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Short-term elevated CO_2 exposure stimulated photochemical performance of a coastal marine diatom

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ABSTRACT

Ocean acidification changes seawater chemistry, with increased CO_2 and decreased pH regarded as the most important factors that impact marine organisms. This study employed an unconventional methodology to distinguish the independent effects of pH versus CO_2. Changes in CO_2 dominated the photochemical responses of the coastal diatom *Phaeodactylum tricornutum* to short-term ocean acidification. Increased CO_2 lowered non-photochemical quenching of excitation and stimulated the electron transport rates of photosynthesis, with the largest effects on both parameters when CO_2 and pH were altered simultaneously. Changes in pH alone did not show significant effects upon non-photochemical quenching (NPQ) nor upon electron transport rates, but can synergistically amplify CO_2 effects under low light. Maximal induction of NPQ after illumination showed only a limited response to increasing CO_2 under stable pH, across a range of increasing light levels, but maximal induced NPQ declined rapidly with increasing CO_2 under variable pH, when measured under exposure to sub-saturating light, but not under saturating light. These findings show that aqueous CO_2 and pH affect different physiological processes independently or interactively, which should be taken into account in future research for better understanding of responses to ocean acidification at the mechanistic level.

1. Introduction

Anthropogenic CO_2-induced ocean acidification has become a worldwide environmental issue (Doney et al., 2009). Ocean acidification has broad impacts on marine organisms (Balch and Fabry, 2008; Chen et al., 2014; Jin et al., 2015), and therefore potentially affects the marine ecosystem (Blackford, 2010; Havenhand, 2012). Increased CO_2 could provide more available carbon for photosynthesis, potentially stimulating the growth rate of photoautotrophs as well as primary production (Mackey et al., 2015; Wu et al., 2010). Decreased pH in seawater could affect the redox-balance and ion-homeostasis of organisms by altering the pH gradient between the cytoplasm and the outer surface of cell, disrupting the calcium carbonate precipitation of calcifying organisms, therefore threatening some potentially fragile species that are sensitive to pH changes (Clarkson et al., 2015; Cornwall et al., 2013; Flynn et al., 2012; Rokitta et al., 2012).

Current coastal oceans are affected by hydro-dynamic factors as well as biological processes, generating a fluctuating carbonate system, with pH variation ranges as large as 1 unit (Cornwall et al., 2013; Hofmann et al., 2011; Kapsenberg et al., 2015). Therefore, fluctuation of carbon supplies already have current impacts on coastal species (Wallace et al., 2014). Recent studies have shown that fluctuating pH reduced the growth rate of some macro-algae (Cornwall et al., 2013). Fluctuating coastal pH is now superimposed with global ocean acidification, that might be a selective pressure, favoring some species while disfavoring others because of the divergent responses of phytoplankton to ocean acidification (Falkowski and Oliver, 2007; Wu et al., 2015).

As the core component in photosynthesis, photosystem II (PSII) converts light energy to electrons, but downstream CO_2 assimilation reactions are the limiting stage of photosynthesis when light goes to saturating levels (Barber and Andersson, 1992; Nelson and Yocum, 2006; Sukenik et al., 1987). Therefore, unutilized light energy has to be dissipated via non-photochemical quenching (NPQ) mechanisms, mainly energy-dependent NPQ, to protect PSII from photo damage (Niyogi et al., 2005). Although carbon assimilation relies upon dissolved CO_2 carbon concentrating mechanisms (CCMs) allow cells to access dissolved bicarbonate (HCO_3^-) from

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seawater through the action of carbonic anhydrase (Giordano et al., 2005). Carbon assimilation is therefore sensitive to changes in both aqueous CO_2 concentration, to changes in total dissolved inorganic carbon (DIC) and to pH dependent changes in DIC speciation (John et al., 2007). Changes in carbon fixation will alter the cellular concentration of ATP and NADPH, which in turn feed back to affect photochemical processes, as well as the energy dissipation pathways (Takahashi and Murata, 2005). Theoretically, an increase in CO_2 provides more substrates for photosynthesis to drain off electrons from electron transport, to alleviate photoinhibition and stimulate PSII activity (Wu et al., 2010, 2014b). These interactions are however complicated because carbon concentrating mechanisms driven by cyclic electron transport have been implicated as sites for energy dissipation under excess light, and down-regulation of carbon concentration mechanisms under elevated CO_2 can thus suppress a mechanism for excitation dissipation (Badger and Price, 2003; MacKenzie et al., 2005; Wu et al., 2010).

