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Phenotypic plasticity of carbon fixation stimulates cyanobacterial blooms at elevated CO2

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Although phenotypic plasticity is a widespread phenomenon, its implications for species responses to climate change are not well understood. For example, toxic cyanobacteria can form dense surface blooms threatening water quality in many eutrophic lakes, yet a theoretical framework to predict how phenotypic plasticity affects bloom development at elevated pCO2 is still lacking. We measured phenotypic plasticity of the carbon fixation rates of the common bloom-forming cyanobacterium Microcystis. Our results revealed a 1.8- to 5-fold increase in the maximum CO2 uptake rate of Microcystis at elevated pCO2, which exceeds CO2 responses reported for other phytoplankton species. The observed plasticity was incorporated into a mathematical model to predict dynamic changes in cyanobacterial abundance. The model was successfully validated by laboratory experiments and predicts that acclimation to high pCO2 will intensify Microcystis blooms in eutrophic lakes. These results indicate that this harmful cyanobacterium is likely to benefit strongly from rising atmospheric pCO2.

INTRODUCTION

Phenotypic plasticity is one of the major challenges in the study of how organisms will respond to environmental change (1–3). Phenotypic plasticity implies that the traits of organisms are not constant, as the same genotype may display different phenotypes depending on the prevailing environmental conditions. For example, the temperature and CO2 response of a species may vary depending on the climatic conditions to which individuals of this species have been previously exposed (3, 4). Taking the phenotypic plasticity of traits into account is therefore essential for accurate predictions of how species will respond to climate change.

Cyanobacterial blooms appear to increase in frequency, intensity, and duration in many eutrophic lakes, reservoirs, and estuaries, often in association with increasing nutrient loads, changes in land use, and global warming (5–8). Dense cyanobacterial blooms can have severe environmental impacts, as bloom-forming species are able to produce a variety of potent toxins affecting birds and mammals including humans, and may therefore negatively affect the use of water for recreation, drinking water, and fisheries (8–10). Recent research indicates that surface blooms of cyanobacteria may benefit not only from high temperatures but also directly from the increase in partial pressure of carbon dioxide (pCO2) in the atmosphere (11–13). The photosynthetic activity of dense cyanobacterial blooms depletes dissolved CO2 concentrations in the upper water column and increases pH (11, 14), thereby shifting the inorganic carbon equilibrium toward bicarbonate (HCO3−) and carbonate (CO32−). Rising atmospheric pCO2 in combination with depletion of the dissolved CO2 concentration by surface blooms will increase the pCO2 gradient across the air-water interface. This may result in an enhanced influx of CO2 into the water column (15) that fuels the photosynthetic carbon fixation of cyanobacterial blooms (11, 13).

Phenotypic plasticity is likely to play an important role in the response of cyanobacterial blooms to rising CO2. Cyanobacteria use a sophisticated CO2-concentrating mechanism (CCM) (16, 17). Most inorganic carbon taken up by the cells is first converted to bicarbonate and then transported to cellular compartments called carboxysomes, where bicarbonate is converted back to CO2 and fixed into organic carbon by the RubisCO enzyme. The cyanobacterial CCM comprises up to five different carbon uptake systems, including two CO2 uptake systems (NDH-13 and NDH-14) and three bicarbonate uptake systems (BCT1, BicA, and SbtA) (18). These uptake systems differ in kinetic properties. For example, BicA has a low affinity for bicarbonate but high flux rate, whereas SbtA has a high affinity but low flux rate (18, 19). Moreover, cyanobacteria may combine and regulate these uptake systems in different ways (12, 18–20). Also, the number of carboxysomes per cell may vary in response to changes in CO2 availability (21). This flexibility of the CCM creates the potential for a high degree of phenotypic plasticity of carbon fixation rates in cyanobacterial blooms.

Thus far, phenotypic plasticity of CO2 and bicarbonate uptake kinetics have been quantified for only a limited number of cyanobacteria. This includes the freshwater laboratory strains Synechocystis PCC 6803 (22) and Synechococcus PCC 7942 (23, 24), as well as the marine cyanobacteria Trichodesmium IMS101 (25) and Prochlorococcus MED4 (26). However, phenotypic plasticity of the carbon uptake kinetics of bloom-forming freshwater cyanobacteria is essentially unknown, and a methodology to incorporate this plasticity into predictive models of cyanobacterial and other harmful algal blooms is still lacking.

