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Warming and oligotrophication cause shifts in freshwater phytoplankton communities

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Abstract

While there is a lot of data on interactive effects of eutrophication and warming, to date, we lack data to generate reliable predictions concerning possible effects of nutrient decrease and temperature increase on community composition and functional responses. In recent years, a wide-ranging trend of nutrient decrease (re-oligotrophication) was reported for freshwater systems. Small lakes and ponds, in particular, show rapid responses to anthropogenic pressures and became model systems to investigate single as well as synergistic effects of warming and fertilization *in situ* and in experiments. Therefore, we set up an experiment to investigate the single as well as the interactive effects of nutrient reduction and gradual temperature increase on a natural freshwater phytoplankton community, using an experimental indoor mesocosm setup. Biomass production initially increased with warming but decreased with nutrient depletion. If nutrient supply was constant, biomass increased further, especially under warming conditions. Under low nutrient supply, we found a sharp transition from initially positive effects of warming to negative effects when resources became scarce. Warming reduced phytoplankton richness and evenness, whereas nutrient reduction at ambient temperature had positive effects on diversity. Our results indicate that temperature effects on freshwater systems will be altered by nutrient availability. These interactive effects of energy increase and resource decrease have major impacts on biodiversity and ecosystem function and thus need to be considered in environmental management plans.

KEYWORDS

biodiversity, climate change, evenness, oligotrophication, Planktotrons, regime shift, species richness, temperature increase, tipping point, warming

1 | INTRODUCTION

Anthropogenic actions cause numerous pressures and changes in ecosystems worldwide (Millennium Ecosystem Assessment, 2005), which endanger their stability (Ives & Carpenter, 2007) and therefore eventually the goods and services these ecosystems provide for humanity (Costanza et al., 1997; Rockström et al., 2009). Many of these pressures—eutrophication (Schindler, 2006), loss of biodiversity (Cardinale et al., 2012), climate change—are global phenomena and affect all types of ecosystems (Rosenzweig et al., 2007; Walther

et al., 2002). Freshwater systems, such as ponds, are especially susceptible to environmental changes as they are comparably small and variable in their morphology, and often undergo rapid changes in biodiversity and ecosystem functions (Adrian et al., 2009; Williamson, Dodds, Kratz, & Palmer, 2008).

It remains uncertain to what extent changes in community composition directly translate into changes in ecosystem function. Ecosystem functions, such as total biomass production, are calculated as the sum of all individual species performances combined

(Nijss & Impens, 2000). Continuous levels of ecosystem function are usually maintained through species asynchrony (Hautier et al., 2014), but this may be disrupted through pulsed disturbances that synchronize species patterns, or through extinctions that reduce the pool of contributing species (Larsen, Williams, & Kremen, 2005). Not all species contribute to all ecosystem functions equally (Hector & Bagchi, 2007; Lefcheck et al., 2015; Shurin, Clasen, Greig, Kratina, & Thompson, 2012), so that the consequences of a reduction in biodiversity depend both on the function and species in question. In general, the maintenance of multiple functions ("functional integrity") requires higher diversity than any single ecosystem function alone (Gamfeldt & Hillebrand, 2008; Hector & Bagchi, 2007; Isbell et al., 2011). As an exemplary ecosystem function, we studied the change in resource use efficiency (RUE, the amount of biomass per unit resource) in relation to nutrient decrease and warming.

Increasing temperatures and changes in nutrient availability are two of the most pervasive abiotic pressures of the last decades (Pomati, Matthews, Jokela, Schildknecht, & Ibelings, 2012; Tadolé, 2010). In aquatic ecosystems, warming influences biogeochemical cycles and nutrient-, oxygen-, and CO₂-availability (O'Reilly, Alin, Plisnier, Cohen, & McKee, 2003). Mediated through these abiotic responses, species' physiology, and population dynamics are strongly influenced by temperature (Cardinale, 2011; Striebel, Schabhtl, Hodapp, Hingsamer, & Hillebrand, 2016); consequently, warming is poised to alter communities and whole ecosystems. Compared to marine phytoplankton, most freshwater algae are adapted to higher temperatures (10–30°C) (Butterwick, Heaney, & Talling, 2005), with differences between species and taxa: optimal temperatures reported for diatoms range between 5–25°C (Butterwick et al., 2005; Soulsby, Houston, Montgomery, Surukip, & Takahashi, 1981), for cyanobacteria between 25–30°C (Jöhnk et al., 2008; Lürling, Eshetu, Faassen, Kosten, & Huszar, 2013), and for chlorophytes optimal temperatures range up to 30°C (Butterwick et al., 2005). Although warming of freshwater systems rarely exceeds the fundamental niche of phytoplankton, the different temperature optima of the various taxonomic groups will induce changes in dominance toward better adapted species (Kosten et al., 2012) at the expense of groups less competitive at higher temperatures (Rosset, Lehmann, & Oertli, 2010).

The precise effects of warming will depend on interactive effects with other factors, for example, pH or nutrient availability. Regarding nutrient availability, an increase in temperature changes the physiological requirements of phytoplankton toward lower phosphorus and higher nitrogen content (Cotner, Makino, & Biddanda, 2006; Toseland et al., 2013; Yvon-Durocher, Dossena, Trimmer, Woodward, & Allen, 2015), potentially switching the system from P- to N-limitation (Gilbert, 2013). Most studies addressing the interaction between warming and altered nutrient requirements focused on high nutrient conditions, revealing synergistic effects of eutrophication and warming (Heino, Virkkala, & Toivonen, 2009; Moss et al., 2003; Rosset et al., 2010). These nutrient-warming interactions may strongly differ in oligotrophic systems, where temperature-dependent nutrient requirements are not easily met by availability. However, few studies explicitly addressed warming effects under lowered nutrient

conditions (De Senerpont Domis, Van De Waal, Helmsing, Van Donk, & Mooij, 2014; Velthuis et al., 2017).

