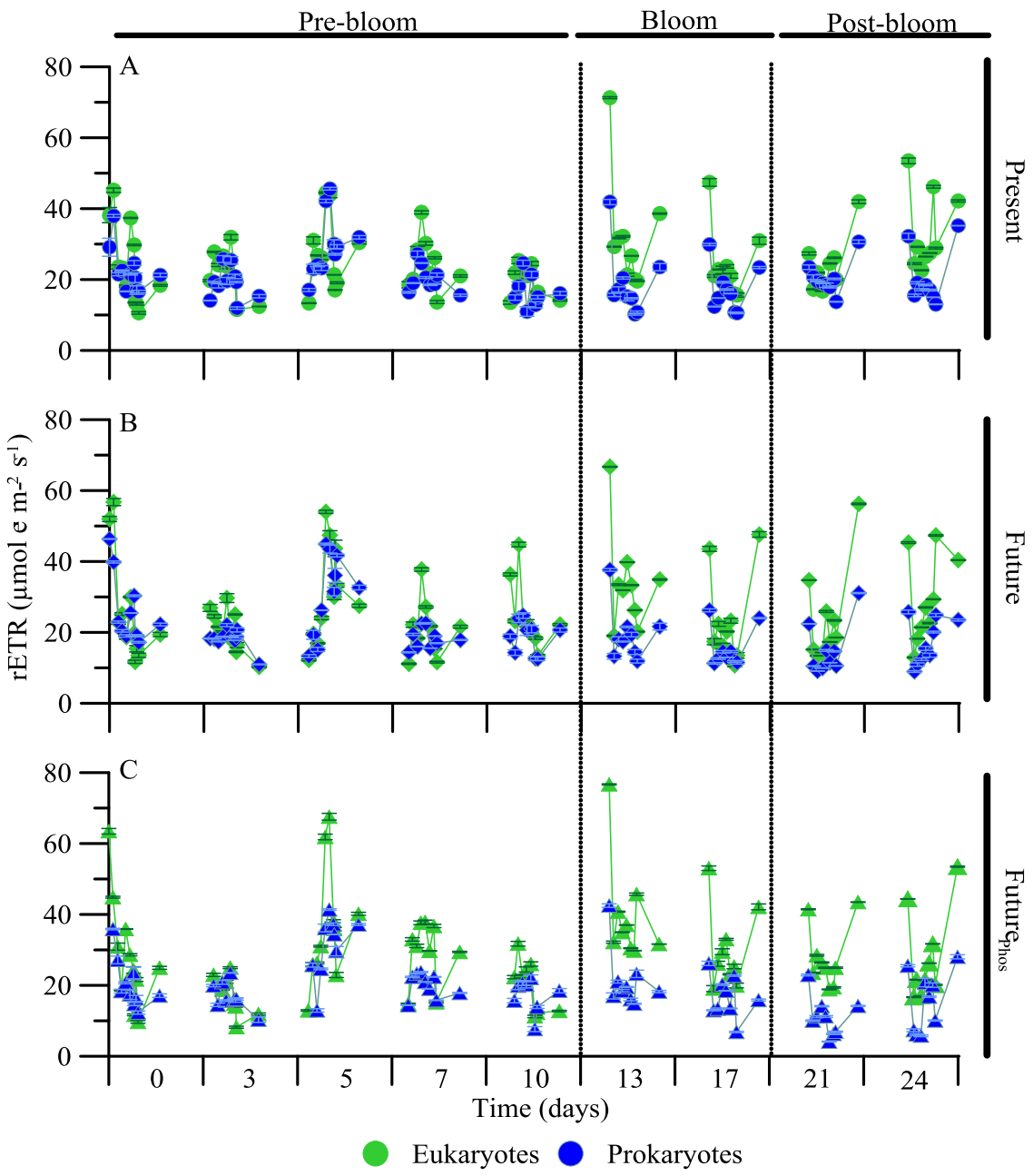

Pre-bloom
Bloom
Post-bloom
A
B
C
Present
Future
Future$_{Phos}$
rETR (µmol e m^{-2} s^{-1})
0
20
40
60
80
0
3
5
7
10
13
17
21
24
Time (days)
Eukaryotes
Prokaryotes

