ECOLOGICAL SOCIETY OF AMERICA

Ecology/Ecological Monographs/Ecological Applications

PREPRINT

This preprint is a PDF of a manuscript that has been accepted for publication in an ESA journal. It is the final version that was uploaded and approved by the author(s). While the paper has been through the usual rigorous peer review process of ESA journals, it has not been copy-edited, nor have the graphics and tables been modified for final publication. Also note that the paper may refer to online Appendices and/or Supplements that are not yet available. We have posted this preliminary version of the manuscript online in the interest of making the scientific findings available for distribution and citation as quickly as possible following acceptance. However, readers should be aware that the final, published version will look different from this version and may also have some differences in content.

The doi for this manuscript and the correct format for citing the paper are given at the top of the online (html) abstract.

Once the final published version of this paper is posted online, it will replace the preliminary version at the specified doi.

Running head: Community stoichiometry in a changing world

Community stoichiometry in a changing world: combined effects of warming and eutrophication on phytoplankton dynamics

Lisette N. De Senerpont Domis^{1,2,4} Dedmer B. Van de Waal,¹ Nico R. Helmsing,¹ Ellen Van Donk^{1,3} and Wolf. M. Mooij^{1,2}

¹Netherlands Institute of Ecology (NIOO-KNAW), Department of Aquatic Ecology,
Droevendaalsesteeg 10, 6708 PB, Wageningen, The Netherlands

²Aquatic Ecology & Water Quality Management Group, Dept. Environmental Sciences,
Wageningen University, Droevendaalsesteeg 3a, 6708 PB, Wageningen, The Netherlands

³Department of Ecology and Biodiversity, University of Utrecht, Utrecht, The Netherlands

⁴ E-mail: l.desenerpontdomis@nioo.knaw.nl

Abstract:

The current changes in our climate will likely have far reaching consequences for aquatic ecosystems. These changes in the climate, however, do not act alone and are often accompanied by additional stressors such as eutrophication. Both global warming and eutrophication have been shown to affect the timing and magnitude of phytoplankton blooms. Little is known about the combined effects of rising temperatures and eutrophication on the stoichiometry of entire phytoplankton communities. We exposed a natural phytoplankton spring community to different warming and phosphorus loading scenarios using a full-factorial design. Our results demonstrate that rising temperatures promote the growth rate of an entire phytoplankton community. Furthermore, both rising temperatures and phosphorus loading stimulated the maximum biomass built up by the phytoplankton community. Rising temperatures led to higher carbon:nutrient stoichiometry of the phytoplankton community under phosphorus limited conditions. Such a shift towards higher carbon:nutrient ratios, in combination with a higher biomass build-up, suggests a temperature-driven increase in nutrient use efficiency of the phytoplankton community. Importantly, higher carbon:nutrient stoichiometry of phytoplankton is generally of poorer nutritional value for zooplankton. Thus, although warming may result in higher phytoplankton biomass, this may be accompanied by a stoichiometric mismatch between phytoplankton and their grazers, with possible consequences for the entire aquatic food web.

Key words: climate change, ecological stoichiometry, eutrophication, multiple stressors, oligotrophication, phytoplankton

INTRODUCTION

Our climate is changing at an unprecedented rate, with rising $p\text{CO}_2$, increasing global temperatures, and regional changes in precipitation causing drought or flooding (Solomon et al. 2007). These changes in climate will have diverse consequences for the structure and functioning of ecosystems. For instance, elevated $p\text{CO}_2$ causes acidification of the oceans, with detrimental consequences for calcifying organisms (Doney et al. 2012). At the same time, higher CO_2 levels may be beneficial for primary production, both in the water (Hein and Sandjensen 1997, Schippers et al. 2004) as well as on land (Nemani et al. 2003). Associated increases in global temperatures may differentially alter the timing and distribution of organisms across ecosystems (Walther et al. 2002, Parmesan and Yohe 2003). In aquatic ecosystems, warming induces enhanced thermal stratification which decreases nutrient concentrations in the upper mixed layer of oceans and deeper lakes (Behrenfeld et al. 2006, Jöhnk et al. 2008), while changes in precipitation may alter the input of carbon (C) and nutrients from terrestrial run-off into rivers, lakes and coastal seas (Hinton et al. 1997, Rabalais et al. 2009). These climate-driven changes in resource availabilities will have consequences for primary production, which in turn may change ecosystem structure and functioning.

Climate change, however, does not act alone and many ecosystems are also affected by stressors such as habitat fragmentation, eutrophication and pollution. Interactive effects of these multiple ‘stressors’ make it difficult to predict impacts based on single-stressor studies (Vinebrooke et al. 2004, Crain et al. 2008). Recent studies have provided evidence for the presence of interactive effects of global warming and other ecological stressors on freshwater ecosystems (Carvalho and Kirika 2003, Mckee et al. 2003, Hall and Cotner 2007, Huber et al. 2008, Pomati et al. 2012). Despite ongoing efforts to reduce anthropogenic input, one of the most ominous stressors on lake ecosystem functioning remains eutrophication (Schindler 2006).

Freshwater phytoplankton communities have been shown to undergo strong changes in response to global warming combined with eutrophication (Winder and Hunter 2008, Hsieh et al. 2010). Furthermore, temperature-driven shifts in phytoplankton community composition towards more cyanobacteria seem nutrient dependent (Elliott et al. 2006). In addition to reducing nutrient availability as result of enhanced thermal stratification, warming may also increase nutrient availability as result of enhanced remineralization (Gudasz et al. 2010) and terrestrial runoff (Jeppesen et al. 2009). Warming may also lower the nutrient loading at which a shallow lake switches from a natural state to a degraded state, indicating that warming could exacerbate the effect of eutrophication (Mooij et al. 2009). The impact of warming on a phytoplankton community does not need to be strong, and in fact may also be smaller than other stressors, such as nutrient loading (Moss et al. 2003). Clearly, global warming and eutrophication both affect phytoplankton communities. Interestingly, these effects can show a similar direction of change with respect to phytoplankton communities, as both may promote the occurrence of cyanobacteria blooms (Paerl and Huisman 2008), enhance phytoplankton biomass and reduce top-down control by herbivores (Huber et al. 2008, Mooij et al. 2009).