Though the effects of ocean acidification on diatoms have been extensively studied, recent debates on laboratory seawater studies indicate that, to better understand how ocean acidification affects diatoms at mechanistic levels, it is critical to differentiate the independent effects of decreased pH and increased CO_2, and how they interactively affect diatoms during ocean acidification (Cressey, 2015; Hurd, 2015). For instance, ocean acidification can suppress primary production under inhibitory light levels due to down regulation of CCM (Gao et al., 2012b), while alleviating UV damage to diatoms under moderate light level (Wu et al., 2014a). A few studies showed that the independent manipulation of pH and CO_2 is a feasible approach to separate pH and CO_2. For instance, the nitrogen fixation of *Trichodesmium* was lowered by a decrease of pH, but was not affected by an increase in CO_2 (Shi et al., 2012), while a coccolithophore was less sensitive to high CO_2 and bicarbonate than to low CO_2 (Bach et al., 2013). To analyze short-term responses of photochemical parameters to environmental change (Adams and Demmig-Adams, 2004), we altered pH or CO_2 across a range covering most variation in coastal areas, to study the independent and interactive effects of pH and CO_2 on the photochemical processes of a model diatom.

2. Materials and methods

2.1. Species and culture conditions

Phaeodactylum tricornutum (CCMA106) was originally isolated from the South China Sea and obtained from the Center for Collection of Marine Bacteria and Phytoplankton (CCMA) of Xiamen University. This species was inoculated in sterilized seawater and enriched according to Aquil medium recipe (Morel et al., 1979). Cultures were maintained in exponential phase by semi-continuous culturing in Erlenmeyer flasks with maximal cell density kept below 3×10^5 mL^{-1}. Cultures were equilibrated with ambient air by aerating at a flow rate of ~300 mL min^{-1} to achieve an aqueous dissolved CO_2 of around 14 μmol l^{-1}. Approximately 70% of the culture volume was replaced daily with fresh medium at the beginning of dark period, then flasks were randomly replaced into a growth chamber. Illumination was provided with cool fluorescent tubes at a photon flux density of ~120 μmol m^{-2} s^{-1}, with the light:dark cycle set as 14:10, with temperature controlled at 20 ± 1 °C.

2.2. Experimental setup

In the middle of the light period, 200 mL of culture were filtered onto polycarbonate membrane at a light vacuum pressure around −0.02 Mpa. Cells were then resuspended off the filters in 200 mL of 20 mmol Tris L^{-1} buffered assay medium with different pH or/and DIC concentrations. The buffered medium imposed 3 categories of CO_2 regimes, each comprising 5 treatment levels and 3 replicates:

1. vCO_2/vpH: variable CO_2 (2.2, 4.3, 8.7, 17.4, 34.8 μmol L^{-1}) with variable pH (7.63, 7.92, 8.20, 8.46, 8.69) at stable DIC (2.0 mmol L^{-1});
2. vCO_2/spH: variable CO_2 (2.2, 4.3, 8.7, 17.4, 34.8 μmol L^{-1}) at stable pH (8.20) with variable DIC (0.5, 1.0, 2.0, 4.0, 8.0 mmol L^{-1});
3. sCO_2/vpH: stable CO_2 (8.7 μmol L^{-1}) with variable pH (7.63, 7.92, 8.20, 8.46, 8.69) and variable DIC (0.5, 1.0, 2.0, 4.0, 8.0 mmol L^{-1}).

To achieve target aqueous CO_2, DIC concentration and pH levels were iteratively calculated with CO2SYS software, based on the target values of CO_2, pH, salinity, and nutrients, the equilibrium constants k_1 and k_2 for carbonic acid dissociation (Roy et al., 1993), and k_B for boric acid (Dickson, 1990). Then artificial seawater (Morel et al., 1979) was prepared with the calculated DIC supplemented with Tris buffer at a final concentration of 20 mmol L^{-1} pH was then altered to the target value by titration with 1 mmol L^{-1} HCl. The key parameters of carbonate system are shown in Table 1.

