Received: 13 May 2021 | Revised: 23 November 2021 | Accepted: 29 November 2021

DOI: 10.1111/fwb.13865

Freshwater Biology WILEY

ORIGINAL ARTICLE

Long-term acclimation might enhance the growth and competitive ability of *Microcystis aeruginosa* in warm environments

Bogdan Drugă[1] | Elisabeth Ramm[2] | Edina Szekeres[1] | Cecilia Chiriac[3] | Adriana Hegedüs[1] | Maria Stockenreiter[4]

[1]Institute of Biological Research (NIRDBS), Cluj-Napoca, Romania

[2]Institute of Meteorology and Climate Research, Atmospheric Environmental Research (IMK-IFU), Karlsruhe Institute of Technology (KIT), Garmisch-Partenkirchen, Germany

[3]Biology Centre of the Czech Academy of Sciences, Institute of Hydrobiology, České Budějovice, Czech Republic

[4]Department Biology II, Experimental Aquatic Ecology, Ludwig-Maximilians-Universität München, Planegg-Martinsried, Germany

Correspondence
Bogdan Drugă, Institute of Biological Research (NIRDBS), 48 Republicii Street, Cluj-Napoca 400015, Romania.
Email: bogdan.druga@icbcluj.ro

Funding information
UEFISCDI; Norway Grants Call 2019; Romanian Ministry of Education and Research, Grant/Award Number: 25N/2019 – BIOSERV; AQUACOSM EU Horizon 2020 INFRAIA, Grant/Award Number: 731065 and 871081; DFG, Grant/Award Number: 1075/1-1; Lehre@LMU Munich

Abstract

1. The positive effect of global warming on the growth of cyanobacteria has been widely predicted, but long-term studies targeting their adaptive potential to higher temperature have not been carried out so far. Predicting the magnitude and impact of cyanobacterial blooms in the future as a response to global warming requires an understanding of how cyanobacteria might change in the long term due to climate change.
2. Here we examined the effect of exposing three *Microcystis aeruginosa* strains isolated in Romania to ambient (22°C) and high (26°C) temperature for 6 months. Then, the competitive ability of the strains after heat acclimation was evaluated, by analysing their impact on plankton community composition.
3. One of the three strains displayed significantly higher growth rates after 6 months of cultivation at higher temperatures. Following inoculation into a natural plankton community, the overall cyanobacterial abundance significantly increased in the cultures inoculated with this heat-acclimated strain of *M. aeruginosa* as compared to the ambient-acclimated version. The structure of eukaryotic communities was impacted by both inoculated cyanobacteria and temperature during the experiments.
4. The results of this study emphasise the high potential of cyanobacteria to respond to stressors, and highlight the fact that previous acclimation to warming is a critical factor in shaping the overall structure of plankton communities.
5. Our study strongly advocates for including a step of culture acclimation to future experimental conditions in research programmes aiming to better understand the long-term impact of climate change on aquatic ecosystems.

KEYWORDS
climate change, cyanobacteria, long-term studies, mesocosms, plankton communities

1 | INTRODUCTION

Freshwater ecosystems are amongst the most biologically diverse on Earth, and they provide irreplaceable services for both nature and human society (Hoekstra & Mekonnen, 2012). However, these ecosystems are increasingly threatened by many human activities, including water pollution, climate change, habitat alteration, and expansion of agricultural and urban landscapes (Albert et al., 2020; Dudgeon, 2019). As a result, freshwater biodiversity continues to decline at an alarming rate (Collen et al., 2014). One of the most recognisable signs of freshwater degradation in recent decades is represented by the severe overgrowth of cyanobacteria as well as their increasingly frequent blooms (Huisman et al., 2018; Wells et al., 2015).

Analysis of sediment cores from over 100 lakes in North America, Europe, and Asia has shown that cyanobacterial harmful algal blooms (CyanoHABs) have increased considerably over the last century and that this increase has accelerated in the last 50 years (Monchamp et al., 2018; Taranu et al., 2015; Yan et al., 2019). Even though in some cases nutrient loading (especially phosphorus) control has temporarily led to a decrease in bloom occurrence in the 1970s and 1980s, continuous global warming during the last 10–15 years has again accelerated this phenomenon (Duan et al., 2009; Guo, 2007; Michalak et al., 2013). An increasingly higher number of CyanoHABs has been reported in recent years in both freshwater and marine environments, some of them occurring at unusual high latitudes (Kahru & Elmgren, 2014; Spatharis et al., 2012). Considering ongoing climate change, this trend is likely to continue in the coming decades (Ullah et al., 2018; Visser et al., 2016). Compared to other phytoplankton groups, cyanobacteria are known to be positively influenced by higher temperatures concerning metabolism, growth, survival, reproduction, and consequently general competitiveness. As a result, the latest studies indicate that warming and other factors (e.g. eutrophication, increasing carbon dioxide levels) are likely to enhance the frequency, duration, and intensity of CyanoHABs in many aquatic ecosystems around the world (Huisman et al., 2018; Mesquita et al., 2020; Symes & van Ogtrop, 2019). Moreover, theoretical modelling analyses have predicted an increase of CyanoHAB occurrence towards the end of the century, which might happen on the expense of chlorophytes (Chapra et al., 2017; Ji et al., 2017).

The cyanobacterial genus *Microcystis* includes species known for being strong competitors of chlorophytes and brown groups of algae (diatoms, dinoflagellates, etc.) for light in eutrophic lakes, especially in connection with elevated temperatures (Drugă et al., 2019; Jöhnk et al., 2008). By contrast, mesocosm tests have indicated that lower nutrient concentrations might alleviate the otherwise positive effect of climate change on cyanobacteria proliferation (Cabrerizo et al., 2020). However, the vast majority of the studies done so far were based on short-term small-scale laboratory experiments, which do not account for the potential of cyanobacteria to long-term acclimate under tested conditions (Ji et al., 2017; Soares et al., 2013). Because of this, models that predict changes in aquatic ecosystem composition and function are limited by the lack of understanding of the physiology (and evolution of that physiology) in key phytoplankton taxa (Huisman et al., 2018; Litchman et al., 2015; Thomas et al., 2012). It is, however, the long-term response of phytoplankton to stressors that we need to understand in order to accurately predict the impact of future environmental changes on aquatic ecosystems (Boyd et al., 2018; Mock et al., 2016; Thomas & Litchman, 2016). Long-term experiments provide valuable information to improve predictive models, especially if this information is combined with data on physiological responses to certain variables such as temperature, nutrient loading, etc. (Burford et al., 2020). Considering the threat represented by the potentially increasing occurrence of CyanoHABs in the future, which also comes with enormous financial costs (Dodds et al., 2009), new knowledge is needed on the ability of certain species to adapt to changing conditions. Moreover, this should be tested in mesocosm experiments to assess the competitive abilities of the cyanobacterial strains within complex natural phytoplankton communities. Upscaling small-volume laboratory experiments to larger mesocosms (including several trophic levels) is desirable, as this approach solves some of the shortfalls that come from using exclusively laboratory experiments (which allow for only *snapshot* types of investigations, a limited volume of laboratory cultures etc.; Stibor et al., 2019).

Here, we conducted a mesocosm study on the role of acclimation of cyanobacteria to higher freshwater temperatures. We have chosen to work with *Microcystis aeruginosa*, which is known for causing some of the most frequent and potentially toxic CyanoHABs in freshwaters around the world (Huisman et al., 2018). We tested the impact of 6-month exposure to a higher temperature than the ambient on natural planktonic communities from a eutrophic lake also subjected to warming. We hypothesised that: (1) *M. aeruginosa* strains can acclimate to elevated temperatures; and (2) heat-adapted strains display a higher competitive ability as compared to ambient-adapted strains. It was assumed that strains might respond differently due to specific genotypes and/or shared environmental effects.

2 | METHODS

2.1 | Biological material and growth conditions

The three *M. aeruginosa* strains (M10, M11, and M12) used in this study were isolated during the summer of 2018 from three eutrophic freshwater lakes in Romania: Lake Ciuperca (45.18°N 28.79°E), Lake Tașaul (44.35°N 28.61°E), and Lake Gorgova (45.15°N 29.18°E). The average water temperature of all three lakes during the summer is 22°C, including natural fluctuations. The strains were then deposited as non-axenic cultures in the Collection of Cyanobacteria and Algae of the Institute of Biological Research in Cluj-Napoca, Romania (Dragoș et al., 1997). Their phylogenetic identity was assessed based on the 16S ribosomal DNA (rDNA) gene that was amplified with specific primers (Frank et al., 2008). The PCR fragments were sequenced by a third-party company (Macrogen Europe), which confirmed that all strains belong to the *M. aeruginosa* species.

For this study, in order to allow for long-term acclimation, the strains were cultured for 6 months in semi-batch conditions, corresponding to *c*. 50 generations, generation time of 2–4 days, which is similar to natural conditions (Wilson et al., 2006) in ultrapure water-based BG11 medium (Allen & Stanier, 1968), under controlled 16-hr:8-hr light:dark conditions provided by white LED lamps (25 μmol photon m^{-2} s^{-1}). They were grown at 22°C (summer mean temperature of lakes of origin) or 26°C, which is the predicted increase of temperature by 2100 (IPCC report, 2018). Triplicate 100-ml samples were placed in glass tubes (Figure S1), each culture being aired daily for 60 min with pre-filtered (0.22 μm; Minisart, Sartorius) atmospheric air. This air supply was added to avoid cell gathering (which would have caused self-shading of cells). It was added for only 60 min per day (instead of continuous bubbling), because continuous mixing would have caused a very fast (and also less environmentally relevant) growth of the cultures, which would have greatly increased the effort to maintain them on the long term. Cultures were transferred to fresh medium every 2–3 weeks.

