**Title:**Ocean acidification modulates expression of genes and physiological performance of a marine diatom

**Author:**Yahe Li1, Shufang Zhuang1, Yaping Wu1, Honglin Ren2, Fangyi Chen1, Xin Lin1, Kejian Wang1\*, John Beardall3, Kunshan Gao1\*

**Institutions:**

1State Key Laboratory of Marine Environmental Science/College of Ocean and Earth Sciences, Xiamen University (Xiang An Campus), Xiamen 361102, China.

2 Key Laboratory of Zoonosis Research, Ministry of Education, Institute of Zoonosis,Jilin University, Changchun 130062, China.

3School of Biological Sciences, Monash University, Clayton, Victoria 3800, Australia

\***Authors for correspondence:** ksgao@xmu.edu.cn; wkjian@xmu.edu.cn

**Abstract**

Ocean Acidification (OA) is known to affect various aspects of the physiological performance of diatoms, but there is little information on the underlining molecular mechanisms involved. Here, we show that in the model diatom *Phaeodactylum tricornutum* expression of the genes related to light harvesting, carbon acquisition and carboxylation, nitrite assimilation and ATP synthesis are modulated by OA. Growth and photosynthetic carbon fixation were enhanced by elevated CO2 (1000 μatm) under both constant indoor and fluctuating outdoor light regimes. The genetic expression of nitrite reductase (*nir*) was up-regulated by OA regardless of light levels and/or regimes. The transcriptional expression of fucoxanthin chlorophyll *a*/*c* protein (lhcf type (FCP)) and mitochondrial ATP synthase (mtATP synthase) genes were also enhanced by OA, but only under high light intensity. OA treatment decreased the expression of β-carbonic anhydrase (*β-ca*) along with down-regulation of CO2 concentrating mechanisms (CCMs). Additionally, the genes for these proteins (NiR, FCP, mtATP synthase, β-CA) showed diel expressions either under constant indoor light or fluctuating sunlight. Thus, OA enhanced photosynthetic and growth rates by stimulating nitrogen assimilation and indirectly by down-regulating the energy-costly inorganic carbon acquisition process.

**Key-words**: diatom, genes, growth, ocean acidification, physiology

**1. Introduction**

Ocean acidification (OA), expressed in milieu as a decline in pH, is driven by rapid increases in CO2 taken up by the oceans from the atmosphere and is altering marine chemical environments with consequences for marine organisms and the biological CO2 pump (Beardall et al., 2014). Although intracellular pH levels of both photosynthetic organisms and animals are known to be below the bulk seawater pH (Flynn et al., 2012), external pH decline is known to affect the physiology of many marine organisms to different extents (Pörtner et al., 2011). For instance the extracellular pH changes can influence the membrane electrochemical potential and enzyme activity (Kramer et al., 2003; Milligan et al., 2009; Wu and Gao, 2009). Responses of genes to OA have been studied in invertebrates (Crawley et al., 2010; O'Donnell et al., 2010; Nakamura et al., 2012; Vidal-Dupiol et al., 2013), the coccolithophore *Emiliania huxleyi* (Richier et al., 2011; Benner et al., 2013) and the diatom *Thalasiossira pseudonana* (Crawfurd et al., 2011; Hennon et al., 2014, 2015). Despite the genetic and physiological performances observed in the diatom *T. pseudonana* grown under OA (Hennon et al., 2015), there is still a need to understand the correlation between physiological performance and molecular responses in different species, which is essential to fully comprehend the mechanisms involved in the responses of marine organisms to OA.

