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Rising CO_2 and increased light exposure synergistically reduce marine primary productivity

METHODS

The parameters of the seawater carbonate system (Suppl. Table 1) were calculated from pH and pCO_2 or measured values of DIC with CO_2 SYS software[1], and cross-checked with DIC or pCO_2, using the equilibrium constants of K_1 and K_2 for carbonic acid dissociation after Roy et al. (1993)[2]. The pH change was determined with a pH meter (pH510, OAKTON) which was calibrated with standard National Bureau of Standards (NBS) buffer solution (Hanna), and the pH_{NBS} values were converted to pH_{Total} (pH_T) using the CO_2 SYS software.

Seawater samples for phytoplankton pigment analysis (8 L) were filtered through 47-mm GF/F glass fiber filters (under a vacuum pressure < 75 mm Hg and in dim light), and then were immediately frozen in liquid nitrogen prior to analysis in the laboratory. Phytoplankton community structure was analyzed based on the fractions of pigments, which were extracted in N,N-dimethylformamide and determined using an Aglient series 1100 HPLC system fitted with a 3.5-mm Eclipse XDB C8 column (100×4.6 mm; Agilent Technologies) based on a modified method [3] according to Mantoura & Llewellyn (1983)[4] and Van Heukelem & Thomas (2001)[5]. The abundance of different phytoplankton groups was determined using CHEMTAX[6].

Photorespiration was estimated as the difference of the net photosynthetic O_2 evolution by the cells at reduced (2%) and ambient (21%) O_2 levels[7]. The tris-buffered seawater (pH 8.2) was flushed with either pure N_2 or air to establish the low (2%) or air-equilibrated (21%) levels of dissolved O_2.

The apparent growth light use efficiency (α) was calculated by assuming that growth rate was zero at zero light and growth saturating point (PARexc) was taken as the light level at which maximal growth rate was observed.

The data were expressed as the means ± standard deviation (SD). Field and lab data were analyzed using two-sample *t*-test or paired *t*-test, respectively, to establish statistical significance ($P < 0.05$).

Supplementary Table 1. Parameters of the seawater carbonate system under the ambient and elevated CO_2 concentrations. Measurements and estimation of the parameters are described in the supplementary methods. Different superscripted letters indicate significant difference ($P < 0.05$) between the high- and low-CO_2 treatment.

	pCO_2 (µatm)	pH_T	DIC (µmol kg^{-1})	HCO_3^- (µmol kg^{-1})	CO_3^{2-} (µmol kg^{-1})	Total alkalinity (µmol kg^{-1})
Lab	390	8.02±0.01[a]	1913.6±57.4[a]	1739.7±48.5[a]	161.2±8.9[a]	2155.3±68.3[a]
	1000	7.68±0.01[b]	2116.3±72.8[b]	2000.5±67.2[b]	83.1±5.5[b]	2217.9±79.6[a]
Field	385	8.04±0.01[a]	1889.7±38.6[a]	1700.8±32.0[a]	176.3±6.6[a]	2134.0±46.5[a]
	800	7.76±0.01[b]	1981.8±34.7[b]	1854.7±34.7[b]	101.0±3.8[b]	2102.8±43.2[a]
	1000	7.69±0.01[c]	2097.2±40.5[c]	1973.3±37.1[c]	91.5±3.4[b]	2196.1±44.7[a]

Supplementary Table 2. Locations of the stations, cruise information, sea surface temperature (SST, °C) and pH_T, $NO_3^-+NO_2$ (N, μmol L^{-1}) and PO_4^{3-} (P, μmol L^{-1}), solar PAR (mean, μmol photons m^{-2} s^{-1}) during ^{14}C-traced incubations, incubation time (h), surface seawater chlorophyll a concentration (Chl a, μg L^{-1}), chlorophyll a concentration (μg L^{-1}) of phytoplankton assemblages grown for 6-7 days under low CO_2 (LC,385 μatm) and high CO_2 (HC, 800 μatm for all stations except SEATS and C3, where 1000 μatm CO_2 was applied), and the primary productivity (PP, triplicate incubations, μg C $L^{-1}h^{-1}$) by the phytoplankton assemblages grown in the low CO_2 microcosms at the end (day 7) of the growth-out in the microcosms. BLQ stands for "below the limit of quantification". The concentrations of the nutrients were determined by the chemistry group of Xiamen Univ. during the cruises. Chlorophyll a concentration in the microcosms at station PN07 was not measured (nd).

