



UvA-DARE (Digital Academic Repository)

Effects of rising CO₂ on the harmful cyanobacterium *Microcystis*

Sandrini, G.

Publication date

2016

Document Version

Final published version

[Link to publication](#)

Citation for published version (APA):

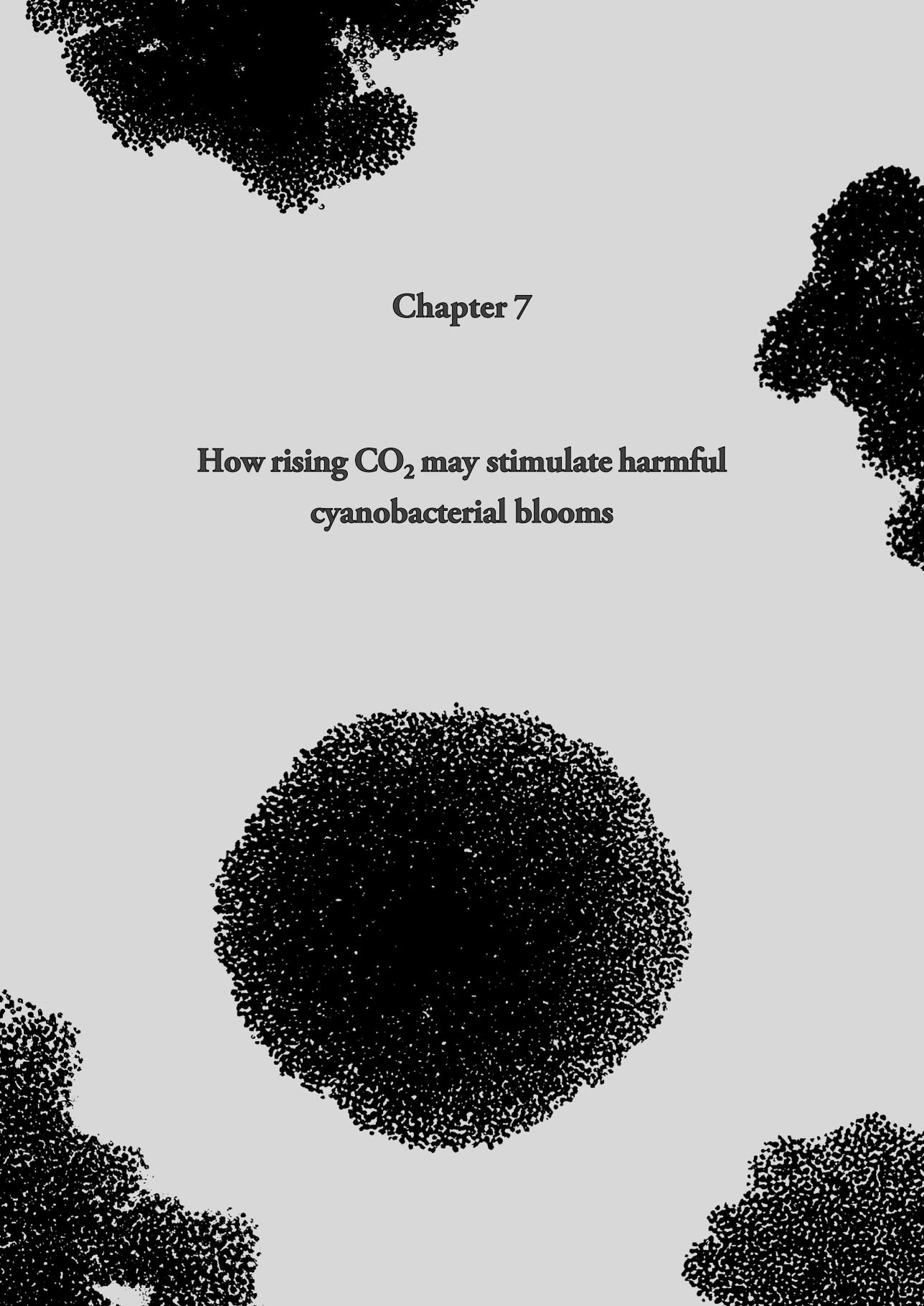
Sandrini, G. (2016). *Effects of rising CO₂ on the harmful cyanobacterium *Microcystis**. [Thesis, fully internal, Universiteit van Amsterdam].