Apart from the effect of temperature on physiological rates, temperature may also change the chemical composition of organisms due to shifts in cellular allocation of elements (Sterner and Elser 2002). For instance, organisms acclimatized to low temperatures seem to exhibit higher nitrogen (N) and phosphorus (P) demands as these organisms need not only to invest into more proteins (containing nitrogen) and RNA (containing phosphorus), but also show an increase in cell size (Woods et al. 2003). At the same time, a temperature driven increase in growth rate may promote the allocation of elements to nutrient rich RNA (Sterner and Elser 2002).

Alterations in the interplay between temperature and nutrient availability under the projected climate change scenarios will presumably affect the chemical composition of organisms,

and thereby have knock-on effects on the whole ecosystem (Sardans et al. 2012). Through enhanced remineralization, higher temperatures may lead to increased nutrient supply (Gudasz et al. 2010), lowering phytoplankton carbon:nutrient stoichiometry. In contrast, enhanced thermal stratification may reduce nutrient concentrations and increase the average light availability, causing an increase in phytoplankton carbon:nutrient stoichiometry (Jäger et al. 2008, Van de Waal et al. 2010). In addition, elevated $p\text{CO}_2$ may raise the carbon:nutrient ratio of phytoplankton, particularly when nutrients are limiting (Urabe et al. 2003, Schoo et al. 2013). Increases in phytoplankton carbon:nutrient stoichiometry may impede the trophic transfer efficiency from primary to secondary producers (Urabe et al. 2003, Elser et al. 2010), and thereby create or enhance stoichiometric bottlenecks in the entire aquatic food web (Van Donk et al. 2008, Van de Waal et al. 2010; Schoo et al. 2013).

To obtain a more mechanistic understanding of the combined effect of warming and eutrophication on the stoichiometry of phytoplankton, we exposed a natural phytoplankton spring community to different warming and phosphorus loading scenarios using a full-factorial design. As bottom-up processes have been argued to be the most important drivers of phytoplankton succession during spring (Sommer et al. 1986, Sommer et al. 2012, De Senerpont Domis et al. 2013), we tested the combined effects of warming and phosphorus loading on biomass build-up and C:N:P stoichiometry of a natural phytoplankton spring community.

MATERIAL AND METHODS

Experimental set-up

Plankton communities were cultured for 48 days in 10 L polycarbonate carboys (NALGENE, Rochester, USA). This period was chosen as it represents the response window of phytoplankton during spring succession. Plankton inoculum was obtained from a shallow eutrophic peat lake in

the northern part of the Netherlands (Tjeukemeer; 52° 5 N, 5° 5E) at March 6, 2006. The plankton inoculum was sampled at 0.5-1.0 m depth. We have chosen Tjeukemeer as our reference lake, because we wanted : (1) a eutrophic lake; (2) we had detailed temperature data from 40 years which allowed us to design our different spring warming scenarios with precision and reality; (3) we had detailed plankton data of our reference lake for a period of 40 years. As we focused on the influence of nutrient loading and warming on the phytoplankton community properties in the absence of herbivores, we removed larger zooplankton species by filtering our initial 500 L plankton inoculum over a 140 μ m mesh. Subsequently, to ensure equal starting conditions among the experimental cultures in terms of nutrient concentrations and phytoplankton species composition, the lake water was concentrated using a commercial separator (Clara 80 Alfa Laval, courtesy of Ingrepo BV, Borculo, The Netherlands), thoroughly mixed overnight and subsequently resuspended in water from oligotrophic Lake Maarsseveen, The Netherlands. Although the effect of ultracentrifuging the lake water was considered detrimental to large algae and zooplankton, a diverse phytoplankton community consisting predominantly of smaller species of the Chlorophyceae (e.g. *Chlamydomonas*, *Chlorella*, *Chlorococcum*, *Desmusdesmus* and different species of *Scenedesmus*) was maintained. Throughout the course of the experiment, zooplankton counts ($> 30 \mu$ m, based on 395 mL sample) showed that the presence of crustacean zooplankton herbivores and large rotifers was at or below detection limit, hence we considered grazing by larger zooplankton negligible.

Using a full-factorial design, the culture vessels were subjected to three spring warming scenarios and three nutrient treatments with quadruple replication of each of the nine treatment combinations. The dilution rate was 0.013 d^{-1} . To ensure homogeneity of the sampling medium and to prevent wall growth, cultures were mixed for 1 min at 100 rpm every two hours, using a magnetic stirrer. Cultures were also stirred manually prior to sampling every day. In combination

with the small surface area to volume ratio of the carboys (16.9 m^{-1}) we deemed the impact of wall growth on experimental conditions on nutrient and phytoplankton dynamics negligible. All experiments were aerated with pressurized air, filtered over a $0.2 \text{ }\mu\text{m}$ bacterial air vent. Philips TLD 30w/33w cool-white fluorescent tubes were used as a light source with an incident light intensity of $120 \text{ }\mu\text{mol photons m}^{-2} \text{ s}^{-1}$, integrated over PAR range. The day-night cycle simulated natural spring conditions in The Netherlands, i.e. daylight was gradually increased from 14 h and 15 min at the start to 16 h and 45 min at the end of the experiment.

Phosphorus loading scenarios

The nutrient treatments represent three different phosphorus (P) loadings, i.e. low P ($1 \text{ }\mu\text{mol L}^{-1}$), intermediate P ($10 \text{ }\mu\text{mol L}^{-1}$) and high P ($100 \text{ }\mu\text{mol L}^{-1}$). These values were chosen to mimic the range of P loading present in Dutch peat lakes, ranging from oligotrophic to hypertrophic conditions. Note that low P loading in these typical Dutch lakes is considerably higher than in shallow lakes in other parts of the world. All other macro and micro nutrients were provided in ample supply, as described in Appendix A, resulting in $\text{NO}_3^-:\text{PO}_4^{3-}$ ratios of 1000:1 (oligotrophic conditions); 100:1 (mesotrophic to eutrophic conditions) and 1:1 (eutrophic-hypertrophic conditions). Nutrient loading of the cultures was realized by replacing the sampled amount with (395 mL) fresh growth medium containing the ratios as described in Appendix A. To mimic the chemical conditions of natural lake water, growth medium was prepared from $0.45 \text{ }\mu\text{m}$ filtered lake water (Whatman GmbH, Dassel, Germany) from the oligotrophic Lake Maarsseveen, with PO_4^{3-} concentrations at or below detection level. As the lake water contained trace elements in non-limiting amounts, these were not added to the medium.

