

Supporting Information

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SI Materials and Methods

Cell counts of lake samples. Lugol's iodine was added to fresh lake samples (1:100 v/v of a 5% solution) to preserve phytoplankton cells for microscopy. The samples were stored at 4°C until identification. Phytoplankton was identified to genus level, and if possible to the species level, and counted according to the Utermöhl-method adjusted to the European standard protocol NEN-EN 15204 using a Leica DM IRB inverted light microscope (Leica Microsystems BV, Rijswijk, The Netherlands). Biovolume was estimated from cellular dimensions and geometry (1). Individual *Microcystis* cells were counted after disintegrating the colonies with KOH (2).

Dissolved inorganic carbon and sodium. Chemostat samples for dissolved inorganic carbon (DIC) measurements were centrifuged for 15 minutes at 4000 g and 4°C, and subsequently the supernatant was filtered using a 47 mm GF/C filter (Whatman, Maidstone, UK) followed by a 0.45 µm pore size 47 mm diameter polyethersulfone membrane filter (Sartorius AG, Göttingen, Germany). Lake samples for analysis of DIC and sodium concentrations were filtered on-site using the same filtration procedure. The filtrates of chemostat and lake samples were transferred to sterile plastic urine analysis tubes (VF-109SURI; Terumo Europe N.V., Leuven, Belgium), which were filled completely and stored at 4°C until further analysis. DIC (3-5 technical replicates per sample) was measured with a TOC-V_{CPH} TOC analyzer (Shimadzu, Kyoto, Japan). Concentrations of CO₂(aq), bicarbonate and carbonate were calculated from DIC, pH and temperature (3) (Table S5). Sodium concentrations were measured using an Optima 8000 ICP-OES Spectrometer (Perkin Elmer, Waltham, MA, USA).

Toxin analysis. For microcystin analysis, lake samples were filtered on-site over 1.2 µm pore size 25 mm diameter GF/C filters (Whatman). The loaded filters were stored at -20°C and subsequently freeze-dried. Microcystins were extracted with 75% MeOH and analyzed by HPLC as described previously (4), using a Shimadzu LC-20AD HPLC system with a SPD-M20A photodiode array detector (Shimadzu, Kyoto, Japan). The two largest microcystin peaks of the

lake samples matched with the retention times and UV-spectra of MC-LR and MC-RR standards kindly provided by the University of Dundee.

DNA isolation. Chemostat samples for gDNA isolation were centrifuged for 15 min at 4000 *g* and 4°C. Subsequently, gDNA was isolated from the pellets using the DNeasy Blood & Tissue kit (Qiagen GmbH, Hilden, Germany) according to the supplier's instructions. Lake samples were filtered on-site over 1.2 µm pore size 25 mm diameter GF/C filters (Whatman), and loaded filters were stored at -20°C. Subsequently, gDNA from lake samples was extracted using the ZR Fungal/Bacterial DNA MiniPrep™ kit (Zymo Research, Orange, CA, USA) according to the supplier's instructions. Both the chemostat and the lake gDNA samples were further purified using the DNA Clean & Concentrator™-25 kit (Zymo Research) according to the supplier's instructions. The quality of the gDNA in the chemostat and lake samples was assessed using a Nanodrop 1000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA), which resulted in A_{260}/A_{280} values above 1.8 for all samples. The gDNA concentrations were quantified using the Nanodrop 1000 spectrophotometer for the lake samples and a Qubit 2.0 Fluorometer (Thermo Fisher Scientific, Waltham, MA, USA) for the chemostat samples.

Primer development and PCR reactions. To detect the presence of different *Microcystis* C_i uptake genotypes, we designed primers targeting *bicA* encoding for a low-affinity but high-flux bicarbonate uptake system, *sbtA* encoding for a high-affinity but low-flux bicarbonate uptake system, and the combined presence of *bicA+sbtA* (Table S1). We also designed primers targeting *sbtB* (a gene often linked with *sbtA* and encoding for a post-translational regulator of SbtA [5]), the sodium/proton antiporter gene *nhaS3* and the LysR-family transcriptional regulator *ccmR2*. In *Microcystis*, the *bicA*, *sbtA*, *sbtB* and *nhaS3* genes are all located on the same operon, and *ccmR2* is the transcriptional regulator located upstream (Fig. 1) (6). Furthermore, to detect the presence of toxic (microcystin-producing) genotypes, we included primers targeting the *mcyB* gene involved in microcystin synthesis. We developed primers targeting the iron stress induced protein gene *isiA*, to distinguish strain CCAP 1450/10 from the other strains (Table S1). Finally, we also developed primers targeting the 16S rDNA genes and the RuBisCO chaperone gene *rbcX*, to quantify the overall *Microcystis* population.