General rights

It is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), other than for strictly personal, individual use, unless the work is under an open content license (like Creative Commons).

Disclaimer/Complaints regulations

If you believe that digital publication of certain material infringes any of your rights or (privacy) interests, please let the Library know, stating your reasons. In case of a legitimate complaint, the Library will make the material inaccessible and/or remove it from the website. Please Ask the Library: <https://uba.uva.nl/en/contact>, or a letter to: Library of the University of Amsterdam, Secretariat, P.O. Box 19185, 1000 GD Amsterdam, The Netherlands. You will be contacted as soon as possible.



Chapter 7

**How rising CO₂ may stimulate harmful
cyanobacterial blooms**

How rising CO₂ may stimulate harmful cyanobacterial blooms

Giovanni Sandrini¹, Jolanda M.H. Verspagen¹, Petra M. Visser¹,
Lucas J. Stal^{1,2}, Hans C.P. Matthijs¹, Timothy W. Davis³,
Hans W. Paerl⁴, Jef Huisman¹

¹*Department of Aquatic Microbiology, Institute for Biodiversity and Ecosystem Dynamics,
University of Amsterdam, Amsterdam, The Netherlands*

²*Department of Marine Microbiology, Royal Netherlands Institute for Sea Research (NIOZ),
Yerseke, The Netherlands*

³*NOAA Great Lakes Environmental Research Laboratory, Ann Arbor, Michigan, USA*

⁴*Institute of Marine Sciences, University of North Carolina at Chapel Hill, Morehead City, North
Carolina, USA*

Keywords: climate change; cyanobacteria; harmful algal blooms; lakes; rising CO₂

This chapter is based on my contribution to a more extensive review paper invited for a special issue of *Harmful Algae* (entitled “The global expansion of harmful cyanobacterial blooms: diversity, ecology, causes, and controls”):

Visser PM, Verspagen JMH, Sandrini G, Stal LJ, Matthijs HCP, Davis TW, Paerl HW, Huisman J. (2016). How rising CO₂ and global warming may stimulate harmful cyanobacterial blooms. *Harmful Algae* (in press)

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; Peeters *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 CO₂ 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 CO₂ levels may stimulate the proliferation of surface-dwelling cyanobacteria by providing them preferential access to a vast and rising pool of atmospheric CO₂ (Paerl and Ustach, 1982; Ibelings and Maberly, 1998; Verspagen *et al.*, 2014b). An increase in atmospheric CO₂ increases its dissolution in water. Enhanced dissolution of CO₂ 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 CO₂ 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 CO₂ supersaturation, *i.e.*, dissolved CO₂ concentrations that greatly exceed equilibrium with the atmosphere (Cole *et al.*, 1994; Sobek *et al.*, 2005). Conversely, in other lakes, CO₂ 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 CO₂ 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 CO₂ concentrations on cyanobacteria will be addressed. First, an overview of physiological and ecological responses to elevated CO₂ concentrations will be provided, with special emphasis on the CO₂-concentrating mechanisms

(CCMs) 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 CO_2 in water is temperature dependent. However, seasonal variation of the measured $\text{CO}_2(\text{aq})$ concentration in Lake Volkerak is much larger, because biological consumption and production of CO_2 act at a much faster rate than the equilibration of CO_2 between water and atmosphere. In winter and spring, the measured $\text{CO}_2(\text{aq})$ concentration in Lake Volkerak largely exceeds the $\text{CO}_2(\text{aq})$ concentration that would be predicted from equilibrium with the atmosphere, and hence in winter and spring the lake is supersaturated with CO_2 . 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 $\text{CO}_2(\text{aq})$ concentration to $1 \mu\text{mol L}^{-1}$ (≈ 30 ppm), such that the lake becomes severely undersaturated with CO_2 in summer while the pH rises above 9 for several months (Figure 7.1). These data illustrate that the $\text{CO}_2(\text{aq})$ concentration in eutrophic lakes can vary from supersaturation in winter to undersaturation in summer.