2.3. Chlorophyll fluorescence measurements

Sub samples were dark adapted for 15 min by covering with aluminum foil, and then measured with a Xenon-PAM (Walz, Germany). After dark adaptation, sub-sample were pipetted and filled into quartz cuvette, which was then covered with a lid to avoid contact with air. Initial F_v/F_m for samples was around 0.58. Time induction curves (a typical trace figure shown as Fig S1 in supplementary material) were then measured by a Xenon-PAM, during 240 s exposure to each actinic light, intensities were set at 0, 410, 840, 1200 or 1650 μmol m^{-2} s^{-1}. During each exposure period a saturating pulse was repeatedly applied every 20 s at ~5000 μmol s^{-2} s^{-1} for a duration of 0.8 s. We used the fluorescence variables from the Xenon-PAM to estimate electron transport rates and non-photochemical quenching under different CO_2 and pH regimes.

2.4. Data analysis

NPQ was calculated as:

$$(F_m - F_{m\prime})/F_{m\prime}$$

while the electron transport rate (ETR), was calculated as:

$$PFD \times 0.5 \times (F_{m\prime} - F_t)/F_{m\prime},$$

where F_m represents the maximal fluorescence yield after dark adaptation, F_m' represents the maximal fluorescence yield under

Table 1
The CO_2 regimes and the key parameters of the seawater carbonate system, DIC and CO_2 concentration was in μmol L^{-1}, while pH was in NBS scale.

CO_2 regimes							
	vCO_2/vpH	DIC	2000	2000	2000	2000	2000
		pH	7.63	7.92	8.20	8.46	8.69
		CO_2	34.8	17.4	8.7	4.3	2.2
	vCO_2/spH	DIC	500	1000	2000	4000	8000
		pH	8.20	8.20	8.20	8.20	8.20
		CO_2	2.2	4.3	8.7	17.4	34.8
	sCO_2/vpH	DIC	500	1000	2000	4000	8000
		pH	7.63	7.92	8.20	8.46	8.69
		CO_2	8.7	8.7	8.7	8.7	8.7

actinic light, and F_t represents the steady-state fluorescence. To summarize the relationship between photochemical performance and CO_2, we identified the maximal NPQ induced during exposure to each of the actinic light levels under a given CO_2/pH regime, which represents the energy not used by photosynthesis, and negatively correlated with photosynthetic processes during actinic light exposure.

The plots of NPQ versus time were fitted with a transformed equation which was originally developed for photosynthesis versus irradiance curve (Eilers and Petters, 1988):

$$\mathrm{NPQ} = (\mathrm{T}) \Big/ \left[\mathrm{a} \times (\mathrm{T})^2 + \mathrm{b} \times (\mathrm{T}) + \mathrm{c}\right]$$

Where T is the time duration (in second) under actinic light, and a, b, c was the adjust parameters.

$$\text{Maximal NPQ} = 1 \Big/ \left[\mathrm{b} + (\mathrm{ac})^{1/2}\right]$$

Maximal NPQ versus CO_2 concentration under different light intensities was then fitted with an exponential decay function:

$$\mathrm{NPQ} = \left((\mathrm{a} - \mathrm{c}) \times \mathrm{e}^{(-[\mathrm{CO_2}] \times \mathrm{b})}\right) + \mathrm{c};$$

where *a* is the amplitude of the fitted curve, *b* is the decay constant, and *c* is the plateau, to determine how changing CO_2 concentrations influenced photochemical performance.

Statistical differences among treatments were analyzed by ANOVA with significance set at $p = 0.05$. Correlation between maximal NPQ and treatment light intensity was tested with Spearman correlation analysis.

3. Results

Under variable CO_2 with variable pH (vCO_2/vpH), non-photochemical quenching (NPQ) was induced once actinic light turned on, increased gradually and reached maximal values after around 80 s across all applied light levels (Fig. 1A–D). Maximal NPQ was positively correlated with light intensity (Correlation coefficient = 0.91), increasing from 0.6 under 410 μmol m^{-2} s^{-1} (Fig 1A) to 2.5 under 1650 μmol m^{-2} s^{-1} (Fig. 1D). Under the lower light levels (410, 840 μmol m^{-2} s^{-1}) NPQ decreased rapidly after peaking at 100 s (Fig. 1A and B), but NPQ was more sustained after reaching peak levels under 1200 and 1650 μmol m^{-2} s^{-1} (Fig. 1C and D). There were significant differences among CO_2 levels for the NPQ induction curves, with lower NPQ for higher CO_2 treatments (Fig. 1A–D). The general patterns of NPQ versus light and time were similar for variable CO_2 with stable pH (Fig. 2) in comparison to variable CO_2 and variable pH. In contrast, stable CO_2 with variable pH did not show significant effects upon NPQ measured at each light level (Fig. 3).

