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Primary producers or consumers? Increasing phytoplankton bacterivory along a gradient of lake warming and browning

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Abstract

Eukaryotic phytoplankton form the basis of aquatic food webs and play a key role in the global carbon cycle. Many of these evolutionarily diverse microalgae are also capable of feeding on other microbes, and hence simultaneously act both as primary producers and consumers. The net ecosystem impact of such mixotrophs depends on their nutritional strategy which is likely to alter with environmental change. Many temperate lakes are currently warming at unprecedented rates and are simultaneously increasing in water color (browning) due to increased run-off of humic substances. We hypothesized that the resulting reduction in light intensity and increased bacterial abundances would favor mixotrophic phytoplankton over obligate autotrophs, while higher temperatures might boost their rates of bacterivory. We tested these hypotheses in a mesocosm experiment simulating a gradient of increasing temperature and water color in temperate shallow lakes as expected to occur over the coming century. Mixotrophs showed a faster increase in abundance under the climate change scenario during spring, when they dominated the phytoplankton community. Furthermore, both bacterial abundances and rates of phytoplankton bacterivory increased under future climate conditions. Bacterivory contributed significantly to phytoplankton resource acquisition under future climate conditions, while remaining negligible throughout most of the season in treatments resembling today's conditions. Hence, to our knowledge, we here provide the first evidence for an increasing importance of bacterivory by phytoplankton in future temperate shallow lakes. Such a change in phytoplankton nutritional strategies will likely impact biogeochemical cycles and highlights the need to conceptually integrate mixotrophy into current ecosystem models.

The photosynthetic microbes, collectively called phytoplankton, form the basis of aquatic food webs and are responsible for about 50% of global primary production (Field et al. 1998). A less well recognized role of eukaryotic

phytoplankton is their major contribution to bacterivory in a wide range of freshwater and marine habitats including oligotrophic lakes and oceans, under ice environments, as well as humic lakes (Berninger et al. 1992; Isaksson et al. 1999; Zubkov and Tarran 2008). Many photosynthetic eukaryotes thus serve as primary producers and consumers at the same time and the net ecosystem function of these mixotrophs will depend on their balance between autotrophic and heterotrophic nutrition, which in turn is sensitive to environmental conditions (Bird and Kalff 1989; Weithoff and Wacker 2007). The diverse anthropogenic impacts that currently alter aquatic ecosystems might therefore also affect the nutritional balance of mixotrophs. This would not only impact rates of carbon fixation directly, but might also alter

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Additional Supporting Information may be found in the online version of this article.

microbial food web dynamics (Wilken et al. 2014b), rates of nutrient remineralization (Rothhaupt 1996), and the nutritional quality of mixotrophs for higher trophic levels (Weithoff and Wacker 2007).

Current changes in aquatic ecosystems include warming of surface waters, shortened periods of ice cover and hence longer growing seasons, as well as altered light and nutrient availability through increased strength of stratification (Adrian et al. 2009; Doney et al. 2012). At the same time, many temperate lakes experience an increase in water color that is predicted to continue over the next century (Hansson et al. 2013; de Wit et al. 2016; Weyhenmeyer et al. 2016). Causes are still debated, but include increasing input of dissolved organic carbon (DOC) in the form of humic and fulvic acids due to altered land-use patterns, changed precipitation frequencies, and reversal from acidification (Hongve et al. 2004; Evans et al. 2005; Kritzberg and Ekström 2012; de Wit et al. 2016). Both warming and browning can have pronounced impacts on aquatic organisms, their metabolism and ecological interactions. Increasing temperatures often lead to changing plankton phenology with earlier development of peak abundances of phytoplankton and zooplankton (Winder and Schindler 2004; Nicolle et al. 2012). The generally stronger response in rates of heterotrophic processes to temperature compared with primary production can strengthen top-down control of phytoplankton biomass, and cause a shift in ecosystem metabolism toward heterotrophy (Yvon-Durocher et al. 2010; Hansson et al. 2013). The direct effects of temperature are modulated by light availability with the onset and magnitude of the phytoplankton spring bloom being enhanced by high light availability due to earlier thermal stratification (Berger et al. 2010; Winder et al. 2012). On the other hand, light limitation of the photoautotrophic community due to browning can cause a decline in benthic primary producers (Vasconcelos et al. 2016), or result in lower peak phytoplankton biomass (Ask et al. 2009; Karlsson et al. 2009; Kritzberg et al. 2014). Higher input of allochthonous DOC underlying the process of browning furthermore supports the heterotrophic metabolism of the bacterial community (Ask et al. 2009; Sanders et al. 2015; Zwart et al. 2016). Increasing water color can thus intensify the shift in ecosystem metabolism toward heterotrophy observed with warming.

The high percentage of mixotrophic phytoplankton often found in humic lakes (Isaksson et al. 1999; Bergström et al. 2003) suggests that the abundance of mixotrophic taxa could increase with lake browning. High abundances of bacterial prey in these lakes provide a substitutable resource for low dissolved nutrient concentrations or low light availability and hence give mixotrophs a competitive advantage over specialist autotrophs (Jansson et al. 1996; Bergström et al. 2003; Pålsson and Granéli 2004). In a subarctic lake, the increased input of allochthonous dissolved organic matter resulted in increasing ratios of mixotrophs to autotrophs, along with a

shift from a food web based on primary production to one based on bacterial production (Forsström et al. 2013). Controlled laboratory experiments suggest that mixotrophic phytoplankton would respond to several expected environmental changes by increasing the heterotrophic component of their metabolism. For instance, low light intensities might favor heterotrophic nutrition to substitute photosynthesis as a source of carbon and energy (Holen 1999). Furthermore, ingestion rates directly depend on prey abundance (Rothhaupt 1996; Wilken et al. 2010) and hence, higher bacterial abundances typically found at elevated DOC concentrations would permit higher ingestion rates. In addition, warming has been shown to shift the nutrition of a mixotrophic chrysophyte toward heterotrophy under saturating prey availability (Wilken et al. 2013). Collectively, these changes suggest a more heterotrophic nutrition of mixotrophs under future climate conditions and an increasing importance of phagotrophy for phytoplankton carbon acquisition.