Warming scenarios

The three warming scenarios reflected the seasonal rise in water temperature during a cold, an average and warm spring, based on long term temperature data of the shallow peat Lake Tjeukemeer (Fig. 1). Temperatures ranged from 9 °C to 11 °C in the cold spring scenario, from 9 °C to 16 °C in the average spring scenario and from 9 °C to 21 °C in the warm spring scenario. These temperature warming scenarios fell all within the bandwidth of spring warming regimes measured in the Tjeukemeer between Julian day 110 and 158 during the period of 1961-2006 (Mooij et al. 2008). Application of temperature scenarios was automated using the Farex SR minisystem (RKC Instruments Inc., Tokyo, Japan), ensuring a temperature control of ± 0.5 °C. Throughout the experiment temperature was recorded every minute.

Sampling

Sampling was carried out at three day intervals and the sampled amount was replaced by fresh medium. By using filters with different mesh size, the sample was immediately divided into three fractions: $> 60 \mu\text{m}$, $< 60 \mu\text{m}$ and $> 30 \mu\text{m}$, and $< 30 \mu\text{m}$. The focus in our study was on the edible part (size fraction $< 30 \mu\text{m}$) of the phytoplankton community (Haney 1973, Cyr and Curtis 1999), as this part typically is consumed by filter-feeding zooplankton and potential changes in this fraction will presumably have consequences for higher trophic levels. The presence of herbivorous zooplankton was checked in the fraction $> 60 \mu\text{m}$, as well as in the fraction < 60 and $> 30 \mu\text{m}$. The fraction $< 30 \mu\text{m}$ was subsampled for chlorophyll-*a* (PHYTO-PAM fluorometer, Walz, Mess- & Regeltechnik GmbH, Effeltrich, Germany), particle counts (CASY cell counter, Schärfe System GmbH, Reutlingen, Germany), seston C, N, and P content, qualitative microscopic identification, and nutrient analyses. Immediately after sampling, nutrient samples were filtered through aquadest rinsed glass microfiber filters (Whatman GF/F, Maidstone, UK). Filtrate samples for soluble N and P analyses were stored in polyethylene containers at -20 °C for later nutrient analysis. Soluble

reactive phosphate (SRP) was determined at 715 nm following Murphy and Riley (1962). Ammonium (NH_4^+) and nitrate (NO_3^-) were determined using a Technicon TV AAcs 800 autoanalyzer. P content in seston retentate was determined by first incinerating the samples for 30 min at 500°C, followed by a 2% persulphate digestion step in the autoclave for 30 min at 121°C. Subsequently, the digested samples were analyzed using a QuAAtro segmented flow analyzer (Seal analytical Inc., Beun de Ronde, Abcoude). C and N content in seston retentate was determined using a FLASH 2000 organic elemental analyzer (Brechtbueler Inc., Interscience B.V., Breda, The Netherlands).

Data analysis

We tested whether the time course of phytoplankton community properties (chlorophyll-*a* and N:P, C:N, and C:P stoichiometry) and nutrient dynamics (SRP, and NO_3^-) showed significant differences between treatments using Analysis of Variance for repeated measurements (rANOVA). Variables were transformed prior to analysis, if so required to meet the assumptions of homogeneity of variance and normality of (r)ANOVA. If the assumption of sphericity of the variance-covariance matrices of the (r)ANOVA analyses was violated, the degrees of freedom (df) were Huyn-Feldt corrected, resulting in an adjustment of the significance of the F-ratio (Huyn and Feldt 1976).

To gain insight in the mechanisms behind the differences in the response of the phytoplankton biomass to the temperature warming and nutrient loading scenarios we fitted a density dependent growth model to the separate timeseries of the replicates:

$$\frac{dN}{dt} = rN \left(\frac{K - N}{K} \right)$$

In this continuous logistic equation, N represent the total phytoplankton biomass ($\mu\text{g Chl-}a \text{ L}^{-1}$), r the maximum rate of net population growth (d^{-1}), and K the carrying capacity of the phytoplankton community. More specifically, K describes the potential maximum biomass that can be sustained by a system under the prevailing conditions. Model fits were obtained by minimization of the residual sum of squares of observed and modeled values of the phytoplankton biomass. Using two-way ANOVA, the independent parameter estimates of each replicate experimental time series were tested for differences in treatment effects. All statistical analyses were carried out using Statistica 8.0 (Stat Soft Inc. 2008, Tulsa, USA).

RESULTS

Nutrient dynamics

Both the soluble reactive phosphate (SRP) and nitrate (NO_3^-), showed a significant response in time (Fig. 2; Table 1). As a logical consequence of the different P loading scenarios, SRP concentrations showed significant differences between the different nutrient loading treatments (Fig. 2a-c; Table 1). Irrespective of the warming scenario, SRP showed a sharp decrease in concentrations until day 9, after which it remained at or close to limiting levels until day 45 of the experiment. The level of decrease depended on the nutrient loading scenario. SRP showed a strong increase at the last day of the experiment.

The differences in P loading scenarios were also reflected in significant differences in the time courses of NO_3^- concentrations (Fig. 2d-f, Table 1). NO_3^- levels showed a steady decrease throughout the course of the experiment, where the rate of decrease depended on both warming and nutrient loading scenarios (Table 1). In general, the rate of decrease was stronger in warmer treatments than in the cold treatment. No significant interactive effects between warming and nutrient loading scenarios were observed (Table 1).

