Supplementary Information

Reversal in competitive dominance of a toxic versus nontoxic cyanobacterium in response to rising CO_2

Dedmer B. Van de Waal, Jolanda M. H. Verspagen, Jan F. Finke, Vasiliki Vournazou, Anne K. Immers, W. Edwin A. Kardinaal, Linda Tonk, Sven Becker, Ellen Van Donk, Petra M. Visser and Jef Huisman

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1. Mathematical model of competition for inorganic carbon and light

We develop a model that considers several phytoplankton species competing for inorganic carbon and light in a well-mixed water column. The population dynamics of the species depend on the assimilation of carbon dioxide and bicarbonate. Uptake of these inorganic carbon sources induces dynamic changes in pH. These changes in pH, in turn, affect the availability of the different carbon sources, which feeds back on population growth. In addition, the growing cyanobacterial populations cast more shade, reducing light available for photosynthesis, and thereby suppressing further carbon assimilation and population growth.

Population dynamics

We assume that the specific growth rates of the competing species depend on their intracellular carbon content (Droop, 1973; Grover, 1991). Let n denote the number of species, let X_i denote the population density of species i, and let Q_i denote its intracellular carbon content. The population dynamics of the competing species, and the dynamic changes in their cellular carbon content, can be summarized by two sets of differential equations:

$$\frac{dX_i}{dt} = \mu_i X_i - m_i X_i \qquad i=1,\ldots,n \qquad (1)$$

$$\frac{dQ_i}{dt} = u_{CO_2,i} + u_{HCO_3^-,i} - r_i - \mu_i Q_i \qquad i=1,\ldots,n \qquad (2)$$

The first set of equations describes the population densities of the competing species, where μ_i is the specific growth rate of species i and m_i is its specific loss rate. The second set of equations describes the cellular carbon contents of the species, which increase through uptake of carbon dioxide ($u_{CO2,i}$) and bicarbonate ($u_{HCO3,i}$), and decrease through respiration (r_i) and through dilution of the cellular carbon by growth.

We assume that the cellular carbon assimilated by each species consists of structural biomass and a transient carbon pool. The relative size of the transient carbon pool, S_i, is:

$$S_i = \frac{Q_i - Q_{\min,i}}{Q_{\max,i} - Q_{\max,i}} \qquad (3)$$

where $Q_{\min,i}$ is the minimum amount of cellular carbon incorporated into the structural biomass of species i, and $Q_{\max,i}$ is its maximum amount of cellular carbon. The transient carbon pool can be invested to make new structural biomass, which contributes to further population growth. The specific growth rate of a species is determined by the size of its transient carbon pool:

$$\mu_i = \mu_{\max,i} S_i = \mu_{\max,i}\left(\frac{Q_i - Q_{\min,i}}{Q_{\max,i} - Q_{\min,i}}\right) \qquad (4)$$

where $\mu_{\max,i}$ is the maximum specific growth rate of species i. Our model formulation resembles Droop's (1973) classic growth model. However, in our model the cellular carbon content is

constrained between $Q_{\min,i}$ and $Q_{\max,i}$, as there are physical limits to the amount of carbon that can be stored inside a cell. The specific growth rate equals zero if the transient carbon pool is exhausted (i.e., $\mu_i = 0$ if $Q_i = Q_{\min,i}$), and reaches its maximum if cells are satiated with carbon (i.e., $\mu_i = \mu_{\max,i}$ if $Q_i = Q_{\max,i}$).