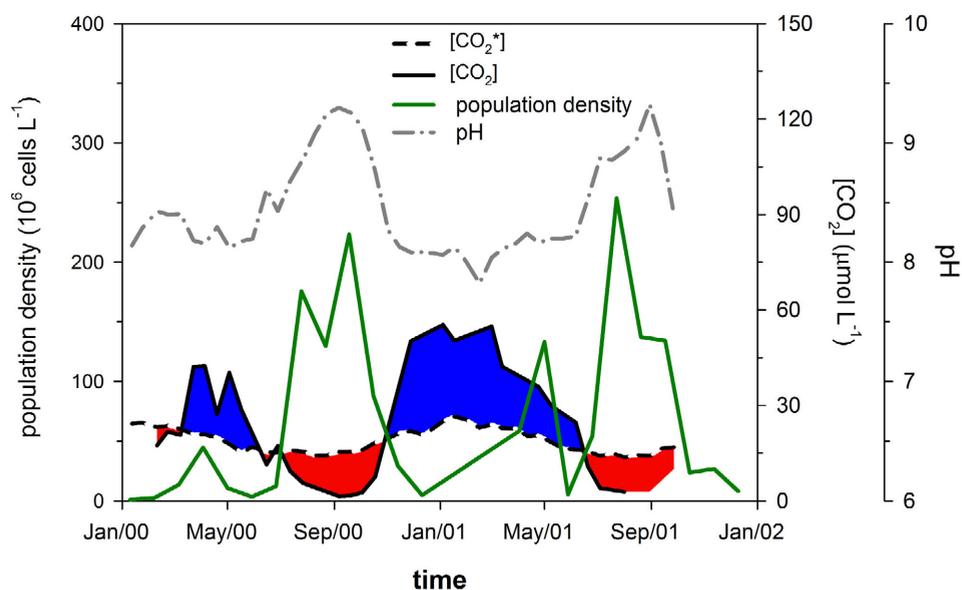


Figure 7.1. Seasonal changes in phytoplankton population density (green line), dissolved CO_2 concentration ($[\text{CO}_2]$, black solid line) and pH (grey dash-dotted line) in Lake Volkerak during two consecutive years. The black dashed line is the expected dissolved CO_2 concentration ($[\text{CO}_2^*]$) when assuming equilibrium with the atmospheric $p\text{CO}_2$ level. Blue shading indicates that the lake is supersaturated with CO_2 , 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 $\text{CO}_2(\text{aq})$ concentration by cyanobacterial blooms turns lakes into a sink for atmospheric CO_2 (Balmer and Downing, 2011). The CO_2 gas influx depends on the CO_2 deficit. More specifically, the CO_2 influx (g_{CO_2}) is proportional to the difference

between the expected concentration of CO₂(aq) in equilibrium with the atmosphere (calculated from Henry's law) and the observed CO₂(aq) concentration (Siegenthaler and Sarmiento, 1993; Cole *et al.*, 2010):

$$g_{CO_2} = v (K_H pCO_2 - CO_2(aq)) \quad (1)$$

where v is the gas transfer velocity (also known as piston velocity) across the air-water interface, K_H is the solubility constant of CO₂ gas in water, and pCO_2 is the partial pressure of CO₂ in the atmosphere. If it is assumed that the dense cyanobacterial bloom has stripped the surface layer of CO₂(aq), this equation simplifies to $g_{CO_2} = v K_H pCO_2$. The gas transfer velocity depends on several parameters, especially wind speed. A typical value for the gas transfer velocity of lakes is $v = 0.02 \text{ m h}^{-1}$ (Crusius and Wanninkhof, 2003; Cole *et al.*, 2010). Hence, assuming an atmosphere with $pCO_2 = 400 \text{ ppm}$, the CO₂ influx during a dense cyanobacterial bloom would amount to $\sim 7 \text{ mmol m}^{-2} \text{ d}^{-1}$. 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 CO₂ concentration, to 800 ppm, would roughly double the CO₂ influx to $\sim 14 \text{ mmol m}^{-2} \text{ d}^{-1}$. Moreover, this might still be an underestimate for dense cyanobacterial blooms. At $\text{pH} > 9$, which is typical for dense blooms, the chemical reaction of CO₂ with the abundant hydroxide ions further increases CO₂ 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 CO₂ 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 CO₂ 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 CO₂(aq) concentration to $0.2 \mu\text{mol L}^{-1}$ 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 CO₂(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).

