Effects of rising CO₂ on the harmful cyanobacterium Microcystis

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How rising CO$_2$ may stimulate harmful cyanobacterial blooms
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Rising CO₂ and cyanobacterial blooms: a review

Abstract
Climate change is likely to stimulate the development of harmful cyanobacterial blooms in eutrophic waters, with negative consequences for water quality of many lakes, reservoirs and brackish ecosystems across the globe. In addition to effects of temperature and eutrophication, recent research has shed new light on the possible implications of rising atmospheric CO₂ concentrations. Depletion of dissolved CO₂ by dense cyanobacterial blooms creates a concentration gradient across the air-water interface. A steeper gradient at elevated atmospheric CO₂ concentrations will lead to a greater influx of CO₂, which can be intercepted by surface-dwelling blooms, thus intensifying cyanobacterial blooms in eutrophic waters. Bloom-forming cyanobacteria display an unexpected diversity in CO₂ responses, because different strains combine their uptake systems for CO₂ and bicarbonate in different ways. The genetic composition of cyanobacterial blooms may therefore shift. In particular, strains with low-affinity uptake systems may benefit from the anticipated rise in inorganic carbon availability. Furthermore, cyanobacteria differ from eukaryotic algae in that they can fix dinitrogen, and new insights show that the nitrogen-fixation activities of some, but not all, diazotrophic cyanobacteria are strongly stimulated at elevated CO₂ levels. However, models and lake studies indicate that the response of cyanobacterial growth to rising CO₂ concentrations can be suppressed by nutrient limitation. Hence, the greatest response of cyanobacterial blooms to climate change is expected to occur in eutrophic and hypertrophic lakes.

Introduction
It is well-established that in addition to anthropogenic nutrient enrichment, changes in the Earth’s climate, specifically rising temperatures and altered hydrologic patterns, strongly influence the frequency, intensity, and duration of harmful cyanobacterial blooms (Robarts and Zohary, 1987; Trenberth, 2005; Peceters et al., 2007; Suikkanen et al., 2007; Wiedner et al., 2007; Jöhnk et al., 2008; Paerl and Huisman, 2008, 2009; Wagner and Adrian, 2009; O’Neil et al., 2012; Paerl and Paul, 2012). An expansion of cyanobacterial blooms is of great societal concern, because harmful cyanobacteria can impair safe drinking, irrigation, fishing and recreational waters that are critical for the growing global human population.

There is convincing evidence that a key driver of climate change is the concentration of atmospheric carbon dioxide (CO₂), which has been shown to modulate the Earth’s surface and water temperatures via the ‘greenhouse effect’ (IPCC 2012). Furthermore, long-term records of atmospheric CO₂ in ice cores and the atmosphere (e.g., at Mauna Loa, Hawaii) have shown that there is a well-defined parallel between increasing CO₂ concentrations and the rise of man-made fossil fuel combustion (Tans et al., 1990).
The relationships between rising atmospheric CO$_2$ levels, global warming and declining water quality are controlled through complex interactions with altered evaporation and rainfall patterns, changing hydrological flows and shifts in chemical and biological processes, all of which interact in non-linear ways (Paerl and Paul, 2012). This creates an enormous challenge in predicting the quantitative and qualitative ramifications for the many types of water bodies that are likely to be impacted. Furthermore, the transport and delivery of nutrients that are critical for development, proliferation and maintenance of cyanobacterial blooms are strongly influenced by climate-driven changes in precipitation patterns and biogeochemical processes (Michalak et al., 2013). All of these factors ultimately control planktonic communities, including cyanobacterial blooms (Mitrovic et al., 2003; Elliott, 2010; Hall et al., 2013; Michalak et al., 2013).

In addition to its influence on global warming, rising atmospheric CO$_2$ levels may stimulate the proliferation of surface-dwelling cyanobacteria by providing them preferential access to a vast and rising pool of atmospheric CO$_2$ (Paerl and Ustach, 1982; Ibelings and Maberly, 1998; Verspagen et al., 2014b). An increase in atmospheric CO$_2$ increases its dissolution in water. Enhanced dissolution of CO$_2$ lowers pH, causing a slow acidification of the oceans (Orr et al., 2005; Doney et al., 2009). In freshwaters, however, the impact of rising atmospheric CO$_2$ appears more complex than in most marine ecosystems. Freshwater systems range widely in pH and alkalinity (Lazzarino et al., 2009; Balmer and Downing, 2011), which affects the speciation of inorganic carbon. Many freshwater ecosystems receive large amounts of organic carbon from terrestrial systems, which may result in CO$_2$ supersaturation, i.e., dissolved CO$_2$ concentrations that greatly exceed equilibrium with the atmosphere (Cole et al., 1994; Sobek et al., 2005). Conversely, in other lakes, CO$_2$ concentrations are strongly depleted as a consequence of the photosynthetic activity of dense phytoplankton blooms (Talling, 1976; Balmer and Downing, 2011; Verspagen et al., 2014b). Similar to the depletion of other resources, depletion of inorganic carbon (C$_i$) can limit growth (Hein, 1997), particularly in dense surface blooms of cyanobacteria (Ibelings and Maberly, 1998). Hence, the natural range of variation in CO$_2$ availability is much larger in lakes than in marine or terrestrial ecosystems, and bloom-forming cyanobacteria must cope with this variability.

This review will focus on the current state of knowledge on effects of climate change on harmful cyanobacteria. Although many reviews have already addressed this topic (e.g. Paerl and Huisman, 2009; Carey et al., 2012; O’Neil et al., 2012), most reviews focused on the direct or indirect effects of increased temperature, often in combination with accelerating eutrophication. In this review, effects of rising CO$_2$ concentrations on cyanobacteria will be addressed. First, an overview of physiological and ecological responses to elevated CO$_2$ concentrations will be provided, with special emphasis on the CO$_2$-concentrating mechanisms...
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(CCMI)s of cyanobacteria. This is followed by a further exploration of interactive effects of rising CO₂ and nutrient availability, and possible effects of elevated CO₂ on cyanotoxin production. Key questions to be addressed are, for instance, whether global change is likely to lead to a proliferation of cyanobacteria at the expense of eukaryotic phytoplankton species, and whether the composition of cyanobacterial blooms may change.