Carbon assimilation

We assume that uptake rates of carbon dioxide and bicarbonate are increasing but saturating functions of resource availability as in Michaelis-Menten kinetics, and are suppressed when cells become satiated with carbon (Morel, 1987; Ducobu et al, 1998). Since carbon assimilation requires energy, we further assume that these uptake rates depend on photosynthetic activity. Uptake rates of carbon dioxide and bicarbonate by the different species can then be described by:

$$u_{CO_2,i} = \left(\frac{u_{\max,CO_2,i}\,[CO_2]}{H_{CO_2,i} + [CO_2]} \right) (1 - S_i)\, P_i \qquad (5a)$$

$$u_{HCO_3^-,i} = \left(\frac{u_{\max,HCO_3^-,i}\,[HCO_3^-]}{H_{HCO_3^-,i} + [HCO_3^-]} \right) (1 - S_i)\, P_i \qquad (5b)$$

where $u_{\max,CO2,i}$ and $u_{\max,HCO3,i}$ are the maximum uptake rates of carbon dioxide and bicarbonate, respectively, $H_{CO2,i}$ and $H_{HCO3,i}$ are the half-saturation constants, S_i is the relative size of the transient carbon pool as defined by equation (3), and P_i is the relative photosynthetic activity of species i (with $0 < P_i < 1$).

The light reaction of photosynthesis determines the amount of energy available for carbon assimilation. We therefore calculate the relative photosynthetic activity of a species from its depth-averaged photosynthetic rate (Huisman & Weissing, 1994; Huisman et al, 1999):

$$P_i = \frac{1}{z_m} \int_0^{z_M} p_i(I(z))\, dz \qquad (6)$$

where $p_i(I(z))$ is the photosynthetic rate of species i, and z_m is the total depth of the water column. The notation $p_i(I(z))$ indicates that the light reaction of photosynthesis is a function p_i of light intensity I, which in turn is a function of depth z.

The photosynthetic rate of a species is described as a Monod function of light intensity:

$$p_i(I) = \frac{p_{\max,i} I}{H_{I,i} + I} \tag{7}$$

where $p_{\max,i}$ is the maximum photosynthetic rate of species i, and $H_{I,i}$ is its half-saturation constant for light. Because the maximum carbon uptake rate is already accounted for in equations (5a,b), we set $p_{\max,i} = 1$ (which constrains P_i to $0 \le P_i \le 1$). The underwater light intensity varies with depth according to Lambert-Beer's law:

$$I(z) = I_{\text{in}} \exp\left(- K_{\text{bg}} z - \sum_{i=1}^{n} k_i X_i z \right) \tag{8}$$

This equation states that the light intensity transmitted through the water column increases with the incident light intensity (I_{in}), but decreases with the depth in the water column (z), the background turbidity of the water itself (K_{bg}), the specific light attenuation coefficients of the competing species (k_i), and the population densities of the species (X_i). We define I_{out} as the light intensity reaching the bottom of the water column (i.e., $I_{\text{out}} = I(z_m)$).

With equations (7) and (8), the depth integral in equation (6) can be solved analytically (Huisman & Weissing, 1994):

$$P_i = \left(\frac{1}{\ln(I_{\text{in}} / I_{\text{out}})} \right) \ln\left(\frac{H_{I,i} + I_{\text{in}}}{H_{I,i} + I_{\text{out}}} \right) \tag{9}$$

Carbon is lost by respiration. We assume that the respiration rate is proportional to the size of the transient carbon pool:

$$r_i = r_{\max,i} S_i \tag{10}$$

where $r_{\max,i}$ is the maximum respiration rate when cells are fully satiated with carbon.

Dissolved inorganic carbon

Carbon dioxide readily dissolves in water, and a small fraction reacts with water forming carbonic acid (H_2CO_3). Carbonic acid may subsequently dissociate into bicarbonate and a proton. The reaction from dissolved carbon dioxide to bicarbonate, and vice versa, depends on pH and is relatively slow (Stumm & Morgan, 1996). Bicarbonate can dissociate further into carbonate and a proton. This is a much faster process, such that the dissociation of bicarbonate into carbonate and its reverse reaction are essentially in equilibrium with alkalinity and pH (Stumm & Morgan, 1996). The chemical reactions of inorganic carbon are summarized in Table S1. In addition to these chemical processes, carbon dioxide and bicarbonate are consumed for photosynthesis by the competing species, and carbon dioxide is released by respiration.