Here, we develop a novel theoretical framework to predict how phenotypic plasticity will affect the proliferation of cyanobacteria in response to rising atmospheric pCO2. For this purpose, we investigate phenotypic plasticity of the carbon uptake kinetics of Microcystis, one of the most ubiquitous and notorious bloom-forming cyanobacteria (8, 27). Two toxic Microcystis strains were cultured in the laboratory to measure their CO2 and bicarbonate uptake kinetics after acclimation to low and to high pCO2 (Fig. 1, A and B). The observed phenotypic plasticity of the kinetic parameters was implemented in a mathematical model to predict cyanobacterial growth and dynamic changes in CO2 uptake and inorganic carbon chemistry (Fig. 1, C and D). The model predictions were validated using controlled laboratory chemostat experiments exposed to low and to
These pCO₂ settings reflect the wide variation in dissolved CO₂ concentrations in lakes, ranging from CO₂-undersaturated to CO₂-supersaturated waters (11, 28). After the chemostats were in steady state for ~20 days, we took samples to measure CO₂ and bicarbonate uptake kinetics of the Microcystis cells with a membrane inlet mass spectrometer (MIMS) using a chemical disequilibrium assay (29, 30). With a dilution rate of 0.2 day⁻¹, the experiments allowed for ~6 generations of physiological acclimation to the imposed CO₂ conditions, which is comparable to the time scale of cyanobacterial bloom development. The results show that the carbon uptake kinetics of Microcystis cells acclimated to high pCO₂ were very different from the uptake kinetics of cells acclimated to low pCO₂ (Fig. 2 and fig. S1 for Microcystis PCC 7806 and PCC 7941, respectively).

To quantify the uptake kinetics, the measured CO₂ and bicarbonate uptake rates (V) were fitted to the Michaelis-Menten equation

\[ V = \frac{V_{\text{max}} [C]}{K_{\text{sat}} + [C]} \]

where [C] is the CO₂ or bicarbonate concentration, \( V_{\text{max}} \) is the maximum uptake rate, and \( K_{\text{sat}} \) is the half-saturation constant (i.e., the carbon concentration at which the uptake rate equals half of \( V_{\text{max}} \)). The ratio \( V_{\text{max}}/K_{\text{sat}} \) represents the initial slope of the uptake kinetics at \([C] = 0\) and provides a measure of the uptake efficiency at low carbon concentration.

For Microcystis PCC 7806, the maximum net CO₂ uptake rate (\( V_{\text{max},\text{CO}_2,\text{net}} \)) was more than five times higher at high pCO₂ than at low pCO₂, whereas the maximum bicarbonate uptake rate (\( V_{\text{max},\text{HCO}_3} \)) was not significantly affected by pCO₂ (Fig. 2; Fig. 3, A and B). Half-saturation constants for CO₂ and bicarbonate uptake were both significantly higher in cells acclimated to high pCO₂ (Fig. 3, C and D). The CO₂ uptake efficiency (i.e., the initial slopes in the insets of Fig. 2, A and B) was not much affected by pCO₂. The bicarbonate uptake efficiency, however, was almost three times lower at high pCO₂ than at low pCO₂ (insets in Fig. 2, C and D).

For Microcystis PCC 7941, \( V_{\text{max},\text{CO}_2,\text{net}} \) was 1.8 times higher, whereas \( V_{\text{max},\text{HCO}_3} \) was ~40% lower at high than at low pCO₂ (fig. S1 and Fig. 3, A and B). Its half-saturation constants for CO₂ and bicarbonate uptake were not significantly affected by pCO₂ (Fig. 3, C and D).

Hence, after acclimation to high pCO₂, both Microcystis strains achieved much higher maximum CO₂ uptake rates, but they became less efficient in bicarbonate uptake through either an increased half-saturation constant or a reduced maximum bicarbonate uptake rate.

**Modeling phenotypic plasticity**

We incorporated the observed plasticity of the carbon uptake kinetics into a mathematical model. The model considers quantitative plastic traits and assumes that trait values dynamically adjust to the prevailing environmental conditions. If \( x \) denotes the value of a plastic trait (such as \( V_{\text{max}} \) and \( K_{\text{sat}} \)), then dynamic changes of this trait value are described as

\[ \frac{dx}{dt} = c[f(CO₂) - x(t)] \]

where the function \( f(CO₂) \) describes the acclimated trait value as a function of the dissolved CO₂ concentration, \( x(t) \) is the actual trait value at time \( t \), and \( c \) is the acclimation rate. The function \( f(CO₂) \) is replaced by \( f[HCO₃⁻] \) for traits involved in bicarbonate uptake.

