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How rising CO_2 and global warming may stimulate harmful cyanobacterial blooms

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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 CO_2 concentrations. Depletion of dissolved CO_2 by dense cyanobacterial blooms creates a concentration gradient across the air-water interface. A steeper gradient at elevated atmospheric CO_2 concentrations will lead to a greater influx of CO_2, which can be intercepted by surface-dwelling blooms, thus intensifying cyanobacterial blooms in eutrophic waters. Bloom-forming cyanobacteria display an unexpected diversity in CO_2 responses, because different strains combine their uptake systems for CO_2 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. Increasing temperatures also stimulate cyanobacterial growth. Many bloom-forming cyanobacteria and also green algae have temperature optima above 25°C, often exceeding the temperature optima of diatoms and dinoflagellates. Analysis of published data suggests that the temperature dependence of the growth rate of cyanobacteria exceeds that of green algae. Indirect effects of elevated temperature, like an earlier onset and longer duration of thermal stratification, may also shift the competitive balance in favor of buoyant cyanobacteria while eukaryotic algae are impaired by higher sedimentation losses. Furthermore, cyanobacteria differ from eukaryotic algae in that they can fix dinitrogen, and new insights show that the nitrogen-fixation activity of heterocystous cyanobacteria is strongly stimulated at elevated temperatures. However, models and lake studies

indicate that the response of cyanobacterial growth to rising CO_2 concentrations and elevated temperatures 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.

Keywords: climate change, cyanobacteria, harmful algal blooms, lakes, rising CO_2, temperature

1. 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 (CO_2), 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 CO_2 in ice cores and the atmosphere (e.g., at Mauna Loa, Hawaii) have shown that there is a well-defined parallel between increasing CO_2 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, 2012), 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,

2012; 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 also be addressed. The mechanistic underpinnings supporting cyanobacterial expansion in an atmospherically-CO_2 enriched, warmer, and nutrient-enriched world will be explored.

Physiological traits vary among species and strains and may direct the response of cyanobacterial species to a changing climate. First, an overview of these responses to elevated CO_2 concentrations will be provided, with special emphasis on CO_2-concentrating mechanisms (CCMs). Then the focus will be on direct and indirect temperature effects on cyanobacterial growth and competition, followed by a further exploration of interactive effects of climate change with nutrient availability. 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.

2. **Response to rising CO_2**

2.1. Does rising CO_2 intensify bloom development?

Rising atmospheric CO_2 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 CO_2 concentrations in freshwater lakes are sufficiently high to cover the carbon demands of phytoplankton populations, because many lakes are "supersaturated" with CO_2 (Cole et al., 1994; Sobek et al., 2005; Jansson et al., 2012), and (2) that changes in CO_2 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 pCO_2 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 CO_2 (Cole & Caraco, 2001; Pacala et al., 2001; Richey et al., 2002; Maberly et al., 2013), which is subsequently mineralized, causing pCO_2 levels that commonly exceed 1,500 ppm. However, even in these "supersaturated waters", the actual concentration of dissolved CO_2 (CO_2(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 pCO_2 of 1,500 ppm. According to Henry's Law, assuming a solubility constant of $K_H = 0.034$ mol L^{-1} atm^{-1}, the CO_2(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 CO_2(aq) concentration in this lake within a few hours (Talling, 1976; Maberly, 1996). In some lakes, the CO_2(aq) concentration can even be drawn down to less than 0.1 µmol L^{-1}, corresponding to a pCO_2 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 1 (Verspagen et al., 2006; 2014b). In this figure, the CO_2(aq) concentration that would be predicted from equilibrium with the atmosphere (i.e., [CO_2*]) has also been indicated. This predicted equilibrium CO_2(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 CO_2(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 CO_2(aq) concentration in Lake Volkerak largely exceeds the CO_2(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 CO_2(aq) concentration to 1 µmol 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 (Fig. 1). These data illustrate that the CO_2(aq) concentration in eutrophic lakes can vary from supersaturation in winter to undersaturation in summer.

The drawdown of the CO_2(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_{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):

$$g_{CO2} = v(K_H pCO_2 - CO_2(aq)) \quad (1)$$

where v is the gas transfer velocity (also known as piston velocity) across the air-water interface, K_H is the solubility constant of CO_2 gas in water, and pCO_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 $g_{CO2} = v\ K_H\ pCO_2$. 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 m h^{-1} (Crusius and Wanninkhof, 2003; Cole et al., 2010). Hence, assuming an atmosphere with pCO_2 = 400 ppm, the CO_2 influx during a dense cyanobacterial bloom would amount to ~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 ~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* CYA140 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 (Fig. 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 (Fig. 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).

The second misconception is that changes in CO_2 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 CO_2 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., 2015b). 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* HUB5-2-4, 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 CO_2 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 pCO_2 levels (0.5 ppm) to saturating pCO_2

levels (2,800 ppm) led to an almost 20-fold increase of the *Microcystis* biomass. Hence, these laboratory experiments show that addition of CO_2 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 CO_2 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 CO_2 on cyanobacterial growth is species specific. Moreover, the next sections will show that there is even tremendous variation in CO_2 response within species.

2.2. The CO_2-concentrating mechanism of cyanobacteria

Phytoplankton use CO_2 and bicarbonate available in the environment for carbon fixation with the RuBisCO enzyme. To overcome the low affinity of RuBisCO for CO_2, most phytoplankton, including cyanobacteria, evolved a CO_2-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 CO_2 and bicarbonate from the environment, conversion of the acquired CO_2 into bicarbonate in the cytoplasm, and subsequent diffusion of the accumulated bicarbonate into specialized compartments called carboxysomes (Fig. 3). In the carboxysomes, carbonic anhydrases convert the accumulated bicarbonate back to CO_2, surrounding RuBisCO by a high CO_2 concentration. RuBisCO incorporates CO_2 into the Calvin-Benson-Bassham 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 (Fig. 3). These uptake systems have different physiological properties (Price et al., 2004; Price, 2011;

228 Sandrini et al., 2015b). Two of the bicarbonate transporters, BicA and SbtA, are sodium-
229 dependent symporters (Shibata et al., 2002; Price et al., 2004). BicA has a low affinity for
230 bicarbonate ($K_{0.5}$ = 70-350 μM bicarbonate) but high flux rate. Conversely, SbtA has a high
231 affinity for bicarbonate ($K_{0.5}$ <5 μM bicarbonate) but low flux rate (Price et al., 2004). The third
232 bicarbonate transporter, BCT1, is ATP-dependent, and similar to SbtA it has a high affinity for
233 bicarbonate ($K_{0.5}$ = 10-15 μM bicarbonate) but a low flux rate (Omata et al., 1999; Omata et al.,
234 2002). All three bicarbonate uptake systems are located in the plasma membrane (Price, 2011).
235 The two CO_2 uptake systems, NDH-I_3 and NDH-I_4, convert CO_2 that passively diffuses into
236 the cell to bicarbonate in a NADPH-dependent reaction (Price et al., 2002; Price, 2011). NDH-I_3
237 has a high affinity for CO_2 ($K_{0.5}$=1–2 μM CO_2) but a low flux rate (Maeda et al., 2002; Price et
238 al., 2002). Conversely, NDH-1_4 has a lower affinity for CO_2 ($K_{0.5}$=10–15 μM CO_2) but a high
239 flux rate (Maeda et al., 2002; Price et al., 2002). This diverse array of C_i uptake systems enables
240 cyanobacteria to respond effectively to changes in C_i availability.
241 Eukaryotic algae can also employ a CCM, but it works differently from the CCM of
242 cyanobacteria. In the green alga *Chlamydomonas reinhardtii*, the CCM is based on a light-driven
243 pH gradient that is set up across the chloroplast thylakoid membrane, converting bicarbonate
244 transported into the thylakoid lumen into CO_2 near the pyrenoids where CO_2 fixation takes place
245 (Moroney and Ynalvez, 2007; Moroney et al., 2011). An interesting selection experiment where
246 *C. reinhardtii* was exposed to elevated CO_2 for 1,000 generations revealed that some cell lines
247 lost the ability to induce high-affinity CO_2 uptake (Collins and Bell, 2004; Collins et al., 2006).
248 This was attributed to mutations in CCM genes. Hence, similar to cyanobacteria, *C. reinhardtii*
249 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.

