Supplementary Materials for

Photoinhibition of the Picophytoplankter *Synechococcus* Is Exacerbated by Ocean Acidification

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Supplementary information

Materials and Methods

The composition of SN15 medium contains 1 L seawater with 1 mL 4.41 M $NaNO_3$, 1 mL 91.1 mM K_2HPO_4, 1 mL 13.7 mM $Na_2EDTA \cdot 2H_2O$, 1 mL 98.1 mM Na_2CO_3, 1 mL 0.74 μM Vitamin B_{12}, 1 mL cyano trace metal solution [400 mL distilled water, 100 mL 297 mM citric acid $\cdot H_2O$, 100 mL 229 mM ferric ammonium citrate, 100 mL 27 mM $MnCl_2 \cdot 4H_2O$, 100 mL 16.1 mM $Na_2MoO_4 \cdot 2H_2O$, 100 mL 859 μM $Co(NO_3)_2 \cdot 6H_2O$, 100 mL 7.72 mM $ZnSO_4 \cdot 7H_2O$].).

Table S1 Carbonate chemistry parameters in the cultures of *Synechococcus* CB0101 grown under different light levels combined with the ambient (AC, 415 μatm) or elevated pCO_2 (HC, 1000 μatm). The values are means ± SD of triplicate cultures. Different superscripted letters indicate significant ($p < 0.05$) differences among the treatments. CO_2-light indicates the growth CO_2 and light levels.

CO_2-light	pH_{NBS}	TA (μM)	DIC (μM)	CO_2 (μM)	HCO_3^- (μM)	CO_3^- (μM)
AC-25	7.94±0.01^a	1259±12^a	1177±13^a	17.16±0.37^a	1120±12^a	40.27±0.12^a
HC-25	7.65±0.01^b	1430±18^a	1396±17^b	39.38±0.34^b	1331±16^b	24.81±0.75^b
AC-50	7.94±0.01^a	1278±6^a	1196±7^a	17.47±0.43^a	1138±7^a	40.81±0.50^a
HC-50	7.66±0.00^b	1375±12^a	1339±13^b	37.02±0.56^b	1278±12^b	24.29±0.13^b
AC-150	7.97±0.01^a	1255±7^a	1168±6^a	15.88±0.28^a	1110±6^a	42.72±0.81^a
HC-150	7.65±0.00^b	1369±22^a	1334±22^b	37.48±0.39^b	1273±21^b	23.82±0.55^b
AC-250	7.99±0.01^a	1267±7^a	1176±8^a	15.13±0.36^a	1115±8^a	45.31±0.51^a
HC-250	7.65±0.02^b	1335±18^a	1300±16^b	36.90±1.06^b	1240±16^b	23.00±1.04^b
AC-400	7.97±0.01^a	1255±7^a	1167±6^a	15.61±0.40^a	1108±6^a	43.36±1.14^a
HC-400	7.65±0.00^b	1320±20^a	1284±20^b	35.98±0.75^b	1226±19^b	23.00±0.0.28^b
AC-800	7.97±0.01^a	1277±6^a	1189±6^a	15.82±0.28^a	1129±6^a	44.37±0.52^a
HC-800	7.65±0.01^b	1317±26^a	1282±25^b	36.48±0.29^b	1223±24^b	22.60±0.86^b

Table S2 The photochemical parameters derived from rapid light curves (Fig. S2) of *Synechococcus* cells grown under different light levels combined with the ambient (AC, 415 μatm) or elevated pCO_2 (HC, 1000 μatm). α (a.u.–arbitrary units), $rETR_{max}$ (a.u.), and I_k (μmol photons m^{-2} s^{-1}) represents photosynthetic light-harvesting efficiency, maximum electron transport rate, and light saturation point, respectively. The values are mean ± SD of triplicate cultures. Different superscripted letters indicate significant ($p < 0.05$) differences among the treatments.