Due to the widespread re-oligotrophication of freshwater systems, the interaction between nutrient limitation and warming is of increased importance (Jeppesen et al., 2005): Globally, eutrophication of freshwater systems has been identified as a severe ecological and economic problem, initializing efforts to reduce nutrient (mainly phosphorus) loading originating from washing detergents and agricultural fertilizers. Targeted management strategies have been devised and implemented in the 1970s and 1980s and efforts to avoid new influx and to reduce already present nutrient loadings have been successful. Consequently, phosphorous concentrations in many lakes have declined immensely (Anneville, Gammeter, & Straile, 2005; Pomati et al., 2012; Van Donk et al., 2008). This has, for example, been well studied in Lake Biwa, Japan, for which both biotic and abiotic variables have been tracked over 32 years, showing an increase in nutrient loading in the 1960s and 1970s, followed by a decline during the 1980s, when management practices showed effect (Hsieh et al., 2010; Tsai et al., 2014). High nutrient concentrations are often characterized by high biomass production by few dominant species (Borge et al., 2004), whereas re-oligotrophication increases species richness and community evenness (Pomati et al., 2012) coupled with a decrease in autotrophic productivity or standing stocks (Finger, Wuest, & Bossard, 2013; Verbeek et al., 2018).

Thus, in nutrient reduced systems, we expect different mechanisms shaping the temperature—productivity relationship, partly through direct physiological responses, partly through altered community composition and dominance in eutrophic and oligotrophic systems, respectively.

To test the effects of oligotrophication and warming, as well as their interaction, on the productivity and diversity of a natural phytoplankton community, we performed a controlled indoor mesocosm experiment. Treatments were chosen based on a field study of small farmland ponds in Belgium (Verbeek et al., 2018), which revealed a strong decrease in nutrient loading over time (especially for phosphorus) with significant change in phyto- and zooplankton biomass and composition. Using an indoor mesocosm facility comprising 12 so-called Planktotrons (Gall et al., 2017), we manipulated temperature (either constant at 20°C or increasing by 2°C per week for a total experiment duration of 7 weeks) and nutrient availability (either ambient or gradually decreasing by 20% per week) in a factorial design. The inoculum for the experiment was a natural phytoplankton community from one of the ponds in Belgium. We used 20°C as "ambient" treatment, as it was close to the ambient temperature at the time of the experiment and is a common temperature for the small farmland ponds particularly in early spring, partially due to their low depth (Staehr & Sand-Jensen, 2006) in the region where we took the inoculum. We chose to increase the temperature by 2°C weekly, as this allowed for changes in species abundance in response to the temperature increase but avoided heat shocks. This reflects natural conditions as field data show that temperatures in ponds can increase by 2°C or more in the course of 1 week during spring (Berger et al., 2007; Huesemann et al., 2016). Phytoplankton species are

able to acclimate to changes in water temperature in the timeframe of a few generations (Coles & Jones, 2000; Staehr & Sand-Jensen, 2006). While our final temperature of 32° is rarely achieved in temperate ponds, it is nevertheless not completely outside of the temperature range of some algae, in particular cyanobacteria or green algae (which are the dominant group in our experimental community), which can have their temperature optimum at 26°C, 35°C or in some cases even higher (Coles & Jones, 2000; Yentsch, 1974). The nutrient reduction was 20% per week in the oligotrophication treatments, whereas the treatments with constant nutrient supply were replenished with 20% of the original nutrient content weekly to maintain constant nutrient conditions. These two nutrient scenarios were chosen to provide a substantial, but not too rapid decrease in nutrient availability for the oligotrophication treatment, and to reflect semi-continuous conditions by the regular exchange in medium for the constant treatment.

Throughout the experiment, we monitored community composition, biomass, and resource use efficiency of the community to test the following hypotheses:

H1: Increasing temperatures and nutrient reduction have interactive effects on algal biomass production. We expect an initial increase in biomass as nutrient supply is high, with the rate of increase being higher at a higher temperature (Figure 1). After the initial growth phase, the control (constant nutrient, no warming) will progress to carrying capacity, whereas the oligotrophication treatment without warming will show decreasing biomass with progressing nutrient loss. For the warming treatments, we expect significant temperature \times nutrient interactions: as soon as warming exceeds threshold temperatures, biomass production potentially declines, with the onset of the decline being earlier with lowered nutrient availability. Thus, we expect a change in the sign of the warming effect over time from an increase in the initial biomass production rate compared to the control (positive effects of warming with high nutrient availability) to a decrease under progressing oligotrophication

(negative effects of warming with nutrient deficiency). **H2:** Algal resource use efficiency (RUE = biomass production per unit resource available) will increase with oligotrophication which is expected to directly reduce excess nutrient supply and indirectly increase RUE by increasing biodiversity (Filstrup, Hillebrand, Heathcote, Harpole, & Downing, 2014; Ptasnik, Solimini, et al., 2008). Consequently, we also expect algal C:nutrient ratios to be altered by both oligotrophication (altered supply of N and P) and temperature (altered demand for N and P). **H3:** As the system is isolated without immigration, the initial diversity of the phytoplankton assemblage will be reduced over time in all treatments (Figure 1). We expect more rapid competitive dominance and exclusion with warming in the closed settings of our experiment (Hillebrand, Burgmer, & Biermann, 2012) leading to reduced species richness and evenness (Figure 1). Reversing the predictions from eutrophication scenarios (Hillebrand et al., 2007), reduced nutrient conditions will show higher diversity (species richness and evenness) than the nutrient constant controls. We expect a significant nutrient \times temperature interaction, as we foresee that reduced nutrient supply mitigates the negative effect of warming on coexistence.