The primers were designed to match sequences of 13 *Microcystis* strains for which the full genome was sequenced, including *Microcystis* NIES-843 (7), *Microcystis* PCC 7806 (8), *Microcystis* PCC 7005 (6) and ten other *Microcystis* strains (9). The primers targeting the *ccmR2*, *bicA*, *sbtA*, *sbtB* and *nhaS3* genes also matched sequences of seven additional strains for which only *ccmR2* and the *bicA-sbtAB-nhaS3* operon were sequenced (6); hence, the primers are based on 20 *Microcystis* strains in total. Sequences of several other (non-*Microcystis*) cyanobacteria were used to ensure that the primer design included several mismatches with these other sequences. Furthermore, discrimination between *Microcystis* and other cyanobacteria is aided by the observation that *Microcystis* is thus far the only known cyanobacterial genus with *bicA* and *sbtA* located next to each other in the same operon (6). Other cyanobacteria with both *bicA* and *sbtA* have these genes placed in separate locations in the genome. Table S3 provides an overview of all primers used in this study.

PCR reactions, to test the developed primers, were done with the GoTaq® Hot Start Polymerase kit (Promega Corporation, Madison, WI, USA) according to the supplier's instructions. After an initial denaturation of 2 min at 95°C, 35 cycles were used that consisted of a denaturation step at 95°C for 45 s, an annealing temperature step at 60°C for 30 s and an extension step at 72°C for 3 min. Subsequently, a final extension step at 72°C was used for 5 min. The reactions contained 0.3 $\mu\text{mol L}^{-1}$ primers and 10 ng gDNA in a total reaction volume of 25 μL . Other reaction components were added as instructed by the supplier. The developed primers were tested with gDNA of various *Microcystis* laboratory strains (CCAP 1450/10, CCAP 1450/11, HUB 5.3, HUB 5.2.4, NIES-843, NIVA-CYA 140, PCC 7005, PCC 7806, PCC 7941, V145 and V163) using PCR and gel electrophoresis, which confirmed that only the targeted gDNA sequences were amplified (no by-products were detected). As negative control, the primers were tested on gDNA of several ubiquitous freshwater cyanobacterial species, *Anabaena circinalis* CCAP 1403/18, *Aphanizomenon flos-aquae* CCAP 1401/7 and *Planktothrix agardhii* CCAP 1460/1, which did not result in amplified PCR products.

Next, PCR reactions and gel electrophoresis were performed to detect the different *Microcystis* C_i uptake genotypes in purified gDNA from Lake Kennemermeer samples. Again the GoTaq® Hot Start Polymerase kit (Promega Corporation) was used, and gDNA of five *Microcystis* laboratory strains was investigated for comparison. All three *Microcystis* C_i uptake

genotypes, *bicA* strains (no *sbtA*), *sbtA* strains (no *bicA*) and *bicA+sbtA* strains, were detected in all lake samples (Table S4). PCR-products of strain NIVA-CYA 140, which has a transposon insert in *bicA* (6), were not detected in Lake Kennemermeer. This indicates that strains with such a transposon insert in *bicA* were not present in the lake.

Quantification of C_i uptake genotypes with qPCR. Relative abundances of the different genotypes of *Microcystis* were quantified using qPCR, by applying the Maxima[®] SYBR Green Master Mix (2x) kit (Thermo Fisher Scientific, Waltham, MA, USA) to purified gDNA according to the supplier's instructions in an ABI 7500 Real-Time PCR system (Applied Biosystems, Foster City, CA, USA). The two-step cycling protocol was used, with a denaturation temperature of 95°C (15 s) and a combined annealing/extension temperature of 60°C (60 s) during 40 cycles. The reactions contained 0.3 $\mu\text{mol L}^{-1}$ primers, with 1 ng gDNA when using chemostat samples and 10 ng gDNA when using lake samples, in a total reaction volume of 25 μL . Other reaction components were added as instructed by the supplier. ROX solution was used to correct for any well-to-well variation and melting curve analysis was performed on all measured samples to rule out non-specific qPCR products. Each qPCR plate contained reference gDNA samples and samples with primers targeting reference genes (see below) to overcome plate effects. The LinRegPCR software tool (version 2012.3) (10, 11) was used for baseline correction, calculation of quantification cycle (C_q) values using linear regression, and calculation of the amplification efficiency of each individual run (Table S3). LinRegPCR did not detect samples without amplification or a plateau (except for negative controls), or samples with a baseline error or noise error, or with deviating amplification efficiencies. To calculate relative ratios between the numbers of gene copies, the comparative C_T method was used (12).

To determine the relative abundance of toxic genotypes, we used the microcystin synthetase gene *mcyB* (primers *mcyB-F* and *mcyB-R*) as 'target gene' and the RuBisCO chaperone gene *rbcX* (primers *rbcX-F* and *rbcX-R*) present in all *Microcystis* strains as 'reference gene'. Purified gDNA of the axenic toxic strains PCC 7806 and PCC 7941 was used as 'reference samples'.