Response to rising CO₂

Does rising CO₂ intensify bloom development?

Rising atmospheric CO₂ levels are often thought to have only minor impacts on bloom development in freshwater ecosystems. This assumption is based on two common misconceptions. It is often argued (1) that the CO₂ concentrations in freshwater lakes are sufficiently high to cover the carbon demands of phytoplankton populations, because many lakes are “supersaturated” with CO₂ (Cole et al., 1994; Sobek et al., 2005; Jansson et al., 2012), and (2) that changes in CO₂ availability have little effect on bloom development, because most cyanobacteria can also utilize bicarbonate as C source.

Concerning the first misconception, it is true that the pCO₂ in many lakes worldwide is well above atmospheric equilibrium (i.e., supersaturated; Cole et al., 1994). Most carbon input in lakes originates from terrestrial primary production in the surrounding watershed and not from atmospheric CO₂ (Cole & Caraco, 2001; Pacala et al., 2001; Richey et al., 2002; Maberly et al., 2013), which is subsequently mineralized, causing pCO₂ levels that commonly exceed 1,500 ppm. However, even in these “supersaturated waters”, the actual concentration of dissolved CO₂ (CO₂(aq)) is still quite low, and cyanobacterial blooms can easily turn a supersaturated lake into an undersaturated lake (Ibelings and Maberly, 1998; Verspagen et al., 2014b). For instance, consider a supersaturated lake with a pCO₂ of 1,500 ppm. According to Henry’s Law, assuming a solubility constant of \( K_H = 0.034 \text{ mol L}^{-1} \text{ atm}^{-1} \), the CO₂(aq) concentration in this lake would be only ~50 µmol L⁻¹. This concentration is certainly not enough to cover the photosynthetic carbon demand of a dense cyanobacterial bloom. The photosynthetic activity of dense blooms can be as high as 12.5 to 50 µmol C L⁻¹ h⁻¹ (Hein et al., 1997), depleting the CO₂(aq) concentration in this lake within a few hours (Talling, 1976; Maberly, 1996). In some lakes, the CO₂(aq) concentration can even be drawn down to less than 0.1 µmol L⁻¹, corresponding to a pCO₂ of only a few ppm (Lazzarino et al., 2009, Balmer and Downing, 2011).

Data from Lake Volkerak, a large eutrophic lake in the Netherlands, is provided in Figure 7.1 (Verspagen et al., 2006; 2014b). In this figure, the CO₂(aq) concentration that would be predicted from equilibrium with the atmosphere (i.e., [CO₂⁺]) has also been indicated. This predicted equilibrium CO₂(aq) concentration shows some variation during the
seasons, as the solubility of CO2 in water is temperature dependent. However, seasonal variation of the measured CO2(aq) concentration in Lake Volkerak is much larger, because biological consumption and production of CO2 act at a much faster rate than the equilibration of CO2 between water and atmosphere. In winter and spring, the measured CO2(aq) concentration in Lake Volkerak largely exceeds the CO2(aq) concentration that would be predicted from equilibrium with the atmosphere, and hence in winter and spring the lake is supersaturated with CO2. Conversely, dense blooms of the harmful cyanobacterium Microcystis occur in Lake Volkerak in summer and early fall. The photosynthetic activity of these blooms depletes the CO2(aq) concentration to 1 µmol L\(^{-1}\) (≈30 ppm), such that the lake becomes severely undersaturated with CO2 in summer while the pH rises above 9 for several months (Figure 7.1). These data illustrate that the CO2(aq) concentration in eutrophic lakes can vary from supersaturation in winter to undersaturation in summer.

Figure 7.1. Seasonal changes in phytoplankton population density (green line), dissolved CO2 concentration ([CO2], black solid line) and pH (grey dash-dotted line) in Lake Volkerak during two consecutive years. The black dashed line is the expected dissolved CO2 concentration ([CO2*]) when assuming equilibrium with the atmospheric pCO2 level. Blue shading indicates that the lake is supersaturated with CO2, whereas red shading indicates undersaturation. In the months July-October, the cyanobacterium Microcystis comprised 75-98% of the phytoplankton population. Adjusted from Verspagen et al. (2014b).

The drawdown of the CO2(aq) concentration by cyanobacterial blooms turns lakes into a sink for atmospheric CO2 (Balmer and Downing, 2011). The CO2 gas influx depends on the CO2 deficit. More specifically, the CO2 influx (\(\dot{g}_{CO2}\)) is proportional to the difference
between the expected concentration of CO$_2$(aq) in equilibrium with the atmosphere (calculated from Henry’s law) and the observed CO$_2$(aq) concentration (Siegenthaler and Sarmiento, 1993; Cole et al., 2010):

$$g_{\text{CO}_2} = v \left( K_H p_{\text{CO}_2} - C_{\text{O}_2}^{\text{aq}} \right)$$