2 | MATERIALS AND METHODS

2.1 | Experimental setup

The experiment was conducted in 12 custom-tailored, stainless steel indoor-mesocosms, the so-called Planktotrons located at the Institute for Chemistry and Biology of the Marine Environment (ICBM) in Wilhelmshaven, Germany (Gall et al., 2017). These tanks are 1.2 m high and have an inner diameter of 0.8 m, resulting in a volume of 600 L. Built-in rotors with silicon lips at the side, top, and bottom, gently rotate in the Planktotrons, to prevent wall growth during the experiment. To ensure homogeneous phytoplankton distribution as well as

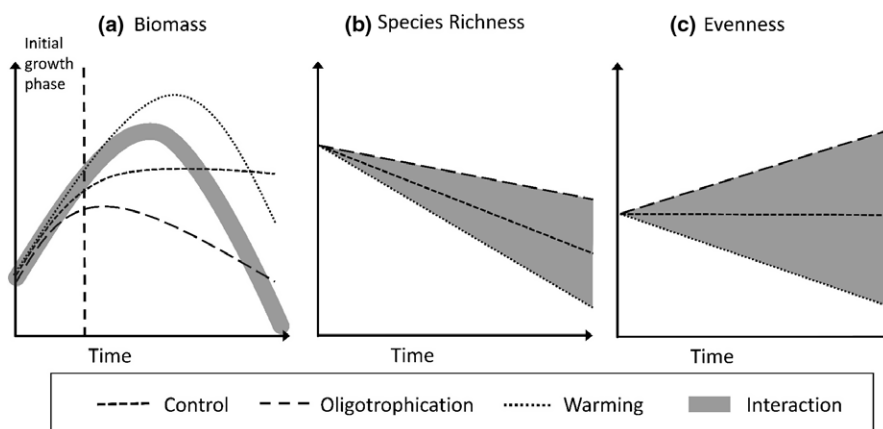


FIGURE 1 Expected dynamics over time for biomass, richness, and evenness of the phytoplankton community for the different treatments, temperature manipulation and changes in nutrient concentrations (full factorial design 2 \times 2 temperature \times nutrients)

equal nutrient conditions throughout the water column, the mesocosms were manually mixed daily using a disk according to Striebel, Kirchmaier, and Hingsamer (2013).

The 2×2 factorial design was run in three replicates each: Two nutrient conditions, “constant nutrient concentrations” and “decreasing nutrient concentrations,” and two temperature treatments, “ambient temperature” and “increasing temperature,” resulted in a total of 12 experimental units. Treatments with “constant nutrient concentrations” were refilled after sampling (20% exchange per week) with a medium including the initial nutrient concentrations. Treatments with “decreasing nutrient concentrations” were refilled with purified water, resulting in stepwise decreasing nutrient conditions. Temperature was kept constant at 20°C for “ambient temperature” treatments during the whole experiment (see Gall et al., 2017 for technical details), while for “increasing temperature” conditions the temperature was raised weekly after each sampling by 2°C (up to a maximum of 32°C during the last week of the experiment). The Planktotrons were filled with an artificial phytoplankton growth medium, according to the WC medium (Anderson, 2005), but with reduced nutrient concentrations resembling the nutrient conditions in the pond, the inoculum originated from (see below and Supporting Information Table S1). The pH was kept constant to avoid confounding effects (mean 7.7 ± 0.37 SD) by adding TES buffer to the medium. Light conditions were kept constant during the experiment using a custom-tailored setup of LED lights. The intensity supplied at the water surface of the Planktotrons was $660 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$ with 16:8 light-dark intervals.

A natural spring phytoplankton community from a eutrophic pond near Leuven in Belgium (Langerode Vijver, $50^{\circ}49'44.1''\text{N } 4^{\circ}38'21.9''\text{E}$), prefiltered using a 20 μm mesh screen to remove zooplankton, was used as inoculum. We focused on phytoplankton as the only trophic level because it is more likely to be affected by interactive effects of temperature and resource supply.

A total of 20 μm mesh size was chosen after pre-experiments showed that larger mesh size did not remove all zooplankton, but 20 μm caused no notable change in phytoplankton community composition based on microscopic determination. The exchanged amount of water was weekly controlled for zooplankton and was free of grazers throughout the entire experiment. All Planktotrons were inoculated with the same initial phytoplankton community and same nutrient conditions and started with equal temperature conditions (20°C). The experiment was conducted for 44 days in total.

2.2 | Sampling and analyses

In vivo chlorophyll *a* concentrations were measured daily after mixing the Planktotrons using a handheld fluorometer (TURNER DESIGNS, AquaFluor™). Water temperature and light intensity were logged continuously in three Planktotrons using data loggers (Onset HOBO Pendant® data logger). All other parameters were measured weekly in association with the 20% medium renewal in each of the Planktotrons. Samples were taken after mixing the Planktotrons (according to Striebel et al., 2013) with disks (one specific disk per

Planktotron to avoid contamination) to ensure a homogeneous contribution within the water column. Water was removed from the top of the water column using beakers and the volume removed was determined and controlled by weighing the water. Samples for nutrient and pigment analyses and phytoplankton determination were bottled and processed immediately. Total phosphorus (TP) concentrations of samples were quantified by persulfate digestion followed by molybdate reaction (Wetzel & Likens, 2000). Samples for particulate organic carbon (POC), nitrogen (PON), and particulate organic phosphorus (POP) were filtered onto precombusted and acid-washed glass-fiber GFC (Whatman) filters. Filters were stored at -80°C until analysis. The CN elemental composition was measured with a CHN analyzer (Thermo, Flash EA 1112) and POP by molybdate reaction after sulfuric acid digestion (Wetzel & Likens, 2000).