237

238 *Biomass build-up*

239 Phytoplankton biomass, as indicated by chlorophyll-*a* concentration, showed a significant
 240 response to both warming and nutrient loading scenarios (Fig. 3, Table 1). In all treatments, the
 241 phytoplankton biomass increased to a stationary phase, of which the timing and magnitude
 242 depended on both the warming and nutrient loading scenarios. Under intermediate and warm
 243 conditions, biomass build-up extended over a longer period resulting in a higher biomass at the
 244 stationary phase. The same pattern could be seen in response to the nutrient loading scenario,
 245 where the higher P loading scenarios resulted in a higher biomass (Fig. 3).

246 The phytoplankton community was dominated by green algae throughout the experiment
 247 (Appendix B). Regardless of treatment, the relative contribution of phytoplankton functional
 248 groups showed a similar succession through the time course of the experiments, with diatoms
 249 initially present until day 15, but rapidly being replaced by green algae). This green algal
 250 community was dominated by different members of the Chlorophyceae, i.e. *Chlamydomonas*,
 251 *Chlorococcum*, *Desmusdesmus* and different species of *Scenedesmus*, and remained largely
 252 unaltered during the remainder of the experiment in all treatments.

253

254 *Elemental stoichiometry*

255 The elemental stoichiometry of seston changed considerably over time, depending on both
 256 warming and nutrient loading scenarios (Fig. 4, Table 1). As seston dry weight showed a strong
 257 correlation with chlorophyll-*a* ($R^2 = 0.70$, $P = 0.000$), we concluded that seston stoichiometry
 258 reflected to a large extent stoichiometry in the phytoplankton community.

259 The phytoplankton nitrogen:phosphorus ratios (N:P) showed significant changes over time,
 260 which depended on the P loading scenario (Fig. 4, Table 1). At the low and medium P treatment

the N:P ratios increased steadily in the initial days of the experiments to remain well above the Redfield ratio for the remainder of the experiment. This indicates that the low availability of P was indeed limiting phytoplankton growth. In the high P treatment, phytoplankton N:P ratios remained below the Redfield ratio throughout the duration of the experiment. This suggests that phytoplankton was not limited by P, but possibly by N. Indeed, the observed low NO_3^- concentration at the intermediate warming scenario (Fig. 2) suggests that N may have been limiting for phytoplankton growth. In the cold and warm scenarios, however, the higher NO_3^- concentrations (Fig. 2) suggest that biomass build-up was limited by another factor, such as temperature, CO_2 or light. Regardless of the P loading scenario, higher temperatures resulted in higher N:P ratios (Table 1).

Carbon:phosphorus ratios of the phytoplankton (C:P) increased steadily over time during the experiment (Fig. 4d-f, Table 1). The different P loading scenarios were reflected in the significant differences in C:P, with highest C:P ratios in the low and intermediate P treatment, and lowest C:P ratios in the high P treatment. In addition, C:P showed a significant response to both P loading and warming over time (Table 1). Importantly, warming seemed to amplify the effect of P limitation on C:P ratios, as the C:P ratios were significantly higher in warmer treatments (Table 1).

A slightly different pattern was observed in the phytoplankton carbon:nitrogen ratios (C:N), where C:N ratios remained largely unaltered during the first week and subsequently increased steadily over time (Fig. 4 g-i, Table 1). In the cold and warm high P treatments, phytoplankton C:N ratios leveled off towards the end of the experiment (Fig. 5f). The different P loading scenarios were also apparent through differences in C:N ratios, which was generally lower with high P loading (Table 4). Interestingly, P limitation also resulted in higher C:N ratios, which was furthermore amplified by temperature (Table 1).

Logistic model output

The additive effect of warming and P loading on phytoplankton biomass build-up suggests that temperature affects different biological properties of the phytoplankton community than P loading does. To get a more mechanistic understanding on how temperature and P availability may act upon phytoplankton biomass, we fitted a logistic growth model on the chlorophyll-*a* data from each treatment. An ANOVA of our model results showed that carrying capacity was not only affected by an increased P loading (Fig. 5b, $F_{2,27} = 17.55$, $P = 0.000$) but also by an increased temperature (Fig. 5a, $F_{2,27} = 3.59$, $P = 0.042$). More specifically, both warming and P loading resulted in a significantly higher maximum phytoplankton biomass, particularly under high P loading. The intrinsic growth rate of the phytoplankton community was not affected by P loading, but did show a clear increase in response to warming (Fig. 5c-d, $F_{2,27} = 4.61$, $P = 0.019$).

DISCUSSION

Phytoplankton dynamics

Our results clearly demonstrate that rising temperatures combined with increases in P loading promote growth and biomass build-up of a natural phytoplankton spring community. Strongest changes in phytoplankton biomass were induced by enhanced P loading, causing about a 4-fold increase (Fig. 3, Fig. 5d). On top of that, rising temperatures further promote the build-up of phytoplankton biomass, thereby yielding highest densities in the warmer treatments. This was particular evident under P rich conditions, where biomass increased up to a 2-fold as result of warming (Fig. 3c). Rising temperatures also cause a strong increase in the phytoplankton community growth rate (Fig. 5a). The different P loading scenarios, however, did not affect growth rate. The observed temperature-driven changes in phytoplankton community growth rate may affect the timing of the phytoplankton spring bloom. An earlier onset of a phytoplankton

spring bloom may, in turn, cause a phenological mismatch with herbivores, with cascading effects throughout the entire food-web (De Senerpont Domis et al. 2007a).

Fluorimetric pigment analyses showed that the succession of functional groups was similar in all treatments, with a relative high contribution of diatoms in the beginning, rapidly being replaced by green algae. Qualitatively, this sequential succession from diatoms to green algae resembled earlier spring warming experiments with natural communities originating from our reference lake (De Senerpont Domis et al. 2007b). Similar to De Senerpont Domis et al. (2007b), from day 20 onwards, the phytoplankton spring biomass was dominated by members of the Chlorophyceae, including *Chlorella*, *Chlorococcum*, *Desmusdesmus* and different species of *Scenedesmus*. The lack of difference in functional group composition between treatments may indicate that the observed responses in biomass build-up and phytoplankton stoichiometry are not caused by changes in the phytoplankton community composition. Changes in nutrient use efficiency, and thereby phytoplankton biomass and stoichiometry, can also be induced by changes in the phenotypic composition. Indeed, it has been shown that higher temperatures promote small unicellular phenotypes of *Scenedesmus*, rather than the larger colony forming phenotypes (Trainor 1993). In our study, however, the average cell size in the $>4\ \mu\text{m}$ fraction, i.e. including all observed phytoplankton species, increased with rising temperatures, rather than decreased (data not shown).