We hypothesized that under future climate conditions expected for northern temperate lakes (1) the combination of increasing temperatures together with decreasing light intensities and higher bacterial abundances coming along with lake browning will cause ingestion rates of bacteria by mixotrophic phytoplankton to increase (2) higher ingestion rates will compensate for lower light intensities, help to cover higher metabolic costs at higher temperatures, and provide a nutrient source, which together will provide mixotrophs with a competitive advantage over specialist autotrophs and therefore cause increased abundances of mixotrophs, and (3) the combination of increasing mixotroph abundances with higher ingestion rates will cause a shift in the ecosystem function of the entire phytoplankton community toward increased bacterivory. To test these hypotheses, we exposed a natural plankton community, originating from the mesotrophic temperate shallow Lake Krankesjön (Southern Sweden), to a gradient of climate change scenarios including both browning and warming in a mesocosm experiment. While most global change studies focus on each environmental forcing separately, we here used a novel approach by combining warming and browning into climate change scenarios based on model predictions of both climate and lake browning. This approach is motivated by the fact, that our future will deliver several environmental threats simultaneously, which are likely to interact. This approach will not separate the mechanisms behind each threat, but will instead provide information on the strength and shape of ecological responses along a climate change gradient. Our approach thereby also provides a predictive tool, which is urgently needed for the understanding of future ecosystem trajectories and potential implications for management and decision making.

Methods

An outdoor mesocosm experiment was performed in Lund, Sweden, from April 2013 to October 2013 with water

from the shallow (mean depth 1.5 m) Lake Krankesjön (for more information about lake properties, see Hansson et al. 2007). This lake can be characterized as meso- to eutrophic based on an average total phosphorus concentration of $42 \mu\text{g L}^{-1}$ and is rich in calcium leading to relatively high pH values (pH 8.3 at start of experiment). The experiment was designed to simulate a temporal gradient of climate change scenarios for temperate shallow lakes expected to occur over the course of the next century. The scenarios consisted of a combination of warming according to temperature projections by the Intergovernmental Panel on Climate Change-IPCC report (Christensen et al. 2007) and increasing water color based on extrapolation of historical absorbance data from several lakes in southern Sweden (Hansson et al. 2013). Since these two factors change in concert throughout the northern hemisphere (Monteith et al. 2007; O'Reilly et al. 2015; Weyhenmeyer et al. 2016), we here combined them in a replicated regression design with correlated increases in both temperature and humic content to allow fine resolution over a gradient of future scenarios. The six experimental treatments were performed in quadruplicates and included a control representing present day conditions and five treatments of gradually increasing water temperature (1°C steps) and water color (50% of control absorbance at 420 nm [$\text{Abs}_{420\text{nm}}$] per step). The five climate change treatments therefore ranged from a modest scenario with 1°C warming and 50% increase in absorbance to the most extreme scenario of 5°C warming and 250% increase in absorbance relative to the control. This latter scenario represents the upper range of warming predictions made by the IPCC-reports, but implies rates of change over the coming century that are well below the fastest rates currently observed for both lake temperature (O'Reilly et al. 2015) and browning (Weyhenmeyer et al. 2016).

Experimental set-up

Twenty-four insulated polyethylene cylinders (diameter 0.7 m, height 1 m) received sediment from Lake Krankesjön and were then filled with 400 L of lake water. A computerized system continually monitored and adjusted the temperature of each mesocosm relative to the average in the control treatment (Nicolle et al. 2012). In order to achieve the desired water color in each treatment a solution of commercially available humic and fulvic substances (HuminFeed[®], Humin-tech GmbH, Düsseldorf, Germany; Rasconi et al. 2015; Urrutia-Cordero et al. 2016) was added, corresponding to concentrations from 0.96 mg L^{-1} (0.42 mgC L^{-1}) in the lowest to 4.68 mg L^{-1} (2.03 mgC L^{-1}) in the treatment with highest humic content. Water color was monitored by weekly measurements of $\text{Abs}_{420\text{nm}}$ and adjusted by addition of humic substances if needed. To maintain phytoplankton productivity, 1 mL of commercially available plant nutrients (Blomstra växtnäring Cederroth, Upplands Väsby, Sweden: 50.1 g L^{-1} nitrogen and 10.0 g L^{-1} phosphorous) was added every

second week starting from 29th May. Evaporation losses were compensated by weekly additions of distilled water. Enclosure walls were scrubbed weekly to prevent periphyton growth. To introduce top-down regulation of the zooplankton community representative for eutrophic lakes (Nürnberg 1996), two juvenile crucian carps (*Carassius carassius*; 1+ yr, mean \pm SD, length: $6.7 \pm 0.3 \text{ cm}$, weight: $3.9 \pm 0.7 \text{ g}$) contained within a net cage were added to each enclosure.

Sampling and analysis

Three depth-integrated samples were taken across the diameter of the enclosures using a 1 m plexiglass tube. These were pooled to get a representative sample for each mesocosm and subsampled for all further analyses. Weekly absorbance measurements at 420 nm were performed on a Shimadzu UV-2600 spectrophotometer after filtration through GF/C glassfiber filters. Chlorophyll *a* (Chl *a*) concentrations were determined using an Algae Lab Analyser (bbe moldaenke, Schwentinental, Germany). This fluorescent based technique was calibrated against ethanol extracted samples analyzed by spectrophotometric measurements according to Jespersen and Christoffersen (1987). Total phosphorus concentrations were measured using an auto analyzer (Skalar, Breda, The Netherlands) after persulfate digestion in an accredited laboratory (ALcontrol Laboratories, Malmö, Sweden). Phytoplankton samples were fixed with Lugol's acid solution and stored at 4°C . For further analysis, samples were settled overnight in sedimentation chambers, and counted on an Olympus CK40 inverted microscope. Phytoplankton were identified to genus level and counted until enumeration of a total of 200 individuals within a random systematic selection of fields of view. For retrieving zooplankton, 10 L of the pooled mesocosm sample were filtered through a $55 \mu\text{m}$ net and zooplankton was preserved in acid Lugol's solution. The filtered water was returned to the mesocosm; that is, a very small volume of water was removed from enclosures during sampling.

For primary production measurements, two 100 mL water samples from each mesocosm received $1 \mu\text{Ci NaH}^{14}\text{CO}_3$ each and were incubated in situ at 0.5 m depth for 4 h. One bottle received natural light, while the second one served as a dark control. After incubation, samples were filtered onto $0.45 \mu\text{m}$ cellulose nitrate membrane filters, which were then placed in scintillation vials and incubated in $500 \mu\text{L}$ 0.1M HCl overnight. Samples received 10 mL of scintillation cocktail and were measured on a Beckmann LS 6500 scintillation counter (Beckman Coulter, Fullerton, California, U.S.A.). pH and alkalinity measurements were performed using a Titration Excellence system (Mettler Toledo, Greifensee, Switzerland). Dissolved inorganic carbon concentrations and primary production were calculated according to Wetzel and Likens (1991) and converted to daily values according to Vollenweider (1965). Primary production measurements from 4 h incubations as used here are typically closer to net than gross rates of primary production, but tend to give an

overestimate of net primary production (Wetzel and Likens 1991).