Marine diatoms, as a key phytoplankton group, contribute about a quarter of the global primary productivity (Granum et al., 2005). Different and controversial responses of diatoms to OA have been reported (as reviewed in Gao and Campbell, 2014). In the model diatom *Phaeodactylum tricornutum*, growth and photosynthetic carbon fixation rate were enhanced when acclimated to 1000 μatm CO2 under indoor low light conditions (Wu et al., 2010; Gao et al., 2012a; Li et al., 2014), but growth was inhibited under the elevated pCO2 level under fluctuating high sunlight levels (Gao et al., 2012a). Additionally, photorespiration of *P. tricornutum* was enhanced by more than 20%, with its CO2 concentrating mechanism (CCM) down-regulated under OA conditions (Gao et al., 2012a), though a doubling of the present CO2 level was shown to reduce the CCM-dependent energy requirement by about 20%, with the saved energy being used to benefit its carbon fixation (Hopkinson et al., 2011).

Responses of diatoms, as well as other organisms, to elevated *p*CO2 must be considered in the context of other ocean changes, such as ocean warming, increased exposure to solar radiation and reduced nutrient availability due to warming-enhanced stratification (see the reviews by Beardall et al., 2009; Riebesell and Tortell, 2011; Gao et al., 2012b; Gao and Campbell, 2014 and literature therein). Although changes in seawater carbonate chemistry due to changed pCO2 interact with light to affect the photophysiology of diatoms (Gao et al., 2012a; Li et al., 2014; Hoppe et al., 2015), little is known about the relationship between physiological and genetic responses under both varying light and altered CO2 conditions. In the present study, *P. tricornutum*,whose genome has been completely sequenced (Bowler et al., 2008), was used to examine the relationship between physiological performance and genetic responses. Thus we demonstrate the variation in physiological parameters (growth, photosynthetic carbon fixation and photochemical performance) and associated expression of key gene products (photosynthesis light harvesting, FCP; Rubisco; β-carbonic anhydrase, β-CA, etc.) after the diatom was acclimated to the projected levels of future ocean acidification under different light treatments.

**2. Materials and methods**

**2.1** **Species and culture conditions**

*Phaeodactylum tricornutum* (strain CCMA 106) was obtained from the Center for Collections of Marine Bacteria and Phytoplankton (CCMBP) of the State Key Laboratory of Marine Environmental Science, Xiamen University and was originally isolated from the South China Sea (SCS) in 2004. Although this is not the strain (CCMP632) used for sequencing of the *Phaeodactylum* genome (Bowler et al.,2008), we used it because of its recent isolation and regional importance. The unialgal culture of this diatom was maintained in filtered (0.22 μm) and sterilized seawater collected from the South China Sea (18° N, 116° E), enriched with Aquil medium (Morel et al., 1979) under 130 μmolm-1 s-1 of Photosynthetic Active Radiation (PAR, L: D = 12: 12) and 20°C before the experiment. During the experimental periods, the cultures were aerated with the air of ambient (390 μatm, LC; from the roof of the building) or elevated CO2 (1000 μatm; HC) concentration, in an illuminated CO2 plant incubator (HP1000G-D, Wuhan Ruihua Instrument & Equipment Ltd, China) under the same light and temperature conditions. The HC was achieved automatically within the CO2 plant chamber with less than 4% variation in the CO2 concentration. Between the dilutions (with dilution every 24 h), cell concentrations were maintained within a range of 7×104 - 2.8×105 cell mL-1 to maintain stable seawater carbonate chemistry (Supplementary Table S1).