Higher temperatures may potentially lead to faster recycling of nutrients, and thereby promote the availability of nutrients. In contrast to other microcosm studies, we found no evidence that temperature enhanced the recycling of phosphorus (Mckee et al. 2003). Only on the last day of the experiment, SRP substantially increased, although these changes do not seem temperature dependent (Fig. 1). The apparent lack of recycling during the largest part of the experiment may thus be a result of the relative short duration of our experiment. Over the course of the experiment,

however, we did observe a decrease in the size distribution of the cell size fraction $< 4 \mu\text{m}$, which is indicative for the bacterioplankton community. Yet, this putative increase in contribution of bacteria did not result in enhanced nutrient availability for the phytoplankton community.

Phytoplankton stoichiometry

The higher maximum biomass (i.e. carrying capacity) observed at higher temperatures and increased P concentrations can be explained by an overall increase in carbon:nutrient ratios. In other words, higher temperatures allow phytoplankton to yield a higher carbon based biomass per unit cellular P or N. This suggests that the nutrient use efficiency of phytoplankton increases with temperature, confirming earlier findings showing that primary productivity increases with temperature (Kerkhoff et al. 2005, Lovelock et al. 2007). Comparable observations of a temperature-driven increase in nutrient use efficiency were reported for the prokaryote *Escherichia coli*, showing a higher C and N based biomass with respect to P, evident from its higher cellular C:P and N:P ratios at higher temperatures (Cotner et al. 2006). Studying a natural phytoplankton spring community rather than single species, we show that temperature may enhance the nutrient use efficiency of an entire community, either through phenotypic adaptation of the community or through shifts towards species with higher resource use efficiencies.

Limitation by P in the low and medium P loading scenarios caused an increase in C:P ratios, which was not only accompanied by an increase in N:P but also in C:N ratios. This implies that the phytoplankton community showed a stronger increase in its C content relative to increases in its N content. With a lower availability of P, phytoplankton may still continue to take up C and N (Sterner and Elser 2002). This luxury consumption appears to be stronger for C than for N, explaining the higher C:N ratios in the P limited treatments. In the high P treatments, C:N and C:P ratios seem to be controlled by different factors. In the cold treatment, the lower temperatures may

have contributed to the lower carbon:nutrient ratios, for instance by reallocation of nutrients to cellular compounds such as RNA and proteins (Cotner et al. 2006). In line with Cotner et al. (2006), we observed a temperature dependent increased in carbon:nutrient ratios in the intermediate temperature scenario. This may be explained by increased allocation towards resource acquisition machinery at higher temperatures (*cf.* Cotner et al. 2006), which is further substantiated by the stronger nitrogen depletion in the intermediate temperature scenario (Fig. 2f). However, this process seems to be impeded in the warmest treatments, most likely a result of limitation by carbon and/or light.

Implications for the food-web

Higher temperatures clearly promote the build-up of phytoplankton biomass, under low P, i.e. oligotrophic conditions, as well as under high P, i.e. eutrophic conditions (Fig. 3). The stoichiometry of this phytoplankton biomass, however, depends on the phosphorus loading of the system. Our results show that under oligotrophic and mesotrophic conditions, temperature-driven increases in carbon:nutrient ratios are prominent. Higher temperatures may promote thermal stratification of the water column, and thereby reduce the nutrient input into the upper mixed layer where phytoplankton typically thrives. Together with elevated $p\text{CO}_2$, the climate-driven decrease in nutrient availability and increase in C availability may cause an increase in phytoplankton carbon:nutrient ratios (Van de Waal et al. 2010). Furthermore, P limitation in phytoplankton may be amplified by enhanced atmospheric N deposition, presumably causing a further increase in their C:P stoichiometry (Elser et al. 2010). Here, we show that higher temperatures can also directly enhance the carbon:nutrient ratios of phytoplankton. These stoichiometric responses, however, do not occur when nutrients are available in excess (Fig. 5c), i.e. under eutrophic conditions.

Despite the higher maximum biomass of the phytoplankton community at higher temperatures, the low nutritional quality (i.e. high carbon:nutrient ratios) of these primary producers may negatively affect higher trophic levels (Sterner and Elser 2002, Urabe et al. 2003, Urabe and Waki 2009). Using *Daphnia galeata x hyalina* clones from our reference lake Tjeukemeer, Bukovinsky et al. (2012) showed that increases in C:P ratio (~400) lead to a strong reduction in growth rate. To what extent a diverse phytoplankton community can mitigate the adverse effects of high carbon:nutrient ratios (Urabe and Waki 2009) needs further investigation. With ongoing re-oligotrophication in many lakes (Jeppesen et al. 2005), decreases in seston food quality are more often reported (Hessen et al. 2006, Sterner et al. 2008, Van Donk et al. 2008). Thus the temperature driven increase in phytoplankton carbon:nutrient ratios may further enhance the stoichiometric mismatch between phytoplankton and their herbivores. Further studies on the intriguing interplay between direct and indirect effects of temperature are needed to further elucidate the potential consequences of climate change for the growth, biomass build-up and stoichiometry of phytoplankton communities and potential knock-on effects on the food web (De Senerpont Domis et al. 2013).

Conclusions

By and large, our data suggest that with rising temperatures, the projected increases in carbon:nutrient stoichiometry may be aggravated by direct impacts of warming on the phytoplankton nutrient use efficiency. Ultimately, this may impede trophic transfer efficiency to higher trophic levels, and affect the structure and functioning of entire aquatic food webs (Sterner et al. 1997, Sterner et al. 1998).