To determine the relative abundances of the different C_i uptake genotypes in lake samples, we used the gene *bicA* (primers *bicA-F2* and *bicA-R1*) and the gene *sbtA* (primers

sbtA-F2 and sbtA-R2) as ‘target genes’. The combined *bicA+sbtA* gene (primers *bicA-F3* and *sbtA-R3*) was detected in all lake samples based on observed PCR product size (Table S4) and served as ‘reference gene’. Purified gDNA of the axenic laboratory strains PCC 7005 and PCC 7941 (both *bicA+sbtA* strains) served as ‘reference samples’, to calculate the relative ratios of (i) the *bicA+sbtA* gene versus the *bicA* gene, and (ii) the *bicA+sbtA* gene versus the *sbtA* gene. We note that the *bicA* gene is present in both *bicA+sbtA* strains and *bicA* strains, and similarly the *sbtA* gene is present in both *bicA+sbtA* strains and *sbtA* strains. Hence, the relative abundances of the different C_i uptake genotypes can be calculated from the above two ratios based on the assumption that the sum of the *bicA+sbtA* strains, *bicA* strains, and *sbtA* strains equals 100%.

To determine the relative abundances of the five *Microcystis* strains in the chemostat experiments, we used the *bicA*, *sbtA*, *bicA+sbtA*, *mcyB* and *isiA* genes as ‘target genes’ to distinguish the different strains (Table S1). The two *sbtA* strains could be distinguished because one of them (strain CCAP 1450/10) lacked the *isiA* gene. Likewise, the two *bicA+sbtA* strains could be distinguished because one of them (strain PCC 7005) lacked the *mcyB* gene. We used the 16S rRNA gene (primers 16S-F and 16S-R) to quantify the overall *Microcystis* population (‘reference gene’). Purified gDNA of monocultures of strain PCC 7941 (which contained all five target genes) served as ‘reference samples’ to calculate the relative abundances of strains with and without *bicA*, *sbtA*, *bicA+sbtA*, *mcyB* and *isiA*, assuming that the sum of the five strains equals 100%.

Validation of qPCR method. To validate the developed method, exponentially growing cultures of the five laboratory strains of *Microcystis* were mixed with equal cell numbers (20% each when mixing five strains, and 50% each when mixing two strains). Cell numbers were counted using a CASY 1 TTC cell counter with a 60 µm capillary (Schärfe System GmbH, Reutlingen, Germany).

The gDNA of the mixtures was extracted and used for relative quantification of the three defined *Microcystis* C_i uptake genotypes using the qPCR approach described above. Non-mixed gDNA of the monocultures of strains PCC 7005, PCC 7806 and PCC 7941 was used as reference samples for the calculations.

In all our validation experiments, the relative abundances of the different genotypes measured by qPCR were very similar to the expected relative abundances (Fig. S4).

Supplementary references

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SI Legends of Figures S1-S4 and Tables S1-S5

Fig. S1. Replacement rates of the C_i uptake genotypes in the chemostat experiments.

(A) Replacement rate of the *bicA* strain by *bicA+sbtA* strains in the low CO₂ chemostats.

(B) Replacement rate of the *sbtA* strains by *bicA+sbtA* strains in the low CO₂ chemostats.

(C) Replacement rate of strain PCC 7005 by strain PCC 7941 in the low CO₂ chemostats.

(D) Replacement rate of *sbtA* strains by *bicA* and *bicA+sbtA* strains in the high CO₂ chemostats; note the biphasic pattern. (E) Replacement rate of strain PCC 7941 by strain PCC 7005 in the high CO₂ chemostats. The rate of replacement was calculated from the slope of the linear regression of $\ln(\text{genotype 1}/\text{genotype 2})$ versus time. Data points show the mean values of three replicated chemostat experiments.

Fig. S2. Temperature and cyanobacterial composition of Lake Kennemermeer. (A) Water temperature. (B) Biomass (expressed as biovolume) of the dominant cyanobacteria in summer and autumn of 2013, including Pseudanabaenaceae (*Pseudanabaena* and *Planktolyngbya*), small Chroococcales (mainly *Cyanonephron* and *Cyanodictyon*), *Anabaenopsis hungarica*, and *Microcystis* spp. (*M. aeruginosa* and *M. flos-aquae*). Early in the season, *Microcystis* spp. also includes some *Woronichinia pusilla*. (C) Relative abundance of toxic *Microcystis* genotypes, based on the *mcyB* gene for microcystin production. (D) Total microcystin concentration increases with the *Microcystis* biomass (expressed as biovolume) (Pearson correlation of log microcystin concentration vs log *Microcystis* biomass: $\rho = 0.92$, $N = 10$, $p < 0.001$). The trend line in (D) is based on linear regression. Error bars represent SD ($N = 3$).

Fig. S3. The replacement rate of *bicA+sbtA* strains by *bicA* strains in the lake study. The rate of replacement was calculated from the slope of the linear regression of $\ln(\text{bicA strains}/\text{bicA+sbtA strains})$ versus time, using the data points of week 29-38 (blue markers). The data points are the mean of three replicate measurements. Regression statistics: $y = 0.0368x - 8.2066$ ($R^2 = 0.89$, $N = 6$, $p < 0.01$).