Rates of bacterivory

On six occasions between 1st May and 14th October grazing incubations were performed with the addition of fluorescently labeled bacteria (FLB) to quantify bacterivory by the phytoplankton community. To obtain FLB, water from the mesocosms was filtered through 1 μm filters, enriched with yeast extract and incubated overnight. The bacterial community was heat-killed and stained with 5-(4,6-dichlorotriazin-2-yl) aminofluorescein (Sherr and Sherr 1993). Grazing incubations were performed in 100 mL glass bottles filled with 150 μm pre-filtered mesocosm water and an addition of FLB below 30% of the natural bacterial abundance. Bottles were incubated in situ for 20 min and samples were taken at the start and end of the incubation. All samples were fixed with a final concentration of 0.17% Lugol's solution, 1.7% glutaraldehyde, and 0.1% thiosulfate. For counts of bacteria and bacterivorous protists, samples were filtered onto 0.2 μm and 1.0 μm nucleopore membranes, respectively, and stained with 4',6'-diamidino-2-phenylindole at a concentration of 100 $\mu\text{g mL}^{-1}$. Cells were counted under an epifluorescence microscope (Nikon[®] Labophot-2) equipped with an UV filter (355/25 nm) and a blue filter (443/3 nm). Flagellated cells were regarded as potential bacterivores and distinction between mixotrophic and heterotrophic protists was based on the presence or absence of pigments. Ingestion rates were calculated from counts of FLB inside bacterivorous protists at the start and end of the incubation period according to Sherr and Sherr (1993). Clearance rates of mixotrophs (Cl_M) were calculated from their ingestion rates (I_M) as: $Cl = I_M/N_B$, where N_B is the bacterial abundance. Total rates of ingestion by the entire mixotrophic population were compared to the standing bacterial population to calculate the fraction of the bacterial population lost through grazing by mixotrophs per day. To express rates of ingestion in units of carbon the sizes of 50 bacterial cells from each mesocosm of both the control and the warmest and brownest treatment were measured and converted to cellular carbon content using an allometric conversion (Loferer-Krößbacher et al. 1998). Quantification of phagotrophy by short term incubations with fluorescently labeled prey are based on assumptions of negligible prey selectivity and constant rates of grazing over the diel cycle, both of which might not always be met (Urabe et al. 2000; Pfandl et al. 2004; Anderson et al. 2017). For this reason, absolute values for phagotrophic carbon acquisition need to be interpreted with care. Comparisons across treatments on the other hand are robust, since there was little variation in bacterial carbon content and dominant mixotrophic taxa across treatments within individual time points (Supporting Information Figs. S1, S2).

Net rates of heterotrophic phytoplankton production (HP_{net}) can be expressed as $HP_{\text{net}} = I_M \times GE$, where GE is the

growth efficiency with which ingested carbon is converted into mixotroph biomass. GE thus summarizes digestion and assimilation efficiencies as well as respirational losses. We used a conservative estimate for a GE of 50% based on values of 40–79% in natural *Dinobryon* populations (Bird and Kalff 1989). The relative contribution of heterotrophic production (HP_{net}) based on bacterivory by the mixotrophic community can finally be compared to total phytoplankton production ($TP = HP_{\text{net}} + PP_{\text{net}}$). Due to the tendency of ¹⁴C-incubations to overestimate net primary production and our conservative estimate of heterotrophic GE, the contribution of the heterotrophic component likely represents a lower boundary.

Data analyses

All data were tested for effects of seasonality and treatment by a repeated-measures general linear model with time as within subject factor and treatment as continuous covariate. In order to improve homoscedasticity and normality of error terms data for Chl *a*, total phosphorus and primary productivity were square- or 4th-root transformed and split into spring (01 April–05 June), summer (06 June–11 August), and fall (12 August–16 October) periods. While this procedure does not permit detection of interactions between treatments and longer term seasonal effects, it prevents bias in the detection of treatment effects from non-normality or heteroscedasticity. Data for the contribution of mixotrophs to total phytoplankton abundance were also split into three periods and square root transformed either directly or after reflection in the case of a left-skewed distribution. Bacterial abundances, bacterial loss rates, ingestion rates by mixotrophs, and the relative contribution of phagotrophy to phytoplankton carbon acquisition were square root transformed prior to analysis.

Results

As intended the six treatments showed the same pattern in water temperature over the season, differing by 1°C increments (Fig. 1a). The differences in water color were slightly more variable with the treatments 1–5 showing on average 135%, 179%, 202%, 236%, and 276% of $Abs_{420\text{nm}}$ in the control (Fig. 1b). Total phosphorus concentrations were characteristic for mesotrophic to eutrophic conditions. The regular nutrient additions lead to an increase of total phosphorus concentrations over the growing season as commonly observed in shallow lakes (Søndergaard et al. 2002; Kolzau et al. 2014) ranging from 15 $\mu\text{g P L}^{-1}$ to 25 $\mu\text{g P L}^{-1}$ during spring up to 92 $\mu\text{g P L}^{-1}$ in October (Fig. 1c). The molar ratio of total N : P started off around 130 at the beginning of the experiments and dropped toward 70 in late June (Fig. 1d). Chl *a* concentrations showed a similar pattern over time as total phosphorus with a small peak in April and early May (Fig. 2a) followed by a clear-water phase and the development of a bloom in late summer (dominated by cyanobacteria; Supporting Information Fig. S3) with Chl *a* concentrations up to