ACKNOWLEDGMENTS

LdeSD thanks Mathew Leibold, Jef Huisman, Bas Ibelings, Alena Gsell and two anonymous reviewers for feedback on earlier versions of the manuscript. Henny Uittenhout designed and constructed the sampling devices. Nienke Lammertse and Denise Rotgans assisted in analyzing nutrients. LdSD was supported by grant 817.01.007/852.00.012 of the Netherlands Organization for Scientific Research (NWO). This is publication 5546 of the Netherlands Institute of Ecology (NIOO-KNAW).

LITERATURE CITED

- Behrenfeld, M. J., R. T. O'Malley, D. A. Siegel, C. R. McClain, J. L. Sarmiento, G. C. Feldman, A. J. Milligan, P. G. Falkowski, R. M. Letelier, and E. S. Boss. 2006. Climate-driven trends in contemporary ocean productivity. *Nature* 444:752-755.
- Bukovinszky, T., A. M. Verschoor, N. R. Helmsing, T. M. Bezemer, E. S. Bakker, M. Vos, and L. N. de Senerpont Domis. 2012. The Good, the bad and the plenty: interactive effects of food quality and quantity on the growth of different *Daphnia* species. *Plos One* 7:e42966.
- Carvalho, L. and A. Kirika. 2003. Changes in shallow lake functioning: response to climate change and nutrient reduction. *Hydrobiologia* 506:789-796.
- Cotner, J. B., W. Makino, and B. A. Biddanda. 2006. Temperature affects stoichiometry and biochemical composition of *Escherichia coli*. *Microbial Ecology* 52:26-33.
- Crain, C. M., K. Kroeker, and B. S. Halpern. 2008. Interactive and cumulative effects of multiple human stressors in marine systems. *Ecology Letters* 11:1304-1315.
- Cyr, H. and J. M. Curtis. 1999. Zooplankton community size structure and taxonomic composition affects size-selective grazing in natural communities. *Oecologia* 118:306-315.
- De Senerpont Domis, L. N., J. J. Elser, A. S. Gsell, V. L. M. Huszar, B. W. Ibelings, E. Jeppesen, S. Kosten, W. M. Mooij, F. Roland, U. Sommer, E. van Donk, M. Winder, and M. Lüring.

- 428 2013. Plankton dynamics under different climatic conditions in space and time. *Freshwater*
429 *Biology* 58:463-482.
- 430 De Senerpont Domis, L. N., W. M. Mooij, S. Hülsmann, E. H. v. Nes, and M. Scheffer. 2007a.
431 Can overwintering versus diapausing strategy in *Daphnia* determine match–mismatch
432 events in zooplankton–algae interactions? *Oecologia* 150:682-698.
- 433 De Senerpont Domis, L. N., W. M. Mooij, and J. Huisman. 2007b. Climate-induced shifts in a
434 phytoplankton community: a mechanistic approach. *Hydrobiologia* 584:403-413.
- 435 Doney, S. C., M. Ruckelshaus, J. E. Duffy, J. P. Barry, F. Chan, C. A. English, H. M. Galindo, J.
436 M. Grebmeier, A. B. Hollowed, N. Knowlton, J. Polovina, N. N. Rabalais, W. J. Sydeman,
437 and L. D. Talley. 2012. Climate Change Impacts on Marine Ecosystems. Pages 11-37 in C.
438 A. Carlson and S. J. Giovannoni, editors. *Annual Review of Marine Science*, Vol 4.
439 *Annual Reviews*, Palo Alto.
- 440 Elliott, J. A., I. D. Jones, and S. J. Thackeray. 2006. Testing the sensitivity of phytoplankton
441 communities to changes in water temperature and nutrient load, in a temperate lake.
442 *Hydrobiologia* 559:401-411.
- 443 Elser, J. J., A. L. Peace, M. Kyle, M. Wojewodzic, M. L. McCrackin, T. Andersen, and D. O.
444 Hessen. 2010. Atmospheric nitrogen deposition is associated with elevated phosphorus
445 limitation of lake zooplankton. *Ecology Letters* 13:1256-1261.
- 446 Gudas, C., D. Bastviken, K. Steger, K. Premke, S. Sobek, and L. J. Tranvik. 2010. Temperature-
447 controlled organic carbon mineralization in lake sediments. *Nature* 466:478-482.
- 448 Hall, E. K. and J. B. Cotner. 2007. Interactive effect of temperature and resources on carbon
449 cycling by freshwater bacterioplankton communities. *Aquatic Microbial Ecology* 49:35-45.
- 450 Haney, J. F. 1973. In-situ examination of grazing activities of natural zooplankton communities.
451 *Archiv Für Hydrobiologie* 72:87-132.

- 452 Hein, M. and K. Sand-Jensen. 1997. CO₂ increases oceanic primary production. *Nature* 388:526-
453 527.
- 454 Hessen, D. O., B. A. Faafeng, P. Brettum, and T. Andersen. 2006. Nutrient enrichment and
455 planktonic biomass ratios in lakes. *Ecosystems* 9:516-527.
- 456 Hinton, M. J., S. L. Schiff, and M. C. English. 1997. The significance of storms for the
457 concentration and export of dissolved organic carbon from two Precambrian Shield
458 catchments. *Biogeochemistry* 36:67-88.
- 459 Hsieh, C. H., K. Ishikawa, Y. Sakai, T. Ishikawa, S. Ichise, Y. Yamamoto, T. C. Kuo, H. D. Park,
460 N. Yamamura, and M. Kumagai. 2010. Phytoplankton community reorganization driven by
461 eutrophication and warming in Lake Biwa. *Aquatic Sciences* 72:467-483.
- 462 Huber, V., R. Adrian, and D. Gerten. 2008. Phytoplankton response to climate warming modified
463 by trophic state. *Limnology and Oceanography* 53:1-13.
- 464 Huynh, H. and L. S. Feldt. 1976. Estimation of the box correction for degrees of freedom from
465 sample data in randomized block and split-plot designs. *Journal of Educational Statistics*
466 1:69-82.
- 467 Jäger, C. G., S. Diehl, and G. M. Schmidt. 2008. Influence of water-column depth and mixing on
468 phytoplankton biomass, community composition, and nutrients *Limnology and*
469 *Oceanography* 53:2361-2373
- 470 Jeppesen, E., M. Sondergaard, J. P. Jensen, K. E. Havens, O. Anneville, L. Carvalho, M. F.
471 Coveney, R. Deneke, M. T. Dokulil, B. Foy, D. Gerdeaux, S. E. Hampton, S. Hilt, K.
472 Kangur, J. Kohler, E. Lammens, T. L. Lauridsen, M. Manca, M. R. Miracle, B. Moss, P.
473 Noges, G. Persson, G. Phillips, R. Portielje, C. L. Schelske, D. Straile, I. Tatnai, E. Willen,
474 and M. Winder. 2005. Lake responses to reduced nutrient loading - an analysis of
475 contemporary long-term data from 35 case studies. *Freshwater Biology* 50:1747-1771.