Dissolved carbon dioxide and carbonic acid cannot be distinguished experimentally. Therefore, let $[CO_2]$ denote the total concentration of dissolved carbon dioxide and carbonic acid. In addition, let [CARB] denote the total concentration of bicarbonate and carbonate. Thus, the total dissolved inorganic carbon (DIC) is defined as:

$$\mathrm{DIC} = [CO_2] + [\mathrm{CARB}] \tag{11}$$

Changes in dissolved inorganic carbon can then be described by (Johnson, 1982; Stumm & Morgan, 1996):

$$\frac{d[CO_2]}{dt} = D([CO_2]_{\mathrm{in}} - [CO_2]) + g_{CO_2} + c_{CO_2} + \sum_{i=1}^{n} r_i X_i - \sum_{i=1}^{n} u_{CO_2,i} X_i \tag{12a}$$

$$\frac{d[\mathrm{CARB}]}{dt} = D([\mathrm{CARB}]_{\mathrm{in}} - [\mathrm{CARB}]) - c_{CO_2} - \sum_{i=1}^{n} u_{HCO_3^-,i} X_i \tag{12b}$$

The first equation describes changes in the concentration of dissolved carbon dioxide through the influx ($[CO_2]_{in}$) and efflux of water containing dissolved CO_2, through gas exchange with atmospheric CO_2 (g_{CO2}), and through the chemical reaction from dissolved CO_2 to bicarbonate and vice versa (c_{CO2}). In addition, the concentration of dissolved carbon dioxide increases through respiration (r_i) and decreases through uptake of CO_2 ($u_{CO2,i}$) by the species. The second

equation describes changes in the summed concentration of bicarbonate and carbonate through in- and efflux of water containing these inorganic carbon species, through the chemical reaction from bicarbonate to dissolved CO_2 and vice versa (c_{CO2}), and through uptake of bicarbonate ($u_{HCO3,i}$) by the species.

Our experiments are continuously aerated with a defined concentration of CO_2. The CO_2 from this gas mixture dissolves in water. We assume that the CO_2 gas influx (g_{CO2}) is proportional to the aeration rate (a), and to the concentration difference between dissolved CO_2 in equilibrium with the gas pressure ($[CO_2^*]$) and the actual dissolved CO_2 concentration (Siegenthaler & Sarmiento, 1993):

$$g_{CO_2} = \gamma a \left([CO_2^*] - [CO_2] \right) \quad (13)$$

where γ is a constant of proportionality. The value of $[CO_2^*]$ is calculated from the partial pressure of CO_2 in the gas inflow (pCO_2) and the solubility of CO_2 gas in water (Table S1).

Dissolved CO_2 reacts with water and subsequently dissociates into HCO_3^- and H^+. This process occurs at a rate k_{CO2} (Table S1). Dissolved CO_2 can also react with OH^- forming HCO_3^-, which occurs at a rate k_{OH}. Conversely, HCO_3^- and H^+ associate to dissolved CO_2 and water at a rate k_H, while HCO_3^- can also react to dissolved CO_2 and OH^- at a rate k_{HCO3}. The overall change in dissolved CO_2 through these chemical reactions (c_{CO2}) can be described as (Johnson, 1982):

$$c_{CO_2} = -\left(k_{CO_2} + k_{OH^-}[OH^-]\right)[CO_2] + \left(k_{H^+}[H^+] + k_{HCO_3^-}\right)[HCO_3^-] \quad (14)$$

Algorithm to calculate bicarbonate and carbonate concentrations

The concentrations of bicarbonate and carbonate can be calculated from [CARB] assuming equilibrium with alkalinity and pH (Portielje & Lijklema, 1995; Stumm & Morgan, 1996). For this purpose, we used an iterative algorithm that is solved at each time step of our model simulations to calculate alkalinity and pH, and from there the bicarbonate and carbonate concentration. This iterative algorithm is described below.