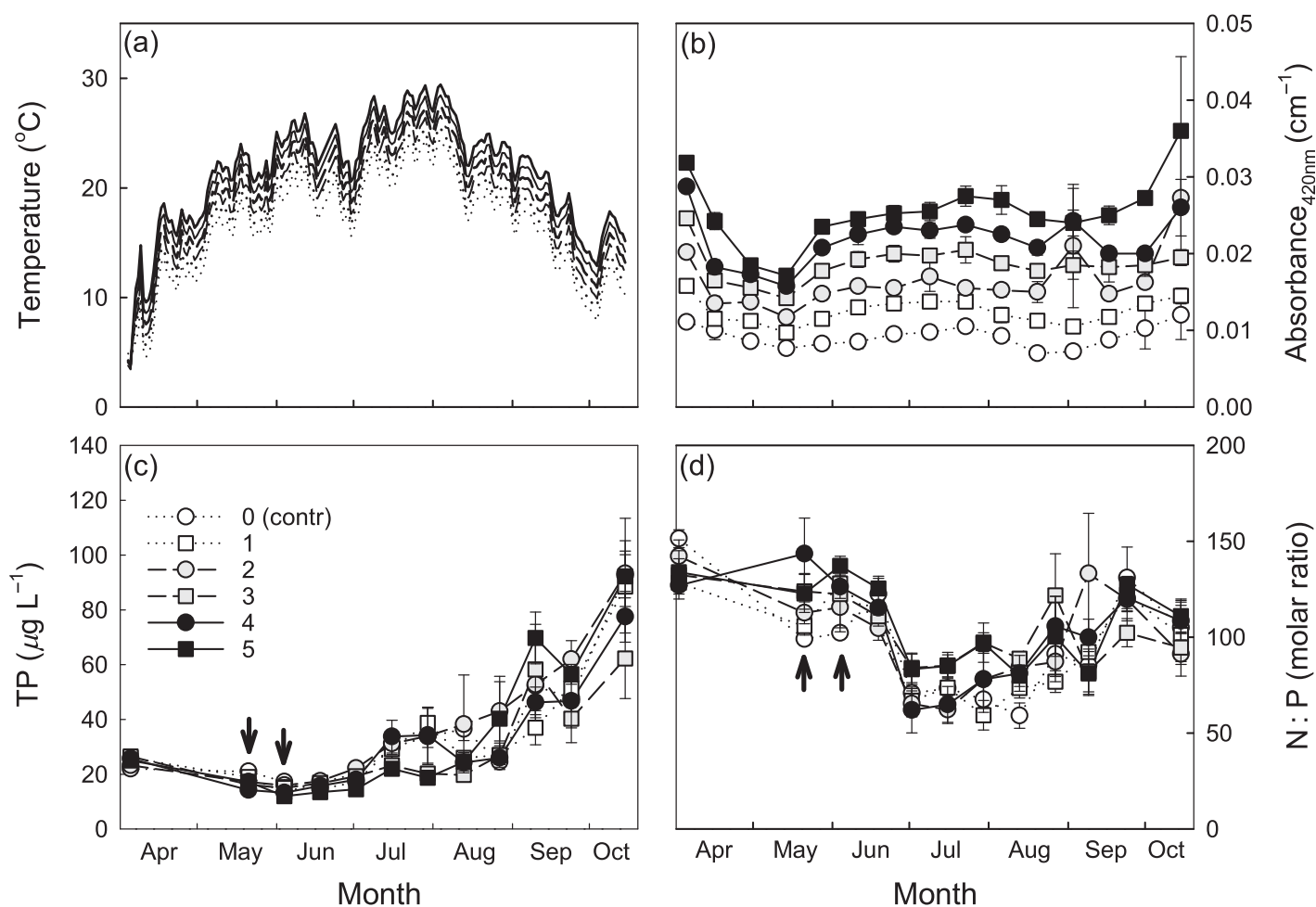


Fig. 1. Time course of temperature (a), absorbance at 420 nm (b), total phosphorus concentration (c), and molar ratio of total nitrogen to total phosphorus (d). Treatments represent the control and the five climate change scenarios (1–5). Error bars give standard errors of treatment means. Arrows indicate direction of a significant treatment effect by the linear model for each time-point with increasing (upward pointing) or decreasing (downward pointing) trend toward future climate conditions.

230 $\mu\text{g L}^{-1}$. The onset of the cyanobacterial bloom happened earliest in the strongest climate change treatment. Chl *a*-specific primary productivity peaked during development of the cyanobacterial bloom in August and early September (Fig. 2b) and also total primary production exhibited a similar pattern, albeit declining less rapidly after the peak of the bloom (Supporting Information Fig. S4). While chlorophyll concentrations remained high throughout October, Chl *a*-specific productivity dropped to low levels again during the late stage of the bloom. Primary productivity was slightly lower under the climate change scenarios from May through July (Fig. 2b).

The total phytoplankton abundance was dominated by mixotrophs during spring and early summer with cellular abundances of up to 2×10^7 cells L^{-1} in early May (Fig. 2c) and up to 90% of the community being mixotrophic during June (Fig. 2d). During this period, the dominance by mixotrophs started earlier and was significantly stronger under the future climate scenarios (Fig. 2d; Supporting Information

Table S1). The relative abundance of mixotrophs decreased in August and despite increasing absolute abundances later in the season (Fig. 2c) remained low during the cyanobacterial bloom for the last 2 months of the experiment. During this time, there was no significant climate change effect on mixotroph abundance. Early in the season, the mixotrophic community was dominated by the chrysophyte *Dinobryon* spp. which contributed to the spring bloom. The cryptophyte *Cryptomonas* spp. became dominant during May (Supporting Information Fig. S1), remained present at lower relative abundances during summer, and reached highest absolute abundances during more eutrophic conditions later in the season (Supporting Information Fig. S1).

Bacterial abundances increased over the season. Especially in late summer, bacteria became threefold more abundant in the warm and brown treatments reaching up to 1.5×10^7 cells mL^{-1} (Fig. 3a; Table 1). Grazing resulted in a significant bacterial mortality during spring when the flagellates grazed