2.2 | Growth rate measurement

Growth rate (μ) of each culture was measured at the beginning of the experiment (day 0) as well as after 2, 4 and 6 months of cultivation. The growth rate at these time points was measured only at 22°C (regardless on whether the strains were previously adapted at 22°C or 26°C), because, in order to observe whether long-term acclimation occurred at 26°C (as compared to 22°C), all cultures should be tested in their original environment. More exactly, subsamples from all cultures (both evolved at 22 and 26°C) were diluted to OD_{600} = 0.1 (*c*. 0.6 × 10^6 cell/ml) and they were then transferred to the control temperature (22°C) for up to 14 days, and their growth rates over the exponential growth period were measured (based on OD measurements done every second day) according to $\mu = (\ln Nd - \ln N0)/d$, where *N0* and *Nd* represent culture density at the beginning and the end of each test, and d is the duration of the test in days. Number of divisions per day (Div/day) was calculated as follows: Div/day = μ/ln2, while generation time (Gen.t) was calculated according to: Gen.t = 1/Div/day. Growth rate measurements were stopped after 14 days because at this time most cultures were already very dense (OD_{600} = 0.9–1.4, corresponding to a density of *c*. 1.16 × 10^7–1.84 × 10^7 cells/ml; Figure S2).

2.3 | Land-based mesocosm experiment

The impact of the three *M. aeruginosa* strains on natural lake communities was tested at the Seeon Limnological Station of the Ludwig-Maximilians-Universität (LMU) Munich, in Bavaria, Germany. Four 1000-L water tanks were used, in which 14 mesocosms made out of translucent plastic (LDPE, Polyverpackung GmbH) were deployed (Figure S3). The tanks were filled with water from the eutrophic lake Bansee (47.964°N 12.44°E; total phosphorus >30 μg/L). Before filling the tanks, the lake water was filtered through a 250-μm gauze to exclude macro- and mesozooplankton; however, microzooplankton (same size class as bigger eukaryotic phytoplankton) grazing was still possible. The temperature in tanks 1 and 3 was not manipulated, while temperature in tanks 2 and 4 was controlled by a program based on a Raspberry Pi computer (Raspberry Pi Foundation) with sensors that measured a reference temperature from tank 3 and switched on or off two heating mats to ensure that these two tanks continuously had 4°C on top of ambient values (Figure S3a). The four tanks were maintained in this way for 14 days, to allow the planktonic resident communities to acclimate to these conditions. Then, the *M. aeruginosa* strains were inoculated into the lake communities, on 19 June 2019. To have three replicates for each experiment, the *M. aeruginosa* strains were not inoculated into the plastic enclosures directly, but into smaller dialysis bags that are permeable for macro- and micronutrients (10–20 kDa pore size; Nadir, Carl Roth, Karlsruhe, Germany). Every enclosure was equipped with three replicate dialysis bags of 1.4 L each that were filled with water from the enclosure (Figure S3b). Before being sealed, each dialysis bag was inoculated with one of the three *M. aeruginosa* strains (initial concentration: 3 × 10^3 cells/ml). Therefore, each strain (ambient- or heat-adapted version) was separately inoculated both into the ambient and heated tanks. Altogether, the experimental setup consisted of 42 dialysis bags distributed into 14 enclosures, including controls (dialysis bags with no added *M. aeruginosa* cells) in both ambient and heated mesocosms. Temperature was recorded every 60 s by the sensors immersed in the tanks, which were connected to the Raspberry Pi computer.

2.4 | Chlorophyll-*a* concentration measurement

Chlorophyll-*a* content (μg/L) was estimated in vivo with an AlgaeLabAnalyser device (bbe Moldaenke GmbH) once a week, for a total of 4 weeks. The AlgaeLabAnalyser is a device made for analysing phytoplankton samples (based on five wavelengths: 470, 525, 570, 590, and 610 nm), simultaneously providing data on the community composition in terms of the proportion of defined functional groups on total chlorophyll-*a* according to their spectral characteristics. Four different major algal groups are distinguished: chlorophytes, cryptophytes, cyanobacteria, and a brown group, which consists mainly of genera that have additional pigments that absorb in the yellow/orange wavelength range (diatoms, crysophytes, dinoflagellates).

2.5 | DNA analysis

The structure of plankton populations in dialysis bags was checked after 4 weeks of field exposure, through DNA metabarcoding. First, the water from every three replicate dialysis bags was united

(*c.* 3 L in total), and centrifuged, and total DNA was isolated using the E.Z.N.A.® Soil DNA Kit (Omega Bio-tek), following manufacturer instructions. DNA concentration/quality was assessed with a NanoDrop™ 2000 Spectrophotometer. A part of the small rDNA subunit (16S for prokaryotes and 18S for eukaryotes) was then amplified through PCR with primers covering a wide range of planktonic taxa (Frank et al., 2008; Hadziavdic et al., 2014). DNA sequencing was done at the LMU Munich. In brief, 5 μl from each PCR were pooled into separate 16S and 18S pools, purified with 0.8× SPRI beads according to a standard protocol and eluted in 200 μl 10 mM Tris pH7.5. Libraries were constructed using the NEBNext UltraTMII DNA library preparation kit, and sequencing was done with a HiSeq 1500 system (Illumina).

2.6 | Statistics

Analyses were conducted in R (version 3.6.0), and the *ggplot2* package (version 3.0.0) was used to produce graphs based on the AlgaeLabAnalyser data. One-way and two-way ANOVAs (on chlorophyll *a* concentrations) with combinations of the factors *time*, *heat adaptation*, and *heated tank* were conducted both for all strains together and for the three strains separately (it is more suitable to reveal differences between strains than to conduct three-way ANOVAs with *heat*, *heat adaptation*, and *strain*). We assured that variance was homogenous with the aid of Fligner tests. For the ANOVAs the control treatments had to be excluded as they contained no added strains (this would lead to the necessity to decode the controls always as *heat adaptation* [no], which would distort the correlations). Prior to the ANOVAs, a linear model that included the interaction of the two factors was defined. Following the ANOVA results, post hoc analysis included mean separation tests for multiple comparisons using Tukey-adjusted comparisons and least square means for main effects. Arithmetic means of the treatments as well as standard deviations (*SD*) of the replicates were calculated. Student *t*-tests (two-sided, equal variance) with 95% confidence interval were conducted in Numbers after assuring that variance was homogenous with the aid of fligner tests. Principal component analysis (PCA) was conducted on chlorophyll *a* concentration using the package *ggfortify* and a code according to Tang et al. (2016).

After DNA sequencing, the base calling and run demultiplexing were conducted using the BaseSpace service (Illumina) meters. Qiime2 was implemented for separating sequences for each sample and OTU picking with its default settings. The sequences were classified based on the taxonomy in the Silva database (97% confidence threshold, version 132). The sequencing data were analysed in R, using ggplot2 and DADA2 for graphical presentation.

Beta diversity was analysed based on DNA data. Rarefaction was performed for 36,000 sequences, followed by β-diversity estimation in QIIME (Caporaso et al., 2010) using Weighted Unifrac distances (Lozupone & Knight, 2005). Visualisation of β-diversity was performed in R version 3.6.3 with the *plot* function of the *graphics* package.

3 | RESULTS

3.1 | Growth rate evolution

The growth rates of the three *M. aeruginosa* strains grown adapted for 6 months at 22 and 26°C were measured in the control environment (22°C). At day 0, all growth rates were similar: $\mu = 0.156–0.159\ day^{-1}$ (Figure 1). In case of strains M10 and M12, a decrease in growth rate occurred after 2 months of exposure to both 22 and 26°C, followed by a recovery after 4 months (except for strain M10 grown at 22°C; Figure 1a,c). After 6 months of cultivation, growth rates of both strains M10 and M12 increased above the original values (day 0). Nevertheless, the differences between growth rates of the strains previously grown at 22 and 26°C were

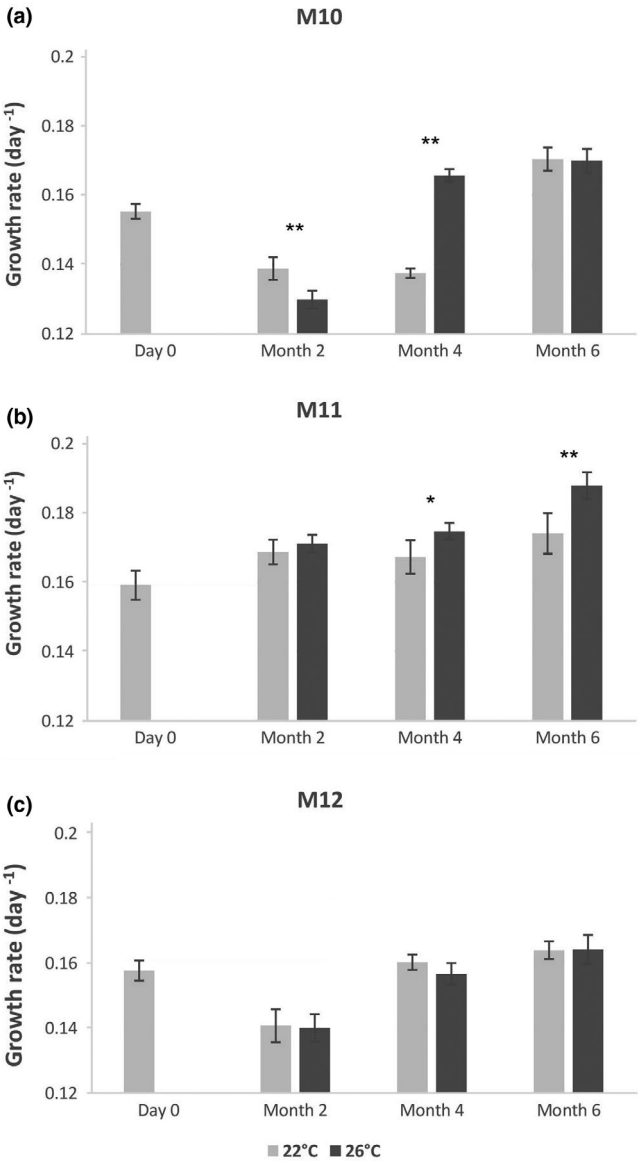

FIGURE 1 Representation of the growth rates during 6 months of adaptation to different temperatures. *$p \leq 0.05$; $t = 2.24$. **$p \leq 0.001$; $t = 3.16$ (*t*-test). Standard deviation presented in the graph; each test was done in three replicates

not statistically significant (*t*-test; $p \geq 0.05$; Figure 1a,c). In the case of strain M11, there was no decrease in growth rate after 2 months. Moreover, growth rates of M11 continued to increase after 4 and 6 months, slightly faster for the strain cultured at 26°C. The differences between growth rates of the M11 cultures previously acclimated at different temperatures (22°C/26°C) were in this case significant ($p \leq 0.001$; Figure 1b). The growth rate of the three strains after 6 months varied from $\mu = 0.165–0.19$, which translates to a generation time of *c*. 3.6–4.2 days, meaning 43–50 generations (growth curves are presented in Figure S4). In case of strain M12, growth rates of both ambient- and heat-grown versions were similar to the values measured at the beginning of the experiment (day 0; Figure 1b).