2.3. 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., 2012). 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 CO_2 uptake systems NDH-I_3 and NDH-I_4 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 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 CO_2 (pCO_2), but perform poorly at high pCO_2 conditions. Conversely, high-flux specialists with *bicA* but without *sbtA* grow poorly at low pCO_2, but perform well at high pCO_2 levels. Finally, C_i uptake generalists containing all five C_i uptake systems grow well across a wide range of pCO_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_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 CYA 140) whereas the other was non-toxic (strain CYA 43). However, the genetic analysis of Sandrini et al. (2014)

296 revealed that these two strains also differed in their C_i uptake systems, which provides a much
297 more parsimonious explanation for the observed reversal in competitive dominance. *Microcystis*
298 strain CYA 140 was a high-affinity specialist (only *sbtA*), and won the competition at low pCO_2
299 levels. In contrast, *Microcystis* strain CYA 43 (= PCC 7005) was a C_i uptake generalist with both
300 *bicA* and *sbtA*, and won the competition at high pCO_2 levels. Hence, while these experiments
301 demonstrate that rising pCO_2 may shift strain dominance, this shift can be attributed to
302 differences in the C_i uptake traits of the strains rather than to differences in their microcystin
303 production. In particular, the results of these competition experiments support the hypothesis that
304 natural selection favors the *sbtA* gene at low CO_2 conditions, whereas *bicA*-containing strains are
305 favored at high CO_2 conditions.
306
307 *2.4. C_i uptake systems of other harmful cyanobacteria*
308 The CCMs of other harmful freshwater cyanobacteria have not been studied in detail, partly
309 because genomic data are still largely lacking. But now the genomes of four *Anabaena* strains
310 (Wang et al., 2012; Shih et al., 2013; Thiel et al., 2014), one *Aphanizomenon* strain (Cao et al.,
311 2014) and nine *Planktothrix* strains (Tooming-Klunderud et al., 2013; Christiansen et al., 2014)
312 have been sequenced. We analyzed the CCM genes present in these genomes, based on high
313 similarity of the protein sequences with the reference protein sequences from *Microcystis* PCC
314 7806, *Microcystis* NIES-843, *Synechocystis* PCC 6803, *Synechococcus* PCC 7002 and
315 *Synechococcus* PCC 7942. This analysis revealed that *Anabaena*, *Aphanizomenon* and
316 *Planktothrix* also display variation in the presence of the *bicA* and *sbtA* genes, whereas the two
317 CO_2 uptake systems and the BCT1 bicarbonate transporter are widespread among all four
318 cyanobacterial genera (Table 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 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 CO_2 conditions but a selective advantage for *bicA*-containing strains in high-CO_2 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 CO_2 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.

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

342 genetic diversity of C_i uptake systems shows that there is major variation in the effectiveness of
343 the cyanobacterial CCM, even among different strains within the same genus. Some
344 cyanobacterial strains perform well at low CO_2, whereas other strains are much better
345 competitors under high CO_2 conditions. This genetic and phenotypic variation in C_i uptake
346 systems provides cyanobacterial communities with the potential for rapid evolutionary
347 adaptation to changing CO_2 conditions, with a major selective advantage for cyanobacteria with
348 high-flux C_i uptake systems in high-CO_2 environments.
349
350 **3. Response to rising temperature**
351 *3.1. Enhanced growth rates*
352 Rising temperatures promote cyanobacterial population dynamics in multiple ways.
353 Temperatures of up to ~25°C directly increase cyanobacterial photosynthesis and growth rate
354 (Robarts and Zohary, 1987; Coles and Jones, 2000; Davis et al., 2009; Mehnert et al., 2010;
355 Lürling et al., 2013). Most phytoplankton species reach their optimum temperature for growth in
356 the range of 20-25°C, although there are exceptions, like the thermophilic cyanobacteria of hot
357 springs (e.g. Allewalt et al., 2006) and stenotherm species (as suggested for *Planktothrix*
358 *rubescens* (Findenegg, 1947)).
359 The general consensus is that cyanobacteria have a higher optimal growth temperature than
360 eukaryotic algae. Paerl and Huisman (2008, 2009) based their ‘Blooms like it hot’ statement on
361 experimental growth rate data of different species by Butterwick et al. (2005) and Reynolds
362 (2006), and of seasonal phytoplankton data in a lake by Jöhnk et al. (2008). As a follow-up, Paerl
363 et al. (2011) and Paerl (2014) showed literature data from several experimental studies, and their
364 graphical compilation of these data also clearly indicate that the temperature optima of

cyanobacteria are higher than those of most algae. The temperature optima of cyanobacteria were in the range of 27-37°C overlapping those of green algae with optima in the range of 27-32°C, while those of dinoflagellates (17-27°C) and diatoms (17-22°C) were distinctly different (Paerl, 2014). An experimental study with different species of cyanobacteria and green algae did not reveal a difference in optimum temperatures between these two taxonomic groups (Lürling et al., 2013). An extensive overview of temperature-dependent growth rates from several other studies yielded a slightly higher optimal temperature for cyanobacteria (27.2°C) than for green algae (26.3°C), but this difference was not significant (Lürling et al., 2013). Summarizing, the temperature optima for cyanobacteria and green algae can overlap, and this is likely dependent on species and culture conditions. However, the difference in temperature optima between cyanobacteria and green algae on one side and dinoflagellates and diatoms on the other appears to be considerable.

In addition to temperature optima, it is of interest to investigate how fast the growth rate increases with temperature. The temperature dependence of the growth rates of species has gained much interest in the context of the metabolic theory of ecology (Gillooly et al., 2001; Brown et al., 2004), and can be calculated from the Arrhenius equation:

$$\mu = c \exp\left(-\frac{E_A}{kT}\right) \quad (2)$$

where μ is the growth rate, c is a normalization constant, E_A is the activation energy, k is Boltzmann's constant (8.62×10^{-5} eV K^{-1}), and T is absolute temperature in Kelvin. The activation energy is a measure of the increase of the growth rate with temperature (below the temperature optimum). It can be estimated from an Arrhenius plot, where the natural logarithm

388 of the growth rates (ln μ) is plotted against the inverse of temperature (1/kT). The value of the
389 activation energy is then obtained from the (negative) slope of a linear regression of ln μ versus
390 1/kT. This approach was applied to the growth data of Lürling et al. (2013). For the
391 cyanobacterium *Aphanizomenon gracile*, for example, this yields an activation energy of E_A =
392 0.64 eV while for the green alga *Scenedesmus acuminatus* it yields 0.11 eV (Fig. 4; Table 2).
393 For the cyanobacterial species investigated by Lürling et al. (2013), E_A ranged from 0.50 to
394 1.23 eV (Table 2). On average, the E_A (± s.d.) of the cyanobacteria was 0.70 (± 0.35) eV,
395 whereas that of green algae was 0.43 (± 0.25) eV. This indicates that the growth rate of
396 cyanobacteria increases faster with temperature than that of green algae, although the variation
397 among species is considerable, and the difference between cyanobacteria and green algae was
398 therefore at best marginally significant (Two-sample Student's *t*-test (for equal variances),
399 df=14, p=0.096). To facilitate comparison with the literature, Q_{10} values (which measure the
400 change in growth rate for a temperature increase of 10°C) were also calculated from the data of
401 Lürling et al. (2013). Over the temperature range from 20 to 27.5°C, this gave Q_{10} values of 2.63
402 ± 0.94 for cyanobacteria and 2.03 ± 1.02 for green algae (Table 2). Other studies also reported
403 high Q_{10} values for cyanobacteria, e.g. a study on seven cyanobacterial species by Mehnert et al.
404 (2010) showed an average Q_{10} of 2.33 ± 0.87. An exceptionally high Q_{10} (~9.6) for the growth-
405 temperature dependence of *Microcystis* was reported by Reynolds (2006).
406 To unravel and understand these differences in temperature sensitivity between cyanobacteria
407 and eukaryotic algae will require further study of the temperature dependencies of the different
408 underlying physiological processes affecting phototrophic growth (e.g., carbon fixation,
409 photorespiration and respiration; Bernacchi et al., 2001; Allen et al., 2005).
410