(1)

where $v$ is the gas transfer velocity (also known as piston velocity) across the air-water interface, $K_H$ is the solubility constant of CO$_2$ gas in water, and $p_{\text{CO}_2}$ is the partial pressure of CO$_2$ in the atmosphere. If it is assumed that the dense cyanobacterial bloom has stripped the surface layer of CO$_2$(aq), this equation simplifies to $g_{\text{CO}_2} = v \times K_H \times p_{\text{CO}_2}$. The gas transfer velocity depends on several parameters, especially wind speed. A typical value for the gas transfer velocity of lakes is $v = 0.02$ m h$^{-1}$ (Crusius and Wanninkhof, 2003; Cole et al., 2010). Hence, assuming an atmosphere with $p_{\text{CO}_2} = 400$ ppm, the CO$_2$ influx during a dense cyanobacterial bloom would amount to $\sim 7$ mmol m$^{-2}$ d$^{-1}$. This influx is substantial and can be intercepted by the surface-dwelling cyanobacterial bloom for supporting photosynthesis (Paerl and Ustach, 1982; Ibelings and Maberly, 1998). A doubling of the atmospheric CO$_2$ concentration, to 800 ppm, would roughly double the CO$_2$ influx to $\sim 14$ mmol m$^{-2}$ d$^{-1}$. Moreover, this might still be an underestimate for dense cyanobacterial blooms. At pH $> 9$, which is typical for dense blooms, the chemical reaction of CO$_2$ with the abundant hydroxide ions further increases CO$_2$ transfer across the air-water surface by a process known as chemically enhanced diffusion (Emerson, 1975; Bade and Cole, 2006). Hence, this simple calculation shows that, in principle, an increase in atmospheric CO$_2$ levels may provide a sufficient influx of C to enable a substantial increase in the productivity of surface-dwelling cyanobacterial blooms.

Models and laboratory experiments have shown that rising CO$_2$ concentrations may indeed exacerbate cyanobacterial blooms (Schippers et al., 2004; Verspagen et al., 2014b). Verspagen et al. (2014b) performed chemostat experiments with *Microcystis* NIVA-CYA 140 under nutrient-saturating conditions. At a low atmospheric pCO$_2$ level of 200 ppm (half the current ambient pCO$_2$), the *Microcystis* population increased until it reached a steady state, at which it had depleted the dissolved CO$_2$(aq) concentration to 0.2 µmol L$^{-1}$ and raised the pH to 10 (Figure 7.2A,C,E). The same experiment was repeated at an elevated atmospheric pCO$_2$ level of 1,200 ppm (three times ambient pCO$_2$), which resulted in a doubling of the *Microcystis* biomass, whereas the CO$_2$(aq) concentration was much less depleted and the pH was raised to only 8.5 (Figure 7.2B,D,F). The model predictions nicely matched the experiments. These results demonstrate, both in theory and lab experiments, that bloom-forming cyanobacteria such as *Microcystis* can become carbon-limited, and that rising pCO$_2$ levels can increase cyanobacterial biomass (Verspagen et al., 2014b).
Figure 7.2. Cyanobacterial growth and inorganic carbon chemistry at two different pCO₂ levels. Left panels: Chemostat experiment with low pCO₂ of 200 ppm in the gas flow and 500 µmol L⁻¹ bicarbonate in the mineral medium. Right panels: Chemostat experiment with high pCO₂ of 1,200 ppm in the gas flow and 2,000 µmol L⁻¹ bicarbonate in the mineral medium. Both chemostats were inoculated with Microcystis NIVA-CYA 140. (A,B) Microcystis biomass (expressed as biovolume) and light intensity penetrating through the chemostat (I_OUT). (C,D) Dissolved CO₂, bicarbonate and carbonate concentrations. (E,F) pH. Symbols represent measurements, lines show model predictions. Adjusted from Verspagen et al. (2014b).
The second misconception is that changes in CO₂ availability have little effect on bloom development, because most cyanobacteria can also utilize bicarbonate. Indeed, it is true that many if not most cyanobacteria can use bicarbonate. However, whereas CO₂ passively diffuses through the cell membrane, utilization of bicarbonate requires investments in sodium-dependent and ATP-dependent bicarbonate uptake systems as well as in sodium antiporters that excrete the sodium accumulated in the cells (Price, 2011; Burnap et al., 2015; Sandrini et al., 2015c). These costs of bicarbonate utilization may have repercussions for the growth rates that can be achieved. For instance, *Synechococcus leopoliensis* grows at 80% of its maximum growth rate when bicarbonate is its main carbon source (Miller et al., 1984). *Microcystis* HUB 5-2-4, which lacks the high-flux bicarbonate transporter BicA but does contain the two high-affinity bicarbonate uptake systems SbtA and BCT1 (see next section), grows at only 35% of its maximum growth rate on bicarbonate alone (Verspagen et al. 2014b). In chemostat experiments, this *Microcystis* strain could barely sustain a small population when CO₂ was largely removed from the gas flow, even though bicarbonate was provided at a saturating concentration of 2,000 µmol L⁻¹ (Verspagen et al., 2014b). An increase from near-zero pCO₂ levels (0.5 ppm) to saturating pCO₂ levels (2,800 ppm) led to an almost 20-fold increase of the *Microcystis* biomass. Hence, these laboratory experiments show that addition of CO₂ may strongly promote cyanobacterial growth even in bicarbonate-rich waters. Yet, other cyanobacterial species such as *Cylindrospermopsis raciborskii* appear to be more effective bicarbonate users, and for these species rising CO₂ concentrations may have a smaller effect on growth rates when bicarbonate is available as an alternative Cᵢ source (Holland et al., 2012). Hence, the effect of rising CO₂ on cyanobacterial growth is species specific. Moreover, the next sections will show that there is even tremendous variation in CO₂ response within species.