AC	25	50	150	250	400	800
α	0.230±0.010	0.228±0.002	0.232±0.009	0.215±0.105	0.203±0.010	0.167±0.013
$rETR_{max}$	98.25±3.77	113.68±4.18	190.08±6.83	194.34±12.19	197.03±3.03	177.26±14.66
I_k	414.6±11.2	498.9±14.2	818.8±5.6	903.2±33.7	974.2±58.9	1063.1±14.0
HC						
α	0.273±0.017	0.242±0.002	0.246±0.003	0.260±0.006	0.241±0.006	0.181±0.013
$rETR_{max}$	118.90±5.80	130.23±1.96	217.27±2.43	231.53±3.05	229.62±7.99	190.53±8.74
I_k	435.0±13.8	537.6±5.59	881.6±14.6	891.5±20.3	952.8±17.0	1051.5±39.0

Table S3. Statistical analyses of physiological traits of *Synechococcus* CB0101 grown under different pCO_2 and light combinations.

Trait	Factor	df	F value	*p* value
Growth rate	pCO_2	1	166.377	<0.001
	light	5	3307.262	<0.001
	pCO_2 * light	5	10.492	<0.001
Chl *a*	pCO_2	1	2.841	0.105
	light	5	313.380	<0.001
	pCO_2 * light	5	11.499	<0.001
Carbon fixation (per cell)	pCO_2	1	66.311	<0.001
	light	5	114.656	<0.001
	pCO_2 * light	5	21.335	<0.001
Carbon fixation (per Chl *a*)	pCO_2	1	34.571	<0.001
	light	5	108.706	<0.001
	pCO_2 * light	5	8.0304	<0.001
Yield	pCO_2	1	120.754	<0.001
	light	5	606.776	<0.001
	pCO_2 * light	5	2.147	0.094
α	pCO_2	1	72.384	<0.001
	light	5	51.776	<0.001
	pCO_2 * light	5	3.270	0.033
rETR	pCO_2	1	101.320	<0.001
	light	5	246.707	<0.001
	pCO_2 * light	5	2.449	0.063
a^*	pCO_2	1	40.565	<0.001
	light	5	60.011	<0.001
	pCO_2 * light	5	14.659	<0.001
POC	pCO_2	1	17.849	<0.001
	light	5	25.968	<0.001
	pCO_2 * light	5	3.809	0.011
PON	pCO_2	1	5.22388	0.031
	light	5	26.69842	<0.001
	pCO_2 * light	5	2.8207	0.038
C/N	pCO_2	1	16.17083	<0.001
	light	5	30.28343	<0.001
	pCO_2 * light	5	5.20364	0.002

Table S4. Percentage inhibition of carbon fixation under elevated pCO_2 under different light levels (μmol photons m^{-2} s^{-1}).

Inhibition (%)	25	50	150	250	400	800
CF(cell)	-21±20%	-4±11%	-10±17%	39±8%	46±1%	38±27%
CF(Chl *a*)	-26±19%	-2±10%	-0±20%	26±12%	32±6%	38±27%