411 *3.2. Increased stability of the water column*

412 Temperature not only has a direct effect on the growth rates of organisms, but also has
413 important indirect effects that can tip the competitive balance between species. In particular, high
414 temperatures increase the stability of the water column, which suppresses turbulent mixing
415 (Peeters et al., 2007; Jöhnk et al., 2008) and extends the duration of thermal stratification (De
416 Stasio et al., 1996; Peeters et al., 2007). Thermal stratification is favorable for buoyant
417 cyanobacteria because weak mixing allows buoyant cyanobacteria to float to the upper water
418 layers, where they have better access to light while shading the non-buoyant phytoplankton
419 below (Reynolds et al., 1987; Huisman et al., 2004; Wagner and Adrian, 2009). Several
420 cyanobacterial species contain gas vesicles, which are hollow protein structures in the cell that
421 are filled with gas (Walsby, 1994). If the buoyancy provided by gas vesicles exceeds the ballast
422 provided by other cell constituents, the cells float upwards.

423 Competition between buoyant cyanobacteria and non-buoyant eukaryotic phytoplankton can
424 be described by a simple model. Consider a highly eutrophic or hypertrophic lake, in which
425 nutrients are in ample supply and phytoplankton species such as diatoms, green algae and
426 buoyant cyanobacteria mainly interact through mutual shading (i.e., competition for light). Let
427 $N_i(z,t)$ denote the concentration of phytoplankton species i at depth z and time t. The population
428 dynamics and vertical distribution of a number of n phytoplankton species can be described by
429 the following set of partial differential equations (Klausmeier and Litchman, 2001; Huisman et
430 al., 2004):

431

432 $$\frac{\partial N_i}{\partial t} = \mu_i(I(z))N_i + v_i\frac{\partial N_i}{\partial z} + D\frac{\partial^2 N_i}{\partial z^2} \qquad i=1,\ldots,n \qquad (3)$$

433

Here, $\mu_i(I(z))$ is the net specific growth rate of species i as function of light intensity I, where light intensity I decreases exponentially with depth z according to Lambert-Beer's law. Moreover, the light intensity at a given depth also depends on the phytoplankton concentrations above that depth, as denser phytoplankton blooms create more turbid conditions. For notational convenience, it was assumed that the net specific growth rate also includes phytoplankton losses due to, e.g., natural mortality, viral lysis and zooplankton grazing. Furthermore, v_i is the vertical sinking or flotation velocity of species i (with $v_i < 0$ for sinking species and $v_i > 0$ for buoyant species), and D is known as the turbulent diffusion coefficient or vertical eddy diffusivity. Zero-flux boundary conditions were assumed.

The model predicts that changes in the turbulent diffusion coefficient D, which is a measure of the mixing intensity, can cause a shift in the outcome of competition (Fig. 5; Huisman et al., 2004). In turbulent waters, vertical mixing dominates over the flotation and sinking rates of the different species. In this case, buoyant cyanobacteria cannot stay in the upper illuminated layers, because they are dispersed throughout the water column by intense vertical mixing. Under these conditions, diatoms and green algae are often superior competitors and displace buoyant cyanobacteria (Visser et al., 1996; Huisman et al., 2004). In contrast, in waters with only weak vertical mixing the flotation rate exceeds the mixing intensity, and hence buoyant cyanobacteria can float upwards and shade all non-buoyant species below. Moreover, in such stagnant waters diatoms and green algae are not entrained but may sink out of the surface layer. Hence, the model predicts that buoyant cyanobacteria displace the diatoms and green algae and dominate the phytoplankton community in waters with weak vertical mixing (Fig. 5).

All three terms in Equation (3) are dependent on temperature. First, the specific growth rates ($\mu_i(I)$) of the different species vary with temperature. As seen in the preceding section, the

growth rates of cyanobacteria tend to respond more strongly to rising temperatures than the growth rates of most eukaryotic phytoplankton. Second, according to Stokes' Law, the vertical flotation and sinking velocities (v_i) of the different species are inversely proportional to the viscosity of water. Since water becomes less viscous at higher temperature, this implies that buoyant cyanobacteria will float upwards faster whereas sinking diatoms will sink faster at higher temperature. Third, higher temperatures favor vertical stratification, which increases the stability of the water column and thereby suppresses vertical mixing by turbulent diffusion (D). Hence, higher temperatures lead to enhanced growth, a higher flotation velocity and a more stable water column, which all tend to favor the development of surface blooms of buoyant cyanobacteria at the expense of sinking diatoms and green algae.

The model predictions are supported by field studies that manipulated the turbulence structure of eutrophic lakes by artificial mixing. In these lake experiments, buoyant cyanobacteria were dominant when the water was stratified, but they were replaced by non-buoyant eukaryotic phytoplankton after the onset of artificial mixing (Reynolds et al., 1983; Visser et al., 1996; Huisman et al., 2004). An experiment in Lake Nieuwe Meer, The Netherlands, investigated an intermittent mixing regime, in which artificial mixing of the entire lake was alternately switched on and off at two-week intervals (Jöhnk et al., 2008). The experiment was performed during the summer of 2003, which turned out to be one of the hottest summers ever recorded in Europe. The increases and decreases in cell number of the buoyant cyanobacterium *Microcystis* during the summer heatwave coincided with the alternations in mixing intensity. When artificial mixing was switched off, the water column stratified and the *Microcystis* concentration in the surface layer rapidly increased both by vertical migration of the colonies and by population growth. And when artificial mixing was switched on again, the water column was well mixed and *Microcystis*

declined. These results demonstrate that, in this lake, the direct effect of high temperatures on cyanobacterial growth rates was by itself not sufficient for cyanobacterial dominance. In addition, high temperatures also increased the stability of the water column, which enhanced the ability of buoyant cyanobacteria to float upwards and shift the competitive balance in their favor (Jöhnk et al., 2008).

3.3. Extension of growing season

Global warming may also affect the annual life cycle of cyanobacteria. Some cyanobacterial species like *Planktothrix agardhii* and *Planktothrix rubescens* form winter blooms or are persistent in the water column throughout the year in temperate lakes (Naselli-Flores et al. 2007; Akçaalan et al. 2014; Anneville et al. 2015). However, most cyanobacterial species decrease in abundance in winter, and several species overwinter as akinetes (specialized cells resistant to cold, desiccation and irradiation) or vegetative cells in lake sediments. N_2-fixing heterocystous cyanobacteria such as *Anabaena*, *Aphanizomenon* and *Gloeotrichia* may form akinetes (Cirés et al., 2010; Sukenik et al., 2012; 2013), whereas *Microcystis* colonies overwinter as vegetative cells (Brunberg and Bostrom, 1992; Misson et al., 2010). The overwintering populations of cyanobacteria in the sediment provide a potential inoculum for spring or summer blooms (Brunberg and Blomqvist, 2003; Cirés et al., 2010; Kravchuk et al., 2011). Model simulations indicate that the absence of recruitment from the sediment would decrease the subsequent summer bloom of *Microcystis* by about 50% (Verspagen et al., 2005). Increasing temperatures may initiate earlier germination of akinetes (Tsujimura and Okubo, 2003; Carey et al., 2014) and recruitment of *Microcystis* colonies (Trimbee and Prepas, 1988; Karlsson-Elfgren et al., 2004; Cao et al., 2008).