Phytoplankton samples for microscopic counts were fixed with 1% Lugol's iodine and counted using an inverted Leica DMIL LED microscope at 200 \times and 400 \times magnification (Utermöhl, 1958). Phytoplankton was counted up to at least 400 cells in total and cell volumes were calculated after approximation to the nearest geometric standard solid (Hillebrand, Dürselen, Kirschtel, Pollinger, & Zohary, 1999). Biovolume proportions of species were used to calculate Pielou's Evenness (Pielou, 1966), additionally, we used richness as diversity estimate.

2.3 | Data analysis

Particulate organic carbon (measured through combustion, see above) and chlorophyll *a* (measured in vivo with a fluorometer) as different proxies for phytoplankton biomass were highly correlated (C~Chl, $r = 0.69$, $p < 0.0001$), so we restricted our analysis to chlorophyll.

We used the nlme package in R to analyze our data. Using the Planktotron identity as a random variable, we performed an analysis separating the fixed effects of temperature and nutrient treatments (and their interaction) from the within-unit analysis of time and time \times treatment effects. We used time as a categorical variable to allow for nonlinear dynamics within the Planktotrons.

Separate Linear Mixed Models (LMMs) were conducted for phytoplankton chlorophyll *a* concentration, total biovolume, evenness, and richness. For phytoplankton, we also measured particulate nutrients (C:N as well as C:P, molar ratios). Correlations between resource use efficiency and diversity (species richness and Pielou's evenness) were assessed through ANOVAs of the final sampling day. In addition, we used resource use efficiency (RUE) (Filstrup et al., 2014; Ptacnik, Solimini, et al., 2008) as a proxy for ecosystem function to track the functional change in relation or reaction to species change. RUE was defined as unit biomass production in chlorophyll *a* ($\mu\text{g/L}$) per unit total phosphorus ($\mu\text{g/L}$).

All data were analyzed with R (R Development Core Team & R Core Team, 2013) using the packages vegan (Oksanen et al., 2013), MASS (Venables & Ripley, 2002), lattice (Sarkar, 2008), ez (Lawrence, 2015), ggplot2 (Wickham, 2009), plyr (Wickham, 2011), reshape (Wickham, 2007), psych (Revelle, 2013), lsr (Navarro, 2015), nlme (Pinheiro, Bates, DebRoy, Sarkar, & R Core Team, 2018), multcomp (Hothorn, Bretz, & Westfall, 2008), and RColorBrewer (Neuwirth, 2014).

3 | RESULTS

3.1 | Treatment effects on algal biomass

Warming and nutrient depletion both had significant effects on algal biomass (significant temperature and nutrient main effects between subjects, LMM, Table 1). Chlorophyll *a* concentrations increased in all treatments within the first half of the experiment (Figure 2a). Especially in combination with warming, Chlorophyll *a* concentrations increased further under constant nutrient concentrations. By contrast, biomass declined after 3 weeks in the nutrient depletion treatments, down to chlorophyll *a* concentrations even lower than the initial concentrations (Figure 2a). Reflected by a significant time \times temperature \times nutrients interaction (LMM, Table 1), the initial positive effect of warming on biomass production turned later into a negative warming effect in the oligotrophication treatment, which resulted in a reduction in biomass below the level reached in the control and a hump-shaped distribution of algae biomass over time.

3.2 | Treatment effects on resource use efficiency (RUE)

RUE changed significantly over time during the experiment (LMM, significant effect of "time," Table 1). Treatment effects were characterized by a significant positive main effect of temperature between subjects and significant within-subject interactions of temperature \times time as well as nutrient \times time (Figure 2b, LMM, Table 1). While the resource use efficiency increased during the whole experimental period under high nutrient conditions, it followed a hump-shaped pattern when decreasing nutrient concentrations and warming interacted (Figure 2b). Initially, RUE for warmed, nutrient-reduced mesocosm was higher than for any other treatment combination (though not significantly so) but decreased below the levels of both the control and the warming treatment with progressing nutrient depletion. At the end of the experiment, maximum RUE was obtained under warmed, constant nutrient conditions.

We did not find any significant effects on C:N ratios throughout the experiment. C:P ratios significantly increased over time in all

treatments (main effect of time, LMM, Table 1, Figure 2c, d), reflecting the incorporation of initially available nutrients in new production of biomass. Significant main effects of nutrients and significant interactions between nutrients \times time and nutrients \times warming reflected that the C:P stoichiometry of phytoplankton was strongly tied to nutrient supply (Table 1). At day 44, C:P was more than twofold higher in the nutrient-depleted treatments compared to the nutrient constant treatments (Figure 2). Temperature effects on C:P dependent on nutrient supply (significant nutrients \times temperature), with warming increasing C:P in nutrient-depleted mesocosms.