### RESULTS

**Phenotypic plasticity of carbon uptake kinetics**

*Microcystis* strains PCC 7806 and PCC 7941 were grown in laboratory chemostats provided with nutrient-rich medium and aerated with either low pCO₂ [100 parts per million (ppm)] or high pCO₂ (1000 ppm).
We assume that the function $f$ has an S-shaped form, such that the acclimated trait value is bound between physiological limits, $x_{\text{low}}$ and $x_{\text{high}}$ (Fig. 1C; see section S2 for details).

All traits significantly affected by $p\text{CO}_2$ in the experiments are considered to be plastic. For instance, in our application, the plastic traits of *Microcystis* PCC 7806 are its maximum CO$_2$ uptake rate and half-saturation constants for CO$_2$ and bicarbonate (Fig. 3). The physiological limits of these traits are set at the trait values observed at low and at high $p\text{CO}_2$.

The resultant description of the phenotypic plasticity of the carbon uptake kinetics was combined with dynamic equations describing population growth and the feedbacks of population growth on inorganic carbon chemistry, light, nutrients, pH, and alkalinity (see section S2 for details).

**Validating the model in laboratory experiments**

To test the model predictions, we ran duplicate chemostat experiments with *Microcystis* PCC 7806 at both low and high $p\text{CO}_2$ (Fig. 1A).

The *Microcystis* populations increased during the first 2 weeks of the experiments, reducing light availability, modifying inorganic carbon chemistry, and increasing pH, after which the cyanobacterial populations and other experimental variables approached a steady state (Fig. 4). The steady states were maintained for several weeks. *Microcystis* PCC 7806 produced much higher steady-state population densities at high than at low $p\text{CO}_2$ (Fig. 4, A and B, and table S1). In the experiments at low $p\text{CO}_2$, the photosynthetic activity of *Microcystis* depleted the dissolved CO$_2$ concentration to the nanomolar range and diminished the bicarbonate concentration to ~$10 \mu\text{M}$, while carbonate became the dominant inorganic carbon species (Fig. 4C). This was accompanied by a strong pH increase to ~11 (Fig. 4E). Conversely, in the experiments at high $p\text{CO}_2$, dissolved CO$_2$ was maintained at ~$10 \mu\text{M}$, bicarbonate increased to ~$3000 \mu\text{M}$, and pH remained <9 (Fig. 4, D and F).

Implementing the CO$_2$ and bicarbonate concentrations observed at steady state (Fig. 4) into the measured carbon uptake kinetics (Fig. 2) shows that, since the dissolved CO$_2$ concentration was...
density if the carbon uptake kinetics were acclimated to low \( \text{CO}_2 \), high \( \text{CO}_2 \), the chemostats quite well, both for the experiments at low and at states of population density, pH, and inorganic carbon chemistry in uptake.

The reaction norms show the plasticity of maximum uptake rates of (\( V_{\text{max,CO}_2} \)) and (\( V_{\text{max,HC}O_3} \)) and the plasticity of half-saturation constants for (\( K_{\text{CO}_2} \)) and (\( K_{\text{HC}O_3} \)) in response to acclimation to either low or high \( \text{CO}_2 \). Data points show parameter values ± SD obtained by Michaelis-Menten fits to replicated measurements of the carbon uptake kinetics (see Fig. 2 and fig. S1; \( n = 4 \) for low \( \text{CO}_2 \) and \( n = 3 \) to 4 for high \( \text{CO}_2 \)). Significant differences between parameter values at low \( \text{CO}_2 \) and high \( \text{CO}_2 \) were assessed using the independent-samples \( t \) test corrected for multiple hypothesis testing (**\(* < 0.001; * < 0.05; \text{n.s.}, \text{not significant})). Statistical details are reported in table S3.

The model predictions captured the time courses and steady states of population density, pH, and inorganic carbon chemistry in the chemostats quite well, both for the experiments at low and at high \( \text{CO}_2 \) (Fig. 4). Similar results were obtained for the other strain, \( \text{Microcystis} \) PCC 7941 (fig. S2). These results provide proof of principle that, at least under controlled laboratory conditions, measurements of the phenotypic plasticity of carbon fixation rates can be used to quantitatively predict the growth of cyanobacteria at different \( \text{CO}_2 \) levels.

The model can be used to quantify how the observed phenotypic plasticity affected cyanobacterial growth. For the experiments at low \( \text{CO}_2 \), model simulations predicted a 14.7% higher population density if the carbon uptake kinetics were acclimated to low \( \text{CO}_2 \) than if they were acclimated to high \( \text{CO}_2 \) (compare green and gray lines in Fig. 4A). Conversely, for the experiments at high \( \text{CO}_2 \), model simulations predicted a 17.3% higher population density if the carbon uptake kinetics were acclimated to high \( \text{CO}_2 \) than if they were acclimated to low \( \text{CO}_2 \) (Fig. 4B). Hence, phenotypic plasticity of the carbon uptake kinetics enhanced the population densities of \( \text{Microcystis} \).