3.2 | Competition experiment

3.2.1 | Temperature

Each *M. aeruginosa* strain was separately inoculated into 1.4-L dialysis bags containing eutrophic lake water (total phosphorus >30 μg/L), which were immersed into four 1000-L mesocosms filled with the same lake water (Figure S3). The water in mesocosms 2 and 4 was heated evenly along the water column. During weeks 2 and 3, when a heatwave was recorded in Bavaria, the water temperature in the heated tanks often exceeded 30°C, and 26°C in the ambient tanks (Figure 2). During the fourth week, temperature frequently dropped below 19°C in the ambient mesocosms and 23°C in the heated tanks.

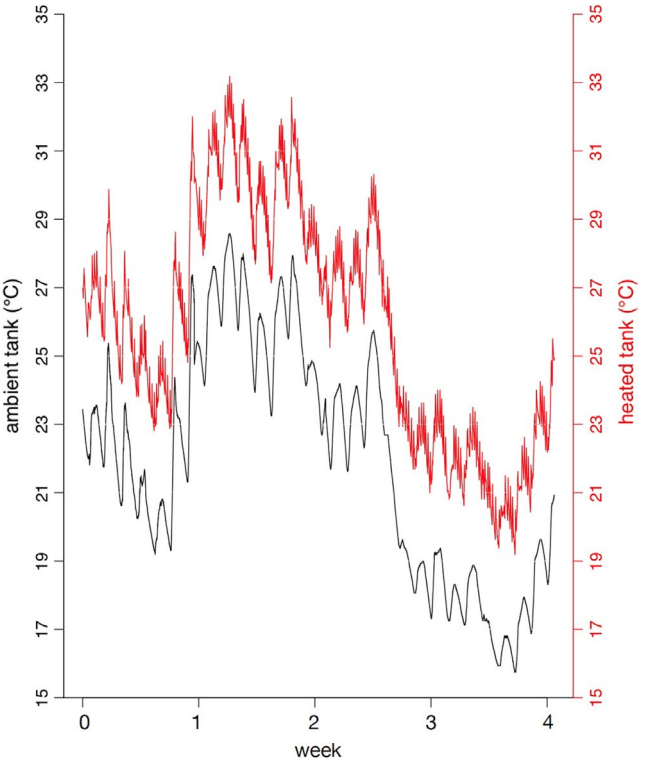

FIGURE 2 Tank temperature for the time of the experiment (mean values of two mesocosms). The black line shows ambient, the red line heated tanks. Visible are day–night fluctuations as well as weather changes

3.2.2 | Phytoplankton group dynamics

The concentration of total chlorophyll-*a* was also different throughout the experiment due to added inoculum and temperature. In general, total chlorophyll-*a* concentration after the first 2 weeks was higher in the heated as compared to ambient tanks (Figure S5). This changed towards the second part of the experiment, when the amount of chlorophyll-*a* in the ambient mesocosms kept rising, while it decreased in most of the heated tanks (Figure S5b). Moreover, total chlorophyll-*a* concentration in ambient mesocosms was significantly higher in the dialysis bags that were inoculated with *M. aeruginosa* M10 and M11 cells as compared to control bags (*t*-test: $p < 0.001$). After 4 weeks, communities that were inoculated with ambient-adapted cyanobacterial cells displayed double the chlorophyll-*a* concentration compared to control communities (Figure S5a,c).

The composition of the phytoplankton communities inside the dialysis bags diverged greatly between the ambient/heated mesocosms. Thus, the chlorophyll-*a* concentration in the dialysis bags (including controls) from the ambient tanks 1 week after the start of the experiment was of *c*. 6 μg/L, while in the bags immersed in the heated tanks it exceeded 10 μg/L (Figure 3). Chlorophytes were the most abundant phytoplankton group in each bag, at every time point. Their concentration in the ambient tanks kept rising with time, while in the heated mesocosms it was constant. Cyanobacteria displayed similar dynamics in time, with an increasing abundance in the ambient tanks and a steady concentration in the heated tanks. Nevertheless, a higher abundance of cyanobacteria was observed (based on chlorophyll measurements) in the communities from the heated tanks that were inoculated with heat-adapted *M. aeruginosa* strains as compared to ambient-adapted strains. Other groups of microalgae were in general not affected by water temperature, nor by inoculum type (Figure S6).

The most noteworthy results were observed in the ambient tanks, regarding the abundance of chlorophytes and cyanobacteria (Figure 3). The highest concentration of chlorophytes was measured in the dialysis bags inoculated with ambient-adapted *M. aeruginosa* cells. The abundance of cyanobacteria increased with time in all bags, but at a higher rate in those inoculated with the strains that were previously adapted to 26°C. The concentration of cyanobacteria in the bags inoculated with heat-adapted strain M11 reached the highest value after 4 weeks (6 μg/L; Figure 3c), while the concentration in the case of strains M10 and M12 reached 4 μg/L at the end of the experiment (Figure 3a,c). The ambient-adapted strain M10 produced similar results as the heat-adapted version (Figure 3a), while ambient-adapted strains M11 and M12 produced a lower cyanobacterial concentration during the four timepoints as compared to their heat-adapted variants (Figure 3c,e; Figure S10). Cyanobacteria, which occur naturally in

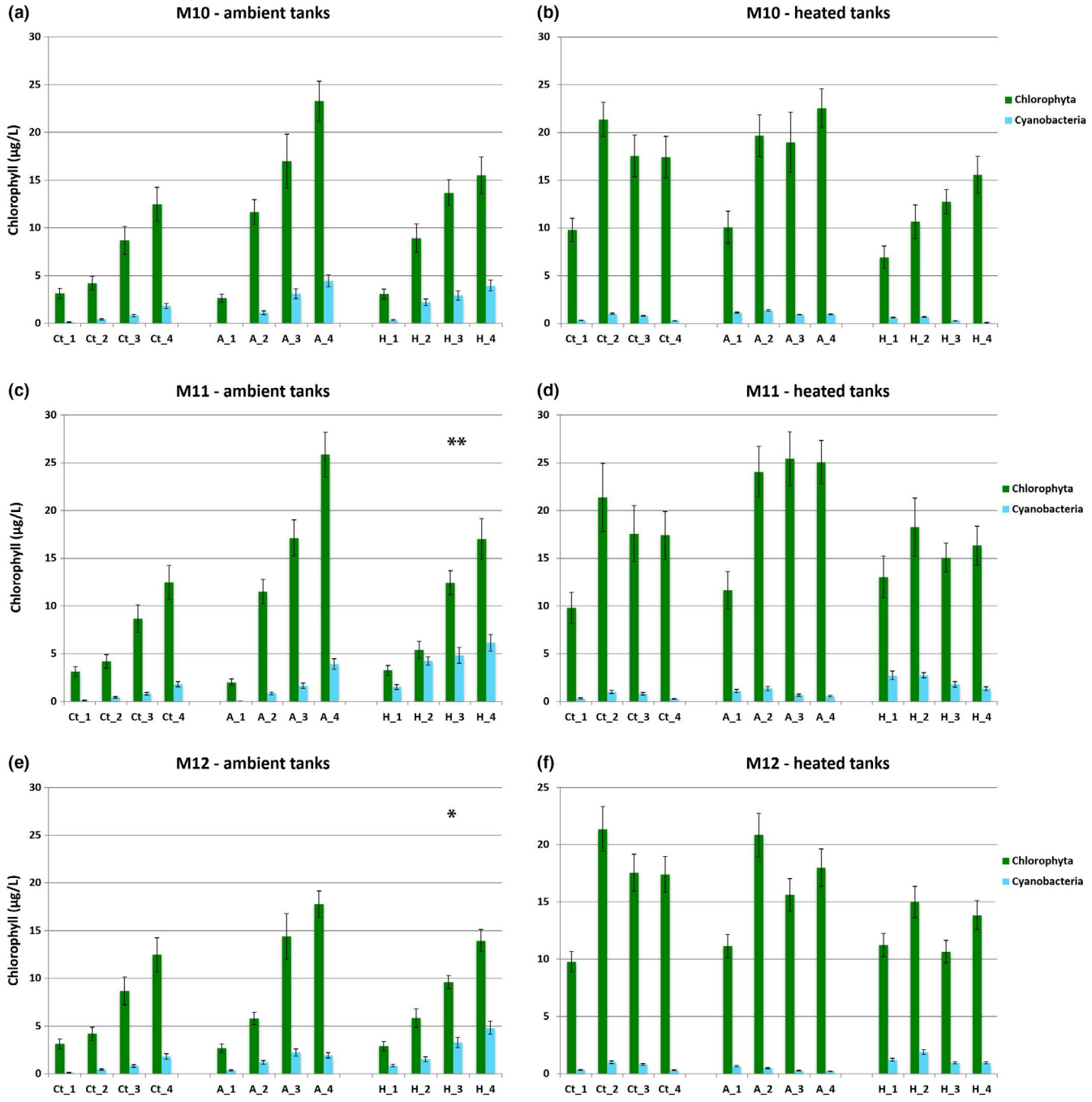

FIGURE 3 Chlorophyll concentration of green algae and cyanobacteria in the ambient and heated tanks. (a and b) strain M10; (c and d) strain M11; (e and f) strain M12; Ct_1–Ct_4 = control dialysis bags (no added cyanobacteria); A_1–A_4 = dialysis bags inoculated with ambient-adapted cyanobacteria; H_1–H_4 = dialysis bags inoculated with heat-adapted cyanobacteria. The numbers (1–4) refer to the week of the experiment when the samples were analysed. Each value represents average chlorophyll-*a* ± *SD*. $^{**}p \leq 0.01$; $^{*}p \leq 0.05$ (for heat-adapted cyanobacteria)

most freshwater lakes from temperate regions, were also present in the control bags, but their concentration throughout the experiment was overall lower than in any of the communities inoculated with *M. aeruginosa*.