Station	Location	Season*	SST	pH_T	N	P	Solar PAR	Incubation time (h)	Chl *a*	Chl *a* (LC)	Chl *a* (HC)	PP
LE04	(18.0°N, 113.0°E)	Summer	29.5	8.03	BLQ	0.014	1681	6	0.05	0.15	0.13	0.10±0.08
PN07	(30.0°N, 124.5°E)	Summer	29.6	8.03	BLQ	0.019	1371	6	0.71	Nd	nd	0.18±0.12
A4	(20.8°N, 115.2°E)	Autumn	25.5	8.04	BLQ	0.156	794	6	0.44	1.08	0.69	2.73±0.32
E606	(18.9°N, 114.1°E)	Autumn	25.3	8.06	BLQ	BLQ	821	6	0.34	0.82	0.20	4.74±0.10
SEATS	(18.0°N, 116.0°E)	Spring	28.7	8.04	BLQ	0.037	1251	12 (24)	0.10	0.49	0.59	2.08±0.14 (19.80±1.09)**
C3	(20.6°N, 114.2°E)	Spring	28.5	8.03	BLQ	0.032	1027	12 (24)	0.21	0.42	0.36	1.83±0.06 (16.28±0.73)**

**summer cruise (July 16 to August 31, 2009), autumn cruise (October 22 to November 25, 2010), spring cruise (April 30 to May 25, 2011). ** the PP values obtained over 24h-incubation period (μg C $L^{-1}d^{-1}$).*

Supplementary Table 3. Statistically significant levels for the comparisons of photosynthetic carbon fixation rates between the low and high CO_2 treatments (Figure 1a,b). The P values were obtained using two-sample t-test.

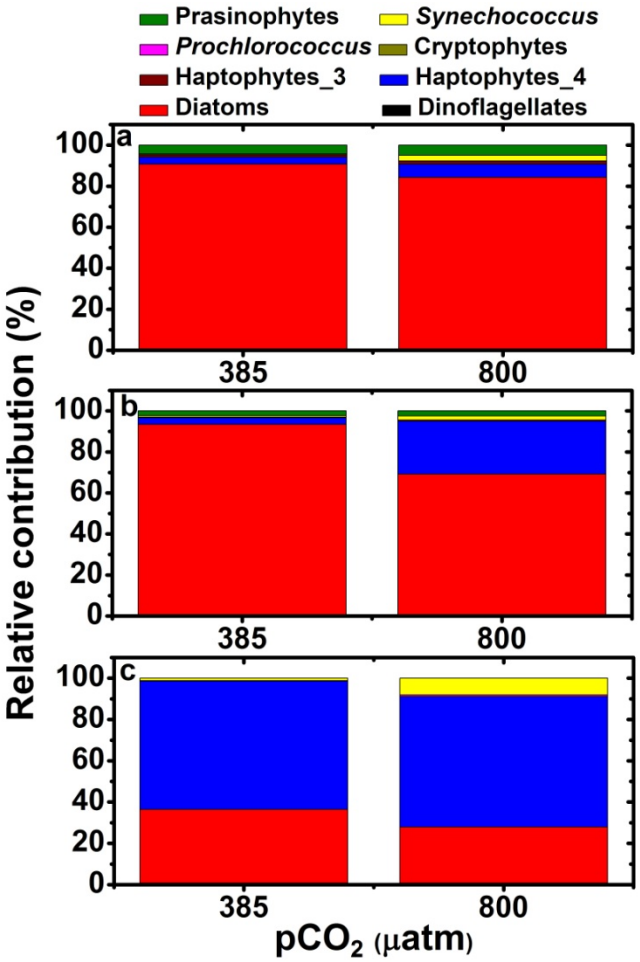
	LE04	PN07	A4	E606	SEATS	C3
Fig1a	0.022	0.016	$6*10^{-4}$	$3*10^{-7}$	0.026	0.002
Fig1b	0.071		0.047	0.002	0.002	0.063
Fig1a inset					$9*10^{-4}$	$2*10^{-4}$
Fig1b inset					$2*10^{-4}$	0.001

Supplementary Table 4. The apparent growth light use efficiency (α, μ perμmol photons^{-1} m^2 s-^{1} d^{-1}or MJ m^{-2}) and PAR threshold (PARexc) of the diatoms beyond which their growth rate declined with increasing light levels in the high CO_2 grown cells or leveled off in the low CO_2 grown ones. The data were derived from Figure 2 and represented as the means ± SD (n=6). The different superscripted letters represent significant (P <0.05) difference between the CO_2 levels. α, or PARexc values are shown either as photon flux density of PAR (μmol photons m^{-2} s^{-1}) or PAR dose (MJ m^{-2}d^{-1}, in parenthesis).