503 In temperate lakes, *Microcystis* has a bloom period in August-September, after which the
504 population settles to the lake sediment (Reynolds et al., 1981; Takamura et al., 1984; Thomas
505 and Walsby, 1986). This loss of buoyancy in autumn has been explained by accumulation of
506 carbohydrates at decreasing temperatures (Thomas and Walsby, 1986; Visser et al., 1995).
507 Akinete formation by filamentous cyanobacteria is often induced by the onset of physiological
508 stress, e.g., by phosphate limitation, light limitation, or decreasing temperature (Sinclair and
509 Whitton, 1977; Adams and Duggan, 1999; Meeks et al., 2002).
510 Since temperature is an important driver of recruitment from the sediment in spring and
511 summer, of subsequent population growth, and of the initiation of the benthic life stages at the
512 end of the season, global warming will likely cause an earlier onset and later cessation of
513 cyanobacterial blooms. Hence, climate change may extend the growing season considerably. In
514 Scandinavian lakes, for example, warmer winters and springs have increased spring and early
515 summer biomass of cyanobacteria (Weyhenmeyer, 2001).
516
517 **4. Interactive effects with nutrient availability**
518 *4.1. Effects of climate change depend on nutrient availability*
519 In many aquatic systems the availability of nutrients determines primary production
520 (Dzialowki et al., 2005; Xu et al., 2010; Lewandowska et al., 2014), and total nitrogen and total
521 phosphorus concentrations are often good predictors of cyanobacterial biomass (Downing et al.,
522 2001; Håkanson et al., 2007). However, at the physiological level, there are still many gaps in
523 our understanding of how nutrient limitation may interact with changes in temperature or CO_2
524 availability (e.g., Spijkerman et al., 2011).

525 Verspagen et al. (2014a) developed a conceptual framework to predict how different nutrient
526 loads may modify effects of rising CO_2 on phytoplankton biomass production. They investigated
527 a stoichiometrically explicit model that describes phytoplankton growth as function of nutrient,
528 CO_2 and light availability. Hence, there are three potentially limiting resources in this model
529 (Fig. 6A). Inorganic carbon becomes limiting at very low pCO_2 levels, nutrients become limiting
530 at very low nutrient loads, and light becomes limiting in dense phytoplankton blooms at high
531 pCO_2 levels and high nutrient loads. Light limitation can of course also be induced by other
532 mechanisms, such as a high background turbidity (due to high concentrations of dissolved
533 organic matter or resuspended sediment particles), deep mixing, or low incident light intensities
534 in winter. The resource limitation pattern in Fig. 6A can be used to sketch to what extent rising
535 pCO_2 levels will increase phytoplankton biomass (Fig. 6B). In oligotrophic waters with low
536 nutrient loads, rising pCO_2 levels will shift phytoplankton growth from carbon-limited to
537 nutrient-limited conditions (black arrow in Fig. 6A). In this case, the higher CO_2 availability
538 stimulates some growth, but the phytoplankton biomass remains constrained by the low nutrient
539 levels in the system (Fig. 6B). Conversely, in hypertrophic waters with high nutrient loads, rising
540 pCO_2 levels will shift phytoplankton growth from carbon- to light-limited conditions (white
541 arrow in Fig. 6A), which will allow a much larger increase in phytoplankton biomass (Fig. 6B).
542 Chemostat experiments with the harmful cyanobacteria *Microcystis* CYA140 and HUB5-2-4
543 confirmed these model predictions (Verspagen et al., 2014a). Hence, the general message
544 emerging from these results is that phytoplankton biomass will respond more strongly to rising
545 pCO_2 levels in eutrophic and hypertrophic than in oligotrophic ecosystems.
546 Similarly, the response of cyanobacteria to temperature depends strongly on nutrient
547 availability (Davis et al., 2009; Wagner and Adrian, 2009; Kosten et al., 2012; Taranu et al.,

548 2012; Beaulieu et al., 2013; Rigosi et al., 2014). For instance, two recent studies analyzed data
549 on cyanobacterial biomass in more than 1,000 lakes in the USA (Beaulieu et al., 2013; Rigosi et
550 al., 2014). They found that the cyanobacterial biomass in these lakes was affected by both
551 nutrients and temperature, where nutrients explained a larger proportion of the variation than
552 temperature. Moreover, the relative importance of nutrients and temperature varied with the
553 nutrient status of the lakes. Nutrient availability had a large impact on cyanobacterial biomass in
554 oligotrophic lakes, whereas temperature was more important in mesotrophic lakes. In eutrophic
555 and hyper-eutrophic lakes, nutrients and temperature had a synergistic effect on cyanobacterial
556 biomass (Rigosi et al., 2014). Synergy of nutrients and temperature implies that in a warmer
557 climate, nutrient concentrations will have to be decreased further in order to control
558 cyanobacterial biomass (Kosten et al., 2012). Furthermore, analysis of this large data set revealed
559 that the response to nutrients and temperature varied among the different cyanobacterial taxa.
560 Some taxa, such as *Anabaena*, are more sensitive to changes in nutrient availability, whereas
561 other taxa, such as *Microcystis*, are more sensitive to changes in temperature (Rigosi et al.,
562 2014).
563
564 *4.2. Effects of climate change on nitrogen fixation*
565 Several genera of harmful cyanobacteria are capable of fixing atmospheric dinitrogen (N_2),
566 including *Anabaena* (nowadays referred to as *Dolichospermum*; Wacklin et al., 2009),
567 *Aphanizomenon*, *Cylindrospermopsis*, *Nodularia*, *Lyngbya* and *Nostoc*. In contrast, other harmful
568 cyanobacterial genera such as *Microcystis* and *Planktothrix* cannot fix N_2. Nitrogen fixation is
569 carried out by nitrogenase (Zehr et al., 2000). This enzyme complex is inhibited by oxygen
570 (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). Heterocysts have a thick glycolipid cell wall. This cell wall serves as a gas diffusion barrier to decrease the diffusion of O_2 into the heterocyst, but should of course allow a sufficient influx of N_2 to enable nitrogen fixation (Walsby, 1985). Hence, the glycolipid layer is not impermeable for gases. The O_2 that unavoidably diffuses into the heterocyst should be respired fast enough to obtain near anoxic conditions inside the heterocyst.

Recently, theory has been developed that uses the different temperature dependencies of O_2 diffusion and respiration to predict how rising temperature will affect nitrogen fixation (Stal, 2009; Brauer et al., 2013). Diffusion of O_2 into the cell depends on the O_2 concentration in the surrounding medium, temperature, and the diffusion properties of the cell wall. All else being equal, the O_2 diffusion rate slightly increases with temperature at a Q_{10} of ~1.1; i.e., with every 10^oC increase of temperature the influx of O_2 into the heterocyst increases only with a factor 1.1 (Stal, 2009). Respiration is an enzymatic process that increases (within its physiological limits) with temperature at a Q_{10} of ~2; i.e. the respiration rate doubles with each 10^oC increase of temperature. The consequence is that at high temperature, the respiration rate may easily keep up with the diffusive influx of O_2. However, at low temperature, when respiration is slow, the influx of O_2 by diffusion may exceed the respiration rate and hence the heterocyst has difficulty to maintain anaerobic conditions. At low temperature, the glycolipid cell wall must therefore be more efficient as a gas diffusion barrier. For instance, in the cold waters of the northern Baltic Sea, heterocysts of *Anabaena* sp. possess a much more efficient gas diffusion barrier than those of *Nodularia spumigena* in the warmer waters of the Bornholm Sea in the southern Baltic (Stal,

2009). The downside of a more efficient gas diffusion barrier is a lower influx of N_2 into the heterocysts, thus suppressing their nitrogen fixation activity. The *Nodularia* blooms in the southern Baltic are indeed more productive in terms of biomass and N_2 fixation activity than the *Anabaena* blooms in the northern Baltic.

These theoretical considerations suggest that increasing temperature will favor heterocysts with glycolipid cell walls that are less rigorous N_2 diffusion barriers and presumably less 'expensive' to synthesize. Therefore, it is likely that increasing temperature will result in a substantially higher N_2 fixation activity and greater competitive advantage for diazotrophic cyanobacteria in nitrogen-limited waters.