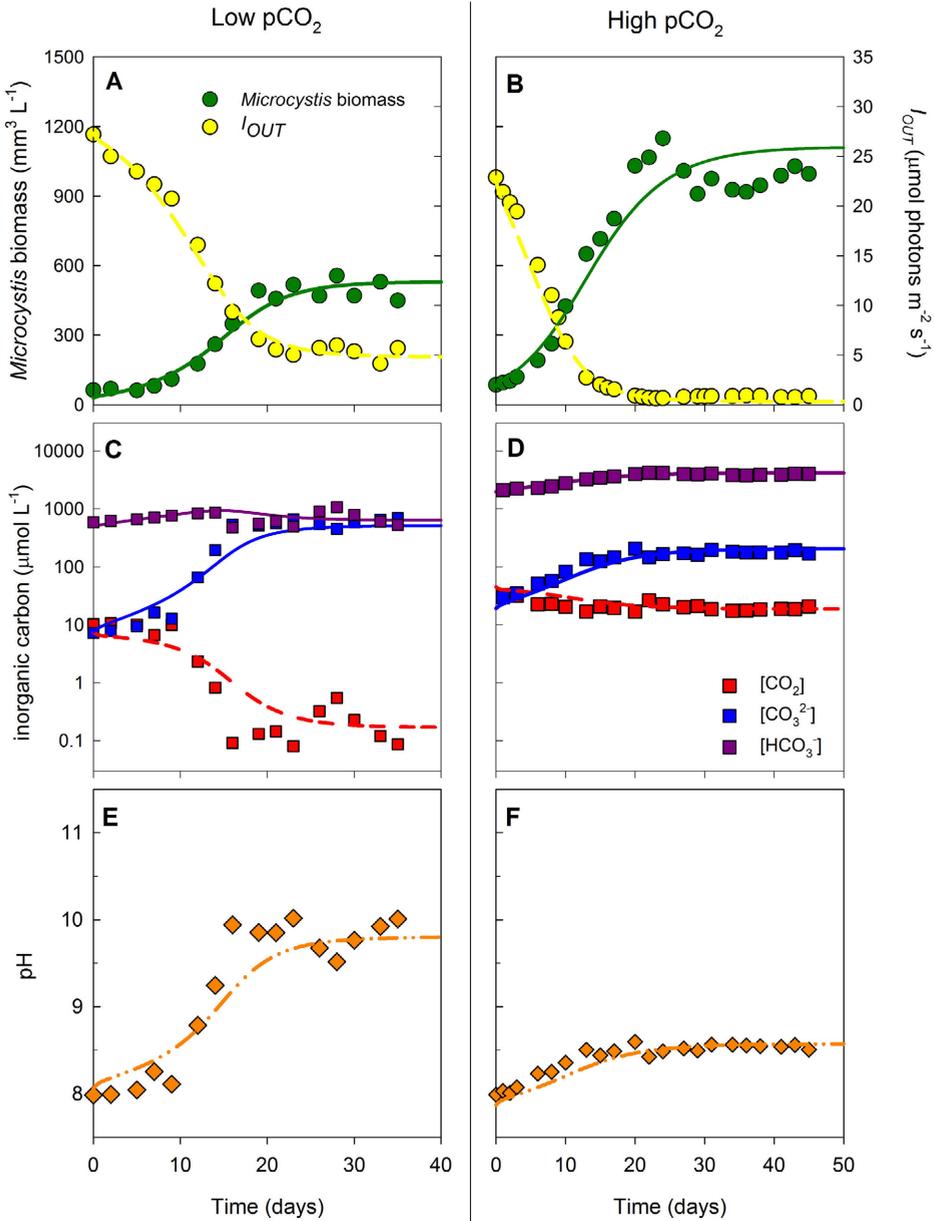


Figure 7.2. Cyanobacterial growth and inorganic carbon chemistry at two different pCO₂ levels. Left panels: Chemostat experiment with low pCO₂ of 200 ppm in the gas flow and 500 μmol L⁻¹ bicarbonate in the mineral medium. Right panels: Chemostat experiment with high pCO₂ of 1,200 ppm in the gas flow 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 C_i 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_i uptake systems have been identified, three for the uptake of bicarbonate and two for the conversion of CO_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 ($K_{0.5} = 70\text{-}350 \mu\text{M}$ bicarbonate) but high flux rate. Conversely, SbtA has a high affinity for bicarbonate ($K_{0.5} < 5 \mu\text{M}$ 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 ($K_{0.5} = 10\text{-}15 \mu\text{M}$ 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_2 uptake systems, NDH-I₃ and NDH-I₄, convert CO_2 that passively diffuses into the cell to bicarbonate in a NADPH-dependent reaction (Price *et al.*, 2002; Price, 2011). NDH-I₃ has a high affinity for CO_2 ($K_{0.5}=1\text{-}2 \mu\text{M}$ CO_2) but a low flux rate (Maeda *et al.*, 2002; Price *et al.*, 2002). Conversely, NDH-I₄ has a lower affinity for CO_2 ($K_{0.5}=10\text{-}15 \mu\text{M}$ CO_2) but a high flux rate (Maeda *et al.*, 2002; Price *et al.*, 2002). This diverse array of C_i uptake systems enables cyanobacteria to respond effectively to changes in C_i availability.