Alkalinity is defined as the acid-neutralizing capacity of water. In our experiments, alkalinity is dominated by dissolved inorganic carbon and inorganic phosphates. Accordingly, the alkalinity is given by (Wolf-Gladrow et al, 2007):

$$\mathrm{ALK} = \left[\mathrm{HCO_3^-}\right] + 2\left[\mathrm{CO_3^{2-}}\right] + \left[\mathrm{HPO_4^{2-}}\right] + 2\left[\mathrm{PO_4^{3-}}\right] + \left[\mathrm{OH^-}\right] - \left[\mathrm{H_3PO_4}\right] - \left[\mathrm{H^+}\right] \quad (15)$$

Alkalinity can be calculated from this equation in two different ways. First, initial estimates of the concentrations of bicarbonate, carbonate, phosphoric acid (H_3PO_4), dihydrogen phosphate ($H_2PO_4^-$), hydrogen phosphate (HPO_4^{2-}), and phosphate (PO_4^{3-}) can be calculated from the summed concentration of bicarbonate and carbonate (CARB), the summed concentration of dissolved inorganic phosphates (R_P, 300 μmol L^{-1} in our experiments), and the proton concentration (H^+) obtained from the pH at the previous time step (pH_{t-1}):

$$\left[\mathrm{HCO_3^-}\right] = \frac{\left[\mathrm{H^+}\right]}{\mathrm{K_2} + \left[\mathrm{H^+}\right]}\left[\mathrm{CARB}\right] \quad (16)$$

$$\left[\mathrm{CO_3^{2-}}\right] = \frac{\mathrm{K_2}}{\mathrm{K_2} + \left[\mathrm{H^+}\right]}\left[\mathrm{CARB}\right] \quad (17)$$

$$\left[\mathrm{H_3PO_4}\right] = \frac{\left[\mathrm{H^+}\right]^3}{\alpha_P} R_P \quad (18)$$

$$\left[\mathrm{H_2PO_4^-}\right] = \frac{\mathrm{K_{P1}}\left[\mathrm{H^+}\right]^2}{\alpha_P} R_P \quad (19)$$

$$\left[\mathrm{HPO_4^{2-}}\right] = \frac{\mathrm{K_{P1}K_{P2}}\left[\mathrm{H^+}\right]}{\alpha_P} R_P \quad (20)$$

$$\left[\mathrm{PO_4^{3-}}\right] = \frac{\mathrm{K_{P1}K_{P2}K_{P3}}}{\alpha_P} R_P \quad (21)$$

where $K_2 = 4.34\times10^{-11}$ is the equilibrium dissociation constant of bicarbonate into carbonate (Table S1), $K_{P1} = 7.11\times10^{-3}$, $K_{P2} = 6.32\times10^{-8}$ and $K_{P3} = 4.47\times10^{-13}$ are the equilibrium constants of the inorganic phosphates, and α_P is calculated as:

$$\alpha_P = \left[\mathrm{H^+}\right]^3 + \mathrm{K_{P1}}\left[\mathrm{H^+}\right]^2 + \mathrm{K_{P1}K_{P2}}\left[\mathrm{H^+}\right] + \mathrm{K_{P1}K_{P2}K_{P3}} \quad (22)$$

Alkalinity is calculated from these initial estimates using equation (15).