Extrapolation to lakes

To estimate how phenotypic plasticity will affect the response of \( \text{Microcystis} \) blooms in lakes to rising atmospheric \( \text{CO}_2 \), we up-scaled the model from laboratory chemostats to eutrophic lakes. For this purpose, we used the measured carbon uptake kinetics of \( \text{Microcystis} \), but adjusted system parameters such as mixing depth, light intensity, and dilution rate to a lake context (section S3 and table S6). We compared model predictions for one plastic and two fixed phenotypes of \( \text{Microcystis} \). The “plastic phenotype” displayed the phenotypic plasticity of \( \text{Microcystis} \) PCC 7806, the “low \( \text{CO}_2 \) phenotype” maintained the uptake kinetics measured in the low \( \text{CO}_2 \) chemostat, and the “high \( \text{CO}_2 \) phenotype” maintained the uptake kinetics measured at high \( \text{CO}_2 \).
and at low atmospheric $pCO_2$, whereas it will display carbon uptake kinetics resembling the high $pCO_2$ phenotype in high-alkaline lakes and at high atmospheric $pCO_2$. In this way, the plastic phenotype is able to maximize its bloom size across the entire $pCO_2$ gradient, with population densities matching those of the low $pCO_2$ phenotype at low $pCO_2$ and those of the high $pCO_2$ phenotype at high $pCO_2$ (Fig. 5A). As a consequence, rising atmospheric $pCO_2$ is predicted to intensify *Microcystis* blooms across a wide range of lakes (Fig. 5E).

**DISCUSSION**

Our results show a 1.8- to 5-fold increase of the maximum CO$_2$ uptake rate of *Microcystis* at elevated $pCO_2$, which greatly exceeds plasticity of the CO$_2$ uptake rates reported for other freshwater cyanobacteria and green algae (Table 1) and a wide variety of marine phytoplankton species (31). This high flexibility in CO$_2$ uptake rates is most likely an adaptation to the major changes in CO$_2$ availability that can be encountered during dense cyanobacterial blooms. Dissolved CO$_2$ concentrations can change from air-equilibrated or even CO$_2$-supersaturated concentrations at the onset of phytoplankton blooms to complete depletion of the available CO$_2$ in fully developed phytoplankton blooms (11, 14, 32). CO$_2$ depletion is accompanied by an increase in pH, which shifts the inorganic carbon chemistry toward bicarbonate. Sustained high carbon fixation rates by very dense cyanobacterial blooms may even decrease the bicarbonate concentration and increase pH to $>$10 (14, 20), shifting the balance from bicarbonate to carbonate as the dominant inorganic carbon species (as in Fig. 4C).

In our chemostat experiments, physiological acclimation occurred within a few weeks, on a similar time scale as the changes in inorganic carbon chemistry induced by the growing cyanobacterial populations. These results imply that the carbon uptake kinetics of cyanobacteria can change drastically during bloom development, from cells with high maximum CO$_2$ uptake rates but low bicarbonate uptake efficiency when dissolved CO$_2$ concentrations are still high at the onset of a cyanobacterial bloom, to cells with low maximum CO$_2$ uptake rates but high bicarbonate uptake efficiency at the peak of the bloom when dissolved CO$_2$ concentrations have been depleted.

The high maximum CO$_2$ uptake rate at elevated $pCO_2$ is offset by down-regulation of the bicarbonate uptake efficiency. Previous results (33) have shown that the high-affinity bicarbonate uptake system BCT1 of *Microcystis* strains is strongly down-regulated at elevated $pCO_2$, which is a sensible response in view of the energetic costs of this adenosine triphosphate (ATP)-dependent uptake system and likely explains the low bicarbonate uptake efficiency at elevated $pCO_2$ in our experiments. The maximum bicarbonate uptake rates of the two *Microcystis* strains in our study responded differently to elevated $pCO_2$, which may be related to the different composition of their bicarbonate uptake systems. Cyanobacteria in which maximum bicarbonate uptake rates were reduced at elevated $pCO_2$ ([*Microcystis* PCC 7941 (19), *Synechocystis* PCC 6803 (22), and *Synechococcus* PCC 7942 (23, 24)]) all contain the low-flux bicarbonate transporter SbtA, whereas cyanobacteria in which maximum bicarbonate uptake rates did not respond to changes in $pCO_2$ ([*Microcystis* PCC 7806 (19) and *Trichodesmium erythraeum* IMS101 (25)]) all contain the high-flux bicarbonate transporter BicA but lack SbtA. Since carbon uptake kinetics have thus far been investigated for only a few cyanobacteria, these associations should be interpreted with some caution. However, down-regulation of the bicarbonate uptake efficiency by a higher
half-saturation constant and/or lower maximum bicarbonate uptake rate has also been reported for green algae (Table 1) and diatoms (34, 35) and, hence, seems a common response to elevated pCO2.