Eukaryotic algae can also employ a CCM, but it works differently from the CCM of cyanobacteria. In the green alga *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_2 near the pyrenoids where CO_2 fixation takes place (Moroney and Ynalvez, 2007; Moroney *et al.*, 2011). An interesting selection experiment where *C. reinhardtii* was exposed to elevated CO_2 for 1,000 generations revealed that some cell lines lost the ability to induce high-affinity CO_2 uptake (Collins and Bell, 2004; Collins *et al.*, 2006). This was attributed to mutations in CCM genes. Hence, similar to cyanobacteria, *C. reinhardtii* likely also possesses high-affinity and low-affinity C_i uptake genes. This experiment demonstrates that eukaryotic algae can evolve in response to elevated CO_2 . Yet, much less is known about the CCM genes and proteins of algae than those of cyanobacteria.

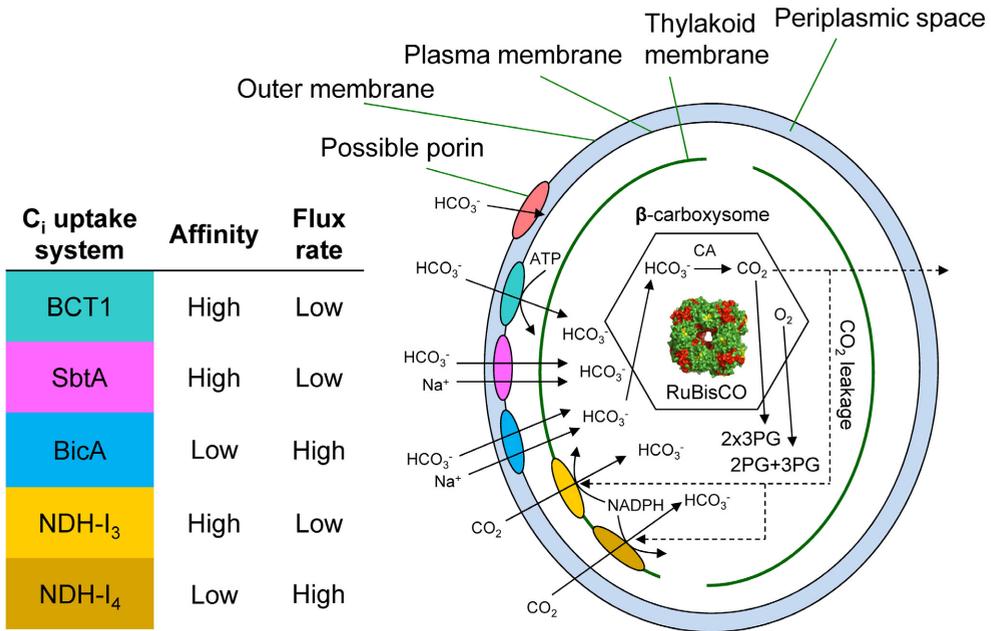


Figure 7.3. Schematic overview of the CCM in cyanobacteria. Five different C_i uptake systems are known in cyanobacteria, including the ATP-dependent bicarbonate uptake system BCT1, two sodium-dependent bicarbonate uptake systems (BicA and SbtA) and two CO₂ uptake systems (NDH-I₃ and NDH-I₄). The C_i 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 C_i 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 C_i 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 C_i uptake genotypes were found (Table 7.1). Some *Microcystis* strains possess all five C_i uptake systems and these are referred to as C_i uptake generalists. Moreover, in *Microcystis*, these C_i 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_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_i uptake genotypes and the presence of microcystin production.