- 476 Jeppesen, E., B. Kronvang, M. Meerhoff, M. Sondergaard, K. M. Hansen, H. E. Andersen, T. L.
 477 Lauridsen, L. Liboriussen, M. Beklioglu, A. Ozen, and J. E. Olesen. 2009. Climate change
 478 effects on runoff, catchment phosphorus loading and lake ecological state, and potential
 479 adaptations. *Journal of Environmental Quality* 38:1930-1941.
- 480 Jöhnk, K. D., J. Huisman, J. Sharples, B. Sommeijer, P. M. Visser, and J. M. Stroom. 2008.
 481 Summer heatwaves promote blooms of harmful cyanobacteria. *Global Change Biology*
 482 14:495-512.
- 483 Kerkhoff, A. J., B. J. Enquist, J. J. Elser, and W. F. Fagan. 2005. Plant allometry, stoichiometry
 484 and the temperature-dependence of primary productivity. *Global Ecology and*
 485 *Biogeography* 14:585-598.
- 486 Lovelock, C. E., I. C. Feller, M. C. Ball, J. Ellis, and B. Sorrell. 2007. Testing the growth rate vs.
 487 geochemical hypothesis for latitudinal variation in plant nutrients. *Ecology Letters*
 488 10:1154-1163.
- 489 Mckee, D., D. Atkinson, S. E. Collings, J. W. Eaton, A. B. Gill, I. Harvey, K. Hatton, T. Heyes, D.
 490 Wilson, and B. Moss. 2003. Response of freshwater microcosm communities to nutrients,
 491 fish, and elevated temperature during winter and summer. *Limnology and Oceanography*
 492 48:702-722.
- 493 Mooij, W. M., L. N. De Senerpont Domis, and S. Hülsmann. 2008. The impact of climate
 494 warming on water temperature, timing of hatching and young-of-the-year growth of fish in
 495 shallow lakes in the Netherlands. *Journal of Sea Research* 60:32-43.
- 496 Mooij, W. M., L. N. De Senerpont Domis, and J. H. Janse. 2009. Linking species- and ecosystem-
 497 level impacts of climate change with simple and complex models. *Ecological Modelling*
 498 220:3011-3020.

- 499 Moss, B., D. Mckee, D. Atkinson, S. E. Collings, J. W. Eaton, A. B. Gill, I. Harvey, K. Hatton, T.
 500 Heyes, and D. Wilson. 2003. How important is climate? Effects of warming, nutrient
 501 addition and fish on phytoplankton in shallow lake microcosms. *Journal of Applied*
 502 *Ecology* 40:782-792.
- 503 Murphy, J. and J. P. Riley. 1962. A modified single solution method for determination of
 504 phosphate in natural waters. 26:31-36.
- 505 Nemani, R. R., C. D. Keeling, H. Hashimoto, W. M. Jolly, S. C. Piper, C. J. Tucker, R. B. Myneni,
 506 and S. W. Running. 2003. Climate-driven increases in global terrestrial net primary
 507 production from 1982 to 1999. *Science* 300:1560-1563.
- 508 Paerl, H. W. and J. Huisman. 2008. CLIMATE: Blooms like it hot. *Science* 320:57-58.
- 509 Parmesan, C. and G. Yohe. 2003. A globally coherent fingerprint of climate change impacts across
 510 natural systems. *Nature* 421:37-42.
- 511 Pomati, F., B. Matthews, J. Jokela, A. Schildknecht, and B. W. Ibelings. 2012. Effects of re-
 512 oligotrophication and climate warming on plankton richness and community stability in a
 513 deep mesotrophic lake. *Oikos* 121:1317-1327.
- 514 Rabalais, N. N., R. E. Turner, R. J. Diaz, and D. Justic. 2009. Global change and eutrophication of
 515 coastal waters. *Ices Journal of Marine Science* 66:1528-1537.
- 516 Sardans, J., A. Rivas-Ubach, and J. Penuelas. 2012. The C:N:P stoichiometry of organisms and
 517 ecosystems in a changing world: A review and perspectives. *Perspectives in Plant Ecology*
 518 *Evolution and Systematics* 14:33-47.
- 519 Schindler, D. W. 2006. Recent advances in the understanding and management of eutrophication.
 520 *Limnology and Oceanography* 51:356-363.
- 521 Schippers, P., M. Lüring, and M. Scheffer. 2004. Increase of atmospheric CO₂ promotes
 522 phytoplankton productivity. *Ecology Letters* 7:446-451.