3.3 | Treatment effects on phytoplankton biodiversity and composition

In all treatments, we observed a decrease in species richness over time (LMM: significant effect of time, Table 1). This decrease was most pronounced in treatments where temperature was increased (Figure 2e), reflected by a significant temperature main effect and time \times temperature interaction (LMM, Table 1). After an initial increase in the first 2 weeks of the experiment, evenness consistently decreased with increasing temperature, but remained high in the interaction treatment until the end of the experiment (Figure 2f). Consequently, temperature was both a significant main effect and a significant interactive effect with time for evenness in the LMM (Table 1). Nutrient effects on evenness were not significant (Table 1). We additionally tested if treatment-mediated effects on resource use efficiency were related to phytoplankton biodiversity at the end of the experiment, however, this was not the case (ANOVA, RUE vs. richness: $p = 0.595$; RUE vs. Pielou's evenness: $p = 0.129$).

By the end of the experiment, chlorophyte and cyanobacteria species dominated the communities, compared to a more balanced community at the start of the experiment (Figure 3). The species composition at the end of the experiment was highly dominated by a single species (*Scenedesmus ecomis*) in the gradually warmed treatments, while the abundances were more evenly distributed in the constant temperature treatments. This insight into species composition explains the strong decline in evenness in the warmed

TABLE 1 Linear Mixed Models, transformations to ensure homogeneity of variance are given in the table. The table gives *F*-values for each test and denotes the *p*-values in brackets. Effects significant at $p < 0.05$ are highlighted in bold

Factor	dfN	dfD	Chl	RUE	C:P	C:N	Richness	Evenness
Transformation			None	None	Log	None	None	None
Between subjects								
Temp	1	8	15.03 (0.005)	8.64 (0.019)	0.98 (0.35)	0.09 (0.764)	9.60 (0.015)	34.59 (<0.001)
Nut	1	8	23.79 (0.001)	0.95 (0.358)	27.83 (<0.001)	0.31 (0.764)	3.92 (0.083)	3.25 (0.109)
Temp \times nut	1	8	0.02 (0.893)	2.19 (0.177)	9.65 (0.015)	0.35 (0.570)	3.59 (0.095)	0.23 (0.641)
Within subjects								
Time	5	40	28.68 (<0.001)	38.14 (<0.001)	21.01 (<0.001)	1.62 (0.176)	28.92 (<0.001)	8.09 (<0.001)
Time \times temp	5	40	6.67 (<0.001)	3.81 (0.006)	0.39 (0.848)	0.44 (0.819)	10.79 (<0.001)	7.11 (<0.001)
Time \times nut	5	40	41.34 (<0.001)	6.17 (<0.001)	7.45 (<0.001)	1.26 (0.300)	1.25 (0.306)	1.07 (0.391)
Time \times nut \times temp	5	40	4.04 (0.005)	0.92 (0.480)	1.64 (0.17)	0.22 (0.953)	0.05 (0.998)	1.65 (0.170)