ANOVA tests were conducted to reveal differences between treatments at the end of the experiment (week 4). Tests were run with the factor *heat adaptation* (yes, no) and the factor *heated tank* (yes, no). There were significant differences in cyanobacteria concentration among bags inoculated with any *Microcystis* strains concerning the factor *heat adaptation* (except for strain M10) and the factor *heated tank*, but not concerning an interaction of the two factors (Table 1). Post hoc analyses (least square means for main effects, Tukey-adjusted comparisons, mean separation test, one-way ANOVA) showed that considering all tanks and strains after 4 weeks, treatments with heat-adapted strains had higher cyanobacterial concentrations, while treatments in ambient tanks

TABLE 1 Two-way ANOVA test on the biomass of cyanobacteria in relation to heat adaptation, tank temperature, time, and their interaction

Factor	*df*	*F* value	*p* value
Heat adaptation (all strains)	1	6.71	**0.01**
Heated tank (all strains)	1	72.58	**<0.001**
Heat adaptation:heated tank (all strains)	1	3.99	0.10
M10—Heat adaptation	1	0.18	0.69
M10—Heated tank	1	33.28	**<0.001**
M10—Heat adaptation:heated tank	1	1.28	0.29
M11—Heat adaptation	1	11.34	**0.01**
M11—Heated tank	1	56.46	**<0.001**
M11—Heat adaptation:heated tank	1	3.39	0.10
M12—Heat adaptation	1	6.23	**<0.05**
M12—Heated tank	1	18.32	**<0.01**
M12—Heat adaptation:heated tank	1	1.33	0.28
Time (M10)	3	3.66	**0.02**
Time (M11)	3	1.62	0.20
Time (M12)	3	1.79	0.16
Time:heat adaptation	3	0.19	0.91
Time:heated tank	3	22.37	**<0.001**

Note: Statistically significant factors are in bold.

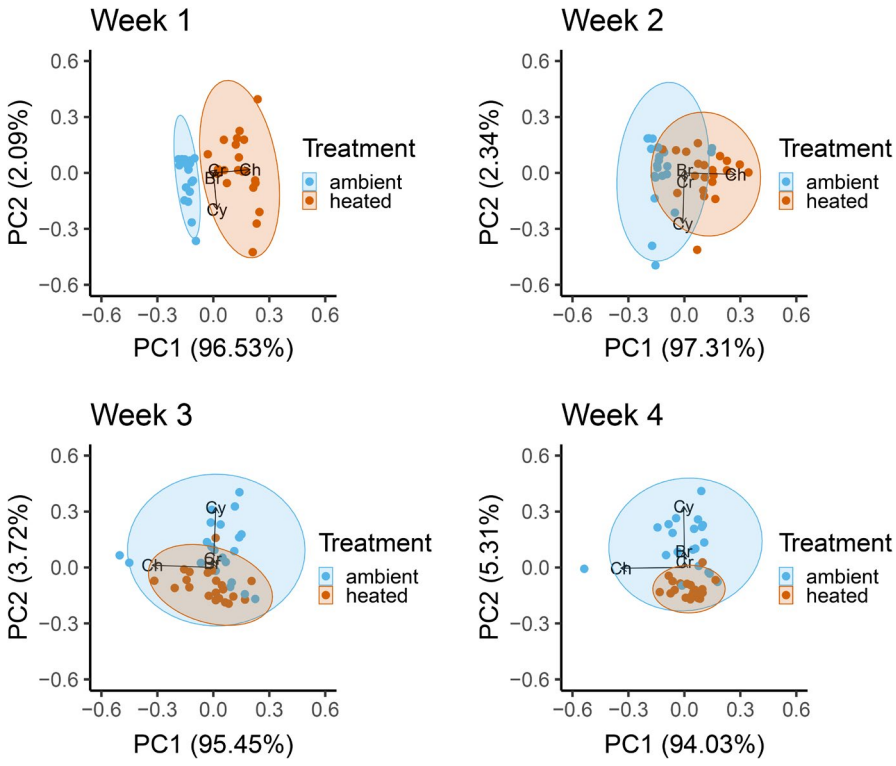

FIGURE 4 Principal component (PC) analysis of phytoplankton group dynamics during the experiment. Principal components are the directions where the plotted data vary the most. The coloured ellipses represent confidence intervals: blue indicates ambient tanks; red indicates heated tanks. Br = Brown groups, Ch = Chlorophyta, Cr = Cryptophyta, Cy = Cyanobacteria

had higher cyanobacterial concentrations as compared to heated tanks (Figure S7; Table 1).

Taken separately, we found significant differences in cyanobacterial concentrations at the end of the experiment (week 4) for each of the three *M. aeruginosa* strains concerning the factor *heated tank*, and also for *heat adaptation* in strains M11 and M12. In case of strain M10, heat adaptation did not produce a significant advantage over the ambient-adapted strain (Table 1). Nevertheless, there were significant differences in M10 cell concentration regarding the factor *time* (week 1, 2, 3, 4), as well as *time:heated tank* (Table 1; Figure S8).

The pigment-based PCA of the phytoplankton communities from the ambient /heated tanks showed clear differences between community structure during the 4 weeks (Figure 4). After week 1 of the experiment, the phytoplankton populations in the two environments

were clearly distinct. Starting with week 2, communities from the ambient tanks started to increase in diversity as compared to those in heated tanks, which continuously decreased in variance until week 4. In the plot, both cyanobacteria and chlorophytes changed direction over time.

3.3 | DNA-based community structure

A total of 2.3 million sequence reads were obtained following the 16S rDNA amplicon sequencing, and 1.2 million for the 18S rDNA. A number of 0.708 million reads (0.575 million for 16s and 0.133 for 18S) passed the processing and filtering steps (sequencing quality and read length). Following the re-assembly, alignment clean up and mapping, the final abundance table in the 14 analysed samples (based on the 16S and 18S sequences) contained 859–1,029 prokaryotic OTUs and 206–283 eukaryotic OTUs.

The most abundant prokaryotic OTUs identified in the samples belong to phylum Proteobacteria (26%–56% of total OTUs; Figure 6). Most of these OTUs were assigned to Alphaproteobacteria (18%–43%) and Gammaproteobacteria (3%–14% of the reads). The main difference between the Proteobacteria in terms of ambient/heated tanks was given by the order Elsterales (Alphaproteobacteria; 11%–26% in the ambient tanks), which were found at a very low concentration in the heated tanks (Figure S9a). In turn, heated tanks displayed 4%–8% of the reads belonging to order Rhizobiales (also Alphaproteobacteria), which were almost absent in ambient tanks. Cyanobacteria was the second well-represented phylum among the prokaryotic OTUs (12%–49%). By far the highest abundance of cyanobacteria was found in the communities inoculated with heat-adapted *M. aeruginosa* strain M11, in both ambient (49% OTUs) and heated tanks (36% OTUs; Figure 5a). This strain version also displayed a superior fitness in lab experiments (Figure S11). Most cyanobacterial DNA reads were assigned to the order Chroococcales (which contains *Microcystis* sp.), but because of sequence ambiguity they could not be allocated to a certain species (Figure S9a). Another cyanobacterial order that was identified only in the ambient mesocosms was Pseudanabaenales (Figure S9a), with 5% of the total reads being found in the control sample (lake water). Sequences indicating order Nostocales were also identified in both control samples (ambient and heated tanks).

Planctomycetes was another abundant phylum in all samples, being slightly more abundant in the heated (18%–36%) than ambient tanks (13%–21%; Figure S9a). Chloroflexi (a phylum of thermophilic bacteria) were identified almost exclusively in the communities from the heated tanks (1%–5% of the total reads), while Verrucomicrobia were found at a higher abundance in ambient conditions (2%–4%) as compared to heated tanks (1% of the reads). Less than 2% of the DNA sequences from ambient tanks could not be assigned to a certain prokaryotic phylum. In turn, a higher proportion of reads from the heated tanks (3%–7%) remained unassigned (Figure 5a).

The eukaryotic populations in heated tanks were dominated by Metazoa (41%–77% of total reads), which were less abundant in the ambient tanks (22%–48% of the reads; Figure 5b). A noticeable difference regarding which metazoan subgroups were present in the ambient/heated tanks was identified: ambient tanks displayed a fairly equal proportion of copepods (genus *Cyclopoida*; 7%–30% of total reads) and gastrotrichs (genus *Chaetonotida*; 7%–33% of reads), while heated tanks were dominated by gastrotrichs (36%–73%), with copepods representing only 1%–7% of the DNA reads (Figure S9b). In turn, chlorophytes were identified in 16%–35% of the reads in the ambient tanks, and only in 5%–15% of the reads from the heated environments. Fungi (mostly belonging to group Cryptomycota) were identified in all samples (3%–9% of the reads), same as phylum Cercozoa (1%–9%; Figure S9b). Dinoflagellates were found mostly in the communities inoculated with *M. aeruginosa* in the ambient tanks (1%–3%). A much higher abundance of dinoflagellates (genus *Woloszynskia*) was observed in the control dialysis bags (24% of the reads; Figure S9b). A variable proportion of reads were unassigned (5%–28% of the reads).

The variation in terms of β diversity was explained, to a large extent, by tank temperature at the time of the experiments, as shown by axis 1, which explained 48.8% of the variance (Figure 6). The impact of inoculated strains, presented on axis 2 (24.7%) was less important in explaining variance except for heat-adapted M11, whose impact on overall community structure was evident.

4 | DISCUSSION

A mechanistical understanding that determines how cyanobacteria might react on the long term to warming, will considerably improve the realism of models describing the impact of climate change on freshwater biodiversity. The goal of this study was to better integrate laboratory culture and field experiments, in order to improve our ability to predict and manage CyanoHABs in a changing world, and therefore to reveal the potential impact of climate change on aquatic ecosystems. We tested the hypothesis that various *M. aeruginosa* strains have different potential to react to higher temperature regimes, leading to an acclimation process that will allow some of the strains to thrive also under competition against other microalgae. According to our results, the hypothesis was confirmed.