The corresponding electron transport rates are presented in the supplementary figures (S2-S4). Increasing CO_2 increases electron transport rates at a given light level, particularly when CO_2 and pH were changed simultaneously (Fig S2). We observed smaller differences in electron transport rates at a given light level for variable CO_2 and stable pH (Fig S3). There were no significant differences among electron transport rates at a given level for stable CO_2 and variable pH (Fig S4).

The Maximal NPQ reached for each actinic light level is plotted (Fig. 4 A, B, C) versus CO_2 or pH for each regime; variable CO_2/variable pH (Fig. 4A), variable CO_2/stable pH (Fig. 4B) and stable CO_2/variable pH conditions (Fig. 4C). As expected, Maximal NPQ increased with actinic light levels under each CO_2/pH regime. Under the variable CO_2 treatments (Fig. 4 A, B) whether pH varied or

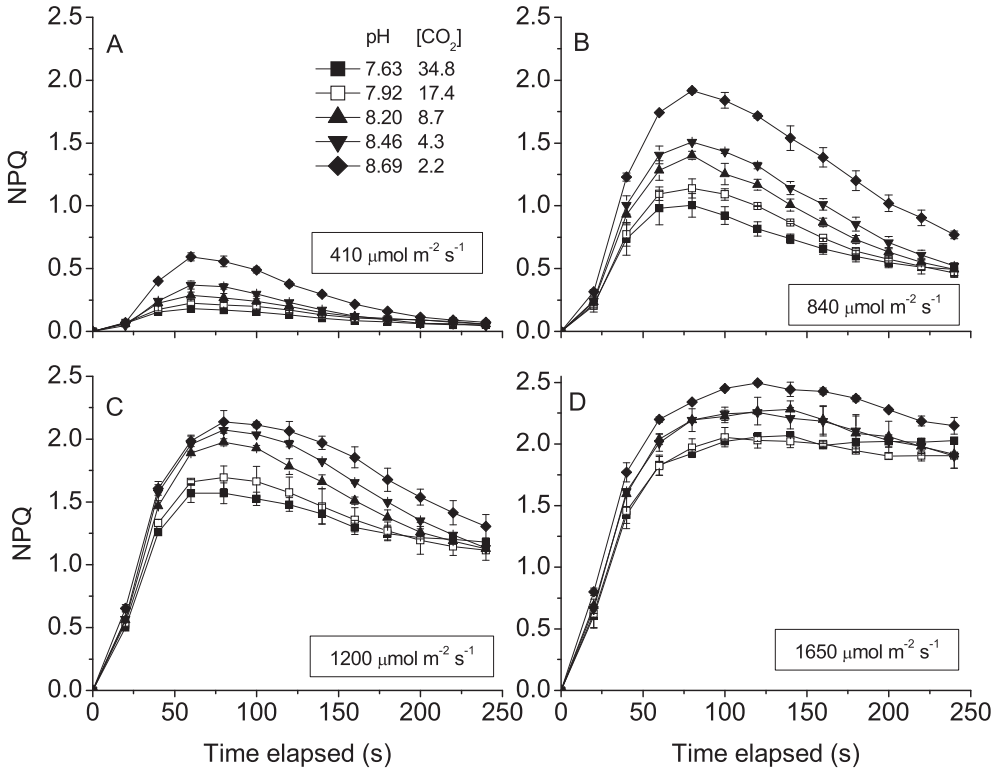

Fig. 1. Time course of NPQ formation of cells grown under ambient CO_2 under different intensities of actinic light (A: 410, B: 840, C: 1200 and D: 1650 μmol m^{-2} s^{-1}) under variable CO_2/variable pH conditions. Vertical bars represent SD, n = 3.