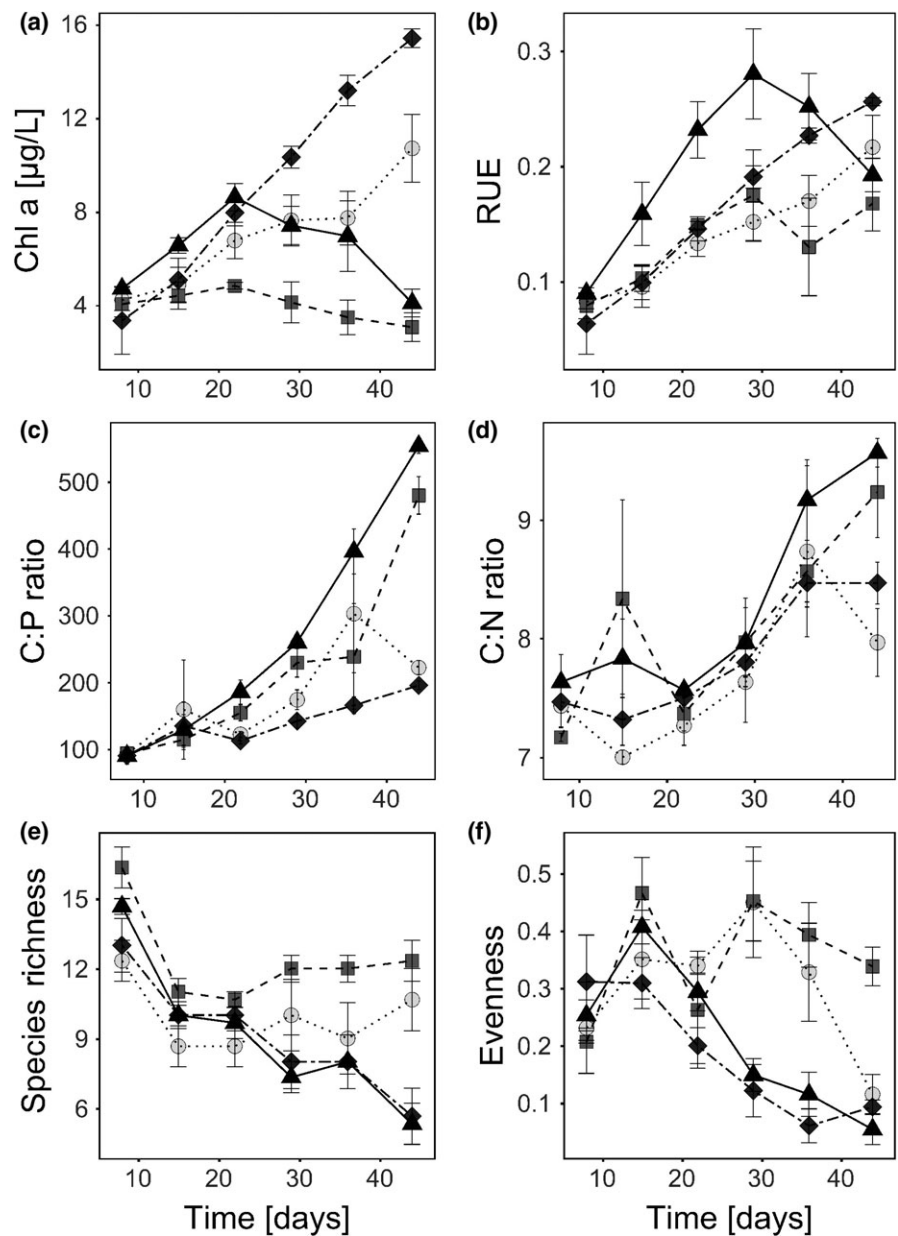


FIGURE 2 Phytoplankton community dynamics during the experiment. Chlorophyll *a* concentration ($\mu\text{g/L}$) (A), resource use efficiency (RUE) (B), molar seston C:P (C) and C:N ratios (D), species richness (E) and Pielou's evenness (F) over the duration of the experiment in days. The different treatments are differentiated through both shape and coloration: ambient temperature and constant nutrients as light gray dots, ambient temperature, and oligotrophication as gray squares, increased temperature and constant nutrients as charcoal diamonds and temperature increase with oligotrophication as black triangles. Data points mark sampling days and are shown as averages of the three replicates including error bars (SE). For C:P ratios one outlier has been excluded and two outliers for C:N ratios

treatments and the 50% reduction in species richness at the end of the experiment, compared to the ambient temperature treatments. At ambient temperature with constant nutrients, *Gonium pectorale* dominated together with *S. eornis*, whereas at ambient temperature and nutrient depletion, *Pteromonas angulosa* and *Achroonema lentum* contributed the largest proportion of biovolume (Figure 3).

4 | DISCUSSION

4.1 | Temperature and nutrient effects on biomass (hypothesis H1)

At the beginning of the experiment, biomass increased in all treatments reflecting the initial availability of light and nutrient. This initial increase was faster at increasing temperatures than at ambient

temperature (Figure 2a), which reflects the positive effects of sub-lethal temperature increases on biochemical reactions and metabolism (Gillooly, Brown, West, Savage, & Charnov, 2001). After 3 weeks, strong nutrient effects and nutrient \times temperature interactions became apparent: in treatments with constant nutrient supply, biomass increased further during the whole experimental period (Figure 2a), whereas biomass decreased in the oligotrophication treatments during the second half of the experiment (Figure 2a). This corresponds to the expected relationship between realized production and resource availability (Gruner et al., 2008; Leibold, 1999). A further reduction in phytoplankton biomass under prolonged phases of oligotrophication can be expected in aquatic ecosystems. The trends in our experiment thus coincide with summer field observations in Belgian ponds over a 10-year sampling period (Verbeek et al., 2018), where nutrient availability in the investigated lakes