- Schoo, K. L., A. M. Malzahn, E. Krause, and M. Boersma. 2013. Increased carbon dioxide availability alters phytoplankton stoichiometry and affects carbon cycling and growth of a marine planktonic herbivore. *Marine Biology* 160:2145-2155.
- Solomon, S., D. Qin, M. Manning, Z. Chen, M. Marquis, K. B. Averyt, M. Tignor, and H. L. Miller, editors. 2007. *Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change*. Cambridge University Press, Cambridge, United Kingdom.
- Sommer, U., Z. M. Gliwicz, W. Lampert, and A. Duncan. 1986. The PEG-Model of seasonal succession of planktonic events in fresh waters. *Archiv Für Hydrobiologie* 106:433-471.
- Sommer, U., R. Adrian, L. N. De Senerpont Domis, J. J. Elser, U. Gaedke, B. W. Ibelings, E. Jeppesen, M. Lüring, J. C. Molinero, W. M. Mooij, E. Van Donk, and M. Winder. 2012. Beyond the PEG model: mechanisms driving plankton succession. *Annual Review of Ecology, Evolution and Systematics* 43:429-448.
- Sterner, R. W., T. Andersen, J. J. Elser, D. O. Hessen, J. M. Hood, E. McCauley, and J. Urabe. 2008. Scale-dependent carbon : nitrogen : phosphorus seston stoichiometry in marine and freshwaters. *Limnology and Oceanography* 53:1169-1180.
- Sterner, R. W., J. J. Elser, E. J. Fee, S. J. Guildford, and T. H. Chrzanowski. 1997. The light:nutrient ratio in lakes: The balance of energy and materials affects ecosystem structure and process. *American Naturalist* 150:663-684.
- Sterner, R. W., J. Clasen, W. Lampert, and T. Weisse. 1998. Carbon : phosphorus stoichiometry and food chain production. *Ecology Letters* 1:146-150.
- Sterner, R. W. and J. J. Elser. 2002. *Ecological stoichiometry. The biology of elements from molecules to the biosphere*. Princeton University Press, New Jersey, USA.

- Trainor, F. R. 1993. Cyclomorphosis in *Scenedesmus subspicatus* (Chlorococcales, Chlorophyta)-stimulation of colony development at low temperature. *Phycologia* 32:429-433.
- Urabe, J., J. Togari, and J. J. Elser. 2003. Stoichiometric impacts of increased carbon dioxide on a planktonic herbivore. *Global Change Biology* 9:818-825.
- Urabe, J. and N. Waki. 2009. Mitigation of adverse effects of rising CO₂ on a planktonic herbivore by mixed algal diets. *Global Change Biology* 15:523-531.
- Van de Waal, D. B., A. M. Verschoor, J. M. H. Verspagen, E. van Donk, and J. Huisman. 2010. Climate-driven changes in the ecological stoichiometry of aquatic ecosystems. *Frontiers in Ecology and the Environment* 8:145-152.
- Van Donk, E., D. O. Hessen, A. M. Verschoor, and R. D. Gulati. 2008. Re-oligotrophication by phosphorus reduction and effects on seston quality in lakes. *Limnologia* 38:189-202.
- Vinebrooke, R. D., K. L. Cottingham, J. Norberg, M. Scheffer, S. I. Dodson, S. C. Maberly, and U. Sommer. 2004. Impacts of multiple stressors on biodiversity and ecosystem functioning: the role of species co-tolerance. *Oikos* 104:451-457.
- Walther, G. R., E. Post, P. Convey, A. Menzel, C. Parmesan, T. J. C. Beebee, J. M. Fromentin, O. Hoegh-Guldberg, and F. Bairlein. 2002. Ecological responses to recent climate change. *Nature* 416:389-395.
- Winder, M. and D. Hunter. 2008. Temporal organization of phytoplankton (Walther et al. 2002) communities linked to physical forcing. *Oecologia* 156:179-192.
- Woods, H. A., W. Makino, J. B. Cotner, S. E. Hobbie, J. F. Harrison, K. Acharya, and J. J. Elser. 2003. Temperature and the chemical composition of poikilothermic organisms. *Functional Ecology* 17:237-245.

570 **SUPPLEMENTAL MATERIAL**

571 **Appendix A:**

572 Compounds added to lake water to construct growth medium (*Ecological Archives xxxx*)

573

574 **Appendix B:**

575 Relative contribution of phytoplankton functional groups to total chlorophyll-*a* biomass

576 (*Ecological Archives xxxx*)

577

Table 1: Summary of rANOVA results on the effect of the different warming (Temp) and nutrient loading (Nut) scenarios on the concentrations ($\mu\text{mol/L}$) of soluble reactive phosphate (SRP), nitrate (NO_3^-), phytoplankton biomass as indicated by chlorophyll-*a* (Biomass), and molar ratios of nitrogen to phosphorus (N:P), carbon to phosphorus (C:P) and carbon to nitrogen (C:N) during the course of the experiment (Time). Values indicate probability levels, values in bold are below significance level = 0.05

Effects	SRP	NO_3^-	Biomass	N:P	C:P	C:N
Temp	0.393	0.039	0.005	0.631	0.346	0.076
Nut	0.000	0.082	0.000	0.000	0.000	0.000
Temp x Nut	0.318	0.280	0.211	0.541	0.327	0.067
Time	0.000	0.000	0.000	0.000	0.000	0.000
Time x Temp	0.659	0.001	0.026	0.030	0.015	0.000
Time x Nut	0.000	0.003	0.000	0.000	0.000	0.000
Time x Temp x Nut	0.262	0.097	0.645	0.199	0.025	0.004

Figure legends

Figure 1: Time course of temperature in the three warming scenarios simulated in the experiments (mean $\pm$ SE.). Upward pointing triangles indicate low temperature treatment (i.e. cold), circles indicate average temperature treatment (i.e. intermediate), and downward pointing triangles indicate high temperature treatment (i.e. warm).

Figure 2: Time series of nutrient dynamics in the three P loading scenarios, with soluble reactive phosphate (SRP, a-c) and nitrate (NO_3^- , d-f). Values represent mean $\pm$ SE. Warming scenarios similar to Figure 1.

Figure 3: Time series of phytoplankton biomass as indicated by chlorophyll-*a* in the three P loading scenarios with low P (a), medium P (b) and high P (c). Values represent mean $\pm$ SE. Warming scenarios similar to Figure 1.

Figure 4: Time series of the seston elemental stoichiometry in the three P loading scenarios, with molar N:P ratio (a-c), molar C:P ratio (d-f), and molar C:N ratio (g-i). Values represent mean $\pm$ SE. Straight line indicates the Redfield ratio. Warming scenarios similar to Figure 1.

Figure 5: Effects of warming and P loading scenario on carrying capacity (a,b) and intrinsic growth rate (c,d). Values represent mean $\pm$ SE. Labels above bars indicate significant differences (two-way ANOVA, $P < 0.05$).

606

607 **Figure 1**

608

Figure 2

Figure 3

611

Figure 4

612

Figure 5