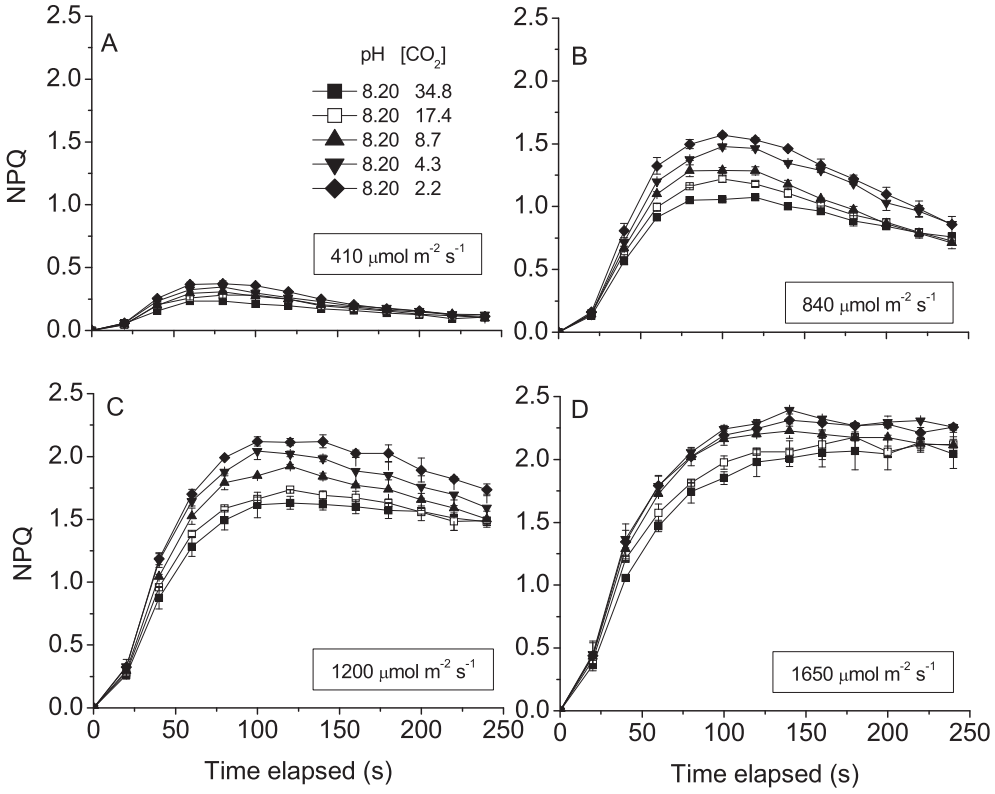

Fig. 2. Time course of NPQ formation of cells grown under ambient CO_2 under different intensities of actinic light (A: 410, B: 840, C: 1200 and D: 1650 μmol m^{-2} s^{-1}) under variable CO_2/stable pH conditions. Vertical bars represent SD, n = 3.