Experiments such as this need to be carefully designed, considering the potential adaptive responses of individual species/strains. While controlled lab conditions allow for various hypotheses regarding cyanobacterial blooms to be precisely tested (Drugă et al., 2019), they can deviate from the natural environment because of certain aspects that cannot be replicated in small volumes indoors. Parameters such as water stratification or synthesis of extracellular polymeric substances by mixtures of strains under certain conditions represent natural elements that are relevant for bloom formation (Dervaux et al., 2015), and this is why lab experiments should be, ideally, confirmed by outdoor studies that reproduce the natural setting as much as possible. There is evidence that phytoplankton cultures adapt to fixed laboratory conditions over years, gradually deviating from those of natural populations of microalgae (Lakeman

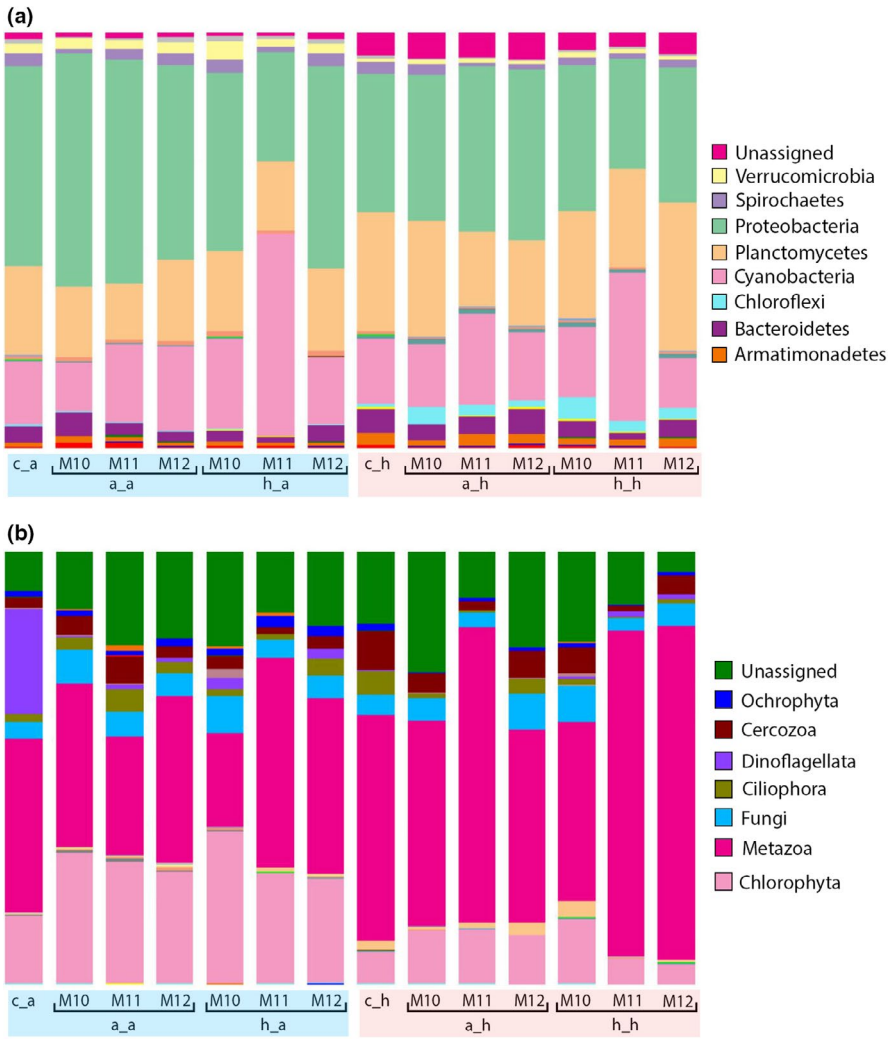

FIGURE 5 Composition of the prokaryotic (a) and eukaryotic (b) communities identified at the end of the experiment (week 4). C_a = control assay, ambient tank; c_h = control assay, heated tank; a_a = ambient-adapted strains inoculated into ambient tank; h_a = heat-adapted strains inoculated into ambient tank; a_h = ambient-adapted strains inoculated into heated tank; h_h = heat-adapted strains inoculated into heated tank. The pale blue and pink backgrounds below each figure represent the ambient and heated mesocosms, respectively

et al., 2009). For such reason we have chosen to work with freshly isolated strains of *Microcystis* sp.; the experiments started less than a year after strain isolation. Moreover, we studied three strains in parallel, as it was previously shown that different genotypes of *Cylindrospermopsis* or *Microcystis* show widely varying responses following short-term exposure to factors such as light, temperature, partial pressure of carbon dioxide (CO_2) or water turbulence (Li et al., 2018; Pierangelini et al., 2015). The growth rate of the three strains during the 6 months of exposure to 22 and 26°C varied, but the differences between the ambient- and heat-acclimated versions were statistically significant only in the case of strain M11. It is also worth mentioning that, during a follow-up experiment, we have found that the optimum temperature for all three strains (measured in the 20–40°C temperature interval) has shifted, for the 26°C-acclimated versions, with *c.* 3°C towards warmer temperatures as compared to the 22°C-acclimated strains. This emphasises the fact that due to the natural variations between strains, and possibly as a consequence of evolutionary adaptation, cyanobacterial species/strains are highly flexible in their response to various temperature conditions. Genetic modifications must, however, be proven to confirm any adaptive evolution. Although differences between strain response are partly due to genome variation (Willis et al., 2018), rapid evolution cannot be ruled out (Bach et al., 2018; Hutchins et al., 2015). So far, this kind of response was not documented in freshwater microalgae, but only in marine phytoplankton (Padfield et al., 2016; Schaum & Collins, 2014). Nevertheless, genomic data would be necessary to fully confirm this hypothesis, in order to disentangle physiological plasticity from genetic evolution.

The competition experiment was run in four 1000-L land-based mesocosms, two of which were continuously heated up for 4°C above ambient temperature. As a result, and also due to a heatwave, the water in the heated tanks often reached values of 32–33°C after 1 week of exposure. This probably pushed the organisms in the heated mesocosms to their limits, since such temperatures are higher than usual in most lakes from temperate areas. Although these values could be seen as very high even in the worst-case scenario of global warming, our results are relevant because they suggest that even if overall phytoplankton concentration could decrease under such high temperatures, some cyanobacteria are adaptable and therefore able to withstand these fluctuations. We were able to show that treatments with heat-acclimated strains can generate higher cyanobacterial concentrations than treatments with ambient-acclimated lineages. This supports hypotheses that: (1) there are *M. aeruginosa* strains that can adjust to elevated temperatures; and (2)

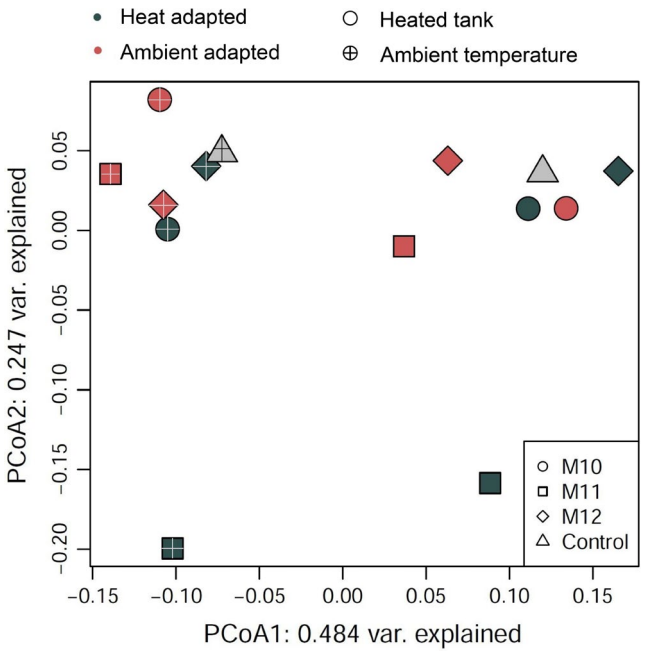

FIGURE 6 Principal coordinates analysis (PCoA) representing the β diversity of the 14 communities at the end of the mesocosm experiment

these strains might display an even higher competitive ability as compared to the ambient-acclimated strains. As for the cyanobacteria in heated mesocosms, decreased growth in weeks 3 and 4 after an initial phase of increased cyanobacterial concentrations in week 1—together with the high temperature profile—indicate that an optimum temperature had been transgressed and cyanobacteria did not recover until the end of the experiment. However, the fact that total chlorophyll-*a* was lower in the heated tanks previously inoculated with heat-adapted cyanobacteria as compared to ambient-adapted strains emphasises the fact that cyanobacterial acclimation to higher temperatures might impact not only on phytoplankton diversity, but also on total algal biomass. This might be a relevant finding considering that microalgae are at the base of aquatic food chain, and requires further study in order to better understand the whole process and its implications. A slightly biased increase of chlorophytes by the AlgaeLabAnalyzer in the ambient tanks cannot be ruled out, since the presence of a different group of algae (belonging to the Zygnematophyceae class) was noticed in some of the dialysis bags displaying the highest concentration of chlorophytes. Nevertheless, the increasing cyanobacterial concentration in the ambient tanks (especially heat-adapted strain M11) shows how plastic cyanobacteria can be. Considering that each strain went, on average, through a series of *c*. 50 generations during the 6-month adaptation experiment, our results suggest either a high evolutionary potential, or an outstanding ability of cyanobacteria to quickly acclimate to high temperatures. Future genomic analyses will have to shed light on this matter. Laboratory experiments have shown that cyanobacterial species typically have optimal growth temperature at higher values than diatoms or dinoflagellates (Griffith & Gobler, 2020). Although there is variation between species, growth rates of cyanobacteria tend also to increase faster with temperature than those of chlorophytes (Visser et al., 2016). This is important, because with global warming, lakes from temperate regions and high latitudes will probably have shorter ice covers during winter, an earlier onset of stratification in spring, warmer summers and prolonged stratification into the autumn (Beaulieu et al., 2013; Michalak et al., 2013). These factors could all contribute to a longer duration and range expansion of CyanoHABs.

The abundance of chlorophytes in the ambient mesocosms increased with time in all dialysis bags, but at a lower degree in those inoculated with heat-acclimated *M. aeruginosa* strains. Thus, the well-growing heat-acclimated *M. aeruginosa* strains seemed to impact the growth of chlorophytes more than the ambient-acclimated strains. This trend was not observed in the heated tanks. This suggests that, while in the heated tanks, temperature was probably the main limiting factor for most microalgae, added cyanobacterial cells had a major influence in chlorophytes concentration in the ambient environments. Since water temperature in ambient mesocosms was on average 2–3°C higher than temperature in the lake (due to the enclosure effect), it is reasonable to assume that cyanobacteria will increase in abundance in the warmer lakes in the future, and that they might play an important role in determining plankton composition of freshwater environments. Other groups of microalgae (cryptophytes, brown groups) did not seem to be affected by the experimental setup, except for dinoflagellates, which were identified only in the control ambient tanks, but not in other treatments.