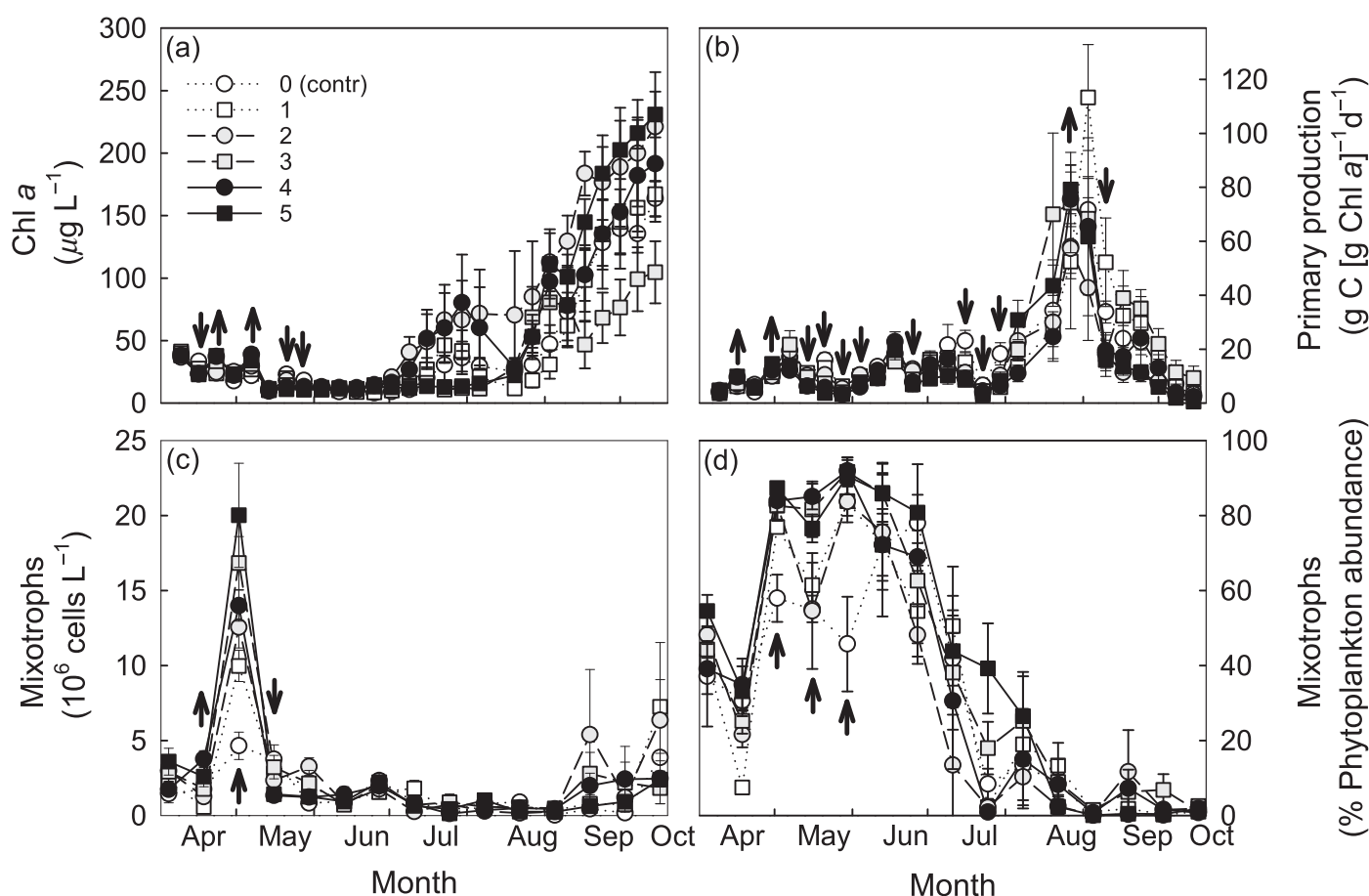


Fig. 2. Time course of Chl *a* concentrations (a), chlorophyll-specific primary production (b), cellular abundances of mixotrophs (c), and the contribution of mixotrophs to total phytoplankton cellular abundance (d) in the mesocosms. Treatments represent the control and the five climate change scenarios (1–5). Error bars give standard errors of treatment means. Arrows indicate direction of a significant treatment effect by the linear model for each time-point with increasing (upward pointing) or decreasing (downward pointing) trend toward future climate conditions.

up to 70% of the standing bacterial population per day (Fig. 3b). Later in the season, when bacterial abundances were high, only a minor fraction of the bacterial population was removed through grazing by mixotrophs. Ingestion and clearance rates by mixotrophic phytoplankton were significantly affected by the climate change treatments ($p < 0.001$; Table 1). The warmest and brownest treatment showed two-fold to threefold higher ingestion rates compared to the control from end of May through August and an up to fivefold increase in September (Fig. 4; Table 1). The higher bacterial abundances together with the higher ingestion rates resulted in a similar percentage of daily bacterial population loss among treatments ($p = 0.255$; Table 1).

The estimated contribution of carbon acquired from bacterivory to total phytoplankton production was low in the control treatment throughout most of the growing season, and reached 10% only in the *Dinobryon* dominated community during early May (Fig. 5a). However, in the climate change treatments increasing ingestion rates led to a significantly higher importance of phytoplankton bacterivory as a

source of carbon acquisition throughout the growing season. Here, bacterivory contributed at least 5–10% to total phytoplankton production both during early summer, when the community was dominated by mixotrophic taxa, but also during autumn when mixotrophs constituted only a minor fraction of the total phytoplankton community (Fig. 5a–f).

Discussion

Predicted climate change is likely to impose considerable impact on aquatic ecosystems, including altered rates in important ecosystem processes, such as primary production and the importance of different carbon acquisition modes in microbial eukaryotes. In our study, the observed climate change responses of the phytoplankton community were largely in line with our hypotheses. Mixotrophs were favored by future conditions during spring, when they showed increased absolute and relative abundances in line with an enhanced competitive advantage (hypothesis 1). Later in the season, relative abundances of mixotrophs decreased

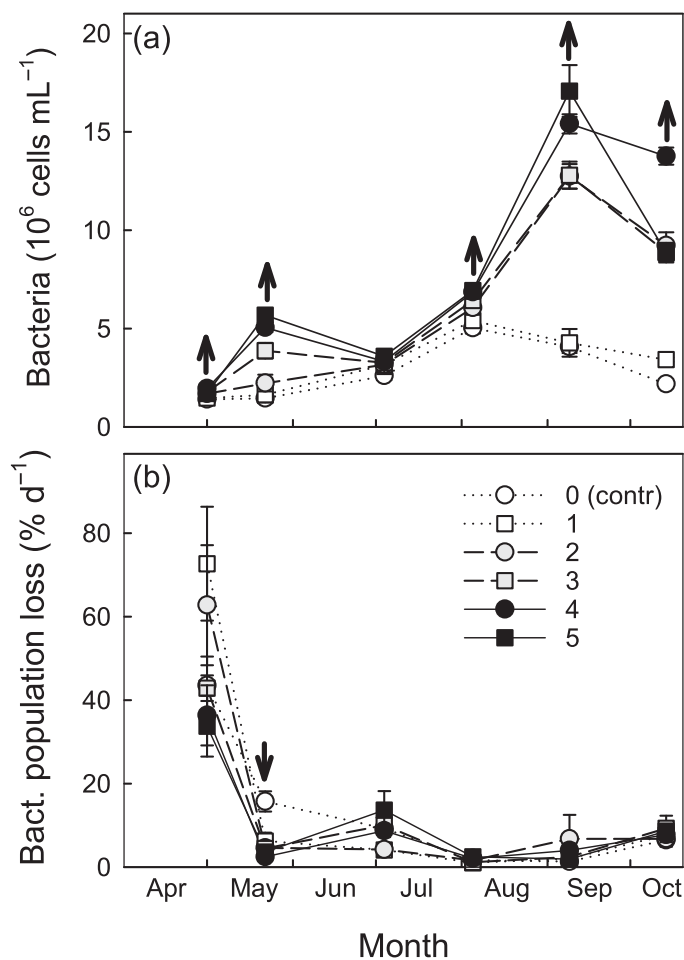


Fig. 3. Bacterial abundance (a), and percentage of standing bacterial population lost per day due to grazing by mixotrophs (b) during the six incubation experiments in the mesocosms. Treatments represent the control and the five climate change scenarios (1–5) with stepwise increased temperature and water color. Error bars give one standard error. Arrows indicate direction of a significant treatment effect by the linear model for each time-point with increasing (upward pointing) or decreasing (downward pointing) trend toward future climate conditions.