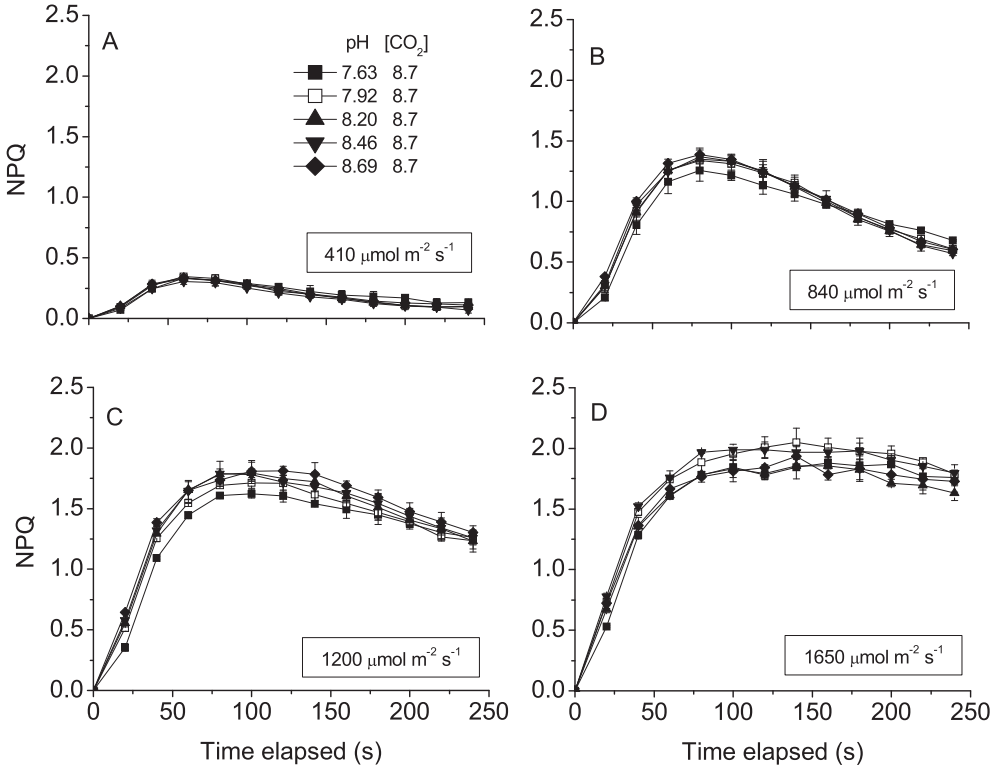

Fig. 3. Time course of NPQ formation of cells grown under ambient CO_2 under different intensities of actinic light (A: 410, B: 840, C: 1200 and D: 1650 μmol m^{-2} s^{-1}) under stable CO_2/variable pH conditions. Vertical bars represent SD, n = 3.

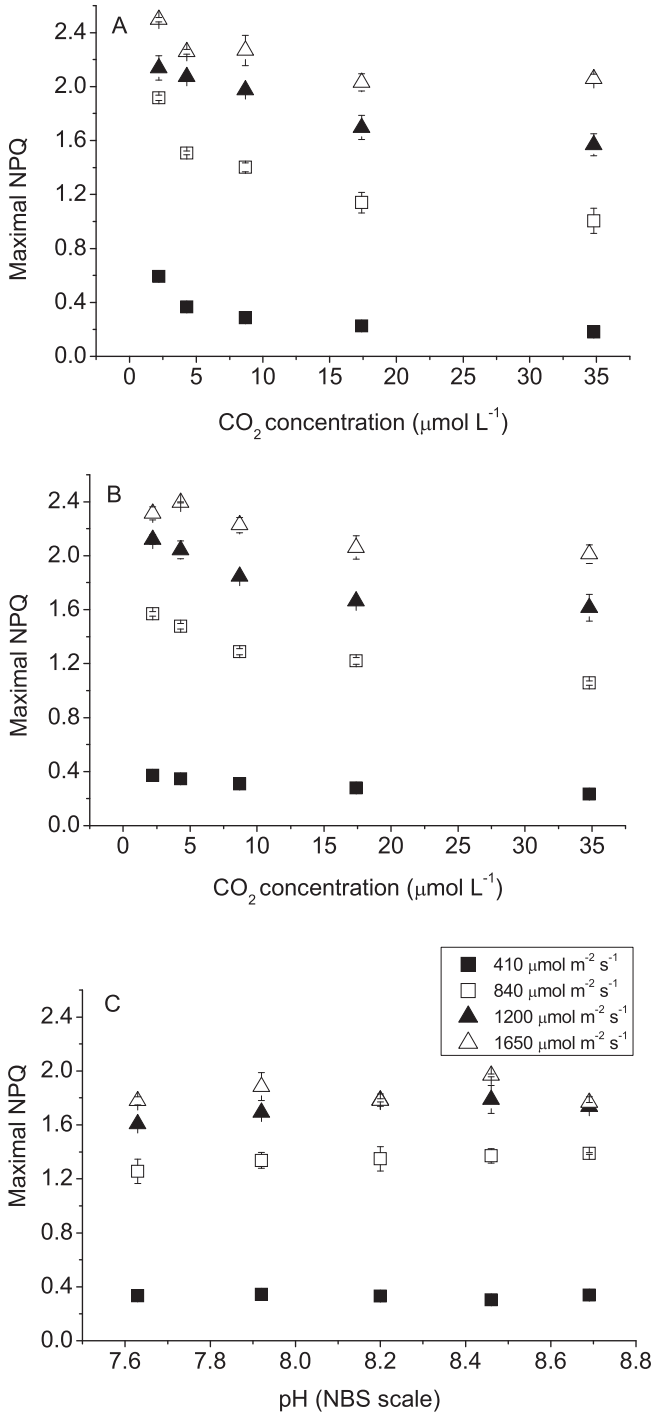

Fig. 4. The maximal NPQ achieved under variable CO_2/variable pH (A), variable CO_2/stable pH (B) and stable CO_2/variable pH conditions (C) at different levels of actinic light (410, 840, 1200 and 1650 μmol m^{-2} s^{-1}). Vertical bars represent SD, n = 3.