Within the C_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_i uptake systems, in this case the loss of BicA. Presumably, in environments with low C_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_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 CO₂ (pCO₂), but perform poorly at high pCO₂ conditions. Conversely, high-flux specialists with *bicA* but without *sbtA* grow poorly at low pCO₂, but perform well at high pCO₂ levels. Finally, C_i uptake generalists containing all five C_i uptake systems grow well across a wide range of pCO₂ levels (from 20 to 10,000 ppm) (Sandrini *et al.*, 2014).

Competition experiments by Van de Waal *et al.* (2011) showed that rising pCO₂ 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_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 pCO₂ levels. In contrast, *Microcystis* strain NIVA-CYA 43 (= PCC 7005) was a C_i uptake generalist with both *bicA* and *sbtA*, and won the competition at high pCO₂ levels. Hence, while these experiments demonstrate that rising pCO₂ may shift

strain dominance, this shift can be attributed to differences in the C_i 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.

C_i 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 “C_i uptake minimalists”.

Hence, similar to *Microcystis*, other genera of harmful cyanobacteria also show genetic variation in their C_i 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 C_i 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.

Strain	Origin	C_i uptake systems					Carboxy- some	MC genes
		Bicarbonate			CO_2			
		BicA ^b	SbtA ^a	BCT1 ^a	NDH-I ₃ ^a	NDH-I ₄ ^b		
8 <i>Microcystis</i> strains	Sandrini <i>et al.</i> (2014)	+	+	+	+	+	+	+/-
<i>Microcystis</i> PCC 7806	Sandrini <i>et al.</i> (2014)	+	-	+	+	+	+	+
11 <i>Microcystis</i> strains	Sandrini <i>et al.</i> (2014)	-	+	+	+	+	+	+/-
<i>Anabaena cylindrica</i> PCC 7122	GBR (Cambridge)	+	-	?	+	+	+	-
<i>Anabaena</i> sp. 90	FIN (Lake Vesijarvi)	-	-	+	+	+	+	+
<i>Anabaena</i> sp. PCC 7108	USA (Moss Beach, California)	-	+	+	+	+	+	-
<i>Anabaena variabilis</i> ATCC 29413	USA (Mississippi)	+, ≠	+	+	+	+	+	-
<i>Aphanizomenon flos-aquae</i> NIES-81	JPN (Lake Kasumigaura)	-	-	+	+	+	+	-
<i>Planktothrix agardhii</i> NIVA-CYA 15	NOR (Lake Kolbotnvatnet)	-	+	+	+	+	+	+
<i>Planktothrix agardhii</i> NIVA-CYA 34	NOR (Lake Kolbotnvatnet)	-	-	+	+	+	+	+
<i>Planktothrix agardhii</i> NIVA-CYA 56/3	FIN (Lake Steinsfjorden)	-	+	+	+	+	+	+
<i>Planktothrix prolifica</i> NIVA-CYA 98	FIN (Lake Steinsfjorden)	-	-	+	+	+	+	+
<i>Planktothrix agardhii</i> NIVA-CYA 126/8	FIN (Lake Langsjon)	+	-	+	+	+	+	+
<i>Planktothrix mougeotii</i> NIVA-CYA 405	FIN (Lake Steinsfjorden)	-	-	+	+	+	+	+
<i>Planktothrix prolifica</i> NIVA-CYA 406	FIN (Lake Steinsfjorden)	-	-	+	+	+	+	+
<i>Planktothrix rubescens</i> NIVA-CYA 407	FIN (Lake Steinsfjorden)	-	-	+	+	+	+	+
<i>Planktothrix prolifica</i> NIVA-CYA 540	FIN (Lake Steinsfjorden)	-	+	+	+	+	+	+
<i>Synechocystis</i> sp. PCC 6803	USA (California)	+	+	+	+	+	+	-
<i>Synechococcus</i> sp. PCC 7002	PRI (Magueyes Island)	+	+	-	+	+	+	-
<i>Synechococcus</i> sp. PCC 7942	USA (Texas)	-	+	+	+	+	+	-

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.