independently of the climate change treatments. As expected mixotrophs showed increased ingestion rates under future conditions (hypothesis 2) and bacterivory by mixotrophs contributed significantly to total phytoplankton production. Under present climate conditions (control treatment), this was mainly the case during spring, when the phytoplankton community was dominated by mixotrophs. However, in the climate change scenarios, bacterivory became increasingly important (hypothesis 3) during several occasions throughout the season, while remaining negligible in the control treatment. Interestingly, bacterivory contributed significantly to phytoplankton production even during autumn, when mixotrophs contributed only a minor part to a late stage of a phytoplankton bloom. This was partly due to rates of primary productivity having already declined sharply at this

Table 1. Results of a general linear model of data from the grazing incubation experiment. The model incorporates time as a repeated-measures within-subject factor and treatment as continuous covariate.

Variable	Factor	df	F	p
Bacterial abundance	Treatment	1	133.95	<0.001
	Time	5	22.44	<0.001
	Time × Treatment	5	32.15	<0.001
Bacterial population loss	Treatment	1	1.368	0.255
	Time	5	54.34	<0.001
	Time × Treatment	5	5.208	<0.001
Clearance rates	Treatment	1	17.97	<0.001
	Time	5	21.63	<0.001
	Time × Treatment	5	9.702	<0.001
Ingestion rates	Treatment	1	108.07	<0.001
	Time	5	9.462	<0.001
	Time × Treatment	5	15.84	<0.001
Phagotrophic C-acquisition	Treatment	1	37.99	<0.001
	Time	5	27.89	<0.001
	Time × Treatment	5	11.50	<0.001

time. The relative abundance of mixotrophic taxa alone is therefore not always a good indicator for the role of bacterivory to total phytoplankton resource acquisition.

Bacterivory as a route of resource acquisition

Our results emphasize the dual functional role of phytoplankton as both primary producers and consumers of bacteria and suggest an increasing importance of their role as bacterivores in future temperate shallow lakes. In contrast to the uptake of individual inorganic resources during photoautotrophic growth, bacterivory provides a pre-defined mixture of elements bound in bacterial biomass. Bacterivory can thus serve as both carbon source to fuel the heterotrophic metabolism and as nutrient source to support an otherwise photosynthetic lifestyle (Stoecker 1998), but a mixotrophic nutrition is now more regarded as a synergistic interaction of nutritional pathways where phagotrophy supplies several resources that will all be utilized to some extent (Mitra et al. 2016). Assimilation efficiencies of both carbon and nutrients from prey can vary with environmental conditions, but usually are relatively high in mixotrophs (40–80%) including *Dinobryon* and can even reach values close to 100% for nutrient assimilation (Bird and Kalff 1989; Caron et al. 1993).

To estimate the potential importance of bacterivory for phytoplankton resource acquisition, we compared the amount of carbon assimilated by mixotrophs from their prey to the primary productivity by the total phytoplankton community. With a minimum estimate of the contribution of bacterivory to total phytoplankton production of up to 5–10% in the climate change scenarios, bacterivory constituted a significant route of resource acquisition at several

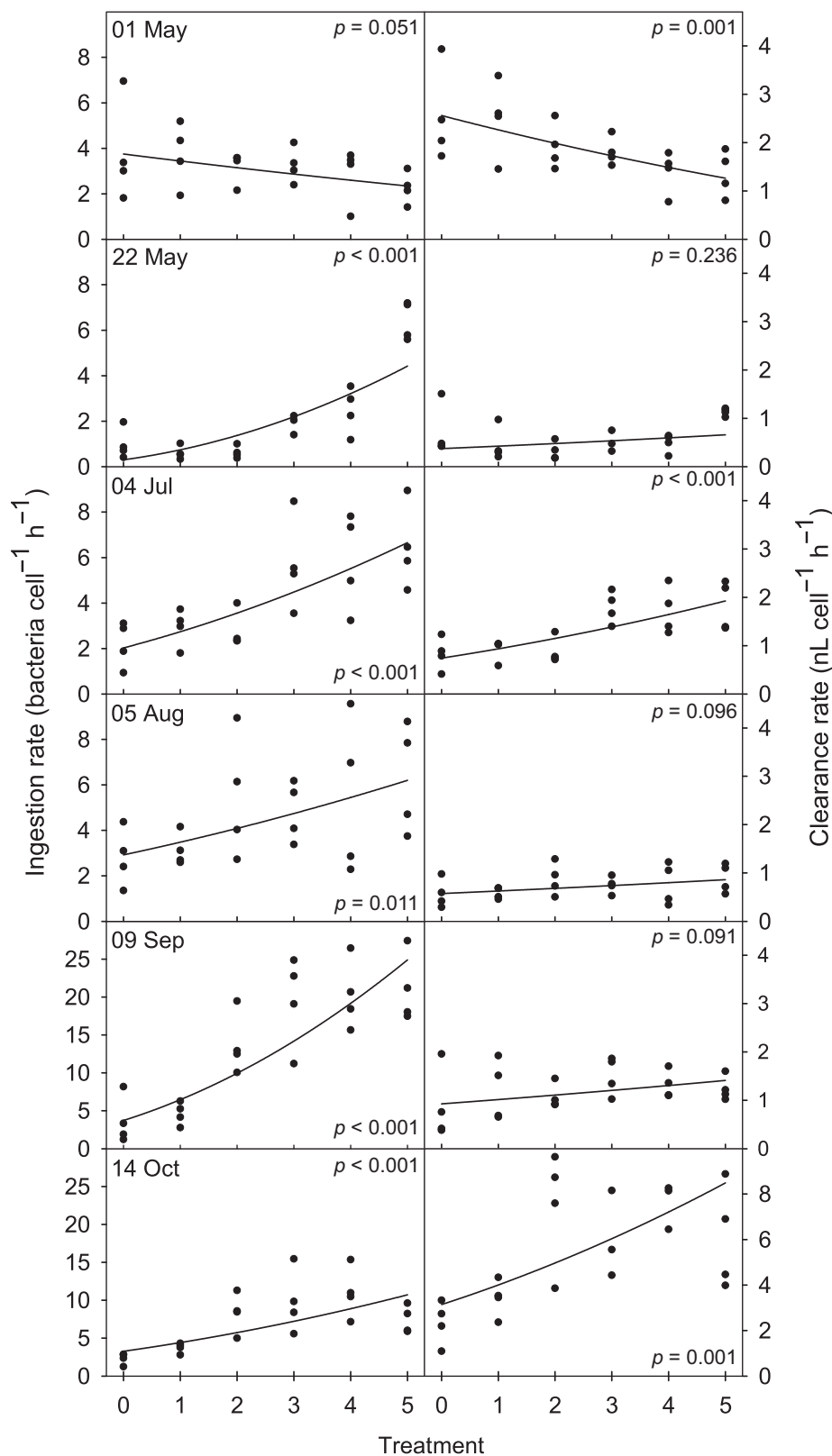


Fig. 4. Ingestion and clearance rates by pigmented flagellates in the mesocosms over the growing season. Data are plotted against the treatments representing the control (0) and the five climate change scenarios (1–5) with stepwise linear increases in temperature and water color. Lines represent regressions as part of a general linear model fitted to the whole data set of square root transformed data.