Alternatively, alkalinity can be calculated from dynamic changes of alkalinity over time. According to equation (15), biological uptake or release of carbon dioxide does not change alkalinity. Furthermore, uptake of bicarbonate for photosynthesis is accompanied by the release of a hydroxide ion or uptake of a proton to maintain charge balance, and therefore bicarbonate uptake does not change alkalinity either. Hence, carbon assimilation by the species does not affect alkalinity. Nitrate, phosphate and sulfate assimilation, however, are accompanied by proton consumption in order to maintain charge balance. Therefore, assimilation of these nutrients increases alkalinity (Brewer & Goldman, 1976; Wolf-Gladrow et al, 2007). More specifically, both nitrate and phosphate uptake increase alkalinity by 1 mole equivalent, whereas sulfate uptake increases alkalinity by 2 mole equivalents (Wolf-Gladrow et al, 2007). Accordingly, changes in alkalinity can be calculated as:

$$\frac{d\mathrm{ALK}}{dt} = D\left(\mathrm{ALK}_{\mathrm{in}} - \mathrm{ALK}\right) + \sum_{i=1}^{n}\left(u_{N,i} + u_{P,i} + 2u_{S,i}\right)X_i \qquad (23)$$

where $\mathrm{ALK}_{\mathrm{in}}$ is the alkalinity of the inflowing mineral medium, and $u_{N,i}$, $u_{P,i}$, and $u_{S,i}$ are the uptake rates of nitrate, phosphate, and sulfate by species i. Because nitrogen, phosphorus and sulfur were not limiting factors in our experiments, we assume for simplicity that the uptake rates of nitrate, phosphate and sulfate are proportional to the net uptake rate of carbon:

$$u_{j,i} = y_{j,i}\left(u_{\mathrm{CO}_2,i} + u_{\mathrm{HCO}_3^-,i} - r_i\right) \qquad \text{with } j = N,P,S \qquad (24)$$

where $y_{N,i}$, $y_{P,i}$ and $y_{S,i}$ are the cellular N:C, P:C and S:C ratios of species i.

The difference, ΔALK, between the alkalinity calculated from equations (16-22) and the alkalinity calculated from equation (23) is used to make a new pH estimate:

$$\mathrm{pH}_{\mathrm{t}} = \mathrm{pH}_{\mathrm{t\text{-}1}} + \Delta\mathrm{pH} \qquad (25)$$

where ΔpH is calculated according to (Stumm & Morgan, 1996):

$$\Delta\mathrm{pH} = \frac{\Delta\mathrm{ALK}}{2.3\begin{pmatrix}\left[\mathrm{H}^+\right] + \left[\mathrm{OH}^-\right] + \alpha_{\mathrm{HCO3}}\alpha_{\mathrm{CO3}}\left[\mathrm{CARB}\right] + \alpha_{01}\alpha_{10}\left[\mathrm{P}_{01}\right] \\ + \alpha_{12}\alpha_{21}\left[\mathrm{P}_{12}\right] + \alpha_{23}\alpha_{32}\left[\mathrm{P}_{23}\right]\end{pmatrix}} \qquad (26)$$

where $\alpha_{HCO3} = [H^+]/([H^+] + K_2)$, $\alpha_{CO3} = K_2/([H^+] + K_2)$, $\alpha_{01} = [H^+]/([H^+] + K_{P1})$, $\alpha_{10} = K_{P1}/([H^+] + K_{P1})$, $\alpha_{12} = [H^+]/([H^+] + K_{P2})$, $\alpha_{21} = K_{P2}/([H^+] + K_{P2})$, $\alpha_{23} = [H^+]/([H^+] + K_{P3})$, $\alpha_{32} = K_{P3}/([H^+] + K_{P3})$, $[P_{01}] = [H_3PO_4] + [H_2PO_4^-]$, $[P_{12}] = [H_2PO_4^-] + [HPO_4^{2-}]$, and $[P_{23}] = [HPO_4^{2-}] + [PO_4^{3-}]$. This new pH estimate is then used to calculate new values for bicarbonate, carbonate and the inorganic phosphates using equations (16-22). This yields a new alkalinity estimate, which gives a new pH, and so on. This iterative procedure is continued until pH and alkalinity have both reached a stable value.

Finally, the bicarbonate and carbonate concentration are calculated from this stable pH value using equations (16) and (17).