Fig. S4. Validation of the qPCR method quantifying the relative abundances of different *Microcystis* genotypes. (A) Mixture of CCAP 1450/10 (*sbtA* strain) and PCC 7806 (*bicA* strain), with 50% of cell numbers each. (B) Mixture of HUB 5-2-4 (*sbtA* strain) and PCC 7941 (*bicA+sbtA* strain), with 50% of cell numbers each. (C,D) Mixture of CCAP 1450/10, HUB 5-2-4, PCC 7005, PCC 7806 and PCC 7941, with 20% of cell numbers each. Quantification of the different genotypes was based on, (A-C) 16S rDNA as reference gene and, (D) *bicA+sbtA* as reference gene. Error bars represent SD obtained from four replicate qPCR measurements ($N=4$).

Table S1. Properties of the five *Microcystis* strains in the competition experiments.

Table S2. Selection coefficients calculated for the chemostat experiments and lake study.

Table S3. Overview of *Microcystis* primer pairs used for PCR analysis.

Table S4. Results of PCR reactions on gDNA from lake samples.

Table S5. Inorganic carbon measurements in the chemostat experiments and lake study.

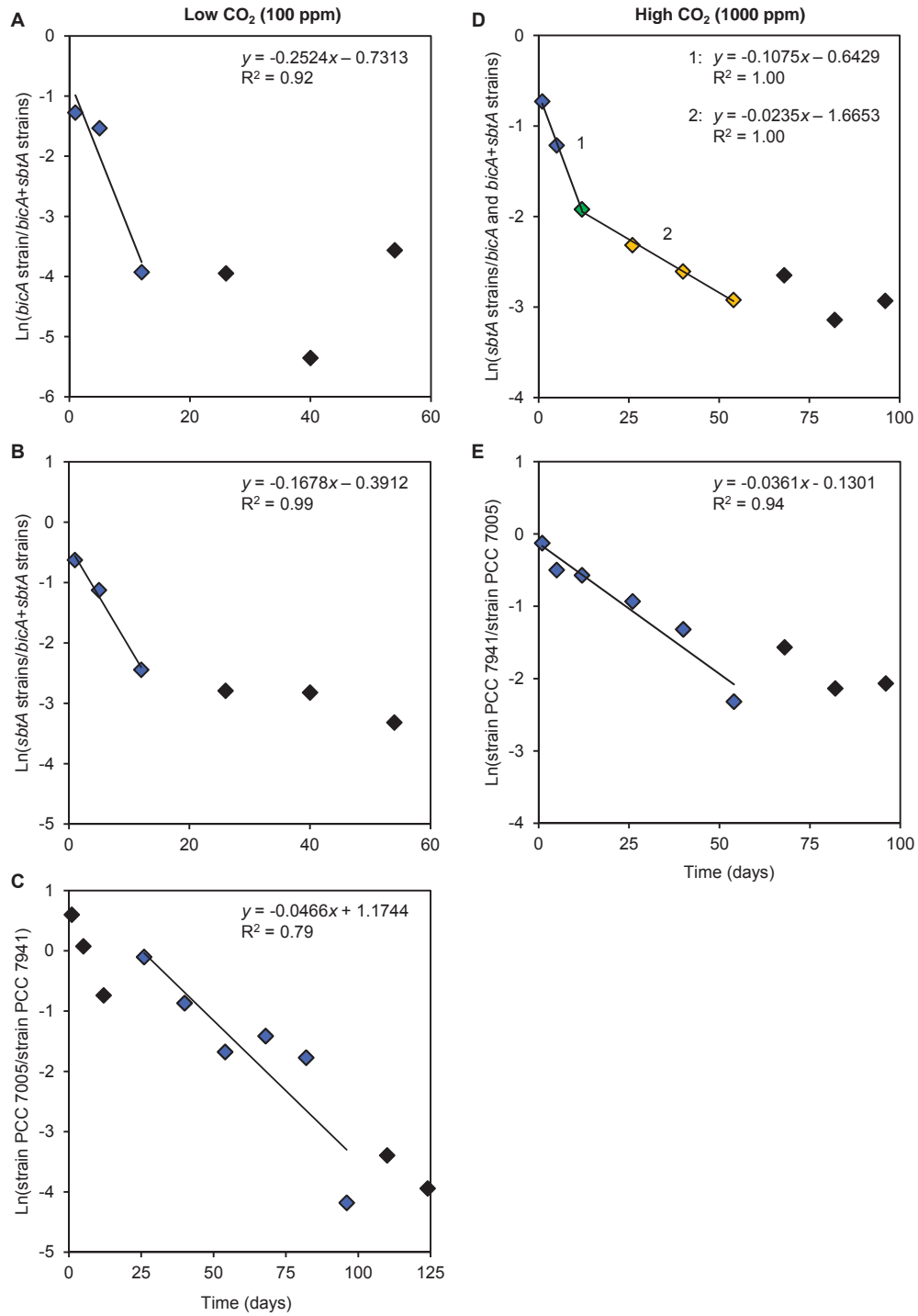


Figure S1.