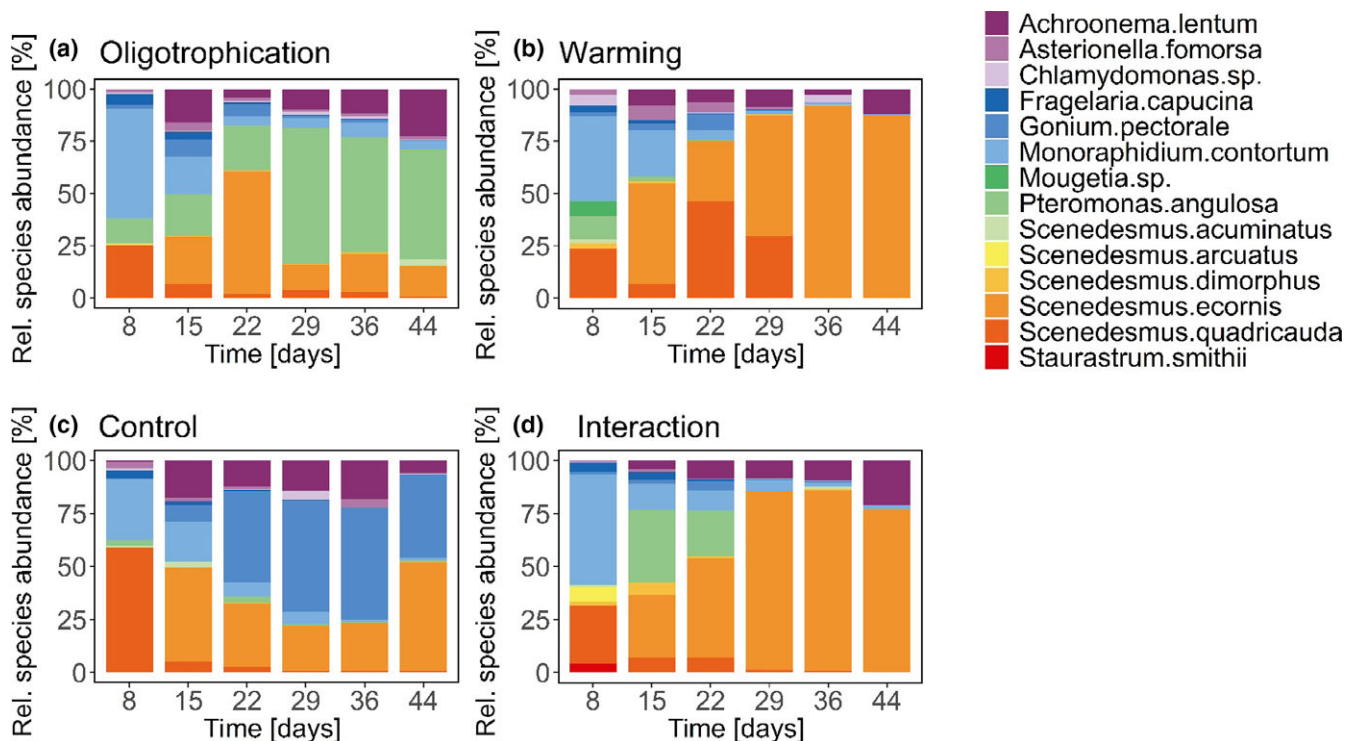


FIGURE 3 Change in relative species abundance in percent in the four treatments over time. Replicates were pooled and only species with at least 1% contribution at any one time are shown. Numbers are based on biovolume [Colour figure can be viewed at wileyonlinelibrary.com]

decreased between 2003 and 2013 (from 1.97 ± 3.29 mg/L TP to 0.49 ± 0.82 mg/L), which was mirrored by a 71.5% decrease in phytoplankton biomass over the same period.

Most importantly, however, we found a clear interaction of warming and oligotrophication effects on algal biomass. At constant nutrient supply, temperature effects mediated between fast (warming) and a slow (ambient temperature) increase in biomass in the second half of the experiment, whereas at reduced nutrient supply, warming led to a substantial decrease in biomass by 50% in the second half of the experiment (Figure 2). Our study shows that warming enhances biomass production if nutrients are abundant, but a lack of nutrient supply might result in detrimental temperature effects. We suppose that warming is connected to enhance nutrient requirements associated with faster growth and if these demands cannot be met, biomass production is impaired.

This interaction between nutrient availability and temperature regime strongly points to an interdependency of energy and matter metabolism in the phytoplankton, as enhanced energy supply can only be converted into higher biomass production if essential resources are available. Namely through an increase in temperature, physiological processes such as photosynthesis, respiration, and protein synthesis are sped up as the rate of biochemical reactions increases. Without a sufficient supply of nutrients, the resulting increased demand for resources to maintain these processes and build necessary molecules and structures cannot be met. This points toward the fundamental links between fluxes of energy and materials in organisms based on the kinetics and elemental compositions of

subcellular structures and processes (Allen & Gillooly, 2009). These energy and matter links are well investigated for light versus nutrient effects (Sterner, Elser, Fee, Guildford, & Chrzanowski, 1997; Urabe & Sterner, 1996), but less so for temperature and nutrients. On a larger spatial and temporal scale, our results are reflected by a time series of Lake Biwa (Hsieh et al., 2010; Tsai et al., 2014). While our experiment can help to reveal the mechanistic background of changes, natural systems are usually subjected to more than two pressures. Ideally, experiments such as this should include data on the community change within the natural system the inoculum was taken from, so that it might be possible to distinguish to which extent the community was shaped by other environmental factors. Another aspect of natural systems not reflected in this experiment is the possibility for new species to immigrate and the subsequent turnover in species composition.

4.2 | Treatment effects on ecosystem function, RUE, and stoichiometry (H2)

We found greater resource use efficiency (RUE) with higher nutrient availability or temperature increase but no significant interactive effect, which is mostly in agreement with findings by De Senerpont Domis et al. (2014). In opposition to our results, some other studies have found that resource use efficiency would increase with lower nutrient availability (Bridgham, Pastor, McLaugherty, & Richardson, 1995; Vitousek, 1982), but this might be due to different community composition and species-specific RUE.

The opposing direction of the two pressures also explains the hump-shaped temporal dynamics of biomass production and RUE. It is possible that biomass and RUE would have declined even further, below the levels of the oligotrophic control over the next weeks, because the temperature stress had also diminished species richness. This increases the probability that the community had lost productive and/or efficient species as well as any buffering capacities of rare species (Corcoran & Boeing, 2012). Without the chance to regain species through regional diversity by immigration (Hillebrand, Soininen, & Snoeijs, 2010), an isolated ecosystem is severely changed and destabilized through the two pressures (Hillebrand et al., 2012). Our results of the interaction treatment could be an indication that ecosystem functions are destabilized in a major way through the interaction of both stressors.

We found a very strong increase in C:P ratios as well as a slight increase in C:N ratios in all treatments, with a significantly higher increase in C:nutrient ratios in the oligotrophication treatments. C:nutrient ratios, in general, have been shown to increase with decreasing nutrient supply (Bridgman et al., 1995; Moreno & Martiny, 2018; Vitousek, 1982) leading to lower food quality of primary producers, the basis of a food web. This can for example be caused by a decrease in nutrient levels in relation to biomass produced at higher metabolic rates at higher temperatures, or by a limitation of nutrient supply, such as phosphorus (Cross, Hood, Benstead, Huryn, & Nelson, 2015). The effects of oligotrophication will therefore become more severe with increasing temperature. Given that oligotrophication is increasingly a phenomenon freshwater systems worldwide (Hsieh et al., 2010; Pomati et al., 2012), this has strong implications for higher trophic levels. Most herbivores are more constrained in their nutrient demands than phytoplankton and higher C:nutrient ratios mean lower food quality for the grazers. With lower food quality, the gross growth efficiency of the grazers decreases, leading to less biomass at higher trophic levels. This pattern is relevant in both aquatic and terrestrial systems (Elser et al., 2000). For the C:N ratio specifically, changes can mostly be explained by temperature effects: an increase in temperature increases the nitrogen-demand in the cells through, for example, higher protein assembly, but because carbon content increases simultaneously through higher biomass production, the C:N ratio remains comparably constant (Cotner et al., 2006). The interaction between lower N-supply and higher productivity then leads to the highest C:N ratios being found in the interaction treatment where both mechanisms occur simultaneously. The effects were, however, not strong enough to be significant.