Phenotypic plasticity complicates assessments of how natural communities will respond to climate change (1–4), because species responses obtained for one set of environmental conditions may deviate from those obtained for other conditions. Our study illustrates that phenotypic plasticity can be built successfully into models. The model predicts an increase in the maximum CO2 uptake rate and that phenotypic plasticity can be built successfully into models. The model reveals the adaptive significance of this phenotypic plasticity, as physiological acclimation to the prevailing CO2 conditions (29, 30). In total, these results provide strong agreement with our experimental results (Figs. 4 and 5). Furthermore, the model reveals the adaptive significance of this phenotypic plasticity, as physiological acclimation to the prevailing pCO2 conditions enhanced the size of the Microcystis population [compare the gray and green lines in Figs. 4 (A and B) and 5A]. Enhanced growth obtained through physiological acclimation may provide a competitive advantage, which likely explains why, in recent competition experiments, Microcystis became a strong competitor in comparison to green algae at elevated pCO2 (36). In total, these results provide strong evidence that the high phenotypic plasticity of the carbon uptake kinetics of Microcystis will stimulate the development of Microcystis blooms at elevated pCO2.

In addition to phenotypic plasticity, evolutionary changes are also likely to affect the carbon uptake kinetics of future cyanobacterial blooms. Shifts in genotype composition have been found in selection experiments with multiple strains of Microcystis (12). Furthermore, evolutionary changes in CO2 uptake rates and growth kinetics have been observed in long-term phytoplankton studies exposed to elevated CO2 for hundreds to thousands of generations (37, 38). The short time span of our single-strain experiments did not provide much time for de novo mutations and subsequent selection, however. Therefore, the observed changes in our study presumably did not involve evolutionary changes in the carbon uptake kinetics but indeed derived from a high phenotypic plasticity of these traits.

While our study focused on the carbon uptake kinetics of one of the most notorious bloom-forming cyanobacteria, other relevant processes known to affect cyanobacterial blooms were left out of the equations. For example, in addition to rising atmospheric pCO2, cyanobacterial blooms will also be affected by global warming (5, 6, 13). Higher temperatures will enhance the growth rate of Microcystis but reduce the solubility of CO2, both in nonlinear ways. How this will play out is difficult to predict without dedicated models. Other important processes affecting bloom development include nutrient limitation, lake stratification, and interactions with viruses and other organisms in the aquatic food web (8). Hence, our model does not attempt to describe the full complexity of the natural world. However, our model and its experimental validation do provide an important first step for the implementation of phenotypic plasticity in ecosystem models that do take these other processes into account. In conclusion, our results demonstrate that incorporation of the phenotypic plasticity of traits improves predictions of species responses to climate change. More specifically, we found that the high

### Table 1. Comparison of the carbon uptake kinetics of freshwater cyanobacteria and green algae measured by MIMS using the chemical disequilibrium assay (29, 30). Units for \( k_{1/2} \) and \( V_{\text{max}} \) are \( \mu \text{M} \) and \( \mu \text{mol C (mg chl \( \alpha \))}^{-1} \) hour\(^{-1} \), respectively.