**2.2** **Experimental set up**

Following the internationally recognized OA research guide (Riebesell et al., 2010), cells grown under both LC and HC conditions for over 10 generations were sampled. To check if there were significant differences in physiological performance and molecular responses to OA, the indoor grown cells were transferred to outdoors (at 20:00 after dilution on the eighth day), exposing them to nearly full sunlight of the normal sinusoidal day. In order to evaluate the effects of elevated CO2 (1000 μatm; HC) during both light and dark periods and/or different times of day, sampling for both physiological and genetic measurements was carried out at various times during the light dark cycle after cells were transferred from LC to HC conditions. Sampling times (with light/dark conditions indicated as l or d respectively) were set as: (1) indoor culture: 4 h-l, 8 h-l, 16 h-d, 28 h-l, 32 h-l, 40 h-d, 76 h-l, 80 h-l, 88 h-d, 172 h-l, 176 h-l, 184 h-d; (2) outdoor culture: 196 h-l, 200 h-l, 208 h-d, 220 h-l, 224 h-l, 232 h-d, 268 h-l,272 h-l and 280 h-d. During the outdoor culture, the water temperature was controlled at 20 ± 0.1°C with a circulating cooler (CTP-300, Eyela, Tokyo, Japan). Solar PAR was monitored every second with a broadband solar radiometer (ELDONET, Real Time Computer, Möhrendorf, Germany). The light levels, including the maximal and mean daytime PAR values are shown in Supplementary Fig.1 and Table 1. Triplicate cultures for each CO2 level were run under the different light regimes.

**2.3 Growth rates**

At the end of the light period, the dilution was performed with the dilution rate about 300% (one part of the culture was added to two parts autoclaved seawater enriched with Aquil medium and pre-equilibrated with the targeted CO2 levels). The cell densities in semi-continuous cultures were counted immediately before and after the dilution (every 24 h) using a particle counter (Z2, Beckman, USA). The specific growth rate (μ, d-1) was calculated as: μ = (ln C1−ln C0) / (t1−t0), where C0is the initial (after dilution) cell concentration and C1 that (before the next dilution) after 24 h.

**2.4 Chlorophyll fluorescence parameters**

The maximal photochemical efficiency, Fv/Fm was measured after 15 minutes dark adaptation using a Xenon-Pulse Amplitude Modulated fluorometer (XE-PAM, Walz, Germany). The relative electron transfer rate (rETR, arbitrary unit) was calculated as: rETR= Fv′/Fm′×0.5×PAR, where Fv'/Fm' represents the effective PSII quantum yield and PAR is the photosynthetically active photon flux density (μmol m-2 s-1) and the coefficient 0.5 takes into account that roughly 50% of all absorbed photons reach PSII. The rapid light curves (RLCs) were measured under eight different PAR levels (every measurement lasted for 10 s). RLCs were fitted as rETR=E/(aE2+bE+c) (Eilers & Petters 1988), where E is PAR (μmol m-2 s-1) and a, b and c are derived parameters. The maximum relative electron transport rate (rETRmax) was expressed as a function of the parameters a, b, and c as follows: rETRmax=1/(b+2(a×c)1/2). The non-photochemical quenching (NPQ) was calculated as: NPQ = (Fm-Fm')/Fm', where Fm was the maximum fluorescence yield after dark adaptation and the Fm', the maximum fluorescence yield under the actinic (growth) light levels.

**2.5 Determination of photosynthetic carbon fixation rate as a function of DIC concentration**

At the eighth day for indoor culture and the first day for outdoor culture, the relationship of photosynthetic carbon fixation rate with external dissolved organic carbon (DIC) concentration in seawater (P-C curve) was determined at 20 °C and 400 μmol photons m-2 s-1 using 14C-labeled sodium bicarbonate (Amersham) as described previously (Gao et al., 2007).

The cells were harvested during the mid-photoperiod by filtering onto hybrid fiber membrane (1μm, Xinya, Shanghai, China), then washing and re-suspending in DIC-free seawater (pre-buffered with 20 mmol L-1 Tris-HCl at pH 8.18) at a final concentration of about 2×105 cells mL-1. The DIC-free seawater was prepared according to Gao et al.,(1993). The DIC concentrations (50-3200 μmol L-1) of the medium were adjusted by adding NaHCO3 solution into cell suspensions prepared with DIC-free seawater. The maximal carbon fixation rate and the K1/2 values for DIC were determined by fitting the rates of photosynthetic carbon fixation at various DIC concentrations to the Michaelis-Menten formula (Michaelis and Menten, 1913).