**The CO₂-concentrating mechanism of harmful cyanobacteria**

Phytoplankton use CO₂ and bicarbonate available in the environment for carbon fixation with the RuBisCO enzyme. To overcome the low affinity of RuBisCO for CO₂, most phytoplankton, including cyanobacteria, evolved a CO₂-concentrating mechanism (CCM) (Kaplan and Reinhold, 1999; Giordano et al., 2005; Badger et al., 2006; Price et al., 2008; Price, 2011). The typical cyanobacterial CCM is based on the uptake of CO₂ and bicarbonate from the environment, conversion of the acquired CO₂ into bicarbonate in the cytoplasm, and subsequent diffusion of the accumulated bicarbonate into specialized compartments called carboxysomes (**Figure 7.3**). In the carboxysomes, carbonic anhydrases convert the accumulated bicarbonate back to CO₂, surrounding RuBisCO by a high CO₂ concentration. RuBisCO incorporates CO₂ into the Calvin-Benson cycle, which assimilates the acquired carbon into organic molecules.
In cyanobacteria, five different C\textsubscript{i} uptake systems have been identified, three for the uptake of bicarbonate and two for the conversion of CO\textsubscript{2}, that diffuses into the cell, to bicarbonate (Figure 3). These uptake systems have different physiological properties (Price et al., 2004; Price, 2011; Sandrini et al., 2015c). Two of the bicarbonate transporters, BicA and SbtA, are sodium-dependent symporters (Shibata et al., 2002; Price et al., 2004). BicA has a low affinity for bicarbonate ($K_{0.5} = 70-350 \mu$M bicarbonate) but high flux rate. Conversely, SbtA has a high affinity for bicarbonate ($K_{0.5} < 5 \mu$M bicarbonate) but low flux rate (Price et al., 2004). The third bicarbonate transporter, BCT1, is ATP-dependent, and similar to SbtA it has a high affinity for bicarbonate ($K_{0.5} = 10-15 \mu$M bicarbonate) but a low flux rate (Omata et al., 1999; Omata et al., 2002). All three bicarbonate uptake systems are located in the plasma membrane (Price, 2011).

The two CO\textsubscript{2} uptake systems, NDH-I\textsubscript{3} and NDH-I\textsubscript{4}, convert CO\textsubscript{2} that passively diffuses into the cell to bicarbonate in a NADPH-dependent reaction (Price et al., 2002; Price, 2011). NDH-I\textsubscript{3} has a high affinity for CO\textsubscript{2} ($K_{0.5} = 1-2 \mu$M CO\textsubscript{2}) but a low flux rate (Maeda et al., 2002; Price et al., 2002). Conversely, NDH-I\textsubscript{4} has a lower affinity for CO\textsubscript{2} ($K_{0.5} = 10-15 \mu$M CO\textsubscript{2}) but a high flux rate (Maeda et al., 2002; Price et al., 2002). This diverse array of C\textsubscript{i} uptake systems enables cyanobacteria to respond effectively to changes in C\textsubscript{i} availability.

Eukaryotic algae can also employ a CCM, but it works differently from the CCM of cyanobacteria. In the green alga \textit{Chlamydomonas reinhardtii}, the CCM is based on a light-driven pH gradient that is set up across the chloroplast thylakoid membrane, converting bicarbonate transported into the thylakoid lumen into CO\textsubscript{2} near the pyrenoids where CO\textsubscript{2} fixation takes place (Moroney and Ynalvez, 2007; Moroney et al., 2011). An interesting selection experiment where \textit{C. reinhardtii} was exposed to elevated CO\textsubscript{2} for 1,000 generations revealed that some cell lines lost the ability to induce high-affinity CO\textsubscript{2} uptake (Collins and Bell, 2004; Collins et al., 2006). This was attributed to mutations in CCM genes. Hence, similar to cyanobacteria, \textit{C. reinhardtii} likely also possesses high-affinity and low-affinity C\textsubscript{i} uptake genes. This experiment demonstrates that eukaryotic algae can evolve in response to elevated CO\textsubscript{2}. Yet, much less is known about the CCM genes and proteins of algae than those of cyanobacteria.
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Figure 7.3. Schematic overview of the CCM in cyanobacteria. Five different Cᵢ uptake systems are known in cyanobacteria, including the ATP-dependent bicarbonate uptake system BCT1, two sodium-dependent bicarbonate uptake systems (BicA and SbtA) and two CO₂ uptake systems (NDH-I₃ and NDH-I₄). The Cᵢ uptake systems differ in their affinities and flux rates. Accumulated bicarbonate is converted to CO₂ by carbonic anhydrases (CA) in the carboxysomes. CO₂ fixation by RuBisCO leads to the formation of 3-phosphoglycerate (3PG), whereas the reaction with O₂ (photorespiration) produces toxic 2-phosphoglycerate (2PG). The dashed lines indicate CO₂ leakage from the carboxysome, which can partly be intercepted by the CO₂ uptake systems.

Genetic diversity of Cᵢ uptake systems in Microcystis

Microcystis is a potentially toxic cyanobacterium that forms dense blooms in eutrophic lakes all over the world (Verspagen et al., 2006; Qin et al., 2010; Michalak et al., 2013), and can produce the hepatotoxin microcystin (Codd et al., 2005; Dittmann et al., 2013). The genomes of 20 strains of Microcystis aeruginosa (Kützing) (sensu Otsuka et al., 2001) were screened recently, which revealed that these strains differ in the combination of Cᵢ uptake systems (Sandrini et al., 2014). Genes encoding the ATP-dependent bicarbonate transporter BCT, and the two CO₂ uptake systems NDH-I₃ and NDH-I₄ were found in all 20 strains. Most other CCM genes are also widespread in Microcystis. However, Microcystis strains differ in the presence of the two sodium-dependent bicarbonate transporters BicA and SbtA. Three Cᵢ uptake genotypes were found (Table 7.1). Some Microcystis strains possess all five Cᵢ uptake systems and these are referred to as Cᵢ uptake generalists. Moreover, in Microcystis, these Cᵢ uptake generalists co-transcribe bicA and sbtA (Sandrini et al., 2014). Other strains contain the
gene *sbtA* encoding for the high-affinity bicarbonate uptake system SbtA but lack the gene *bicA*, and hence will be referred to as high-affinity specialists. And again other strains contain the gene *bicA* encoding for the low-affinity but high-flux bicarbonate uptake system BicA, but lack the gene *sbtA*. These strains will be called high-flux specialists.