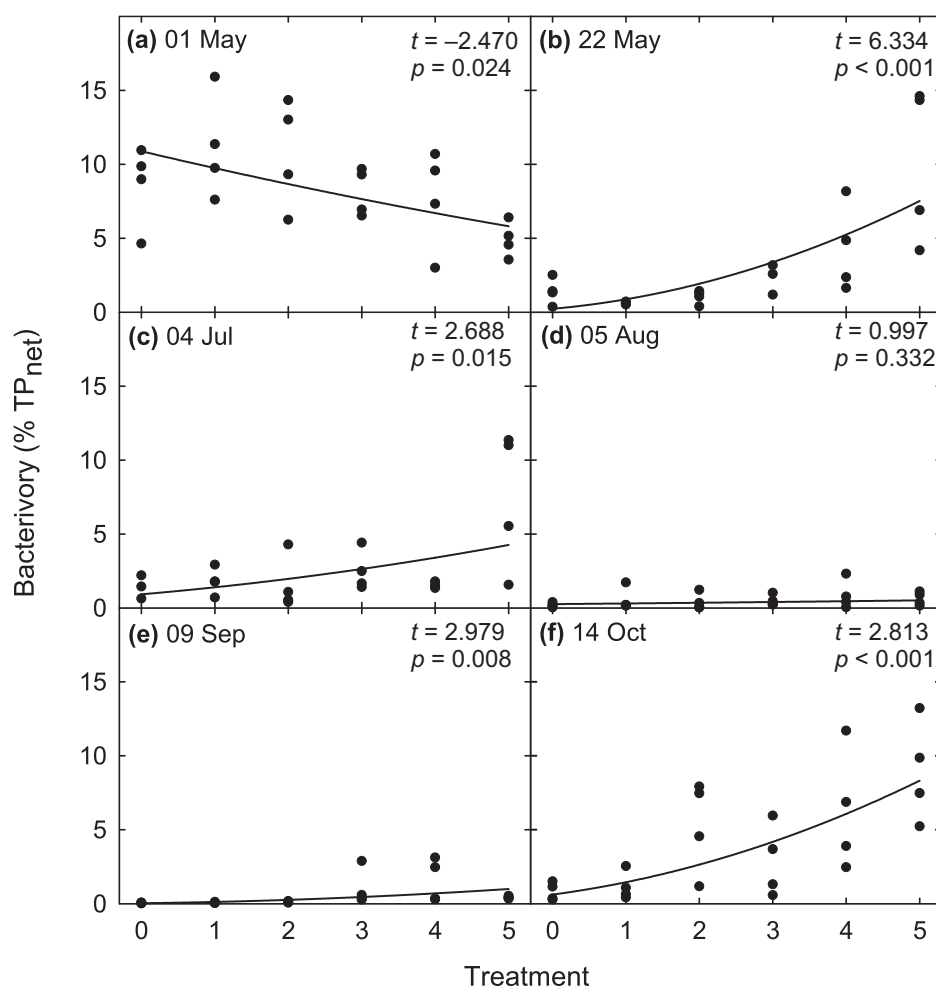


Fig. 5. Estimated relative contribution of bacterivory to total net phytoplankton carbon acquisition at six time points over the growing season (a–f). Data are plotted against the treatments representing the control (0) and the five climate change scenarios (1–5) with stepwise linear increases in temperature and water color. Lines represent the general linear model fitted to the whole data set of square root transformed data.

occasions (Fig. 5). This increasing importance of bacterivory was both due to a decreasing trend in primary production and increasing rates of ingestion by mixotrophic bacterivores. Next to providing carbon, bacterivory might provide a considerable fraction of nutrients to the mixotrophic phytoplankton community. Assimilation efficiencies of nutrients from prey by mixotrophic predators are typically higher than for carbon and bacterial C : P ratios are typically two-fold lower compared to nutrient-replete phytoplankton (Goldman et al. 1987), with an even larger difference under nutrient depletion. Together these comparisons suggest the relative importance of bacterivory as a source of nutrients to be approximately fourfold higher than its contribution to the carbon requirement of the phytoplankton community, thus reaching values of at least 20–40% in spring and under climate change conditions later in the season. Although we cannot track the change in the relative contribution of phago- and phototrophy to growth and elemental requirements of individual taxa, the strongly increased ingestion

rates by these flagellates in the climate change scenario suggest a shift toward a more heterotrophic lifestyle in the future.

Environmental drivers and seasonal dynamics

While our experimental approach with correlated environmental drivers helps to quantitatively assess consequences of climate change, it does not allow identification of individual drivers causing the shift in community composition and increase in phytoplankton phagotrophy. Temperature, light, nutrient availability, and DOC as a resource for bacterial production may all contribute and interact in the control of mixotrophic phytoplankton. Furthermore, mixotrophic species vary widely in their nutritional strategies ranging from primarily autotrophic to primarily heterotrophic nutrition with differing degrees of phenotypic plasticity in response to environmental factors. This diversity of nutritional strategies complicates identification of individual drivers in a specific ecosystem and further complexity is added

by seasonally changing environmental conditions causing rapid succession of the plankton community.