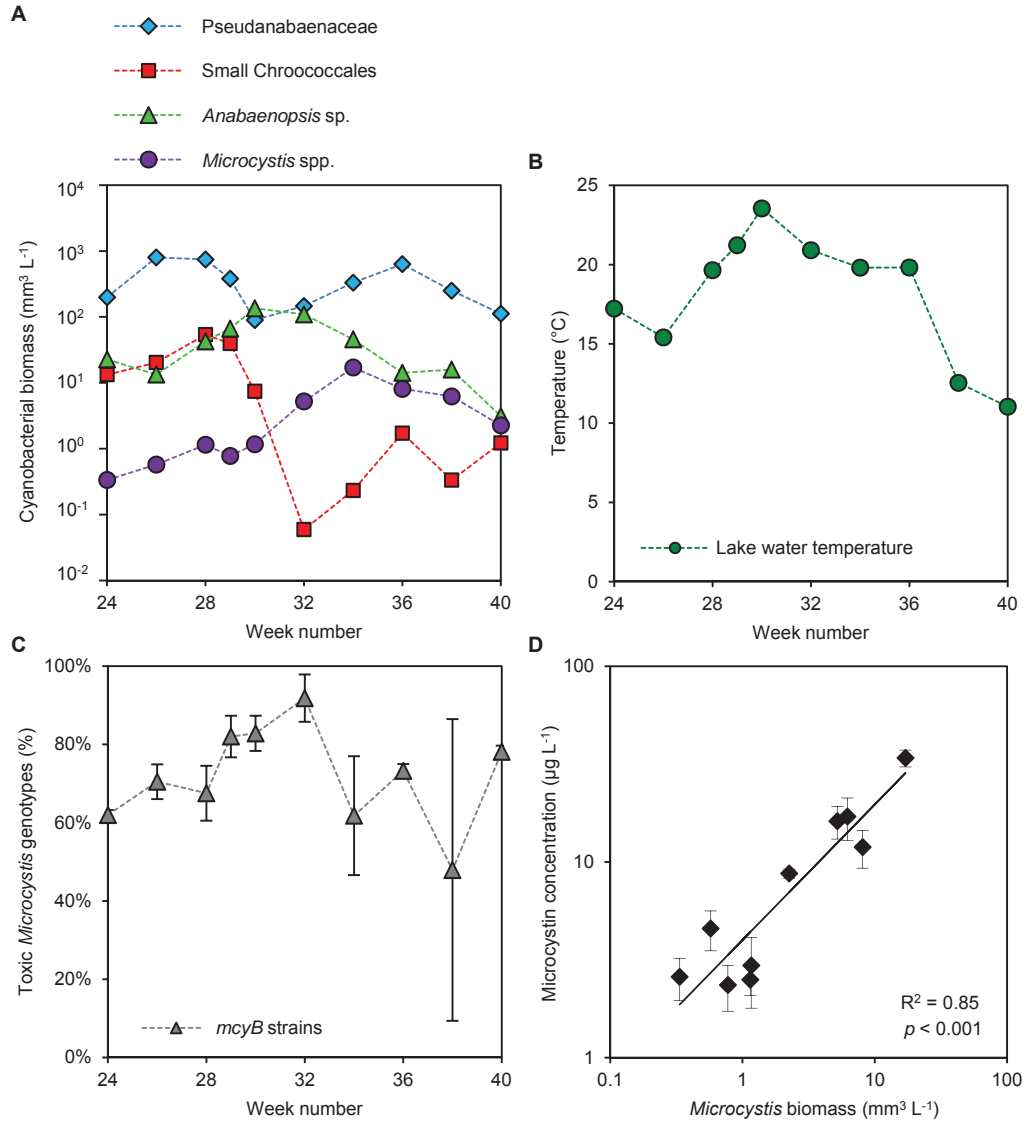


Figure S2.

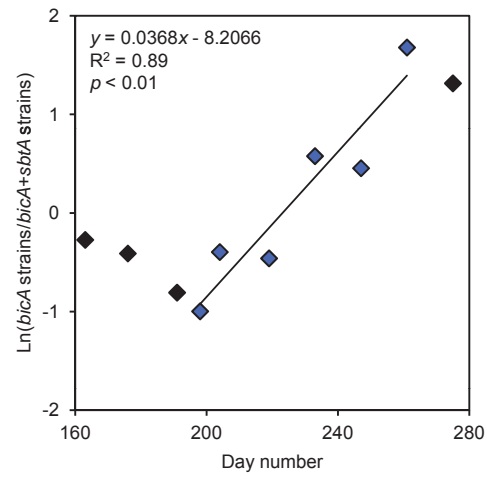


Figure S3.