Rising CO_2 concentrations may also 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 CO_2 response, suggesting that individual strains of these diazotrophs are adapted to grow and fix nitrogen at different CO_2 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 CO_2 responses of *Microcystis* (Sandrini et al., 2014; 2015b). For example, *Trichodesmium erythraeum* IMS101 possesses only the high-flux CO_2 uptake system NDH-I_4 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 pCO_2 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 CO_2 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 N_2 fixation activity in response to elevated CO_2 (Paul et al., 2015). To what extent the N_2 fixation rates of harmful cyanobacteria in lakes and reservoirs will respond to rising CO_2 is still largely an open question. Comparative studies of the genetic and phenotypic variation in CO_2 responses among diazotrophs may shed more light on this important gap in our knowledge.

5. 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 highest variation in cyanotoxin concentration in aquatic ecosystems: the more cyanobacterial biomass, the higher the toxin concentration. Cyanobacterial biomass is affected by CO_2, temperature, nutrients, and light, as has been described in the preceding sections when impacts on cyanobacterial growth were discussed.

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 CO_2 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 CO_2 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). The available studies indicate that nodularin production by *Nodularia spumigena* was stimulated at elevated temperature (Lehtimäki et al., 1997; Hobson and Fallowfield, 2003). Saxitoxin production by *Aphanizomenon* sp. LMECYA was higher at 28°C than at 22°C (Dias et al., 2002), but saxitoxin production by *Cylindrospermopsis raciborskii* strain C10 was lower at 25°C than at 19°C (Castro et al., 2004). Anatoxin production by *Anabaena* and *Aphanizomenon* decreased at high temperature (Rapala et al., 1993). Hence, each of these cyanotoxins shows a different temperature response, which indicates that rising temperatures may alter the toxin composition of cyanobacterial blooms.

6. 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 CO_2 levels on freshwater ecosystems have received surprisingly little attention thus far. Models and laboratory experiments provide arguments that rising CO_2 levels are likely to stimulate cyanobacterial blooms. However, field evidence is still limited, and the extent to which cyanobacterial blooms can sequester atmospheric CO_2 is still largely unexplored. Hence, there is

686 a need for lake studies on the coupling of cyanobacterial blooms with seasonal and diurnal
687 dynamics of the dissolved inorganic carbon, and how these dynamics interact with exchanges of
688 CO_2 with the atmosphere.

689 Furthermore, during recent years much more has become known about the molecular
690 functioning and genetic diversity of cyanobacterial CCMs, both in model cyanobacteria such as
691 *Synechocystis* PCC 6803 (Price, 2011; Burnap et al., 2015) and in environmentally relevant
692 cyanobacteria such as *Microcystis* (Sandrini et al., 2014, 2015b). However, little is known about
693 the abundance, succession and geographical distribution of different C_i uptake genotypes in
694 natural waters, or about evolutionary adaptation of cyanobacterial CCMs following prolonged
695 exposure to elevated CO_2 concentrations. Hence, there is a need for biogeographical and eco-
696 evolutionary studies investigating adaptive responses of cyanobacteria to changes in CO_2
697 availability.

698 Although effects of environmental conditions on microcystin production in *Microcystis* have
699 been extensively investigated, there are many other toxins produced by many other species that
700 have yet to be examined. Furthermore, the toxin concentrations in cyanobacteria-dominated
701 lakes are largely determined by the relative abundances of toxic versus non-toxic strains. Yet,
702 only a few studies have investigated how the competition between toxic and non-toxic strains is
703 altered at elevated temperature (Davis et al., 2009) and elevated CO_2 (Van de Waal et al., 2011).
704 Hence, there is a need for studies assessing how climate change will affect the toxicity of
705 cyanobacterial blooms, and in particular under which circumstances toxic strains are able to
706 outperform non-toxic strains and vice versa.

707 Cyanobacteria and eukaryotic algae may respond differently to climate change, which can
708 lead to large changes in phytoplankton community composition. Yet, only a few studies have

compared growth responses to temperature or CO_2 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 CO_2 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 CO_2 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 CO_2 concentrations, similar comparative lake studies should be carried out that focus on CO_2 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 climate change on cyanobacteria are multifaceted and can be quite complex. However, there is broad consensus in the scientific literature that rising atmospheric CO_2 concentrations and global warming are likely to increase the occurrence, intensity and duration of harmful cyanobacterial blooms in eutrophic lakes. Additionally, the microcystin production of cyanobacteria will probably increase at elevated temperature and high CO_2 levels. 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 changes in climate and land use will cause a further deterioration of the water quality in many areas of the world generates a societal responsibility for scientists, water managers and

731 policy makers to take further steps in our ability to understand, predict and mitigate the
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733
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743

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Table 1. The presence/absence of genes for C_i uptake systems and microcystin synthesis in cyanobacteria.

Strain	Origin	C_i uptake systems					microcystin genes
		Bicarbonate			CO_2		
		BicA	SbtA	BCT1	NDH-I_3	NDH-I_4	
8 *Microcystis* strains	Sandrini et al. (2014)	+	+	+	+	+	+/–
Microcystis PCC 7806	Sandrini et al. (2014)	+	–	+	+	+	+
11 *Microcystis* strains	Sandrini et al. (2014)	–	+	+	+	+	+/–
Anabaena cylindrica PCC 7122	GBR (Cambridge)	+	–	?	+	+	–
Anabaena sp. 90	FIN (Lake Vesijarvi)	–	–	+	+	+	+
Anabaena sp. PCC 7108	USA (Moss Beach, California)	–	+	+	+	+	–
Anabaena variabilis ATCC 29413	USA (Mississippi)	+, ≠	+	+	+	+	–
Aphanizomenon flos-aquae NIES-81	JPN (Lake Kasumigaura)	–	–	+	+	+	–
Planktothrix agardhii NIVA-CYA 15	NOR (Lake Kolbotnvatnet)	–	+	+	+	+	+
Planktothrix agardhii NIVA-CYA 34	NOR (Lake Kolbotnvatnet)	–	–	+	+	+	+
Planktothrix agardhii NIVA-CYA 56/3	FIN (Lake Steinsfjorden)	–	+	+	+	+	+
Planktothrix prolifica NIVA-CYA 98	FIN (Lake Steinsfjorden)	–	–	+	+	+	+
Planktothrix agardhii NIVA-CYA 126/8	FIN (Lake Langsjon)	+	–	+	+	+	+
Planktothrix mougeotii NIVA-CYA 405	FIN (Lake Steinsfjorden)	–	–	+	+	+	+
Planktothrix prolifica NIVA-CYA 406	FIN (Lake Steinsfjorden)	–	–	+	+	+	+
Planktothrix rubescens NIVA-CYA 407	FIN (Lake Steinsfjorden)	–	–	+	+	+	+
Planktothrix prolifica NIVA-CYA 540	FIN (Lake Steinsfjorden)	–	+	+	+	+	+
Synechocystis sp. PCC 6803	USA (California)	+	+	+	+	+	–
Synechococcus sp. PCC 7002	PRI (Magueyes Island)	+	+	–	+	+	–
Synechococcus sp. PCC 7942	USA (Texas)	–	+	+	+	+	–

The C_i uptake systems SbtA, BCT1 and NDH-I_3 have a high substrate affinity and low flux rate, whereas BicA and NDH-I_4 have a low substrate affinity and high flux rate.

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.

+ indicates that the gene is present. - 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 a-3).

The presence of C_i uptake 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 indicates potentially toxic strains.