not, Maximal NPQ was negatively correlated with CO_2, showing an exponential decay to a plateau, which increased with actinic light level ($0.72 < R^2 < 0.99$). Under stable CO_2 while pH varied (Fig. 4C) Maximal NPQ was flat across changing pH levels, while increasing with light level (Fig. 4 C).

We then plotted the resulting decay constant (CO_2^{-1}) from the exponential decay functions fitted to Fig. 4 across the actinic light applied (Fig. 5). NPQ response to CO_2 was greatest for variable CO_2/variable pH treatments under the lowest actinic light. This NPQ response to CO_2 decreased with increasing actinic light. For variable CO_2/stable pH treatments, the NPQ response to CO_2 was generally smaller and showed limited change with increasing light.

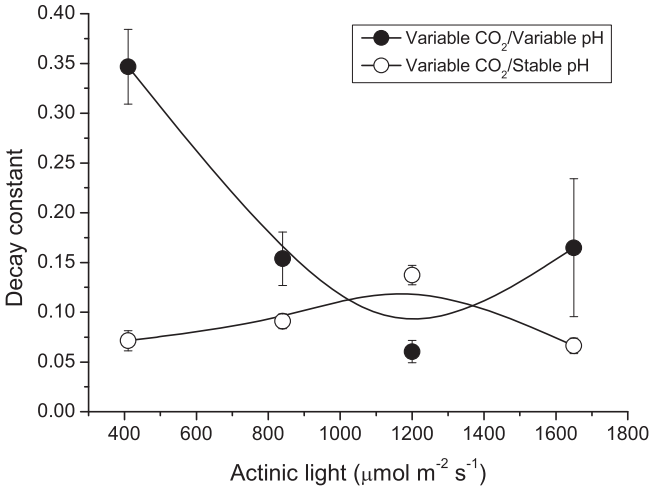

Fig. 5. The exponential decay constant of maximal NPQ versus CO_2 concentration, measured under different light intensities, under variable CO_2/variable pH (closed circles) or variable CO_2/stable pH (open circles) treatments, vertical lines represent SD, n = 3.

4. Discussion

Ocean acidification is characterized by decreasing pH with increasing dissolution of anthropogenic CO_2, with associated changes of other parameters in the seawater chemistry (Feely et al., 2004; Raven, 2005). The increased CO_2 is a major factor that may influence phytoplankton due to its crucial role in photosynthesis (Riebesell, 2004). The often-used methodologies for ocean acidification studies do not generally distinguish the independent effects of pH or CO_2, due to the concurrent changes for both parameters during perturbation (Riebesell et al., 2010). In this study, by means of an unconventional method, we found CO_2 affected the photochemical performance of *P. tricornutum* in a light-dependent manner, and the simultaneous changes of CO_2 and pH had more pronounced effects than manipulation of CO_2 or pH individually, indicating that high CO_2 and low pH could synergistically favor some species, with implications for the phytoplankton community structure in the future ocean (Falkowski and Oliver, 2007; Wu et al., 2015). In contrast, short-term (in minutes) changes in pH alone had little effect upon the excitation dissipation or the effective quantum yield of this coastal diatom species.

As the energy source of photosynthesis, light is the major factor that affects phytoplankton (Falkowski, 2015; Korbee et al., 2005; Marra and Heinemann, 1982; Morel, 1978). Below the light saturation point, increasing light induces higher photosynthesis, as electron transport rate increases, but effective photochemical yield decreases (Wu et al., 2010). Once light energy exceeds requirements, part of the captured light energy has to be dissipated through non-photochemical quenching, to protect photosystems from damage and to limit production of damaging Reactive Oxygen Species (Behrenfeld et al., 2004; Rohacek et al., 2014). As shown in previous works, NPQ was modest under low light, e.g. 410 μmol m^{-2} s^{-1} in this study, a sub-saturating level for photosynthesis in this strain (Wu et al., 2010). NPQ increased greatly when light exceeded 840 μmol m^{-2} s^{-1} as electron transport rate saturated, since this species has a high capacity for photoprotection (Bailleul et al., 2010).