^a C_i uptake system with a high substrate affinity and low flux rate.

^b C_i uptake system with a low substrate affinity and high flux rate.

It is often argued that cyanobacteria have a very effective CCM, and are therefore particularly strong competitors at low CO₂ levels in comparison to eukaryotic phytoplankton (Shapiro, 1990). If so, one might expect that low CO₂ concentrations will favor cyanobacteria, whereas eukaryotic phytoplankton tend to become more dominant at elevated CO₂ 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 CO₂ but not at elevated CO₂ concentrations (Verschoor *et al.*, 2013). Indeed, the new insights reviewed above indicate that not all cyanobacteria are strong competitors at low CO₂. 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 CO₂, whereas other strains are much better competitors under high CO₂ conditions. This genetic and phenotypic variation in C_i uptake systems provides cyanobacterial communities with the potential for rapid evolutionary adaptation to changing CO₂ conditions, with a major selective advantage for cyanobacteria with high-flux C_i uptake systems in high-CO₂ 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 CO₂ 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 CO₂ on phytoplankton biomass production. They investigated a stoichiometrically explicit model that describes phytoplankton growth as function of nutrient, CO₂ and light availability. Hence, there are three potentially limiting resources in this model (**Figure 7.4A**). Inorganic carbon becomes limiting at very low pCO₂ levels, nutrients become limiting at very low nutrient loads, and light becomes limiting in dense phytoplankton blooms at high pCO₂ 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 pCO₂ levels will increase phytoplankton

biomass (**Figure 7.4B**). In oligotrophic waters with low nutrient loads, rising $p\text{CO}_2$ levels will shift phytoplankton growth from carbon-limited to nutrient-limited conditions (black arrow in **Figure 7.4A**). In this case, the higher CO_2 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 $p\text{CO}_2$ 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 $p\text{CO}_2$ levels in eutrophic and hypertrophic than in oligotrophic ecosystems.

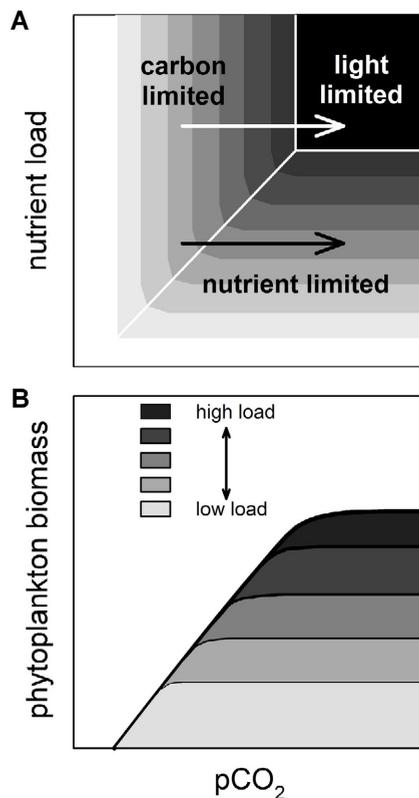


Figure 7.4. Model predictions illustrating how different nutrient loads may modify effects of rising CO_2 on resource limitation and phytoplankton biomass. (A) Hypothesized patterns of resource limitation, at different atmospheric CO_2 levels and nutrient loads. The arrows indicate that rising atmospheric CO_2 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 CO_2 levels will depend on the nutrient load. Adjusted from Verspagen *et al.* (2014a).

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 *Crocospaera* (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 C_i 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 significant 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 Gehring 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; Gehring 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 C_i 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.

Acknowledgements

We thank the two reviewers for their constructive comments. G. Sandrini was supported by the Division of Earth and Life Sciences (ALW) of the Netherlands Organization for Scientific Research (NWO); J.M.H. Verspagen was supported by the Amsterdam Water Science program of the Amsterdam Academic Alliance; H.W. Paerl was supported by US National Science Foundation Grants CBET 0826819, 1230543, and Dimensions of Biodiversity 1240851). We acknowledge the COST Action ES 1105 'CYANOCOST - Cyanobacterial blooms and toxins in water resources: Occurrence, impacts and management' for the opportunity to exchange ideas with co-authors and other scientists.