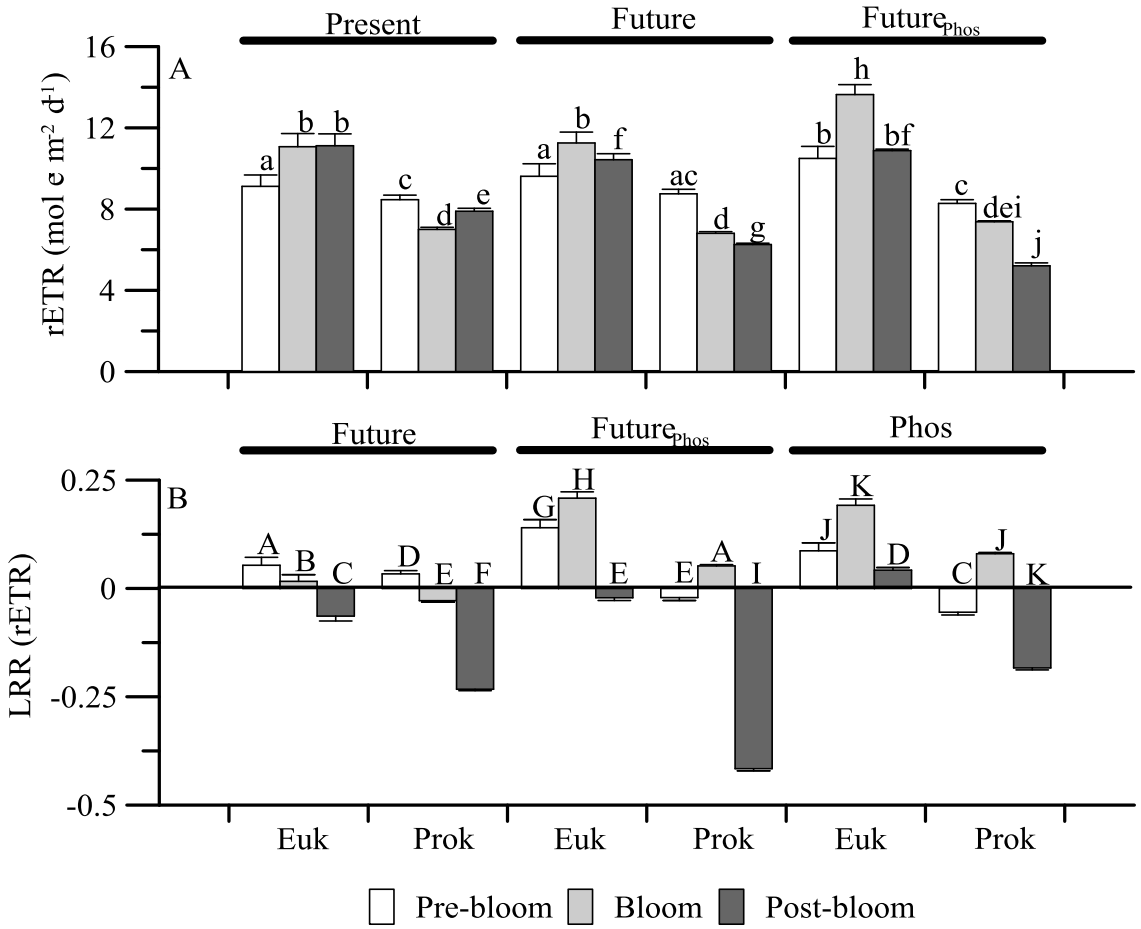
Figure 2

840

841

842

843



844
845 Figure 3

846

847

848

849

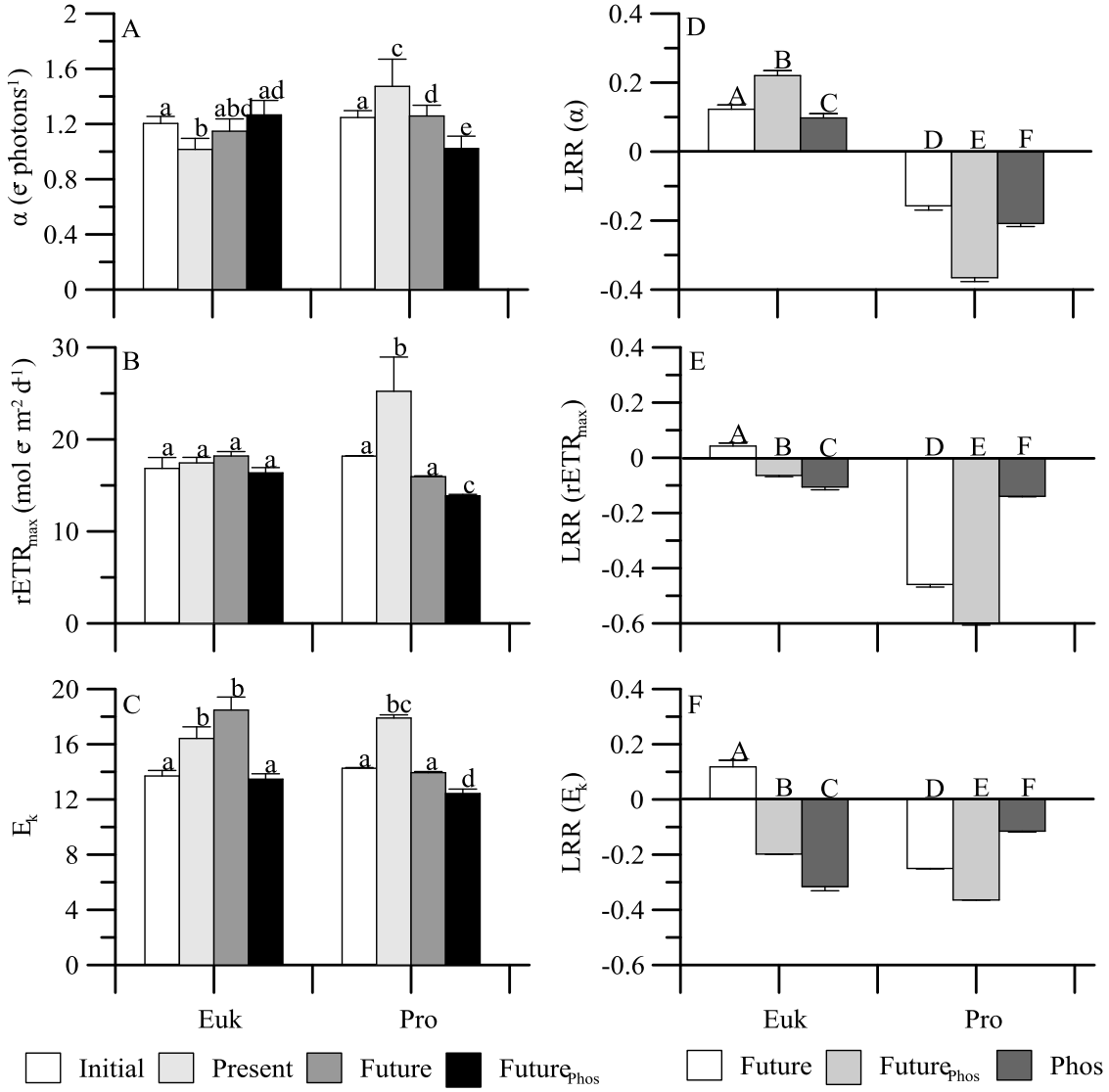

A
B
C
D
E
F
α (ϵ photons-1)
rETRmax (mol ϵ m-2 d-1)
Ek
LRR (α)
LRR (rETRmax)
LRR (Ek)
Euk
Pro
Initial
Present
Future
FuturePhos
Phos

Figure 4