Eleven of the 20 investigated *Microcystis* strains produced the hepatotoxin microcystin. Microcystin-producing strains were found among the C\textsubscript{i} uptake generalists, high-affinity specialists and high-flux specialists, and did not form distinct clusters in phylogenetic trees based on the *bicA* and *sbtAB* sequences (Sandrini *et al.*, 2014). Hence, there is no relationship between the C\textsubscript{i} uptake genotypes and the presence of microcystin production.

Within the C\textsubscript{i} uptake genotypes, several genetic variants were discovered. For instance, one of the strains had a functional *sbtA* gene but a defective *bicA* gene caused by a transposon insert, and other strains combined *sbtA* with only a small remaining fragment of the *bicA* gene (Sandrini *et al.*, 2014). These strains were classified among the high-affinity specialists, because their *bicA* gene is no longer functional. These results indicate that during the course of evolution some strains may have lost the ability to produce specific C\textsubscript{i} uptake systems, in this case the loss of BicA. Presumably, in environments with low C\textsubscript{i} availability the production of this low-affinity but high-flux bicarbonate transporter is an unnecessary burden, and its loss may therefore offer a selective advantage.

Consistent with these evolutionary considerations, laboratory experiments confirmed that the genetic variation in C\textsubscript{i} uptake systems of *Microcystis* has phenotypic consequences (Sandrini *et al.*, 2014). High-affinity specialists with *sbtA* but without *bicA* grow better at a low partial pressure of CO\textsubscript{2} (pCO\textsubscript{2}), but perform poorly at high pCO\textsubscript{2} conditions. Conversely, high-flux specialists with *bicA* but without *sbtA* grow poorly at low pCO\textsubscript{2}, but perform well at high pCO\textsubscript{2} levels. Finally, C\textsubscript{i} uptake generalists containing all five C\textsubscript{i} uptake systems grow well across a wide range of pCO\textsubscript{2} levels (from 20 to 10,000 ppm) (Sandrini *et al.*, 2014).

Competition experiments by Van de Waal *et al.* (2011) showed that rising pCO\textsubscript{2} levels can lead to a reversal in competitive dominance among *Microcystis* strains. These authors interpreted this result by differences in toxin production between the two strains, because one of the strains used in the experiments produced the hepatotoxin microcystin (strain NIVA-CYA 140) whereas the other was non-toxic (strain NIVA-CYA 43). However, the genetic analysis of Sandrini *et al.* (2014) revealed that these two strains also differed in their C\textsubscript{i} uptake systems, which provides a much more parsimonious explanation for the observed reversal in competitive dominance. *Microcystis* strain NIVA-CYA 140 was a high-affinity specialist (only *sbtA*), and won the competition at low pCO\textsubscript{2} levels. In contrast, *Microcystis* strain NIVA-CYA 43 (= PCC 7005) was a C\textsubscript{i} uptake generalist with both *bicA* and *sbtA*, and won the competition at high pCO\textsubscript{2} levels. Hence, while these experiments demonstrate that rising pCO\textsubscript{2} may shift
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strain dominance, this shift can be attributed to differences in the Cᵣ uptake traits of the strains rather than to differences in their microcystin production. In particular, the results of these competition experiments support the hypothesis that natural selection favors the sbtA gene at low CO₂ conditions, whereas bicA-containing strains are favored at high CO₂ conditions.

Cᵣ uptake systems of other harmful cyanobacteria
The CCMs of other harmful freshwater cyanobacteria have not been studied in detail, partly because genomic data are still largely lacking. But now the genomes of four Anabaena strains (Wang et al., 2012; Shih et al., 2013; Thiel et al., 2014), one Aphanizomenon strain (Cao et al., 2014) and nine Planktothrix strains (Tooming-Klunderud et al., 2013; Christiansen et al., 2014) have been sequenced. We analyzed the CCM genes present in these genomes, based on high similarity of the protein sequences with the reference protein sequences from Microcystis PCC 7806, Microcystis NIES-843, Synechocystis PCC 6803, Synechococcus PCC 7002 and Synechococcus PCC 7942. This analysis revealed that Anabaena, Aphanizomenon and Planktothrix also display variation in the presence of the bicA and sbtA genes, whereas the two CO₂ uptake systems and the BCT1 bicarbonate transporter are widespread among all four cyanobacterial genera (Table 7.1). Interestingly, in addition to the three genotypes described in Microcystis, a fourth genotype that lacks both bicA and sbtA was detected in Anabaena, Aphanizomenon and Planktothrix (Table 7.1). Strains with this strategy might be called “Cᵣ uptake minimalists”.