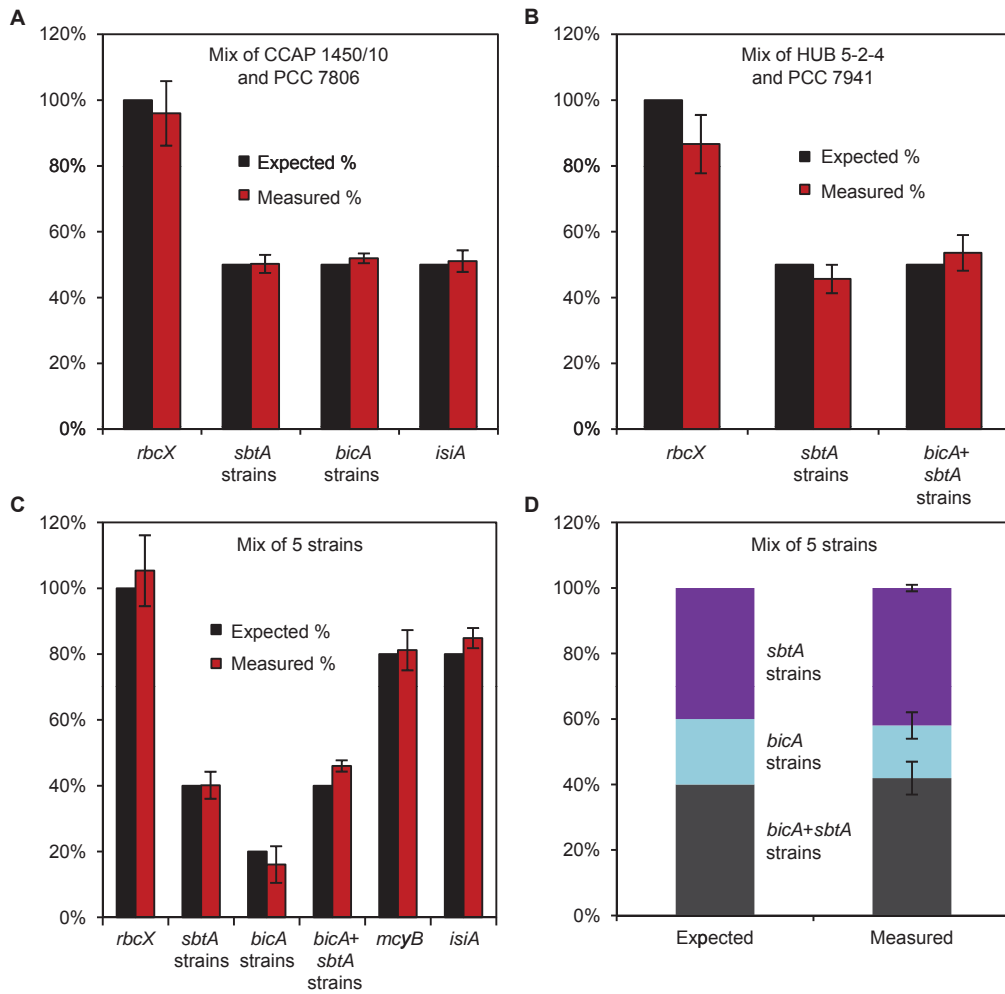


Figure S4.

Table S1. Properties of the five *Microcystis* strains in the competition experiments.

Strain	Origin	C _i uptake genotype	Genes present						Microcystin production
			16S rRNA	<i>rbcX</i>	<i>bicA</i>	<i>sbtA</i>	<i>mcyB</i>	<i>isiA</i>	
CCAP 1450/10	GBR (Lake Blelham Tarn)	<i>sbtA</i>	+	+	-	+	+	-	+
HUB 5-2-4	DEU (Lake Pehlitzsee)	<i>sbtA</i>	+	+	-	+	+	+	+
PCC 7806	NLD (Braakman)	<i>bicA</i>	+	+	+	-	+	+	+
PCC 7005	USA (Lake Mendota)	<i>bicA+sbtA</i>	+	+	+	+	-	+	-
PCC 7941	CAN (Little Rideau Lake)	<i>bicA+sbtA</i>	+	+	+	+	+	+	+

The origins of the strains are indicated with three-letter ISO codes of the different countries. The plus sign (+) or minus sign (-) indicates the presence or absence of a specific gene or microcystin production.

Table S2. Selection coefficients calculated for the chemostat experiments and lake study.

Strain replacement	Replacement rate (day ⁻¹)	Generation time (days)	Selection coefficient (per generation)
<i>Low CO₂ chemostats:</i>			
<i>bicA</i> by <i>bicA+sbtA</i> strains	0.252	2.5	0.62
<i>sbtA</i> by <i>bicA+sbtA</i> strains	0.168	2.5	0.41
PCC 7005 by PCC 7941	0.047	3.5	0.16
<i>High CO₂ chemostats:</i>			
<i>sbtA</i> by <i>bicA+sbtA</i> and <i>bicA</i> strains (first 12 days)	0.108	1.9	0.20
<i>sbtA</i> by <i>bicA+sbtA</i> and <i>bicA</i> strains (next 40 days)	0.024	3.5	0.08
PCC 7941 by PCC 7005	0.036	3.5	0.13
<i>Lake study:</i>			
<i>bicA+sbtA</i> by <i>bicA</i> strains	0.037	1.5-5.2	0.06-0.19

Replacement rates were estimated from the slope of the linear regression of $\ln(x_1/x_2)$ versus time, where x_1 and x_2 are the relative frequencies of two genotypes (Figures S1 and S3).