Non-photochemical quenching, which represents the dissipated energy not used by photosynthesis, is a key parameter that reflects the physiological status, with the interpretation depending upon the time scale (Rohacek et al., 2014). Previous studies found that NPQ could be higher for phytoplankton assemblages grown under ocean acidification conditions under natural sunlight, and thus NPQ was considered as an indicator of stress (Gao et al., 2012b). Under short time scales (e.g. minutes), changes in NPQ mainly reflect the instantaneous response of photochemical processes to environmental changes (Niyogi and Truong, 2013). As CO_2 was manipulated in this study, NPQ at a given light level negatively correlated with increasing CO_2, consistent with rapid responses of CO_2 dependent photochemical processes to environmental changes (Wu et al., 2015).

The synthesis of organic carbon is the primary sink of the electrons generated in photosystem II, so changes in carboxylation often affect photochemical parameters (Stitt, 1986). Theoretically, PSII activity would be stimulated under ocean acidification due to the increased supplies of substrate for Calvin cycle (Wu et al., 2010), while the photosynthesis of a calcifying phytoplankton mainly responded to pH rather than CO_2 (Kottmeier et al., 2016), indicating the complexity of species-specific responses. For the non-calcifying species in the present study, increasing CO_2 had major effects, which significantly enhanced electron transport rates with the greatest stimulation under low light levels. There are diverse results on whether increases in CO_2 will actually stimulate photosynthesis across species (Gao and Campbell, 2014; Torstensson et al., 2012; Wu et al., 2010). A recent meta-analysis found that the fertilization effect of CO_2 was taxon-specific, and favored diazotrophs more than other groups, suggesting that ocean acidification might change the structure of phytoplankton assemblages (Dutkiewicz et al., 2015; Mackey et al., 2015).

The effects of decreased pH might be size-dependent with respect to the micro-environment and the extracellular enzymes around cell surfaces (Flynn et al., 2012; Shen and Hopkinson, 2015). In this study, we found that a simultaneous decrease in pH and increase in CO_2 synergistically stimulated the electron transport rates and lowered NPQ, suggesting that the decrease of pH indeed could favor some species, at least over short timescales. Moreover, the response of NPQ to CO_2 under stable pH showed a convex curvilinear pattern with increasing light levels, while the response of NPQ to CO_2 under varied pH showed a much larger concave curvilinear pattern (Fig. 5), implying that pH might be a critical factor mediating the interactive effects of ocean acidification and high light (Gao et al., 2012b). In addition to fluctuations in coastal waters, on tidal flats where biological activity is very high, drastic changes in CO_2, pH and light could drive microphytobenthos to evolve wider acclimatory scope to accommodate fluctuations (Laviale et al., 2015; Liu et al., 2016). Even in open ocean areas upwelling currents and typhoon induced deep mixing bring low pH/high CO_2 waters to the surface, that could alter the photochemical performance of phytoplankton assemblages over short timescales, with potential implications on primary production (Li et al., 2009).

Phytoplankton experience multiple stressors in natural habitats. Some recent studies have revealed that ocean acidification will affect phytoplankton interactively with other factors, with the overall outcomes synergistic, independent or antagonistic (Boyd, 2011; Gao et al., 2012a, 2012b). These interactive effects impede the prediction of ocean acidification effects on marine organisms and therefore the ecosystem, which has caused debates on the laboratory studies of ocean acidification due to the complexity of the results (Cressey, 2015; Hurd, 2015). As ocean acidification itself is a series of changes in seawater chemistry, which could potentially affect marine organisms in different aspects, the interactive effect of multiple changes in seawater due to ocean acidification, might be critical for the evaluation and prediction of ocean acidification effects. In the case of the coastal diatom *Phaeodactylum tricornutum* increasing CO_2 stimulates photochemistry, at least in the short-term, while decreasing pH has a smaller, but synergistic effect.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.marenvres.2016.12.001.

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