Hence, similar to Microcystis, other genera of harmful cyanobacteria also show genetic variation in their Cᵣ uptake systems. Presumably, this genetic diversity produces a phenotypic variation similar to Microcystis, with a selective advantage for sbtA-containing strains at low CO₂ conditions but a selective advantage for bicA-containing strains in high-CO₂ environments. The phenotypic niche of Anabaena, Aphanizomenon and Planktothrix strains that lack both bicA and sbtA is intriguing, and has not yet been investigated. The absence of both sodium-bicarbonate symporters might imply that bicarbonate uptake has been taken over by the ATP-dependent bicarbonate transporter BCT1, as an adaptation to environments with low sodium concentrations. It is also possible that these Cᵣ uptake minimalists are largely specialized in CO₂ uptake and have only a very limited capacity for bicarbonate uptake, and hence are mainly found in soft waters with pH<6 where bicarbonate uptake is of little advantage.
**Table 7.1. The presence of specific CCM genes in cyanobacteria.**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Origin</th>
<th>Bicarbonate</th>
<th>Carboxysome</th>
<th>CO₂</th>
<th>MC genes</th>
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</thead>
<tbody>
<tr>
<td>8 Microcystis strains</td>
<td>Sandrini et al. (2014)</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Microcystis PCC 7806</td>
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<td>+</td>
<td>-</td>
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<tr>
<td>11 Microcystis strains</td>
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<td>Anabaena cylindrica PCC 7122</td>
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<td>+</td>
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<tr>
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<tr>
<td>Aphanizomenon fluo-aquae NIES-81</td>
<td>JPN (Lake Kasumigaura)</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Planktothrix agarbatis NIVA-CYA 15</td>
<td>NOR (Lake Kolbotnvatnet)</td>
<td>-</td>
<td>+</td>
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</tr>
<tr>
<td>Planktothrix agarbatis NIVA-CYA 34</td>
<td>NOR (Lake Kolbotnvatnet)</td>
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<tr>
<td>Planktothrix agarbatis NIVA-CYA 56/3</td>
<td>FIN (Lake Steinsfjorden)</td>
<td>-</td>
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</tr>
<tr>
<td>Planktothrix prolifica NIVA-CYA 98</td>
<td>FIN (Lake Steinsfjorden)</td>
<td>-</td>
<td>-</td>
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<td>+</td>
</tr>
<tr>
<td>Planktothrix agarbatis NIVA-CYA 126/8</td>
<td>FIN (Lake Langsjon)</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
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<tr>
<td>Planktothrix prolifica NIVA-CYA 406</td>
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<td>-</td>
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</tr>
<tr>
<td>Planktothrix prolifica NIVA-CYA 540</td>
<td>FIN (Lake Steinsfjorden)</td>
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<tr>
<td>Synechocystis sp. PCC 6803</td>
<td>USA (California)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Synechococcus sp. PCC 7002</td>
<td>PRI (Magueyes Island)</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Synechococcus sp. PCC 7942</td>
<td>USA (Texas)</td>
<td>-</td>
<td>+</td>
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</table>

The model cyanobacteria *Synechocystis* PCC 6803, *Synechococcus* PCC 7002 and *Synechococcus* PCC 7942 are shown for comparison with sequenced *Microcystis*, *Anabaena*, *Aphanizomenon*, and *Planktothrix* strains. A plus sign (+) indicates that the gene is present and a dash (−) indicates that the gene is absent. ≠ indicates that only a small fragment of the gene is present. ? indicates that a similar gene is present, but it is not clear if it encodes for the BCT1 bicarbonate transporter (cmpABCD), or possibly a different transporter. The origins of the strains are indicated with three-letter codes of the different countries (ISO 3166-1 α-3). The presence of CCM genes is based on high similarity of the protein sequences with the reference protein sequences in *Microcystis* PCC 7806, *Microcystis* NIES-843, *Synechocystis* PCC 6803, *Synechococcus* PCC 7002 and *Synechococcus* PCC 7942. The presence of microcystin genes (MC) indicates potentially toxic strains.

- C, uptake system with a high substrate affinity and low flux rate.
- C, uptake system with a low substrate affinity and high flux rate.

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Chapter 7
It is often argued that cyanobacteria have a very effective CCM, and are therefore particularly strong competitors at low CO$_2$ levels in comparison to eukaryotic phytoplankton (Shapiro, 1990). If so, one might expect that low CO$_2$ concentrations will favor cyanobacteria, whereas eukaryotic phytoplankton tend to become more dominant at elevated CO$_2$ concentrations. A number of competition experiments between cyanobacteria and eukaryotic phytoplankton seems to support this hypothesis (Shapiro, 1997; Caraco and Miller, 1998; Low-Décarie et al., 2011, 2015). In other experiments, however, eukaryotic phytoplankton dominated over cyanobacteria at low CO$_2$ but not at elevated CO$_2$ concentrations (Verschoor et al., 2013). Indeed, the new insights reviewed above indicate that not all cyanobacteria are strong competitors at low CO$_2$. The genetic diversity of C$_i$ uptake systems shows that there is major variation in the effectiveness of the cyanobacterial CCM, even among different strains within the same genus. Some cyanobacterial strains perform well at low CO$_2$, whereas other strains are much better competitors under high CO$_2$ conditions. This genetic and phenotypic variation in C$_i$ uptake systems provides cyanobacterial communities with the potential for rapid evolutionary adaptation to changing CO$_2$ conditions, with a major selective advantage for cyanobacteria with high-flux C$_i$ uptake systems in high-CO$_2$ environments.

**Interactive effects with nutrient availability**

**Effects of climate change depend on nutrient availability**

In many aquatic systems the availability of nutrients determines primary production (Dzialowki et al., 2005; Xu et al., 2010; Lewandowska et al., 2014), and total nitrogen and total phosphorus concentrations are often good predictors of cyanobacterial biomass (Downing et al., 2001; Håkanson et al., 2007). However, at the physiological level, there are still many gaps in our understanding of how nutrient limitation may interact with changes in temperature or CO$_2$ availability (e.g., Spijkerman et al., 2011).

Verspagen et al. (2014a) developed a conceptual framework to predict how different nutrient loads may modify effects of rising CO$_2$ on phytoplankton biomass production. They investigated a stoichiometrically explicit model that describes phytoplankton growth as function of nutrient, CO$_2$ and light availability. Hence, there are three potentially limiting resources in this model (Figure 7.4A). Inorganic carbon becomes limiting at very low pCO$_2$ levels, nutrients become limiting at very low nutrient loads, and light becomes limiting in dense phytoplankton blooms at high pCO$_2$ levels and high nutrient loads. Light limitation can of course also be induced by other mechanisms, such as a high background turbidity (due to high concentrations of dissolved organic matter or resuspended sediment particles), deep mixing, or low incident light intensities in winter. The resource limitation pattern in Figure 7.4A can be used to sketch to what extent rising pCO$_2$ levels will increase phytoplankton
biomass (Figure 7.4B). In oligotrophic waters with low nutrient loads, rising pCO₂ levels will shift phytoplankton growth from carbon-limited to nutrient-limited conditions (black arrow in Figure 7.4A). In this case, the higher CO₂ availability stimulates some growth, but the phytoplankton biomass remains constrained by the low nutrient levels in the system (Figure 7.4B). Conversely, in hypertrophic waters with high nutrient loads, rising pCO₂ levels will shift phytoplankton growth from carbon- to light-limited conditions (white arrow in Figure 7.4A), which will allow a much larger increase in phytoplankton biomass (Figure 7.4B). Chemostat experiments with the harmful cyanobacteria Microcystis NIVA-CYA 140 and HUB 5-2-4 confirmed these model predictions (Verspagen et al., 2014a). Hence, the general message emerging from these results is that phytoplankton biomass will respond more strongly to rising pCO₂ levels in eutrophic and hypertrophic than in oligotrophic ecosystems.