Generation times were calculated as $t_d = \ln(2)/\mu$, where μ is the growth rate of the total *Microcystis* population (including the dilution rate).

Selection coefficients were calculated as products of the replacement rates and generation times.

Table S3. Overview of *Microcystis* primer pairs used for PCR analyses.

Primer name	Sequence 5'→ 3' (length)	Target gene(s)	Locus tag	Accession no. (Genbank)	Expected product size (bp)	Amplification efficiency (E) ± SD	Normal PCR (1) or qPCR (2)	Reference
bicA-F1	CCTTGCGGATCATCTTCCTG (20)	<i>bicA</i>	IPF_4911	AM778949.1	902/2123	nd	1	This study
bicA-R1	AGGCACATCACTCAAGTCCA (20)							13
bicA-F1	CCTTGCGGATCATCTTCCTG (20)	<i>bicA+</i>	IPF_4911	AM778949.1	1443	nd	1	This study
sbtA-R1	TTGGTCAGCAGCATGAAGAC (20)	<i>sbtA</i>	MAE_62090	AP009552.1				This study
ccmR2-F1	TGGTGAAACTGCCATGACGA (20)	<i>ccmR2+</i>	MAE_62070	AP009552.1	843/2231/	nd	1	This study
sbtA-R1	TTGGTCAGCAGCATGAAGAC (20)	<i>sbtA</i>	MAE_62090	AP009552.1	2351			This study
bicA-R2	CAAGCTAACGGTCGCATCAT (20)	<i>bicA+</i>	IPF_4911	AM778949.1	1109/	nd	1	13
nhaS3-R	CTTCGAGGTAATACCGATACTGGT (24)	<i>nhaS3</i>	MAE_62110	AP009552.1	2789			6
sbtA-F1	GTCTTCATGCTGCTGACCAA (20)	<i>sbtA+</i>	MAE_62090	AP009552.1	1335	nd	1	This study
sbtB-R	ATAGATCATGCCCGCAAATTGAG (24)	<i>sbtB</i>	MAE-62100					This study
16S-F	GTCGAACGGGAATCTTCGGAT (21)	<i>16S rDNA</i>	IPF_5548	AM778951.1	157	1.90±0.02	2	13
16S-R	GCTAATCAGACGCAAGCTCTTC (22)							13
bicA-F2	CAAGCTAACGGTCGCATCAT (20)	<i>bicA</i>	IPF_4911	AM778949.1	132	1.89±0.02	2	13
bicA-R1	AGGCACATCACTCAAGTCCA (20)							13
sbtA-F2	CTGGCCTTTTTGATTGGTGG (20)	<i>sbtA</i>	MAE_62090	AP009552.1	143	1.88±0.02	2	14
sbtA-R2	AGGTTGGAATTGCCGATGG (19)							14
bicA-F3	TCAAGACCCATCCTCACCA (19)	<i>bicA+</i>	IPF_4911	AM778949.1	264	1.86±0.02	2	This study
sbtA-R3	CCACCAATCAAAAAGGCCAG (20)	<i>sbtA</i>	MAE_62090	AP009552.1				This study
rbcX-F	CGGATCATGACGGTAAGAGAACA (23)	<i>rbcX</i>	IPF_2531	AM778933.1	157	1.88±0.02	2	14
rbcX-R	ATTCCGATGTCTCTGGTTGACT (22)							14
mcyB-F	ATCCCATGCTCAGAGACGTT (20)	<i>mcyB</i>	IPF_375	AM778952.1	163	1.87±0.02	2	6
mcyB-R	AGATGTCCGCAGGGATTCAT (20)							6
isiA-F	CTTTAGGCTTTGGAGTCGGAGA (22)	<i>isiA</i>	IPF_5322	AM778872.1	117	1.89±0.01	2	This study
isiA-R	GGTGAAATAAGGCTCCTGCTC (21)							This study

The locus tags are from *Microcystis* PCC 7806 (IPF) and *Microcystis* NIES-843 (MAE). The amplification efficiency E was based on 50-65 amplification curves for each of the primer sets.
nd: not determined.

Table S4. Results of PCR reactions on gDNA from lake samples.