According to the statistical analyses, a synergistic effect of heat acclimation and tank temperature could not be established after 4 weeks of incubation in mesocosms, probably because the water inside heated mesocosms during weeks 2 and 3 was too warm for all phytoplankton in general. Nevertheless, the factor *heat adaptation* interacted significantly with the factor *time* in shaping the cyanobacterial concentration. Taking all strains together, treatments with heat-acclimated strains displayed significantly higher cyanobacterial concentration than those with ambient-acclimated strains at the end of the experiment ($F = 6.71$, $p = 0.01$; post hoc analyses). Also, treatments in ambient tanks had significantly higher cyanobacterial concentrations than those in heated tanks ($F = 72.58$, $p < 0.001$—Table 1). PCA of the community composition showed clear differences between ambient and heated tank treatments at the beginning of the experiment and their subsequent mixing by the end of the test. The range of algal groups growing in heated mesocosms decreased in size and finally overlapped with the wider range of algal groups growing in ambient tanks by the end of the experiment. This means that heat represented a strong stressor. While distribution of chlorophytes points to the negative direction of the x-axis in weeks 1 and 2, it points to the positive direction in weeks 3 and 4. This suggests that the response of this group to the tank treatments changed fundamentally. Such a change could be a result of added cyanobacteria, as the direction of this group in the PCA plot changed between weeks 2 and 4. However, the distribution of cyanobacteria only accounted for 2%–5% (*y*-axis) of the variance. Hence, chlorophytes reacted even more to temperature than cyanobacteria, this being in

line with previous findings indicating higher growth rates for chlorophytes under certain temperature regimes (Lürling et al., 2013).

DNA metabarcoding data largely supported the phytoplankton grouping based on chlorophyll concentration. A total overlap was however not expected, since the two techniques respond to different questions based on different kind of data. As for cyanobacterial abundance, it was obvious that strain M11 is more flexible than M10 and M12, and that the 26°C-acclimated version can successfully withstand temperatures that are considerably higher than natural values (31–32°C). It is important to mention that strain M11 also had the highest growth rate at the beginning of this project, prior to starting the 6-month growth experiment. Considering this, and also the fact that this was the only strain displaying significant differences in growth rate evolution at two different temperatures, suggests that physiological plasticity might predict the adaptive potential in some cyanobacteria. To the best of our knowledge, this is the first time when a relationship between short-term plasticity and long-term response was observed in cyanobacteria. A similar phenomenon was previously detected in the marine alga *Ostreococcus* sp., where strains with larger plastic response to stressors were shown to increase in abundance in the short term and also evolve more in the long term (Schaum & Collins, 2014).

Proteobacteria, a major phylum of Gram-negative bacteria, dominated the bacterial populations in all dialysis bags, except for those inoculated with heat-acclimated *M. aeruginosa* strain M11, which was in this case dominant. Presence of proteobacteria was expected, as they are widespread in most environments, from freshwater to marine ecosystems, soil, wastewaters, etc. (Drugă et al., 2018; Lokesh & Kiron, 2016). The main difference between ambient and heated tanks, in terms of Proteobacteria, was given by order Elsterales. This is a newly proposed group of microorganisms that is still poorly described. It was detected before in freshwaters, but at much lower concentrations than in our samples (Tee et al., 2020). We identified it almost exclusively in the ambient tanks (up to 26% of total OTUs), suggesting that Elsterales do not acclimate easily to warm environments. By contrast, OTUs belonging to phylum Chloroflexi were found almost exclusively in the heated tanks. This group are both aerobic and anaerobic thermophilic prokaryotes, which could explain their absence from the ambient mesocosms (Sutcliffe, 2010).

We observed a decreased eukaryote species richness in the heated mesocosms, which were largely dominated by gastrotrichs. Zooplankton was present in all samples despite filtering the water through a 250-μm gauze in the beginning of the experiment. Therefore, it cannot be ruled out that zooplankton grazing, such as copepods, or non-selective feeders in ambient mesocosms, (e.g. *Daphnia* sp.) had some effect on the overall taxonomic composition of microalgal communities (Drugă et al., 2016). However, the eukaryotic community composition differed less between treatments with heat/ambient-acclimated strains, and more between treatments in heated/unheated tanks. The high proportion of cyclopoids in ambient mesocosms can be associated to both their plasticity in relation to food (higher biodiversity in ambient tanks), and to physical conditions (temperature that was closer to their natural environment). The increased number of gastrotrichs (which are rather benthic organisms) in the heated mesocosms might be considered as unexpected. This can, however, be explained by the fact that all biomass from dialysis bags was collected for DNA isolation, therefore including benthic organisms. Other data have also pointed out that abundances of freshwater invertebrates tend to abruptly decrease as a result of global warming (Sánchez-Bayo & Wyckhuys, 2019). The DNA data-based PCA plot indicated obvious differences in the overall communities between ambient/heated mesocosms, temperature at the time of experiments being, therefore, the main driver of biodiversity. Nevertheless, the heat-acclimated version of strain M11 also clearly impacted on community structure, this confirming the chlorophyll-related results. Our study therefore emphasises the idea that because of warming, freshwater biodiversity might be under threat at many trophic levels, and the increased abundance of cyanobacteria will probably accelerate this phenomenon.

According to some studies, climate change will act as a single-edged sword, resulting in large increases of community biomass (but not biodiversity) and nutrient use efficiency (Mesquita et al., 2020; Ullah et al., 2018). However, after a closer look into community structure in a warmer climate, the second edge appears, meaning mostly negative effects, in the form of biodiversity loss and an increase in cyanobacteria abundance. The rates of biodiversity loss are already greater in freshwater than terrestrial ecosystems due to human activities (Turak et al., 2017). This is why an increasingly higher abundance of freshwater cyanobacteria triggered by global change might be extremely dangerous for both nature and human society.

Here, a single organism was adapted to increased temperature before being inoculated into a natural community that was not previously exposed to heat. In theory, this might offer the target organism a competitive advantage over the resident community. Thus, it is plausible that the effects of adaptation would have been alleviated in a scenario where all members of the community were previously acclimated. To check for that hypothesis, several other microalgae (e.g. chlorophytes, diatoms) should also be exposed to elevated temperatures before being inoculated into mixed communities. This is the next logical step in such an experimental setup. Nevertheless, the results achieved here are highly relevant as they confirm that potentially toxic cyanobacteria, which already pose a serious threat to freshwater ecosystems, might further increase their dominance in the coming decades.

Importantly, all three strains tested in this study were acclimated to both 22 and 26°C long after the end of the experiments presented here. Thus, after 18 months of heat acclimation (autumn of year 2020), strain M11 could be successfully grown at 38°C, while the ambient-acclimated version did not grow under these conditions. This shows that, over time, increased temperature has an evident positive effect on some *Microcystis* sp. lineages. Considering the much higher number of strains in natural environments, it is reasonable to predict that global warming will have an important positive impact on freshwater cyanobacteria in the future. Our data is also important for informing modellers on the realistic potential on

M. aeruginosa to cope with temperature change, which could be used for more accurate predictions of the ecological niches/regions that might be dominated by these organisms in the future.

Apart from being potentially toxic, cyanobacteria also represent low-quality food for zooplankton. Although variable tolerance to toxic cyanobacteria was previously observed in some zooplankton species (Drugă et al., 2016), it is reasonable to assume that the overall structure of zooplankton populations might be seriously impacted by an increased cyanobacterial concentration in freshwaters. There still remains the question whether cyanobacteria will thrive and impact zooplankton in oligotrophic lakes in the future. Even though recent data suggest that cyanobacterial growth is impaired in low-nutrient environments (Cabrerizo et al., 2020), it was also shown that the ability of some cyanobacteria to utilise nutrients can be improved by increasing CO_2 (Ma et al., 2019). Considering this, together with the predicted increase in CO_2 concentration from 400 ppm in the present up to 1,000 ppm by the end of the 21st century (IPCC Report, 2018), as well as the increased potential of *M. aeruginosa* to cope with change, as shown in this study, it can be expected that cyanobacteria will also adapt to low-nutrient environments. Nevertheless, this hypothesis should be further tested.

This study emphasises the importance of running experiments spanning over several months (and even years) in order to have a grasp of the adaptive potential of microalgae. During short-term experiments, the organisms have limited time to acclimate to the new environments. This, together with the fact that lab conditions might act as a stress factor during the first weeks, might lead to unexpected results that are not environmentally relevant (as observed in this study, with strains M10 and M12 displaying a lower growth rate after 2 months). We have shown that the responses of cyanobacteria to higher temperature differ greatly between strains if they are exposed to these conditions for *c*. 50 generations, and it is likely that these differences would be even stronger after a longer period. Considering all these, perhaps the best way forward is to acknowledge and include such mid/long-term studies into future research programmes. Taking the magnitude of threats by CyanoHABs into account, and also considering the ability of cyanobacteria to react to change in the environment, increasing scientific attention for this topic is highly justified.

In summary, *M. aeruginosa* has a strong potential to cope with warm environments, and this is a strain-specific process. Moreover, the strains with higher physiological plasticity are likely to adjust better in the long term. Unlike ambient-acclimated cyanobacteria, the concentration of heat-acclimated strain M11 was significantly higher 4 weeks after being inoculated into phytoplankton communities. Therefore, apart from the temperature at the time of the experiment, previous exposure to warming is a critical factor in shaping the overall structure of plankton communities. Our results demonstrate the adaptative potential of cyanobacteria to stressors and emphasise the need to consider such experiments for a better understanding of the long-term impact of climate change on aquatic ecosystems.