2. Estimation of model parameters

The model parameters were estimated from the experiments. For this purpose, it is useful to distinguish between system parameters and species parameters. System parameters are under experimental control, and included incident light intensity (I_{in}), mixing depth of the chemostats (z_m), dilution rate (D), the composition of the mineral medium (e.g., $[CO_2]_{in}$, $[CARB]_{in}$), and the CO_2 concentration in the gas flow (pCO_2). We assume that the specific loss rates of the species are governed by the dilution rate of the chemostat (i.e., $m_i = D$ for all i). Background turbidity (K_{bg}) was determined from measurements of I_{in} and I_{out} in chemostats filled with mineral medium but without cyanobacteria. According to Lambert-Beer's law, in equation (8), the background turbidity can then be calculated as $K_{bg} = \ln(I_{in}/I_{out})/z_m$. The values of the system parameters are summarized in Table 1 of the main text of this article.

The species parameters were estimated from the monoculture experiments, by fitting the time courses predicted by the model to the time courses of the experimental variables measured in the monoculture experiments. These experimental variables included population density (X_i), total dissolved inorganic carbon, pH, alkalinity, and light penetration through the culture vessel

(I_{out}). Following the same procedure as in earlier studies (Huisman et al, 1999; Passarge et al, 2006), measured data were first log-transformed to homogenize the variances. Subsequently, log-transformed values were normalized, using the total sum of squares of each experimental variable as weighting factor. Parameter estimates were obtained by minimization of the residual sum of squares between observed and predicted values of these log-transformed normalized data, using the Gauss-Marquardt-Levenberg algorithm in the software package PEST (Watermark Numerical Computing, Brisbane, Australia). The values of the species parameters are summarized in Table 2 of the main text.

The parameters estimated from the monoculture experiments were used to predict dynamic changes of species abundances, inorganic carbon concentrations, pH and microcystin concentrations in the competition experiments.

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Table S1 Reactions and equilibrium constants of dissolved inorganic carbon in water.

Reactions	Equilibrium constants	Description	Value [(1)]	Units
$[H_2O] \leftrightarrow [H^+] + [OH^-]$	$[H^+][OH^-] = K_W$	Equilibrium constant of water	7.71×10^{-15}	-
$pCO_2 + [H_2O] \leftrightarrow [CO_2]$	$\frac{[CO_2^*]}{pCO_2} = K_0$	Solubility of CO_2 gas in water	3.73×10^{-2}	mol L^{-1} atm^{-1}
$[CO_2] \leftrightarrow [H^+] + [HCO_3^-]$	$\frac{[H^+][HCO_3^-]}{[CO_2]} = K_1$	Dissociation constant of CO_2	4.25×10^{-7}	-
$[HCO_3^-] \leftrightarrow [H^+] + [CO_3^{2-}]$	$\frac{[H^+][CO_3^{2-}]}{[HCO_3^-]} = K_2$	Dissociation constant of HCO_3^-	4.34×10^{-11}	-
$[H_2O] + [CO_2] \rightarrow [HCO_3^-] + [H^+]$	k_{CO2}	Reaction rate of H_2O and CO_2	2.68×10^{-2}	s^{-1}
$[OH^-] + [CO_2] \rightarrow [HCO_3^-]$	k_{OH}	Reaction rate of OH^- and CO_2	6.32×10^{4}	s^{-1}
$[HCO_3^-] + [H^+] \rightarrow [H_2O] + [CO_2]$	k_{HCO3}	Reaction rate of HCO_3^- and H^+	1.36×10^{-4}	s^{-1}
$[HCO_3^-] \rightarrow [OH^-] + [CO_2]$	k_H	Reaction rate of the dissociation of HCO_3^-	7.47×10^{3}	s^{-1}

(1) Values of the equilibrium constants and reaction rates assume a temperature of 21.5 ^oC and a pressure of 1 atm. The solubility of CO_2 in water and dissociation constants are based on Stumm & Morgan (1996); reaction rates are based on Welch et al (1969).