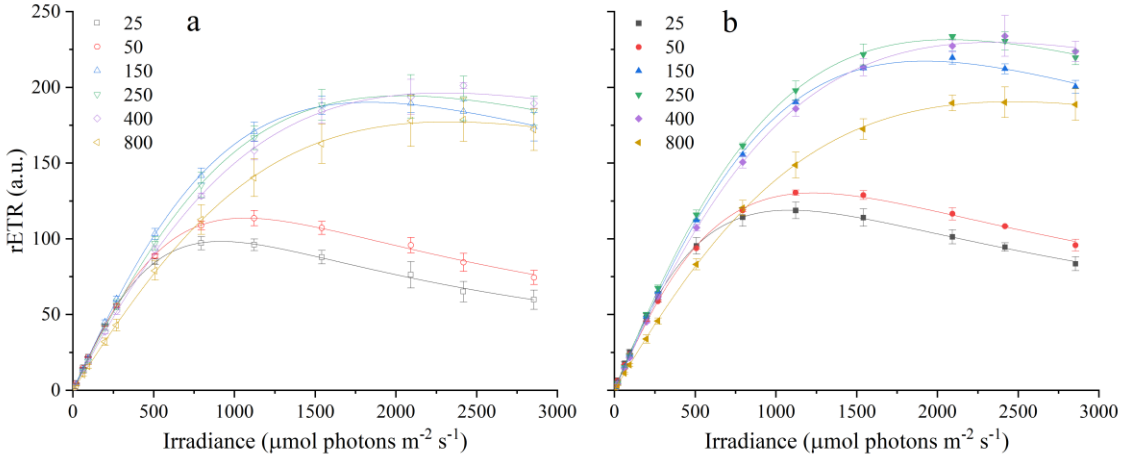

Figure S1 Rapid light curve (RLC) of *Synechococcus* CB0101 grown under different light levels combined with the ambient (AC, 415 µatm) (**a**) or elevated pCO_2 (HC, 1000 µatm) (**b**). The values are mean ± SD of triplicate cultures (n = 3). Principal component analysis (PCA) of the RLC data showed a strong clustering along PC1, which explains 96.54% of the variance in the data set (Figure S4, upper panel). It demonstrated that rETR is higher for HC conditions except at 800 µmol photons m^{-2} s^{-1}.

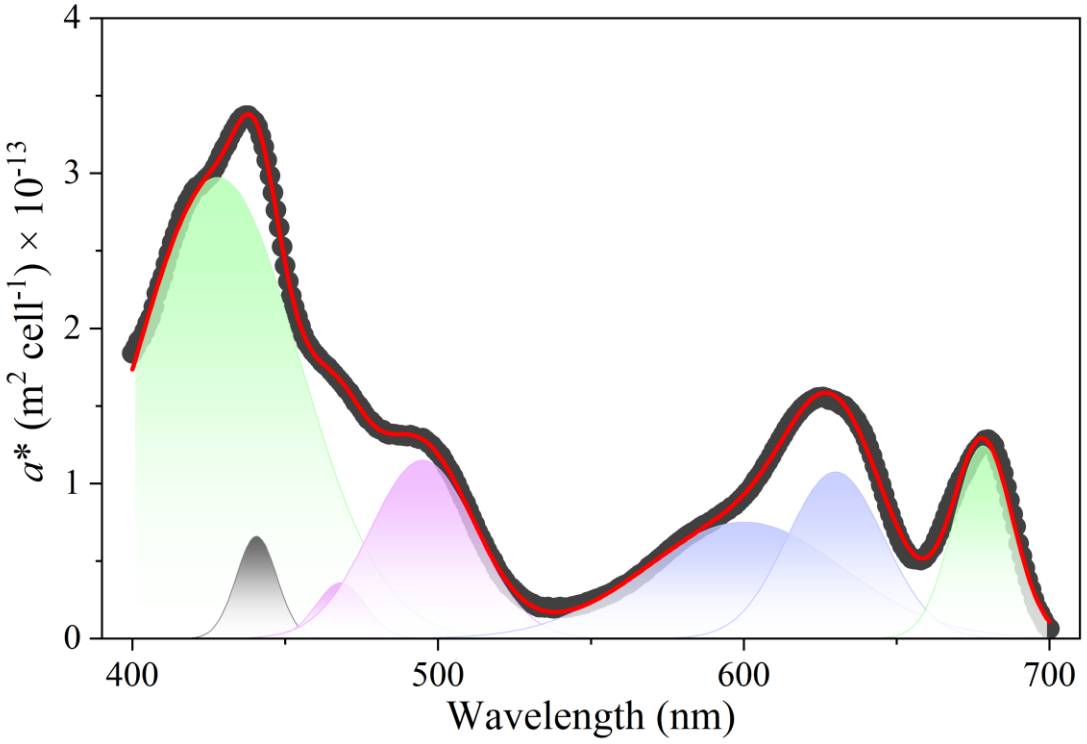

Figure S2 An example of decomposition of the absorption spectra $a^*(\lambda)$ by a series of Gaussian curves. Black dots are measured values of a^*, different colors are the Gauss peak spectra used to decompose the a^* spectrum, and the red line is the sum of all Gaussian curves.