**2.6 Determination of gene expression**

Expression levels of the targeted genes were validated by quantitative reverse transcriptase-polymerase chain reaction (q-RT–PCR), which was performed in a 7500 real-time PCR system (Applied Biosystems). Total RNAs were extracted using RNeasy@ Plant Mini Kit following the manufacturer’s instructions (QIAGEN) and quantified with a NanoDrop 2000 microvolume spectrophotometer (Thermo Scientific). One microgram of total RNAs for each group was separately reverse-transcribed in a final volume of 20 μL using a PrimeScriptTM RT reagent kit (Perfect Real Time) (TaKaRa) following the manufacturer's instructions. Real-time PCR was performed in a reaction mixture of total transcribed cDNA, gene-specific primer and Power SYBR Green PCR Master Mix (Applied Biosystems, UK). The primers for β-carbonic anhydrase (*β-ca*; ID:Phatr2\_51305), fucoxanthin chlorophyll *a*/*c* protein, lhcf type (*fcp*, annotated as *lhcf 3* in the *P. tricornutum* JGI database; ID: Phatr2\_50705), ribulose-1, 5-bisphosphate carboxylase/oxygenase large subunit gene (*rbcl*; Chloroplast gene) mitochondrial ATP synthase (*mtATP*; ID: Phatr2\_14618), peroxisomal membrane protein-related (*pmp*; ID:phatr2\_22819), nitrite reductase (*nir*; not found in JGI ) and NADH dehydrogenase subunit 2 (*ndh2*; Mitochondrial gene) are shown in Table S2. The standard cycling conditions were 95 °C for 10 min, followed by 40 cycles of 95 °C for 15 s, 60 °C for 25 s, and 72 °C for 40 s. Raw relative data quantification was carried out using the 7500 system SDS software version 1.3.1.21, and the housekeeping histone H4 gene was employed as the internal standard (Siaut et al., 2007). The first sampling point in the Low CO2 group (LC group) was used as the calibrator.

**2.7 Data analysis**

Three independent replicate cultures for each CO2/light condition were used in all experiments, and the data are plotted as mean ± SD (standard deviation). Interactive effects of the two CO2 and light conditions on the growth rate were statistically analyzed using two-way ANOVA, and two sample t-tests were used to establish differences among the LC and HC treatments at each sampling time point. All significance tests were done using a 95% confidence level.

**3. Results**

**3.1** **Growth and photosynthetic performance**

For the indoor culture, the specific growth rates (µ) were obtained from day 3 to day 8; for the outdoor growth, the rate was only estimated at day 1. The specific growth rates of the diatom *P. tricornutum* grown under fluctuating sunlight levels (daytime mean PAR of 305 μmol m-2 s-1; Table 1) were not significantly different from those of cells grown under the indoor constant light condition (PAR of 130 μmol m-2 s-1), at both low (LC) and high (HC) CO2 levels (LC: *p* = 0.32; HC: *p* = 0.65; Fig. 1). Nevertheless, the growth was slightly enhanced by the elevated CO2 under either fluctuating sunlight (*p* = 0.02) or indoor constant light (*p* = 0.004) (Fig. 1). The maximal photochemical yield of PSII, Fv/Fm, showed insignificant (*p* = 0.145) differences between the LC and HC-grown cells under the indoor light regimes. When transferred and acclimated to fluctuating sunlight, the yield increased slightly (by 1.4%; *p* = 0.36) in the HC-grown cells, compared to those grown in LC conditions (Fig. 2a). The maximal electron transport rate (rETRmax) showed marked diurnal oscillations under all conditions (Fig. 2b) but was not significantly different (*p* = 0.3 for LC, 0.8 for HC), in either the LC- or HC-grown cells, under the indoor constant irradiance compared to values under fluctuating sunlight. On the other hand, after acclimation to the elevated CO2 concentration for ~8 generations, the HC cells showed slightly higher (by 3-11%) rETRmax (*p* = 0.03) compared to the LC-grown cultures under the indoor light condition, whereas elevated CO2 had no significant effect (*p* = 0.2) under the outdoor fluctuating light conditions. Additionally, the rETR obviously decreased on the last day under fluctuating sunlight (Fig. 2b). However, the cells grown under sunlight conditions (Table 1) showed higher non-photochemical quenching (NPQ) with the highest values occurring during noontime, an effect which was further increased in the HC-grown cells (*p* = 0.02; Fig.2c).