The two key mixotrophic taxa in our experiment, *Dinobryon* and *Cryptomonas*, seem to primarily rely on photosynthesis for their nutrition (Tranvik et al. 1989; Caron et al. 1993), but ingestion rates vary over the diel cycle in *Cryptomonas* and in response to light intensity and temperature in *Dinobryon* (Urabe et al. 2000; Pålsson and Granéli 2003; Heinze et al. 2013). The faster increase in the relative contribution of mixotrophs to total phytoplankton biomass under the climate change scenarios during spring was dominated by *Dinobryon* and occurred at a time when rates of primary production were reduced in the climate change treatments. Bacterivory can constitute the major source of carbon under light limited conditions in *Dinobryon* (Bird and Kalff 1987, 1989) and the higher bacterial abundances might thus have served as a substitutable resource for light to *Dinobryon*, giving it an advantage over its purely autotrophic competitors. A shift in the metabolism of *Dinobryon* from net autotrophy to net heterotrophy across small-scale spatial variations has previously been observed in lakes (Bird and Kalff 1987, 1989) and might occur in a similar fashion with climate change. Additionally, bacterivory can also fully cover the phosphorus needs of *Dinobryon* under oligotrophic conditions (Kamjunke et al. 2007) and hence also the acquisition of nutrients from prey might have contributed to the dominance of *Dinobryon* during spring, when total phosphorus concentrations were still relatively low and high N : P ratios indicated phosphorus to be potentially limiting. During this time, increased competition for nutrients by the phytoplankton community with bacteria under the climate change scenario might have resulted in lower primary production by autotrophs and nutrient acquisition via bacterivory might have contributed to a faster increase in *Dinobryon* abundances.

The shift from a *Dinobryon* dominated spring community to a *Cryptomonas* dominated community in early summer coincided with a decline in Chl *a* concentrations and might have been driven by increasing grazing pressure through zooplankton at this time (Supporting Information Fig. S5). The decline in the contribution of mixotrophs to total phytoplankton during summer is in agreement with their temporal pattern previously observed in humic lakes (Isaksson et al. 1999; Bergström et al. 2000; Bergström et al. 2003) and was not counteracted by the climate change scenario. Throughout this time and until fall when conditions became more eutrophic *Cryptomonas* was the dominant mixotroph in our experiment. In early summer, phagotrophic nutrient acquisition might have helped in becoming the dominant phytoplankton community member, but *Cryptomonas* reached its highest absolute abundances during the most eutrophic conditions in fall. Both the high total phosphorus concentrations during this time and the occurrence of cyanobacterial blooms are in line with earlier reports from Lake Krankesjön (Hargeby et al. 2007; Zhang et al. 2015) and represent a temporal pattern more

frequently observed in shallow lakes (Søndergaard et al. 2002), but are not typically considered favorable conditions for mixotrophs. Nevertheless, *Cryptomonas* also showed relatively higher importance in more eutrophic lakes in South America (Saad et al. 2016) indicating that nutrient limitation is not necessarily the major driver for phagotrophy in this species.

Next to the direct impact of environmental conditions on mixotrophs, an indirect effect of higher DOC concentrations causing increased bacterial abundances under climate change conditions has likely contributed to the higher rates of bacterivory. This is consistent with the positive relationship between mixotroph abundance and bacterial production previously observed in humic lakes (Bergström et al. 2003). The high bacterial abundances might also be the reason for ingestion rates by *Cryptomonas* in the climate change treatment in September (20–25 bacteria cell⁻¹ h⁻¹) being higher than what has been reported previously for this genus (Urabe et al. 2000; Saad et al. 2016). The impact of higher DOC concentrations and bacterial biomass as resources for heterotrophic growth could have been further amplified by increased metabolic rates through warming to cause higher phytoplankton bacterivory (Wilken et al. 2013). In order to understand the metabolic response of specific organisms mechanistically, future studies will need to disentangle the role of individual environmental drivers.

Non-pigmented, heterotrophic flagellates were only of minor importance throughout most of the experiment and therefore could not be quantified accurately. Only in September did heterotrophic flagellates reach higher abundances and contributed significantly to bacterial population loss particularly in the warmer and browner treatments (up to 50% of total bacterial grazing; data not shown). Our methodology was optimized for organisms in the pico- and nanoplankton size range typical for bacterivorous phytoplankton, but might not allow quantification of larger bacterivores, such as ciliates.

Ecosystem consequences

Our experimental design allowed us to resolve the response in phytoplankton ecosystem function to the expected trajectory of environmental change over the course of the next century. The increasing rate of bacterivory and its role as carbon source for the phytoplankton community could be described by a quadratic increase with climate change conditions. The effect was relatively moderate for the politically consented target of less than 2°C warming until the end of the century combined with a modest degree of browning, but might become much stronger for a further increase in temperature and water color. A failure to constrain climate change consequences below this threshold might thus cause phytoplankton communities in future shallow lakes to become more heterotrophic with implications for biogeochemical cycles and food web processes.

The synergistic role of phagotrophy and photosynthesis in the nutrition of mixotrophs is expected to increase trophic transfer efficiency by constituting a shortcut compared to the

traditional microbial loop (Mitra et al. 2014). Because next to organic carbon, phagotrophy provides nutrients to support photosynthetic growth, bacterial production can directly support primary production by mixotrophs, while remineralization is minimized (Rothhaupt 1997; Mitra et al. 2014). If the photosynthetic machinery furthermore provides energy to fuel the metabolism, the organic carbon acquired by phagotrophy could be efficiently incorporated into biomass with only minor respirational losses. Such a tendency toward photoheterotrophy has been described in a freshwater chrysophyte (Wilken et al. 2014a), and could provide an efficient link from bacterial to phytoplankton biomass. Increased trophic transfer efficiency in a food web dominated by mixotrophs is hence predicted to support a higher biomass at high trophic levels (Ward and Follows 2016). A similar mechanism might be at work in humic lakes, where allochthonous carbon supports secondary production by zooplankton (Carpenter et al. 2005; Cole et al. 2011), despite its generally low food quality (Brett et al. 2009). In this case, phytoplankton bacterivory could thus represent an important trophic shunt from bacteria utilizing allochthonous carbon (Kritzbeg et al. 2006) to higher trophic levels. Mixotrophs are often of higher food quality relative to their bacterial prey, presumably due to their production of sterols and poly-unsaturated fatty acids (Boëchat et al. 2007; Weithoff and Wacker 2007; Jäger et al. 2014). *Cryptomonas* for example is of such high food quality to the crustacean *Daphnia* that small quantities of this mixotroph in the food can substantially improve *Daphnia* growth on otherwise low quality allochthonous material (Brett et al. 2009). Already small increases in the relative importance of mixotrophs as food sources might therefore help to maintain zooplankton populations on otherwise high levels of allochthonous carbon sources as often observed in humic lakes (Berggren et al. 2015).

In conclusion, our mesocosm experiment showed a significant contribution of bacterivory to phytoplankton production during spring and suggests an increasing importance of this route for phytoplankton resource acquisition in future temperate shallow lakes also later in the growing season. The shift toward heterotrophy often observed in plankton communities of warming and browning waters (Yvon-Durocher et al. 2010; Ask et al. 2012) might therefore also take place at the level of the primary producers themselves. Increasing phytoplankton phagotrophy will have consequences for carbon flow through the microbial loop and might increase the transfer efficiency of allochthonous carbon to higher trophic levels.

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Conflict of Interest

None declared.

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