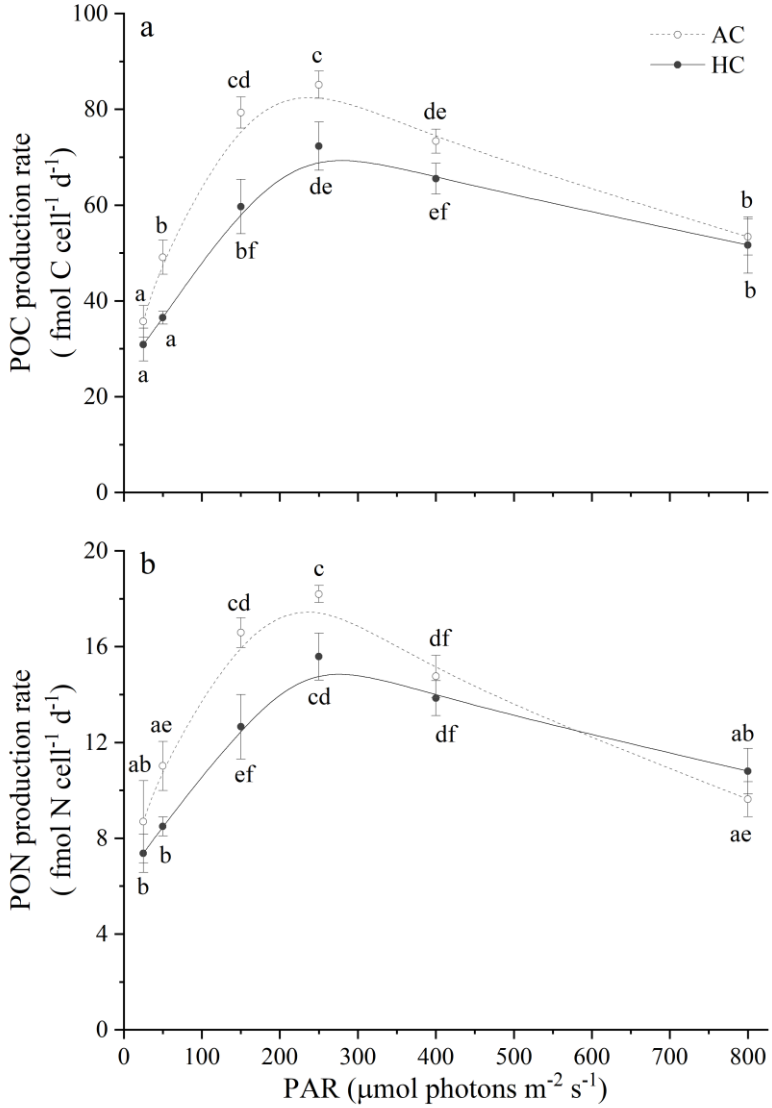

Figure S3 Particulate organic carbon (POC) (**a**) and nitrogen (PON) production rates (**b**) of *Synechococcus* cells grown under different light levels combined with the ambient (AC, 415 μatm) or elevated pCO_2 (HC, 1000 μatm). The production rates of POC or PON were calculated by multiplying the cellular quota by corresponding specific growth rates (d^{-1}). The values are means ± SD of triplicate cultures. Different letters indicate significant ($p < 0.05$) differences among the treatments.

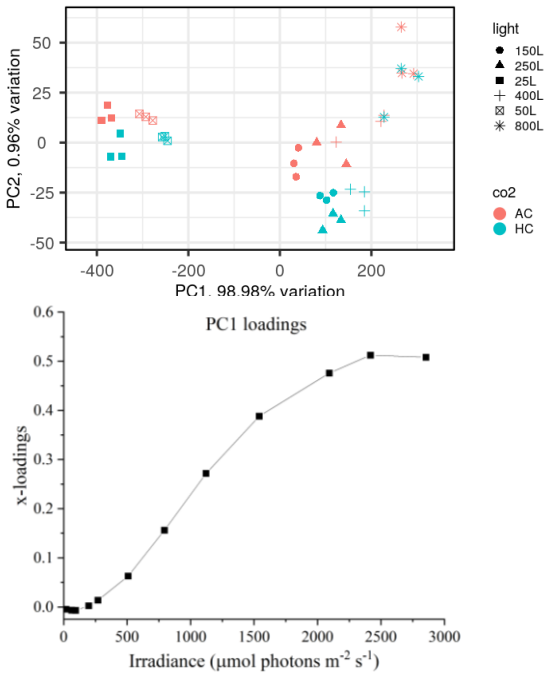

Figure S4 PCA analysis of rETR vs Irradiance data from Fig. S1 for all treatments. The upper panel (scores plot) shows clustering of treatments indicating effects of CO_2 levels in all light intensities except 800 µmol photons m^{-2} s^{-1}. The loadings plot (lower) indicates that the clustering in the scores was due mostly to values at higher irradiance.