Primers	Target genes	Expected PCR product size (bp)	Observed PCR product size (bp)					Samples from Lake Kennemermeer
			PCC 7806	NIES-843	CCAP 1450/10	NIVA-CYA 140	PCC 7005	
bicA-F1 & bicA-R1	<i>bicA</i>	902/2123	902	-	-	2123	902	902
bicA-F1 & sbtA-R1	<i>bicA+</i> <i>sbtA</i>	1443	-	-	-	1443	1443	1443
ccmR2-F1 & sbtA-R1	<i>ccmR2+</i> <i>sbtA</i>	843/2231/ 2351/3572	-	843	2231	-	2351	843+2351
bicA-R2 & nhaS3-R	<i>bicA+</i> <i>nhaS3</i>	1109/2789	1109	-	-	2789	2789	1109
sbtA-F1 & sbtB-R	<i>sbtA+</i> <i>sbtB</i>	1335	-	1335	1335	1335	1335	1335

The PCR reactions on gDNA were used to investigate the presence of the different C_i uptake genotypes in lake samples (last column). For comparison, five isolated *Microcystis* strains were used as reference. A minus sign (-) indicates that no PCR product was detected because the gene was absent in that strain.

Table S5. DIC, pH and temperature (\pm SD) measured in the chemostat experiments and lake study.

Time	DIC ($\mu\text{mol L}^{-1}$)	pH	Temperature ($^{\circ}\text{C}$)
<i>Lake study</i>			
Week 24	1742 \pm 28	9.57 \pm 0.02	17.23 \pm 0.01
Week 26	1632 \pm 74	9.77 \pm 0.02	15.41 \pm 0.15
Week 28	1117 \pm 40	10.00 \pm 0.00	19.65 \pm 0.01
Week 29	931 \pm 25	9.84 \pm 0.03	21.22 \pm 0.16
Week 30	816 \pm 57	9.90 \pm 0.01	23.55 \pm 0.01
Week 32	1793 \pm 34	9.09 \pm 0.01	20.91 \pm 0.02
Week 34	1914 \pm 56	9.46 \pm 0.00	19.81 \pm 0.01
Week 36	1750 \pm 37	9.88 \pm 0.04	19.83 \pm 0.40
Week 38	2823 \pm 125	8.68 \pm 0.04	12.54 \pm 0.04
Week 40	3054 \pm 70	8.86 \pm 0.01	11.03 \pm 0.01
<i>Chemostat experiments at 100 ppm CO₂</i>			
Day 1	-	8.17 \pm 0.38	24.90 \pm 0.10
Day 5	237 \pm 106	10.27 \pm 0.59	25.20 \pm 0.00
Day 12	283 \pm 39	10.87 \pm 0.14	24.98 \pm 0.21
Day 26	233 \pm 61	10.83 \pm 0.05	25.32 \pm 0.28
Day 40	236 \pm 47	11.06 \pm 0.02	24.90 \pm 0.05
Day 54	243 \pm 62	11.16 \pm 0.10	25.33 \pm 0.06
Day 68	277 \pm 13	11.13 \pm 0.12	25.33 \pm 0.13
Day 82	256 \pm 55	11.24 \pm 0.05	25.00 \pm 0.13
Day 96	200 \pm 33	11.09 \pm 0.04	24.80 \pm 0.09
Day 110	264 \pm 10	11.17 \pm 0.05	24.48 \pm 0.16
Day 124	-	10.99 \pm 0.05	24.82 \pm 0.12
Day 138	243 \pm 18	11.15 \pm 0.06	25.03 \pm 0.03
Day 152	-	11.18 \pm 0.10	25.15 \pm 0.05
Day 166	-	11.45 \pm 0.08	24.73 \pm 0.20
Day 173	265 \pm 20	11.23 \pm 0.04	24.67 \pm 0.06
<i>Chemostat experiments at 1000 ppm CO₂</i>			
Day 1	-	7.60 \pm 0.37	24.50 \pm 0.35
Day 5	1038 \pm 52	8.18 \pm 0.08	25.02 \pm 0.30
Day 12	2306 \pm 60	8.55 \pm 0.02	24.80 \pm 0.23
Day 26	2853 \pm 61	8.60 \pm 0.03	25.00 \pm 0.58
Day 40	2899 \pm 34	8.62 \pm 0.06	24.82 \pm 0.19
Day 54	2977 \pm 49	8.90 \pm 0.04	25.47 \pm 0.06
Day 68	2951 \pm 74	8.69 \pm 0.05	25.37 \pm 0.03
Day 82	2874 \pm 142	8.67 \pm 0.03	24.95 \pm 0.10
Day 96	3450 \pm 177	8.66 \pm 0.20	24.80 \pm 0.13
Day 110	3186 \pm 334	8.65 \pm 0.07	24.17 \pm 0.26
Day 124	-	8.50 \pm 0.05	24.57 \pm 0.45
Day 138	3258 \pm 77	8.57 \pm 0.03	24.53 \pm 0.20
Day 152	-	8.63 \pm 0.02	24.88 \pm 0.23
Day 166	-	8.79 \pm 0.05	24.53 \pm 0.32
Day 173	3411 \pm 77	8.71 \pm 0.02	25.05 \pm 0.31

The standard deviations (SD) are based on three different lake samples or three replicate chemostats. Concentrations of CO₂(aq), bicarbonate and carbonate were calculated from DIC, pH and temperature (3).