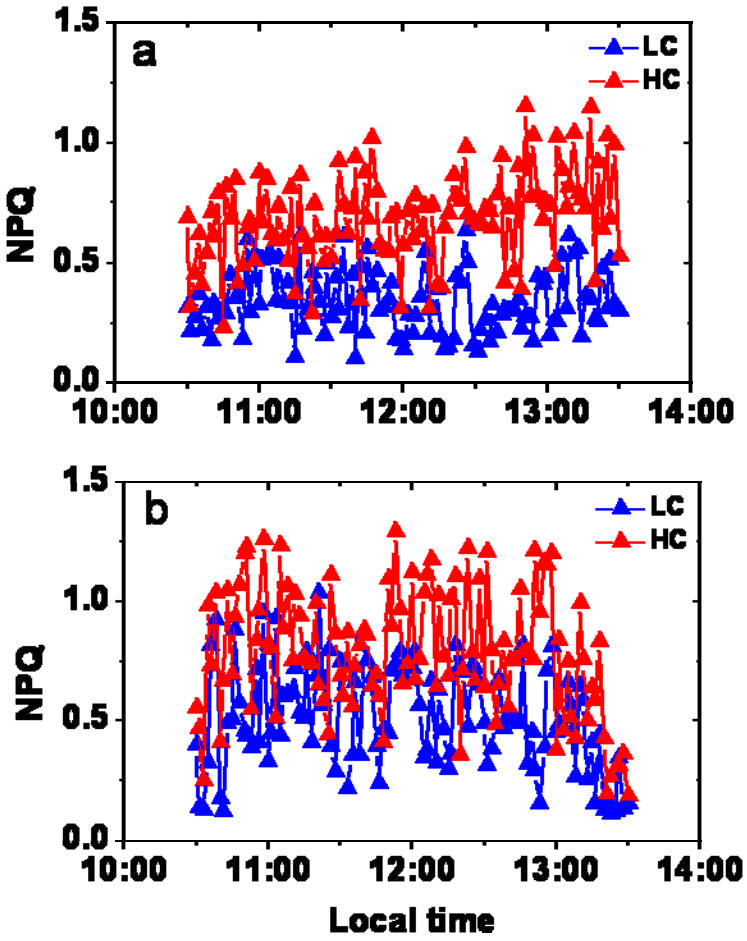
	CO_2	*P. tricornutum*	*T. pseudonana*	*S. costatum*
α	HC	0.020±0.002^a (2.24±0.25^a)	0.054±0.023^a (5.23±1.91^a)	0.030±0.014^a (2.82±1.29^a)
	LC	0.016±0.002^b (1.84±0.23^b)	0.044±0.012^b (4.24±0.97^b)	0.028±0.012^b (2.64±1.15^b)
PARexc	HC	159.5±66.5^a (1.43±0.59^a)	125.0±22.8^a (1.37±0.35^a)	178.1±105.2^a (2.04±1.34^a)
	LC	315.3±117.4^b (2.82±1.05^b)	226.7±46.7^b (2.30±0.39^b)	359.6±143.5^b (3.95±1.45^b)

Supplementary Table 5. Photorespiration (fmol O_2 $cell^{-1}$ h^{-1}) of T. pseudonana and P. tricornutum measured at ambient CO_2 level for the cells grown semi-continuously under the ambient (390 µatm, LC) and elevated (1000 µatm, HC) CO_2 levels for >20 generations. Difference letters represent the significant ($P<0.05$) difference among the treatments.

	T. pseudonana	*P. tricomutum*
LC	59 ± 8^{a}	43 ± 18^{a}
HC	73 ± 3^{b}	54 ± 16^{b}
Ratio (HC:LC)	1.23	1.26



Supplementary Figure 1. Composition of phytoplankton assemblages grown under LC (pCO_2 385 µatm) and HC (pCO_2 800 µatm) levels. a. *Station A4, January 2010, 7-day CO_2 pre-conditioning;* ***b.*** *Station of A4, November 2010, 7-day CO_2 pre-conditioning;* ***c.*** *Station E606, November 2010, 6-day CO_2 pre-conditioning.*



Supplementary Figure 2. Non photochemical quenching (NPQ) of phytoplankton assemblages grown under low pCO_2 (385 µatm, blue triangle) and high pCO_2 (1000 µatm, red triangle). a. *Station SEATS (18^o N, 116^o E), May 2011;* ***b.*** *Station C3 (20.55^o N, 114.20^o E), May 2011. NPQ was determined from 10:30 to 13:30 on May 15th, 23rd, 2011, at day 7 after the CO_2 perturbation, for the two stations, respectively. The mean levels of PAR during the measuring periods were 1938 and 1684 µmol photons m^{-2} s^{-1} for SEATS and C3, respectively.*

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