Figure 7.4. Model predictions illustrating how different nutrient loads may modify effects of rising CO₂ on resource limitation and phytoplankton biomass. (A) Hypothesized patterns of resource limitation, at different atmospheric CO₂ levels and nutrient loads. The arrows indicate that rising atmospheric CO₂ levels will cause a shift from carbon to nutrient limitation in systems with a low nutrient load (black arrow), but from carbon to light limitation in systems with a high nutrient load (white arrow). (B) The extent to which phytoplankton biomass will increase with rising CO₂ levels will depend on the nutrient load. Adjusted from Verspagen et al. (2014a).
Rising CO₂ and cyanobacterial blooms: a review

Effects of climate change on nitrogen fixation

Several genera of harmful cyanobacteria are capable of fixing atmospheric dinitrogen (N₂), including *Anabaena* (nowadays referred to as *Dolichospermum*; Wacklin et al., 2009), *Aphanizomenon*, *Cylindrospermopsis*, *Nodularia*, *Lyngbya* and *Nostoc*. In contrast, other harmful cyanobacterial genera such as *Microcystis* and *Planktothrix* cannot fix N₂. Nitrogen fixation is carried out by nitrogenase (Zehr et al., 2000). This enzyme complex is inhibited by oxygen (Gallon, 1992). Since photosynthesis produces oxygen, cyanobacteria need special adaptations to protect nitrogenase from oxygen inactivation. In freshwater ecosystems, diazotrophic cyanobacteria have developed a spatial separation of photosynthesis and nitrogen fixation by differentiating special cells, known as heterocysts (Muro-Pastor and Hess, 2012). In marine ecosystems, most diazotrophic cyanobacteria lack heterocysts, but have found other ways to avoid oxygen inhibition, for instance by fixing nitrogen at night (Brauer et al., 2013).

Rising CO₂ concentrations may enhance nitrogen fixation rates, as has been reported for non-heterocystous marine cyanobacteria such as *Trichodesmium* and *Crocosphaera* (Hutchins et al., 2007; Levitan et al., 2007; Fu et al., 2008). However, there are large strain-specific differences in CO₂ response, suggesting that individual strains of these diazotrophs are adapted to grow and fix nitrogen at different CO₂ concentrations (Hutchins et al., 2013). These strain-specific differences might again be related to variation in the presence and expression of different C₄ uptake systems, similar to the genetic and phenotypic variation in CO₂ responses of *Microcystis* (Sandrini et al., 2014, 2015c). For example, *Trichodesmium erythraeum* IMS101 possesses only the high-flux CO₂ uptake system NDH-I₄ and the high-flux bicarbonate uptake system BicA (Kranz et al., 2011), which may explain why the nitrogen fixation and growth rate of this high-flux specialist increase strongly with a rise in ambient pCO₂ levels (Hutchins et al., 2007; Levitan et al., 2007; Kranz et al., 2009).

Nitrogen fixation rates in *Nodularia spumigena*, a heterocystous diazotroph from the Baltic Sea, showed contrasting CO₂ responses in different laboratory experiments (Czerny et al., 2009; Wannicke et al., 2012; Eichner et al., 2014). Part of this variation might be attributed to different growth conditions, as some experiments were performed under phosphate-limited conditions (Wannicke et al., 2012) whereas others used phosphate-replete conditions (Czerny et al., 2009; Eichner et al., 2014). Mesocosms with natural phytoplankton assemblages from the Baltic Sea, including *Nodularia* and *Aphanizomenon* species, did not reveal any signification change in N₂ fixation activity in response to elevated CO₂ (Paul et al., 2015). To what extent the N₂ fixation rates of harmful cyanobacteria in lakes and reservoirs will respond to rising CO₂ is still largely an open question. Comparative studies of the genetic and phenotypic variation in CO₂ responses among diazotrophs may shed more light on this important gap in our knowledge.
**Effects of climate change on cyanobacterial toxins**

Cyanobacteria produce a range of bioactive compounds (Welker and Von Döhren, 1998; Leão et al., 2012). Microcystins are the most well-known and most abundant ones in lakes and are toxic to animals (Metcalf and Codd, 2012). In predicting the effects of climate change on microcystin concentrations in a lake, one should focus on the effect of environmental conditions on: 1) cyanobacterial biomass, 2) the ratio of toxic (microcystin-producing) to non-toxic cyanobacteria, and 3) the microcystin production per cell.

Variation in cyanobacterial biomass causes the largest variation in cyanotoxin concentrations in aquatic ecosystems: more cyanobacteria tend to produce more toxins. Cyanobacterial biomass is affected by, e.g., CO₂, temperature, nutrients and light.

The ratio of toxic to non-toxic strains is also a major determinant of the microcystin concentration in lakes (Kardinaal and Visser, 2005). Davis et al. (2009) found that during field experiments in four lakes in the northeast USA, toxic strains of Microcystis grew faster than their non-toxic counterparts when water temperatures were increased 4°C above ambient (average of the four lakes was 24°C). Furthermore, they found that the interaction of increasing temperature and nutrients produced the highest growth rates in toxic strains, potentially leading to larger blooms with higher toxin contents.