1231 **Table 2.** Activation energies (E_A) and Q_{10} values of the growth rates of eight cyanobacteria and
1232 eight green algae. The values are calculated from the increase of growth rate with temperature, in
1233 the range of 20-27.5°C, based on data from Lürling et al. (2013).
1234

Cyanobacteria	E_A	Q_{10}
Anabaena sp. PCC7122	0.58	1.92
Aphanizomenon gracile	0.64	2.40
Cylindrospermopsis raciborskii CIRF-01	0.75	2.56
Microcystis aeruginosa PCC7941	0.54	2.21
Microcystis aeruginosa CYA140	1.23	4.63
Planktothrix agardhii CYA116	0.51	1.93
Planktothrix agardhii CYA126	0.50	
Synechococcus elongatus PCC6301	0.83	2.75
Green algae		
Ankistrodesmus falcatus CHL8	1.03	1.35
Chlamydomonas reinhardtii CHL13	0.85	1.26
Desmodesmus bicellularis CCAP276/14	0.37	1.92
Desmodesmus quadricauda UTEX614	0.46	2.99
Monoraphidium minutum	0.21	1.83
Scenedesmus acuminatus UTEX415	0.11	1.69
Scenedesmus maximus SAG39.81	0.37	4.09
Scenedesmus obliquus SAG276/3a	0.05	1.10

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1236

Figure legends

Fig. 1. Seasonal changes in phytoplankton population density (green line), dissolved CO_2 concentration ([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 ([CO_2*]) when assuming equilibrium with the atmospheric pCO_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).

Fig. 2. Cyanobacterial growth and inorganic carbon chemistry at two different pCO_2 levels. Left panels: Chemostat experiment with low pCO_2 of 200 ppm in the gas flow and 500 μmol L^{-1} bicarbonate in the mineral medium. Right panels: Chemostat experiment with high pCO_2 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* CYA140. (A,B) *Microcystis* biomass (expressed as biovolume) and light intensity penetrating through the chemostat (I_{OUT}). (C,D) Dissolved CO_2, bicarbonate and carbonate concentrations. (E,F) pH. Symbols represent measurements, lines show model predictions. Adjusted from Verspagen et al. (2014b).

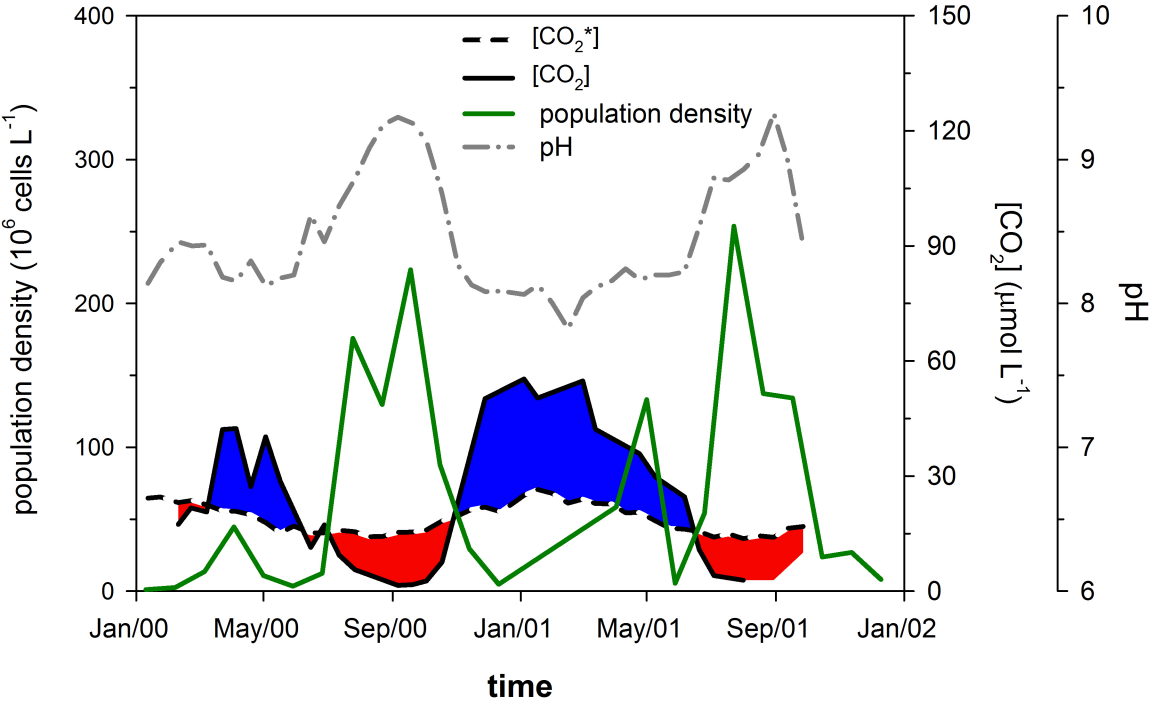
Fig. 3. Schematic overview of the CCM in cyanobacteria. Five different C_i uptake systems are known in cyanobacteria, including the ATP-dependent bicarbonate uptake system BCT1, two sodium-dependent bicarbonate uptake systems (BicA and SbtA) and two CO_2 uptake systems (NDH-I_3 and NDH-I_4). The C_i uptake systems differ in their affinities and flux rates. Accumulated bicarbonate is converted to CO_2 by carbonic anhydrases (CA) in the carboxysomes. CO_2 fixation by RuBisCO leads to the formation of 3-phosphoglycerate (3PG), whereas the reaction with O_2 (photorespiration) produces toxic 2-phosphoglycolate (2PG). The dashed lines indicate CO_2 leakage from the carboxysome, which can partly be intercepted by the CO_2 uptake systems.

Fig. 4. Effect of temperature (expressed as 1/(kT), where k is Boltzmann's constant and T is absolute temperature in degrees Kelvin) on the growth rate of the cyanobacterium *Aphanizomenon gracile* (squares) and the green alga *Scenedesmus acuminatus* UTEX415 (circles) as determined in batch cultures (data from Lürling et al. 2013).

Fig. 5. Model prediction of competition between buoyant cyanobacteria and sinking diatoms and green algae, as a function of water-column depth and turbulent diffusion. The boundary line between the region of cyanobacterial dominance and the region where diatoms and green algae win depends on the ratio of the time scale of turbulent mixing and the vertical flotation velocity of the buoyant cyanobacteria. Hence, the exact position of this boundary line will vary among different species of cyanobacteria. For details, see Huisman et al. (2004).

Fig. 6. (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).