For the P-content of the cells, however, oligotrophication had stronger effects than temperature changes in our experiment. As phosphorous is considered to be the main limiting nutrient in many freshwater systems (but see Elser et al., 2007), the limitation becomes more severe with ongoing oligotrophication. Other studies, such as Van Donk et al. (2008) have found similar patterns of increasing C:P ratios with decreasing resource availability. This trend continues upward into higher trophic levels, as higher organisms often have higher P-demand (e.g. for skeletal structures) and less possibility of stoichiometric flexibility (Elser et al., 2000).

4.3 | Temperature and nutrient effects on species richness and evenness (H3)

Being disconnected from the regional species pool, initial species richness could only decline over time. While we see some loss of species in all treatments, the treatments with ambient temperature had continuously higher species richness than those with increased temperature (Figure 2e), and species loss was much stronger in the treatments with increased temperature. One explanation would be stronger interspecies competition for the limiting resources. Following this, we would expect faster competitive exclusion (Hillebrand, 2011; Hillebrand et al., 2012). Unless there is a possibility of new species immigrating into an ecosystem from a larger, regional meta-community system, local extirpation means that many rare species are permanently lost, which endangers ecosystem function, goods, and services (Jain et al., 2014; Lyons, Brigham, Traut, & Schwartz, 2005; Mouillot et al., 2013). If the system were part of a metacommunity, this would lead to higher turnover, but as we worked in closed systems the effect was merely species loss. It has been shown that low evenness and a strong dominance of single species and general loss of more rare species is correlated (Hillebrand, 2011). With continuing oligotrophication, there was less possibility of any one species out-competing all other species to the point of extinction, as it was unlikely that any one species could realize its full growth potential. Instead, there was strong competition for the limited available nutrients in a more balanced community in terms of abundance. Our results show the inverse effect of fertilization studies such as Ptacnik, Solimini, et al. (2008) or Gamfeldt and Hillebrand (2008)—instead of increasing nutrients resulting in decreasing evenness, we see an increase in evenness with decreasing nutrient loadings (Figure 2e).

In the treatments with increasing temperature, the decrease in species richness could be caused through only a few species being able to cope with the high temperature, as generally, the optimal temperature for phytoplankton is between 10–20°C (Butterwick et al., 2005; Jöhnk et al., 2008). However, even the highest temperatures reached in the experiment are unlikely to have been lethal for entire populations. Instead, species that were too far out of their optimal range to compete and went locally extinct. Hence, the best-adapted species could come to dominate the community (Jensen, Jeppesen, Olrik, & Kristensen, 1994). This is supported by the community composition at the end of the experiment, as we found especially green algae dominating the warmed treatment which are considered to be better adapted to higher temperatures than for example diatoms (which are more abundant in the control). Following Litchman et al. (2015), this can be considered as a result of shifts in trait distribution. It may even become a global pattern with climate change, which favors species with wider temperature ranges or higher temperature optima, such as green algae and cyanobacteria species.

Although there was a trend toward higher RUE with lower diversity (data not shown) which is opposed to trends found in other studies, for example (Ptacnik, Lepistö, et al., 2008; Striebel et al., 2009), we did not see a significant change in resource use efficiency. Instead, we

found high levels of biomass in the treatments with finally very low species numbers. This is in agreement with results from a study of 131 lakes (Filstrup et al., 2014), which shows a strongly negative relationship between community evenness and resource use efficiency. The reason might either be a sampling effect: fast-growing species adapted to high temperature were present and dominated the system, hence also the low evenness (Hillebrand et al., 2007, 2012), or an effect of selection: the species with the greatest competitive strength under the increasing stress conditions is also the one with highest resource use efficiency. This would result in both low evenness and high biomass production based on very little resources. More often, a decrease in diversity has been associated with a general decrease in ecosystem functionality as soon as more than a single function is considered (Gamfeldt, Hillebrand, & Jonsson, 2008; Gamfeldt et al., 2013; Mouillot et al., 2013). To maintain a number of ecosystem functions multiple species with different traits are necessary (Hector & Bagchi, 2007; Mouillot et al., 2013). However, to achieve high levels of a single function it is not necessary to have a large number of species (Moss, 2000). Regarding management practices, our results raise the question whether a decrease in nutrient input will be beneficial to desired ecosystem states such as high diversity. If a pond has been in a eutrophic state for long periods of time, the community is adapted to the high nutrient levels and (re) oligotrophication would act as a stressor, changing community composition and ecosystem functions. If dispersal from other diverse communities is limited, the change in abiotic environment is likely to decrease diversity and productivity of the original community, even more so with globally increasing temperatures.

Our experiment revealed an interdependency of the effects of warming and nutrient availability on phytoplankton biomass. The initial positive effects of temperature increase on biomass production remained positive if enough resources were available, but turned negative under decreasing nutrient supply through oligotrophication. This led to hump-shaped temporal patterns in biomass production, resource use efficiency and strong decreases in diversity measures. Oligotrophication allowed for a more even, species-rich community while increasing temperature stress resulted in a significant reduction in species richness and evenness. Particularly with ongoing problems like high global extinction rates, eutrophication, and climate change, it is necessary to understand what stabilizes an ecosystem and to find out which factors are the most important ones to assure the desired functions (e.g. conservation vs. high productivity, Moss, 2000).

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CONFLICTS OF INTEREST

The authors have no conflict of interest to declare.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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