Changes in microcystin production can be responsible for up to a fourfold variation of the microcystin content per cell (Wiedner et al., 2003; Kardinaal and Visser, 2005; Van de Waal et al., 2009). Many studies have investigated the impact of environmental variables on the microcystin production of toxic cells. The review of Gehringer and Wannicke (2014) indicates that microcystin production is stimulated by an ample supply of nutrients in combination with suitable temperature and light conditions for optimal growth. Under nutrient-rich conditions, elevated CO₂ levels stimulate a further increase of the microcystin content in Microcystis cells (Van de Waal et al., 2009; Sandrini et al., 2015a). Furthermore, in a strain producing several different microcystin variants, elevated CO₂ levels in combination with high nitrogen concentrations shifted the microcystin composition towards the more N-rich but less toxic variant microcystin-RR (Van de Waal et al., 2009).

Almost all previous research on the effects of environmental conditions on microcystin production (reviewed by, e.g., Sivonen and Jones, 1999; Gehringer and Wannicke, 2014) has been performed on free microcystins in the cells, while it is now known that a large fraction is covalently bound to proteins (Zilliges et al., 2011; Meissner et al., 2013, 2015). These bound microcystins cannot be extracted using methanol. The fraction of bound microcystins is variable and dependent on the environmental conditions, e.g., the binding to proteins is associated with oxidative stress caused by high light (Meissner et al., 2013). This raises questions regarding the validity of previous studies as well as the potential toxicity of
bound microcystins. Further research on the binding of microcystins to proteins is therefore recommended.

Cyanobacteria can also produce a variety of other toxins, including the hepatotoxins nodularin and cylindrospermopsin and the neurotoxins anatoxin and saxitoxin. These cyanotoxins are less widespread than microcystin, and only a few studies have investigated how their production is affected by environmental conditions (reviewed by Neilan et al., 2013; Boopathi and Ki, 2014).

Future research needs and conclusions
One of the key points emphasized in this review is that dissolved inorganic carbon concentrations in eutrophic lakes can change dramatically on seasonal time scales, from supersaturation in winter to undersaturation in summer. Yet, the possible impacts of rising atmospheric CO2 levels on freshwater ecosystems have received surprisingly little attention thus far. Models and laboratory experiments provide arguments that rising CO2 levels are likely to stimulate cyanobacterial blooms. However, field evidence is still limited, and the extent to which cyanobacterial blooms can sequester atmospheric CO2 is still largely unexplored. Hence, there is a need for lake studies on the coupling of cyanobacterial blooms with seasonal and diurnal dynamics of the dissolved inorganic carbon, and how these dynamics interact with exchanges of CO2 with the atmosphere.

Furthermore, during recent years much more has become known about the molecular functioning and genetic diversity of cyanobacterial CCMs, both in model cyanobacteria such as Synechocystis PCC 6803 (Price, 2011; Burnap et al., 2015) and in environmentally relevant cyanobacteria such as Microcystis (Sandrini et al., 2014, 2015c). However, little is known about the abundance, succession and geographical distribution of different C4 uptake genotypes in natural waters, or about evolutionary adaptation of cyanobacterial CCMs following prolonged exposure to elevated CO2 concentrations. Hence, there is a need for biogeographical and eco-evolutionary studies investigating adaptive responses of cyanobacteria to changes in CO2 availability.

Although effects of environmental conditions on microcystin production in Microcystis have been extensively investigated, there are many other toxins produced by many other species that have yet to be examined. Furthermore, the toxin concentrations in cyanobacteria-dominated lakes are largely determined by the relative abundances of toxic versus non-toxic strains. Yet, only a few studies have investigated how the competition between toxic and non-toxic strains is altered at elevated temperature (Davis et al., 2009) and elevated CO2 (Van de Waal et al., 2011). Hence, there is a need for studies assessing how climate change will affect the toxicity of cyanobacterial blooms, and in particular under which circumstances toxic
strains are able to outperform non-toxic strains and *vice versa*.

Cyanobacteria and eukaryotic algae may respond differently to climate change, which can lead to large changes in phytoplankton community composition. Yet, only a few studies have compared growth responses to temperature or CO₂ across a wide range of species (e.g., Butterwick *et al.*, 2005; Lürling *et al.*, 2013). The available studies provide little information on the impact of limiting resources (N, P, carbon, light) on the temperature-growth responses, and possible synergistic effects of rising CO₂ and elevated temperature have rarely been investigated (Fu *et al.*, 2007; Karlberg and Wulff, 2013). Hence, to understand changes in community composition, there is a great need for controlled studies that compare growth responses to rising CO₂ and global warming across different species.

Comparative lake data have been analyzed to study the impact of temperature on cyanobacterial dominance across large geographical gradients (Kosten *et al.*, 2012; Taranu *et al.*, 2012; Beaulieu *et al.*, 2013; Rigosi *et al.*, 2014). To predict the impact of rising CO₂ concentrations, similar comparative lake studies should be carried out that focus on CO₂ dynamics and pH in relation to phytoplankton community composition. Furthermore, there is a particular need for long-term lake studies, so that changes over time can be quantified.

In conclusion, the effects of rising atmospheric CO₂ concentrations on cyanobacteria are multifaceted and can be quite complex. There are still many intriguing open questions and uncertainties. Hence, there is a clear need for more laboratory and field research across a range of spatiotemporal scales. The risk that rising CO₂ levels will cause a further deterioration of the water quality in many areas of the world generates a societal responsibility for scientists, water managers and policy makers to take further steps in our ability to understand, predict and mitigate the occurrence of toxic blooms in the surface waters across the changing landscapes of our planet.

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