ACKNOWLEDGMENTS

This work was supported by UEFISCDI, Project Numbers PN-III-P1-1.1 TE 72/2018, Norway Grants Call 2019 (RO-NO 2019) – Collaborative Research Projects, no. 28/2020, and Romanian Ministry of Education and Research (core programme PN2019–2022 – BIODIVERS 3, grant 25N/2019 – BIOSERV). It was also partly funded by a Transnational Access granted to BD through AQUACOSM EU Horizon 2020 INFRAIA-project No 731065 (CyanoWarm), by AQUACOSM-plus (Horizon 2020-EU.1.4.1.2, Grant agreement ID: 871081) and by a DFG grant to M.S.: 1075/1-1 within SPP Dynatrait 1704. E.R. was supported by Lehre@LMU Munich. Dr Andreas Brachman (from the Biocenter of the LMU Munich) is gratefully acknowledged for the technical assistance and the help concerning DNA sequencing. We also thank Dr Charlotte Briddon for the useful comments and for proofreading the manuscript.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ORCID

Bogdan Drugă https://orcid.org/0000-0001-9468-0323

REFERENCES

Albert, J. S., Destouni, G., Duke-Sylvester, S. M., Magurran, A. E., Oberdorff, T., Reis, R. E., Winemiller K. O., & Ripple, W. J. (2020). Scientists' warning to humanity on the freshwater biodiversity crisis. *Ambio*, *50*, 85–94. https://doi.org/10.1007/s13280-020-01318-8

Allen, M. M., & Stanier, R. Y. (1968). Growth and division of some unicellular blue-green algae. *Journal of General Microbiology*, *51*(2), 199–202. https://doi.org/10.1099/00221287-51-2-199

Bach, L. T., Lohbeck, K. T., Reusch, T. B. H., & Riebesell, U. (2018). Rapid evolution of highly variable competitive abilities in a key phytoplankton species. *Nature Ecology and Evolution*, *2*, 611–613. https://doi.org/10.1038/s41559-018-0474-x

Beaulieu, M., Pick, F., & Gregory-Eaves, I. (2013). Nutrients and water temperature are significant predictors of cyanobacterial biomass in a 1147 lakes data set. *Limnology and Oceanography*, *58*, 1736–1746. https://doi.org/10.4319/lo.2013.58.5.1736

Boyd, P. W., Collins, S., Dupont, S., Fabricius, K., Gattuso, J.-P., Havenhand, J., Hutchins, D. A., Riebesell, U., Rintoul, M. S., Vichi, M., Biswas, H., Ciotti, A., Gao, K., Gehlen, M., Hurd, C. L., Kurihara, H., McGraw, C. M., Navarro, J. M., Nilsson, G. E., … Pörtner, H.-O. (2018). Experimental strategies to assess the biological ramifications of multiple drivers of global ocean change—A review. *Global Change Biology*, *24*(6), 2239–2261. https://doi.org/10.1111/gcb.14102

Burford, M. A., Carey, C. C., Hamilton, D. P., Huisman, J., Paerl, H. W., Wood, S. A., & Wulff, A. (2020). Perspective: Advancing the research agenda for improving understanding of cyanobacteria in a future of global change. *Harmful Algae*, *91*, 101601. https://doi.org/10.1016/j.hal.2019.04.004

Cabrerizo, M. J., Álvarez-Manzaneda, M. I., León-Palmero, E., Guerrero-Jiménez, G., de Senerpont Domis, L. N., Teurlincx, S., & González-Olalla, J. M. (2020). Warming and CO_2 effects under oligotrophication on temperate phytoplankton communities. *Water Research*, *173*, 115579. https://doi.org/10.1016/j.watres.2020.115579

Caporaso, J. G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F. D., Costello, E. K., Fierer, N., Gonzalez Peña, A., Goodrich, J. K., Gordon, J. I., Huttley, G. A., Kelley, S. T., Knights, D., Koenig, J. E.,

Ley, R. E., Lozupone, C. A., McDonald, D., Muegge, B. D., Pirrung, M, … Knight, R (2010). Correspondence QIIME allows analysis of high- throughput community sequencing data Intensity normalization improves color calling in SOLiD sequencing. *Nature Methods*, *7*, 335–336. https://doi.org/10.1038/nmeth0510-335

Chapra, S. C., Boehlert, B., Fant, C., Bierman, V. J., Henderson, J., Mills, D., Mas D. M. L., Rennels, L., Jantarasami, L., Martinich, J., Strzepek, K. M., & Paerl, H. W. (2017). Climate change impacts on harmful algal blooms in U.S. freshwaters: A screening-level assessment. *Environmental Science and Technology*, *51*(16), 8933–8943. https://doi.org/10.1021/acs.est.7b01498

Collen, B., Whitton, F., Dyer, E. E., Baillie, J. E. M., Cumberlidge, N., Darwall, W. R. T., Pollock C., Richman, N. I., Soulsby, A. M., & Böhm, M. (2014). Global patterns of freshwater species diversity, threat and endemism. *Global Ecology and Biogeography*, *23*(1), 40–51. https://doi.org/10.1111/geb.12096

Dervaux, J., Mejean, A., & Brunet, P. (2015). Irreversible collective migration of cyanobacteria in eutrophic conditions. *PLoS One*, *10*, 1–16. https://doi.org/10.1371/journal.pone.0120906

Dodds, W. K., Bouska, W. W., Eitzmann, J. L., Pilger, T. J., Pitts, K. L., Riley, A. J., Schloesser J. T. Thornbrugh, D. J. (2009). Eutrophication of U.S. freshwaters: Analysis of potential economic damages. *Environmental Science and Technology*, *43*(1), 12–19. http://dx.doi.org/10.1021/es801217q

Dragoș, N., Peterfi, L. Ş., Momeu, L., & Popescu, C. (1997). *An introduction to the algae and the culture collection of algae at the Institute of Biological Research*. Cluj University Press.

Drugă, B., Buda, D. M., Szekeres, E., Chiş, C., Chiş, I., & Sicora, C. (2019). The impact of cation concentration on *Microcystis* (cyanobacteria) scum formation. *Scientific Reports*, *9*, 1–10. https://doi.org/10.1038/s41598-019-39619-y

Drugă, B., Turko, P., Spaak, P., & Pomati, F. (2016). Cyanobacteria affect fitness and genetic structure of experimental *Daphnia* populations. *Environmental Science and Technology*, *50*, 3416–3424. https://doi.org/10.1021/acs.est.5b05973

Drugă, B., Ukrainczyk, N., Weise, K., Koenders, E., & Lackner, S. (2018). Interaction between wastewater microorganisms and geopolymer or cementitious materials: Biofilm characterization and deterioration characteristics of mortars. *International Biodeterioration and Biodegradation*, *134*, 58–67. http://dx.doi.org/10.1016/j.ibiod.2018.08.005

Duan, H., Ma, R., Xu, X., Kong, F., Zhang, S., Kong, W., Hao J., & Shang, L. (2009). Two-decade reconstruction of algal blooms in China's Lake Taihu. *Environmental Science and Technology*, *43*, 3522–3528. https://doi.org/10.1021/es8031852

Dudgeon, D. (2019). Multiple threats imperil freshwater biodiversity in the Anthropocene. *Current Biology*, *29*, R960–R967. https://doi.org/10.1016/j.cub.2019.08.002

Frank, J. A., Reich, C. I., Sharma, S., Weisbaum, J. S., Wilson, B. A., & Olsen, G. J. (2008). Critical evaluation of two primers commonly used for amplification of bacterial 16S rRNA genes. *Applied and Environmental Microbiology*, *74*, 2461–2470. https://doi.org/10.1128/AEM.02272-07

Griffith, A. W., & Gobler, C. J. (2020). Harmful algal blooms: A climate change co-stressor in marine and freshwater ecosystems. *Harmful Algae*, *91*, 101590. https://doi.org/10.1016/j.hal.2019.03.008

Guo, L. (2007). Doing battle with the Green Monster of Taihu Lake. *Ecology*, *317*, 1166. https://doi.org/10.1126/science.317.5842.1166

Hadziavdic, K., Lekang, K., Lanzen, A., Jonassen, I., Thompson, E. M., & Troedsson, C. (2014). Characterization of the 18s rRNA gene for designing universal eukaryote specific primers. *PLoS One*, *9*, e87624. https://doi.org/10.1371/journal.pone.0087624

Hoekstra, A. Y., & Mekonnen, M. M. (2012). The water footprint of humanity. *Proceedings of the National Academy of Sciences of the United States of America*, *109*, 3232–3237. https://doi.org/10.1073/pnas.1109936109

Huisman, J., Codd, G. A., Paerl, H. W., Ibelings, B. W., Verspagen, J. M. H., & Visser, P. M. (2018). Cyanobacterial blooms. *Nature Reviews Microbiology*, *16*, 471–483. https://doi.org/10.1038/s41579-018-0040-1

Hutchins, D. A., Walworth, N. G., Webb, E. A., Saito, M. A., Moran, D., McIlvin, M. R., Gale J., & Fu, F. X. (2015). Irreversibly increased nitrogen fixation in *Trichodesmium* experimentally adapted to elevated carbon dioxide. *Nature Communications*, *6*(1), 1–7. http://dx.doi.org/10.1038/ncomms9155

IPCC. (2018). Summary for Policymakers. In: Global warming of 1.5°C. An IPCC Special Report on the impacts of global warming of 1.5°C above pre-industrial levels and related global greenhouse gas emission pathways, in the context of strengthening the global response to the threat of climate change, sustainable development, and efforts to eradicate poverty. World Meteorological Organization, Geneva, Switzerland.