<table>
<thead>
<tr>
<th>Species</th>
<th>Net CO2 uptake</th>
<th>Bicarbonate uptake</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( k_{1/2,CO2} ) &amp; ( V_{\text{max,CO2,net}} )</td>
<td>( k_{1/2,HCO3} ) &amp; ( V_{\text{max,HCO3}} )</td>
</tr>
<tr>
<td></td>
<td>Low pCO2 &amp; High pCO2</td>
<td>Low pCO2 &amp; High pCO2</td>
</tr>
<tr>
<td><strong>Cyanobacteria</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Microcystis aeruginosa</em> PCC 7806</td>
<td>0.4 &amp; 1.6 &amp; 23 &amp; 118</td>
<td>5 &amp; 12 &amp; 112 &amp; 103</td>
</tr>
<tr>
<td><em>Microcystis aeruginosa</em> PCC 7941</td>
<td>0.6 &amp; 0.9 &amp; 73 &amp; 134</td>
<td>8 &amp; 9 &amp; 92 &amp; 55</td>
</tr>
<tr>
<td><em>Synechococcus</em> PCC 7942</td>
<td>0.4 &amp; 5.6 &amp; 165 &amp; 110</td>
<td>15 &amp; 270 &amp; 224 &amp; 126</td>
</tr>
<tr>
<td><em>Synechococcus</em> PCC 7942</td>
<td>0.5 &amp; 3.6 &amp; 103 &amp; 116</td>
<td>9 &amp; 248 &amp; 230 &amp; 206</td>
</tr>
<tr>
<td><em>Synechocystis</em> PCC 6803</td>
<td>1 &amp; 3.5 &amp; 145 &amp; 173</td>
<td>4.8 &amp; 86 &amp; 281 &amp; 218</td>
</tr>
<tr>
<td><strong>Green algae</strong></td>
<td></td>
<td></td>
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<tr>
<td><em>Chlamydomonas</em> noctigama</td>
<td>3.1 &amp; 8.1 &amp; 37 &amp; 39</td>
<td>75 &amp; 379 &amp; 41 &amp; 37</td>
</tr>
<tr>
<td><em>Chlamydomonas</em> reinhardtii</td>
<td>2 &amp; 15 &amp; 46 &amp; 36</td>
<td>26 &amp; 104 &amp; 38 &amp; 60</td>
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<tr>
<td><em>Chlamydomonas</em> reinhardtii</td>
<td>0.9 &amp; 8.3 &amp; 113 &amp; 82</td>
<td>78 &amp; 316 &amp; 92 &amp; 75</td>
</tr>
<tr>
<td><em>Scenedesmus obliquus</em></td>
<td>0.3 &amp; 4.5 &amp; 107 &amp; 105</td>
<td>13 &amp; 55 &amp; 34 &amp; 35</td>
</tr>
<tr>
<td><em>Tetraedron minimum</em></td>
<td>2.1 &amp; 9.4 &amp; 11 &amp; 7</td>
<td>279 &amp; 841 &amp; 60 &amp; 53</td>
</tr>
</tbody>
</table>
phenotypic plasticity of its CO₂ uptake rate provides the bloom-forming cyanobacterium Microcystis with an exceptionally strong capacity to respond to rising pCO₂ levels in comparison to other phytoplankton species. Microcystis blooms already cause major water quality problems in Lake Erie (USA) (39), Lake Taihu (China) (40), Lake Victoria (Africa) (41), and many other eutrophic and hypertrophic lakes worldwide. Our findings warn that rising atmospheric pCO₂ will further intensify surface blooms of Microcystis in the coming decades.

MATERIALS AND METHODS
Species and culture conditions
We studied the toxic (microcystin-producing) cyanobacteria Microcystis aeruginosa PCC 7806 and PCC 7941. Both Microcystis strains contain the two known CO₂ uptake systems (NDH-1³ and NDH-1⁴). In addition, Microcystis PCC 7941 contains all three known bicarbonate uptake systems (BCT1, SbtA, and BicA), whereas Microcystis PCC 7806 has only two of them (BCT1 and BicA) (19).

The Microcystis strains were cultured as unialgal but nonaxenic strains, in CO₂-controlled chemostats specifically designed to study the population dynamics of phytoplankton species (11, 12, 42) (Fig. 1A). The chemostatost allowed full control of light intensity, temperature, nutrient input, and pCO₂ of the aeration gas. Each chemostat consisted of a flat culture vessel with an optimal path length (mixing depth) of zmax = 5 cm and a working volume of ~1.7 liters. The vessel was illuminated from one side to create a unidirectional light gradient, using a constant incident light intensity of 40 μmol photons m⁻² s⁻¹ provided by white fluorescent tubes (Philips PL-L 24W/840/4P, Philips Lighting, Eindhoven, The Netherlands). The temperature was maintained at 25±1°C with a stainless steel cooling finger inside each chemostat and connected to a Colora thermocryostat. To avoid nutrient limitation, we provided a nutrient-rich mineral medium [modified BG-11 medium, (43)], with 8 mM NaNO₃ and 175 μM KH₂PO₄·3H₂O but without addition of Na₂CO₃ or NaHCO₃, at a dilution rate of 0.2 day⁻¹.

The chemostats were aerated with pressurized air containing either 100 ppm pCO₂ ("low pCO₂") or 1000 ppm pCO₂ ("high pCO₂") at a gas flow rate of 30 liters hour⁻¹. At these settings, Microcystis growth is mainly limited by inorganic carbon at low pCO₂ and by light at high pCO₂ (table S1; see also 11, 36). The pCO₂ in the gas flow was checked regularly using an Environmental Gas Monitor (EGM-4; PP Systems, Amesbury, MA, USA). The chemostats were sampled every 2 to 3 days to measure population densities, inorganic carbon, nutrients, and light. The chemostats were considered to be in steady state when the coefficient of variation of the population density was less than 10% for at least four consecutive time points.