[CO2*]
[CO2]
population density
pH
population density (10^6 cells L^-1)
[CO2] (μmol L^-1)
pH
time
400
300
200
100
0
150
120
90
60
30
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10
9
8
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Jan/00
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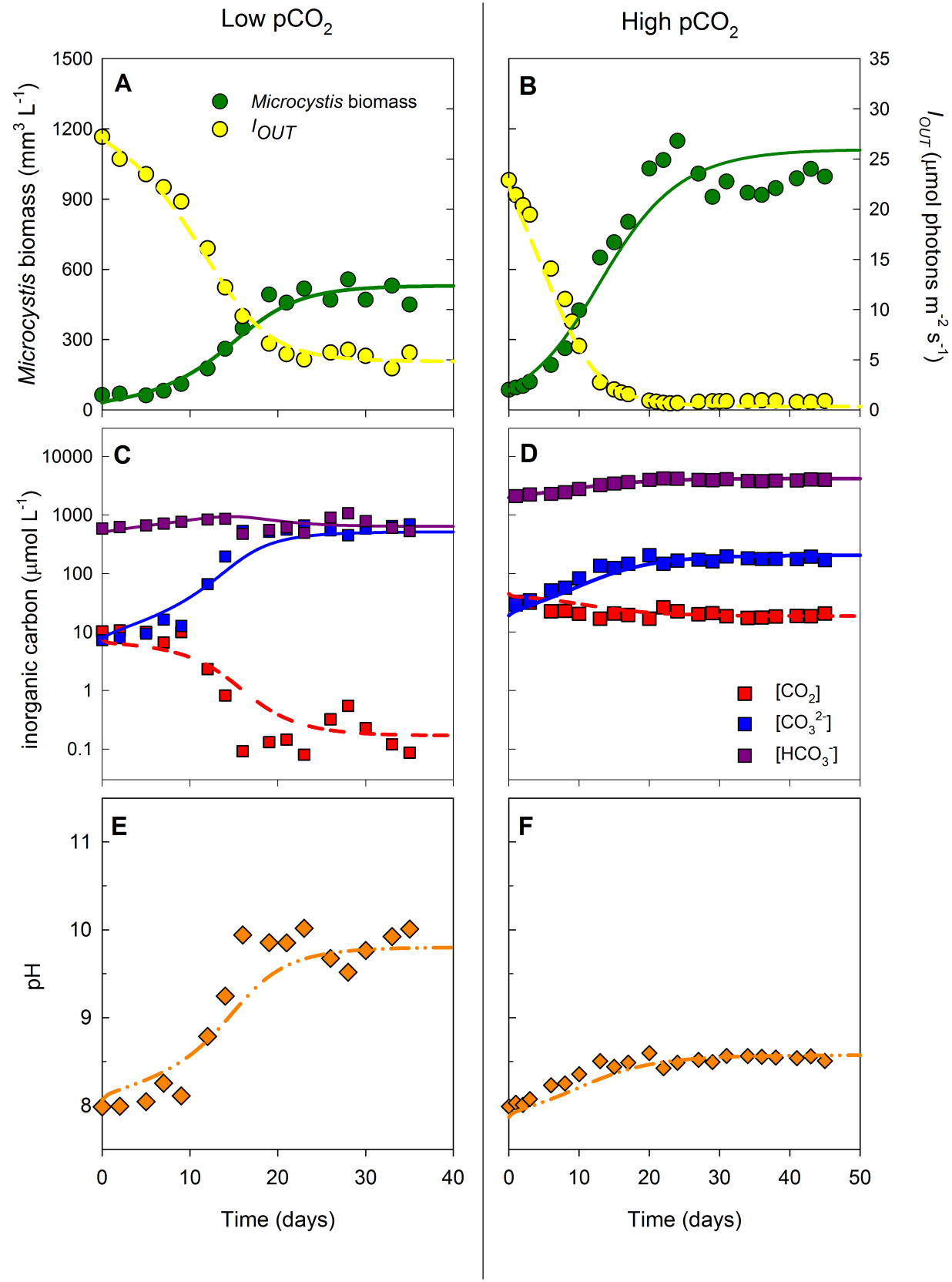
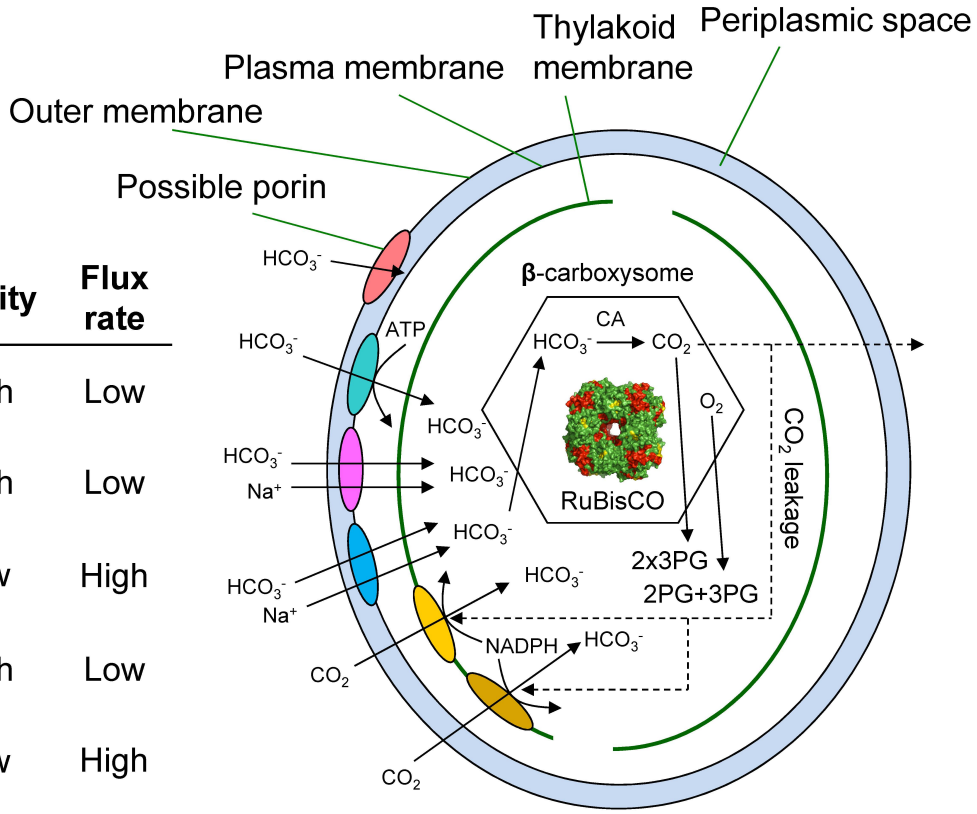
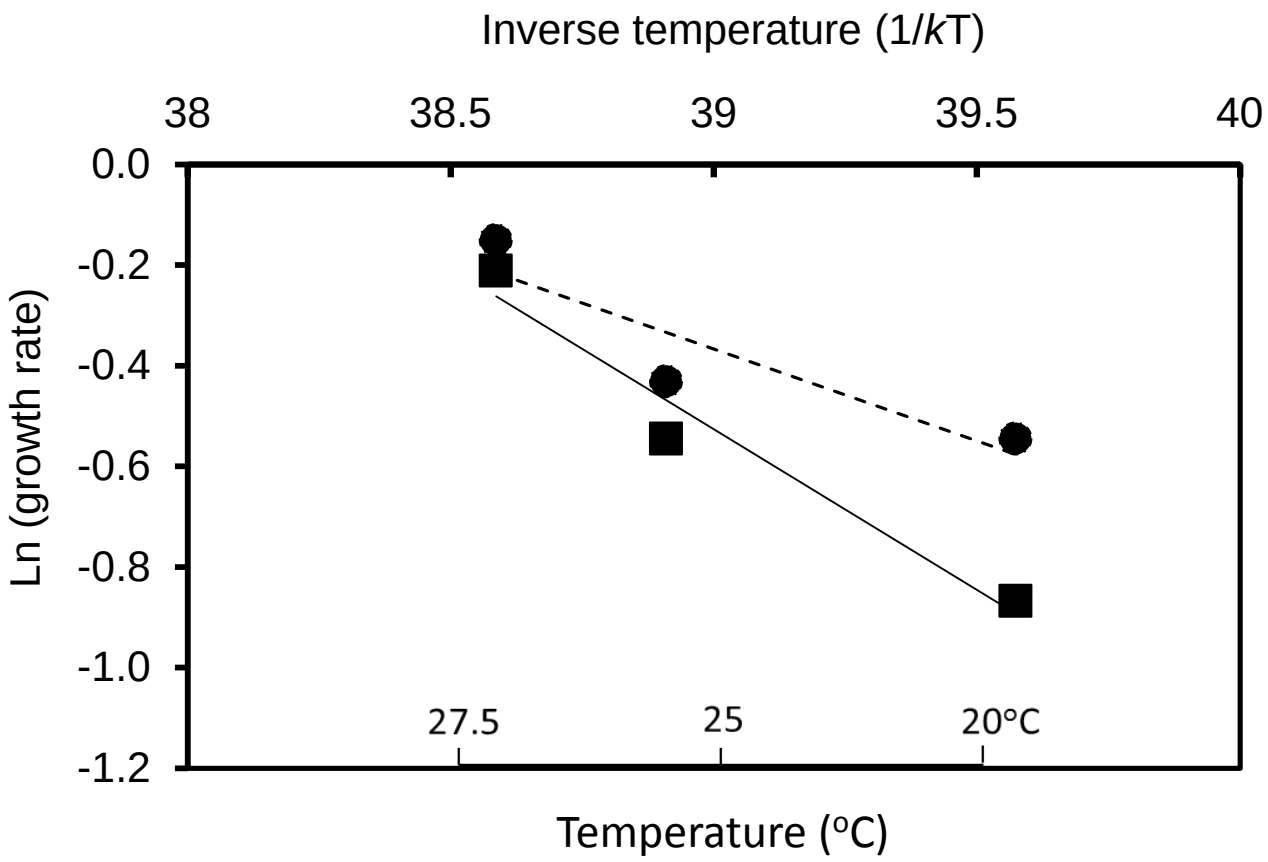
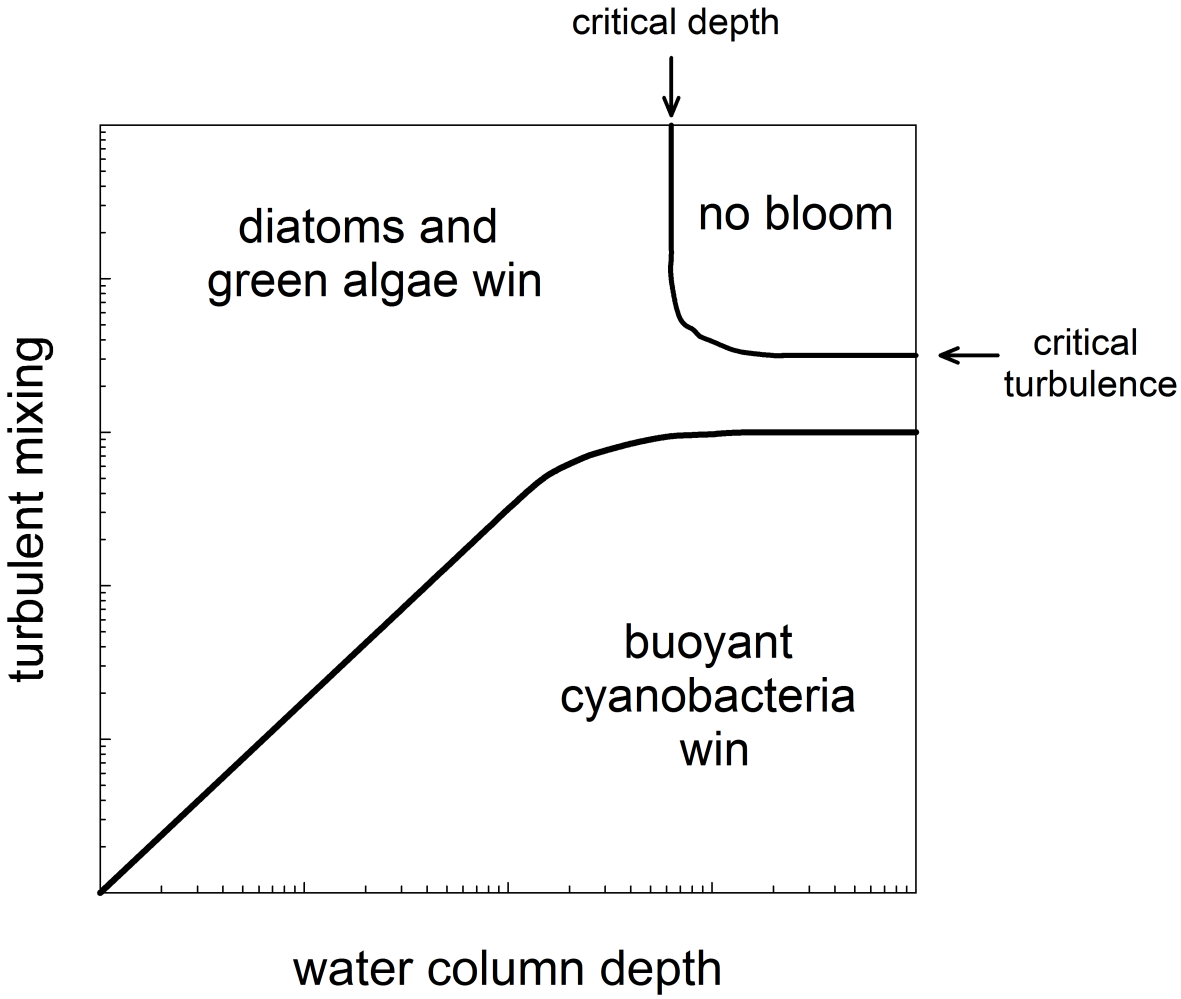
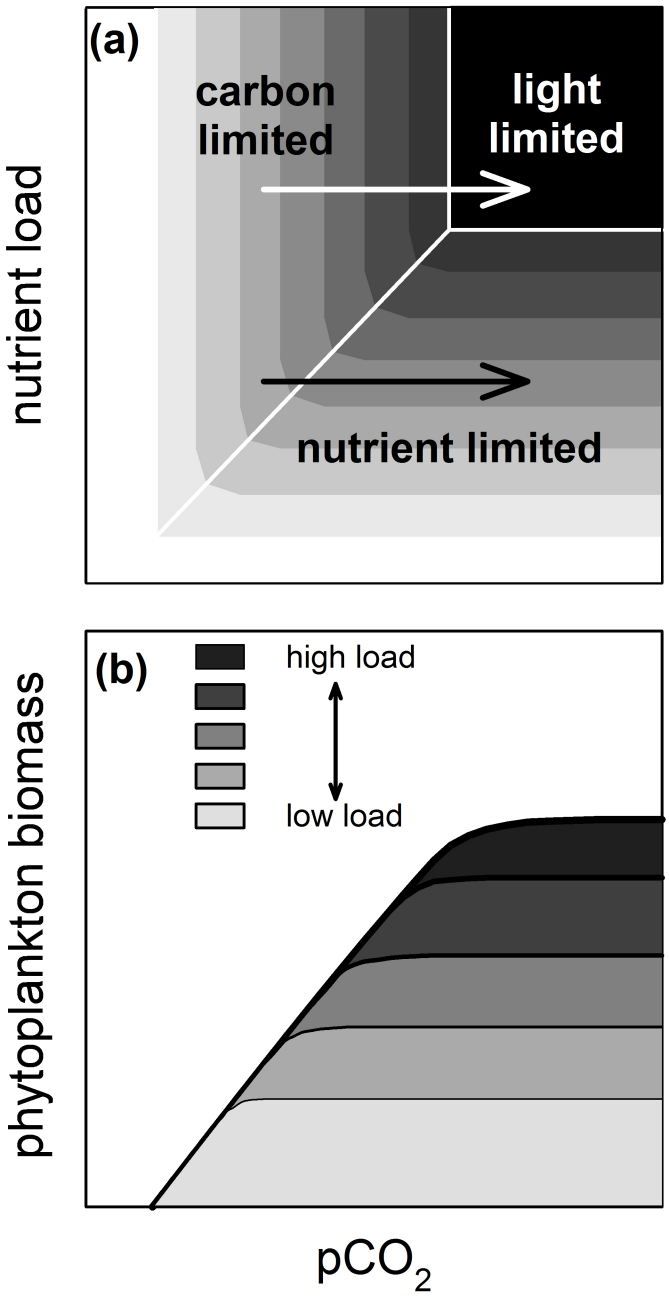

Low pCO2
High pCO2
A
B
C
D
E
F
Microcystis biomass
IOUT
Microcystis biomass (mm3 L-1)
IOUT (µmol photons m-2 s-1)
inorganic carbon (µmol L-1)
pH
[CO2]
[CO3 2-]
[HCO3-]
Time (days)
Time (days)

C_i uptake system	Affinity	Flux rate
BCT1	High	Low
SbtA	High	Low
BicA	Low	High
NDH-I_3	High	Low
NDH-I_4	Low	High




Inverse temperature (1/kT)
38
38.5
39
39.5
40
0.0
-0.2
-0.4
-0.6
-0.8
-1.0
-1.2
Ln (growth rate)
27.5
25
20°C
Temperature (°C)


critical depth
diatoms and
green algae win
no bloom
critical
turbulence
turbulent mixing
buoyant
cyanobacteria
win
water column depth


(a)
carbon limited
light limited
nutrient limited
nutrient load
(b)
high load
low load
phytoplankton biomass
pCO2