Ji, X., Verspagen, J. M. H., Stomp, M., & Huisman, J. (2017). Competition between cyanobacteria and green algae at low versus elevated CO_2: Who will win, and why? *Journal of Experimental Botany*, *68*, 3815–3828. https://doi.org/10.1093/jxb/erx027

Jöhnk, K. D., Huisman, J., Sharples, J., Sommeijer, B., Visser, P. M., & Stroom, J. M. (2008). Summer heatwaves promote blooms of harmful cyanobacteria. *Global Change Biology*, *14*, 495–512. https://doi.org/10.1111/j.1365-2486.2007.01510.x

Kahru, M., & Elmgren, R. (2014). Multidecadal time series of satellite-detected accumulations of cyanobacteria in the Baltic Sea. *Biogeosciences*, *11*, 3619–3633. https://doi.org/10.5194/bg-11-3619-2014

Lakeman, M. B., von Dassow, P., & Cattolico, R. A. (2009). The strain concept in phytoplankton ecology. *Harmful Algae*, *8*, 746–758. https://doi.org/10.1016/j.hal.2008.11.011

Li, M., Xiao, M., Zhang, P., & Hamilton, D. P. (2018). Morphospecies-dependent disaggregation of colonies of the cyanobacterium *Microcystis* under high turbulent mixing. *Water Research*, *141*, 340–348. https://doi.org/10.1016/j.watres.2018.05.017

Litchman, E., de Tezanos Pinto, P., Edwards, K. F., Klausmeier, C. A., Kremer, C. T., & Thomas, M. K. (2015). Global biogeochemical impacts of phytoplankton: A trait-based perspective. *Journal of Ecology*, *103*, 1384–1396. https://doi.org/10.1111/1365-2745.12438

Lokesh, J., & Kiron, V. (2016). Transition from freshwater to seawater reshapes the skin-associated microbiota of Atlantic salmon. *Scientific Reports*, *6*, 1–10. https://doi.org/10.1038/srep19707

Lozupone, C., & Knight, R. (2005). UniFrac: A new phylogenetic method for comparing microbial communities. *Applied and Environmental Microbiology*, *71*, 8228–8235. https://doi.org/10.1128/AEM.71.12.8228-8235.2005

Lürling, M., Eshetu, F., Faassen, E. J., Kosten, S., & Huszar, V. L. M. (2013). Comparison of cyanobacterial and green algal growth rates at different temperatures. *Freshwater Biology*, *58*, 552–559. https://doi.org/10.1111/j.1365-2427.2012.02866.x

Ma, J., Wang, P., Wang, X., Xu, Y., & Paerl, H. W. (2019). Cyanobacteria in eutrophic waters benefit from rising atmospheric CO_2 concentrations. *Science of the Total Environment*, *691*, 1144–1154. https://doi.org/10.1016/j.scitotenv.2019.07.056

Mesquita, M. C. B., Prestes, A. C. C., Gomes, A. M. A., & Marinho, M. M. (2020). Direct effects of temperature on growth of different tropical phytoplankton species. *Microbial Ecology*, *79*, 1–11. https://doi.org/10.1007/s00248-019-01384-w

Michalak, A. M., Anderson, E. J., Beletsky, D., Boland, S., Bosch, N. S., Bridgeman, T. B., Chaffin, J. D., Cho, K., Confesor, R., Daloglu, I., DePinto, J. V., Evans, M. A., Fahnenstiel, G. L., He, L., Ho, J. C., Jenkins, L., Johengen, T. H., Kuo, K. C., LaPorte, E., … Zagorski, M. A. (2013). Record-setting algal bloom in Lake Erie caused by agricultural and meteorological trends consistent with expected future conditions. *Proceedings of the National Academy of Sciences of the United States of America*, *110*(16), 6448–6452. http://dx.doi.org/10.1073/pnas.1216006110

Mock, T., Daines, S. J., Geider, R., Collins, S., Metodiev, M., Millar, A. J., Moulton V., & Lenton, T. M. (2016). Bridging the gap between omics and earth system science to better understand how environmental change impacts marine microbes. *Global Change Biology*, *22*(1), 61–75. http://dx.doi.org/10.1111/gcb.12983

Monchamp, M. E., Spaak, P., Domaizon, I., Dubois, N., Bouffard, D., & Pomati, F. (2018). Homogenization of lake cyanobacterial communities over a century of climate change and eutrophication. *Nature Ecology and Evolution*, *2*, 317–324. https://doi.org/10.1038/s41559-017-0407-0

Padfield, D., Yvon-Durocher, G., Buckling, A., Jennings, S., & Yvon-Durocher, G. (2016). Rapid evolution of metabolic traits explains thermal adaptation in phytoplankton. *Ecology Letters*, *19*, 133–142. https://doi.org/10.1111/ele.12545

Pierangelini, M., Sinha, R., Willis, A., Burford, M. A., Orr, P. T., Beardall, J., Neilan, B. A. (2015). Constitutive cylindrospermopsin pool size in *Cylindrospermopsis raciborskii* under different light and CO_2 partial pressure conditions. *Applied and Environmental Microbiology*, *81*, 3069–3076. https://doi.org/10.1128/AEM.03556-14

Sánchez-Bayo, F., & Wyckhuys, K. A. G. (2019). Worldwide decline of the entomofauna: A review of its drivers. *Biological Conservation*, *232*, 8–27. https://doi.org/10.1016/j.biocon.2019.01.020

Schaum, E., & Collins, S. (2014). Plasticity predicts evolution in a marine alga. *Proceedings of the Royal Society B: Biological Sciences*, *281*, 20141486. https://doi.org/10.1098/rspb.2014.1486

Soares, M. C. S., Lürling, M., & Huszar, V. L. M. (2013). Growth and temperature-related phenotypic plasticity in the cyanobacterium *Cylindrospermopsis raciborskii*. *Phycological Research*, *61*, 61–67. https://doi.org/10.1111/pre.12001

Spatharis, S., Skliris, N., Meziti, A., & Kormas, K. A. (2012). First record of a *Trichodesmium erythraeum* bloom in the Mediterranean Sea. *Canadian Journal of Fisheries and Aquatic Sciences*, *69*, 1444–1455. https://doi.org/10.1139/F2012-020

Stibor, H., Stockenreiter, M., Nejstgaard, J. C., Ptacnik, R., & Sommer, U. (2019). Trophic switches in pelagic systems. *Current Opinion in Systems Biology*, *13*, 108–114. https://doi.org/10.1016/j.coisb.2018.11.006

Sutcliffe, I. C. (2010). A phylum level perspective on bacterial cell envelope architecture. *Trends in Microbiology*, *18*, 464–470. https://doi.org/10.1016/j.tim.2010.06.005

Symes, E., & van Ogtrop, F. F. (2019). The effect of pre-industrial and predicted atmospheric CO_2 concentrations on the development of diazotrophic and non-diazotrophic cyanobacterium: *Dolichospermum circinale* and *Microcystis aeruginosa*. *Harmful Algae*, *88*, 101536. https://doi.org/10.1016/j.hal.2018.10.005

Tang, Y., Horikoshi, M., & Li, W. (2016). Ggfortify: Unified interface to visualize statistical results of popular R packages. *The R Journal*, *8*, 478–489. https://doi.org/10.32614/rj-2016-060

Taranu, Z. E., Gregory-Eaves, I., Leavitt, P. R., Bunting, L., Buchaca, T., Catalan, J., Domaizon I., Guilizzoni, P., Lami, A., McGowan, S., Moorhouse, H., Morabito, G., Pick, F. R., Stevenson, M. A., Thompson, P. L., & Vinebrooke, R. D. (2015). Acceleration of cyanobacterial dominance in north temperate-subarctic lakes during the Anthropocene. *Ecology Letters*, *18*(4), 375–384. http://dx.doi.org/10.1111/ele.12420

Tee, H. S., Waite, D., Payne, L., Middleditch, M., Wood, S., & Handley, K. M. (2020). Tools for successful proliferation: Diverse strategies of nutrient acquisition by a benthic cyanobacterium. *ISME Journal*, *14*, 2164–2178. https://doi.org/10.1038/s41396-020-0676-5

Thomas, M. K., Kremer, C. T., Klausmeier, C. A., & Litchman, E. (2012). A global pattern of thermal adaptation in marine phytoplankton. *Science*, *338*, 1085–1088. https://doi.org/10.1126/science.1224836

Thomas, M. K., & Litchman, E. (2016). Effects of temperature and nitrogen availability on the growth of invasive and native cyanobacteria. *Hydrobiologia*, *763*, 357–369. https://doi.org/10.1007/s10750-015-2390-2

Turak, E., Harrison, I., Dudgeon, D., Abell, R., Bush, A., Darwall, W., Finlayson C. M., Ferrier, S., Freyhof, J., Hermoso, V., Juffe-Bignoli, D., Linke, S., Nel, J., Patricio, H. C., Pittock, J., Raghavan, R., Revenga, C., Simaika, J. P., & De Wever, A. (2017). Essential biodiversity variables for measuring change in global freshwater biodiversity. *Biological Conservation*, *213*, 272–279. http://dx.doi.org/10.1016/j.biocon.2016.09.005

Ullah, H., Nagelkerken, I., Goldenberg, S. U., & Fordham, D. A. (2018). Climate change could drive marine food web collapse through altered trophic flows and cyanobacterial proliferation. *PLoS Biology*, *16*, 1–21. https://doi.org/10.1371/journal.pbio.2003446

Visser, P. M., Verspagen, J. M. H., Sandrini, G., Stal, L. J., Matthijs, H. C. P., Davis, T. W., Paerl H. W., & Huisman, J. (2016). How rising CO_2 and global warming may stimulate harmful cyanobacterial blooms. *Harmful Algae*, *54*, 145–159. http://dx.doi.org/10.1016/j.hal.2015.12.006

Wells, M. L., Trainer, V. L., Smayda, T. J., Karlson, B. S. O., Trick, C. G., Kudela, R. M., Ishikawa A., Bernard, S., Wulff, A., Anderson, D. M., & Cochlan, W. P. (2015). Harmful algal blooms and climate change: Learning from the past and present to forecast the future. *Harmful Algae*, *49*, 68–93. http://dx.doi.org/10.1016/j.hal.2015.07.009

Willis, A., Woodhouse, J. N., Ongley, S. E., Jex, A. R., Burford, M. A., & Neilan, B. A. (2018). Genome variation in nine co-occurring toxic *Cylindrospermopsis raciborskii* strains. *Harmful Algae*, *73*, 157–166. https://doi.org/10.1016/j.hal.2018.03.001

Wilson, A. E., Wilson, W. A., & Hay, M. E. (2006). Intraspecific variation in growth and morphology of the bloom-forming cyanobacterium *Microcystis aeruginosa*. *Applied and Environmental Microbiology*, *72*, 7386–7389. https://doi.org/10.1128/AEM.00834-06

Yan, D., Xu, H., Yang, M., Lan, J., Hou, W., Wang, F., Zhang J., Zhou, K., An, Z., & Goldsmith, Y. (2019). Responses of cyanobacteria to climate and human activities at Lake Chenghai over the past 100 years. *Ecological Indicators*, *104*, 755–763. http://dx.doi.org/10.1016/j.ecolind.2019.03.019

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

How to cite this article: Drugă, B., Ramm, E., Szekeres, E., Chiriac, C., Hegedüs, A., & Stockenreiter, M. (2021). Long-term acclimation might enhance the growth and competitive ability of *Microcystis aeruginosa* in warm environments. *Freshw Biol*, 00, 1–14. https://doi.org/10.1111/fwb.13865