Carbon uptake kinetics
Carbon uptake kinetics of the two Microcystis strains were determined with a MIMS (HPR40, Hiden Analytical Ltd., UK) using the chemical disequilibrium assay (29, 30) after they had grown in the chemostats at either low or high pCO₂ for ~40 days. At this time point, the chemostats were in steady state for ~20 days. Before each assay, fresh Microcystis cells were sampled from the steady-state chemostats and concentrated by gentle centrifugation for 5 min at 600g. Then, the supernatant was discarded and the pelleted Microcystis cells were resuspended in mineral medium without dissolved inorganic carbon (DIC) and nitrate. The medium was adjusted to pH 8.0 ± 0.1 using 50 mM Hepes buffer. To ensure similar assay conditions, the resuspended Microcystis cells were diluted to an OD₇₅₀ (optical density at 750 nm) of 0.3, which corresponds to a Microcystis biovolume of ~400 mm³ liter⁻¹. The suspension was aerated with N₂ gas for at least 1 hour at a temperature of 25°C to remove any residual CO₂. Subsequently, we transferred the Microcystis suspension to a 10-ml MIMS cuvette. We added a final concentration of 50 μM membrane-impermeable dextran-bound sulfonamide (Syntelec AB, Lund, Sweden) to inhibit any possible extracellular carbonic anhydrase activity. The MIMS cuvette was provided with the same light intensity (40 μmol photons m⁻² s⁻¹) using the same white fluorescent tubes and was kept at the same temperature (25°C) as the chemostat experiments.

With the MIMS, we simultaneously measured O₂ and CO₂ fluxes during consecutive light-dark intervals (5 min each) after adding known CO₂ and bicarbonate concentrations during each dark phase. Net C fixation rates and respiration rates (r) were measured as rates of O₂ production in the light and O₂ consumption in the dark, respectively, assuming a photosynthetic quotient of 1.0 (i.e., O₂ production equals net C fixation). Net CO₂ uptake rates (VCO₂,net) were calculated from CO₂ consumption in the light period, corrected for CO₂/bicarbonate interconversion in the medium. Bicarbonate uptake rates (VHCO₃) were calculated from the difference between net C fixation and net CO₂ uptake rates.

The measured CO₂ and bicarbonate uptake rates were fitted to Michaelis-Menten equations

\[
V_{CO₂,net} = \frac{V_{max,CO₂,gross}[CO₂]}{K_{v,CO₂} + [CO₂]} - r \tag{3}
\]

\[
V_{HCO₃} = \frac{V_{max,HCO₃}[HCO₃]}{K_{v,HCO₃} + [HCO₃]} \tag{4}
\]

where [CO₂] and [HCO₃] are the dissolved CO₂ and bicarbonate concentration, Vmax,CO₂,gross and Vmax,HCO₃ are the maximum uptake rates of gross CO₂ and bicarbonate, and K1/2,CO₂ and K1/2,HCO₃ are the half-saturation constants. The maximum net CO₂ uptake rate was calculated as Vmax,CO₂,net = Vmax,CO₂,gross − r. For Microcystis PCC 7941, we extended Eq. 4 with an inhibition term to capture the asymptotic decrease of its bicarbonate uptake rate at high bicarbonate concentrations (section S1 and fig. S1, C and D).

Differences between Michaelis-Menten parameters at low pCO₂ versus high pCO₂ were tested for significance using the independent samples t test, corrected for unequal variances when necessary and for multiple hypothesis testing using the false discovery rate (44).

Sampling and analysis
In each sample, the population density of Microcystis (expressed by the biovolume of the population) was measured in triplicate with a CASY TIC automated cell counter with a 60-μm capillary (OLS OMNI Life Science, Bremen, Germany). Light intensity transmitted through the chemostat (Iout) was measured at the back surface of the chemostat vessel with a LI-COR LI-250 quantum photometer (LI-COR Biosciences, Lincoln, NE, USA). pH was measured with a SCHOTT pH meter (SCHOTT AG, Mainz, Germany) immediately after sampling. DIC was measured as CO₂ after addition of 25% phosphoric acid using a Model 700 TOC Analyzer (OI Corp., College Station, TX, USA). From DIC and pH, we calculated CO₂(aq), bicarbonate, and carbonate concentrations, based on the dissociation constants of inorganic carbon corrected for temperature and salinity (45). Nutrient uptake by cyanobacteria affects alkalinity (11, 46). Our model therefore
required measurement of cellular nutrients, which were determined by gently washing filtered *Microcystis* cells twice with a nutrient-free 15 mM NaCl solution. The washed filters were stored at −20°C until further analysis. For cellular C, N, and S contents, one set of pre-weighted filters were freeze dried, wrapped in tin foil discs (30 mm, Sercon Ltd., Crewe, UK), and analyzed using a Vario EL Elemental Analyzer (Elementar Analysensysteme GmbH, Hanau, Germany). For the cellular C content, another set of the same filters were put into glass tubes that contained 10 ml of Milli-Q water, 0.2 ml of 5.5 M HSO$_4$, and 1.5 ml of 8% (NH$_4$)$_2$SO$_4$ solvent, and the sealed tubes were autoclaved for 1 hour at 121°C, to convert all organic P to orthophosphate. Orthophosphate concentrations were measured with a Skalar SA 400 autoanalyzer (Skalar Analytical B.V., Breda, The Netherlands). Samples for chlorophyll a analysis were filtered on 25-mm glass fiber filters (GF/C, 1.2-μm pore size, Whatman GmbH, Dassel, Germany) and freeze dried for at least 2 hours. Chlorophyll a was extracted with $N,N$-dimethylformamide and measured spectrophotometrically (47).

Mathematical model

We developed a dynamical model to describe the carbon uptake kinetics and population dynamics of cyanobacteria as a function of $p$CO$_2$ and light availability. The model combines previous theoretical and experimental work on phytoplankton growth under light-limited (42, 48) and carbon-limited conditions (11, 36). In short, the model assumes eutrophic conditions, in which all nutrients are in ample supply and, hence, do not limit phytoplankton growth. The CO$_2$ and bicarbonate uptake kinetics and phenotypic plasticity of the uptake parameters are described by Eqs. 1 to 4. The carbon uptake rates and light availability, in turn, determine the growth rate of the cyanobacterial population. Furthermore, uptake of CO$_2$, bicarbonate, and nutrients induces changes in pH and alkalinity. These changes in pH and alkalinity affect the speciation of inorganic carbon, which, in turn, feeds back on carbon uptake and growth of the cyanobacteria. The expanding cyanobacterial population also increases the turbidity of the water column, thereby diminishing light available for further photosynthesis and growth. The model is described in full detail in section S2.

The model parameters were measured experimentally and include system parameters (e.g., incident light intensity, $p$CO$_2$ level in the gas flow, and dilution rate of the chemostats; table S4) and species parameters (e.g., maximum uptake rates and half-saturation constants; table S5). In particular, the carbon uptake kinetics and respiration rates measured by MIMS (Fig. 2 and tables S2 and S3) served as input to predict the carbon uptake and concomitant changes in inorganic carbon chemistry at both low and high $p$CO$_2$. The model and its parameterization are described in detail in section S2.

For the lake model (section S3), we choose parameter values representative for the summer situation in eutrophic lakes dominated by dense *Microcystis* blooms. The species parameters are identical to those of *Microcystis* PCC 7806 (table S5), where we distinguished between a plastic phenotype, low $p$CO$_2$ phenotype, and high $p$CO$_2$ phenotype as described in Results. The system parameters for the lake model are summarized in table S6.

SUPPLEMENTARY MATERIALS

Supplementary material for this article is available at http://advances.sciencemag.org/cgi/content/full/6/8/eaax2926/DC1

Section S1. Description of bicarbonate uptake of *Microcystis* PCC 7941

Section S2. Description of the mathematical model

Section S3. Extrapolation of the model to lakes

Fig. S1. Carbon uptake kinetics of *Microcystis* PCC 7941 acclimated to either low or high $p$CO$_2$

Fig. S2. Population density, inorganic carbon chemistry, and pH in chemostat experiments with *Microcystis* PCC 7941 at low and at high $p$CO$_2$

Table S1. Steady-state characteristics of the chemostat experiments with *Microcystis* PCC 7806 and *Microcystis* PCC 7941.

Table S2. Kinetic parameters estimated from the carbon uptake experiments with *Microcystis* PCC 7941 and its parameterization are described in detail in section S2.

Table S3. Tests of significant differences between kinetic parameters estimated at low versus high $p$CO$_2$.

Table S4. System parameters applied in the chemostat experiments.

Table S5. Species parameters measured experimentally.

Table S6. System parameters applied in the lake model.

References (S2–S9)

REFERENCES AND NOTES


Phenotypic plasticity of carbon fixation stimulates cyanobacterial blooms at elevated CO$_2$

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