**3.2 CCM activity**

The HC-grown cells did not show significantly higher values of K1/2DIC and K1/2CO2, compared to the LC-grown ones (*p* = 0.56 and 0.11 under low and high light levels, respectively; Fig. 3a and 3b). The cells grown under the low light level showed higher maximal photosynthetic rate (Pmax) than those grown under high and fluctuating sunlight levels (*p* < 0.01; Fig. 3c).

**3.3 Gene expression**

RNA extraction from samples taken at 220 h-l, 224 h-l failed, so the RT-PCR was skipped for these two points. The expression levels of targeted genes showed obvious diel changes in both LC and HC-grown cells, especially for β-carbonic anhydrase (*β-ca*) (Fig. 4 and 5). The expression of *β-ca* gene decreased in the HC-grown cells either under low (indoor culture; by up to 80%; *p* = 0.005) or high/fluctuating irradiance (by up to 75%; *p* = 0.03), with the minima observed during the dark period (Fig. 4a). Compared to the indoor constant light level, cells grown under high/fluctuating sunlight levels (Table 1) showed higher expression of the genes for *β-ca* and *lhcf 3* (Fig. 4a, b). The gene expression for *lhcf* 3, encoding the antennae in the light harvesting complex (LHC), was significantly down-regulated (*p* = 0.04) after the cells had acclimated to HC for a period of 4–48 h, with the highest values obtained 8 h after the onset of light and the lowest values 4 h after the onset of darkness. Significantly decreased expression of *lhcf* 3 was observed with time. In the cells exposed to sunlight for 1 day, slightly increased expression was observed, especially for LC-grown cells (Fig. 4b). The expression of the gene for *rbcl* was initially significantly lower in the HC-grown cells (*p* = 0.04), though with increased time span of acclimation to HC, the decrease became insignificant (*p* = 0.9, Fig.4c). When transferred from the indoor condition to the fluctuating sunlight, the expression of this gene was initially reduced and then increased steadily with time. The expression of the mitochondrial ATP synthase (mtATP synthase) gene decreased significantly (*p* = 0.03) when transferred to HC at most of the measurement times except for that at 176 h (indoor grown HC-cells) and after growth under fluctuating light regimes for about 72 h (Fig. 5a). For the peroxisomal membrane protein-related (PMP) gene, slight diel oscillations and both down- or up-regulated expression by elevated CO2 were observed, with down-regulation found under high light and up-regulation under low light levels (Fig. 5b; Fig. S1)**.** However, the expression of the nitrite reductase (NiR) gene was significantly up-regulated by HC under either indoor (*p* = 0.03) or outdoor (*p* = 0.04) growth conditions, but this was independent of the light regimes (Fig. 5c), while an inverse trend was observed in the gene encoding NADH dehydrogenase subunit 2 (NDH2), with the highest down-regulation by about 78% under the OA conditions (Fig. 5d).

**4. Discussion**

Phytoplankton cells within the upper mixing layers of the oceans are exposed to both increasing pCO2 and higher solar radiation due to enhanced thermal stratification. Interactions of these two key factors are crucial for predictions of the biological consequences of global change in the oceans. Here, we first revealed that the marine diatom *P. tricornutum* under elevated CO2 up-regulated its genes related to nitrogen assimilation while it down-regulated its CCM-related genes, so that the cells would have sufficient energy to enhance metabolism and support homeostasis to cope with pH perturbations in milieu.

Changes in growth rate reflect genomic plasticity (Dubinsky and Schofield, 2010). The effects of CO2 on growth has been shown to be mediated by light levels (Gao et al., 2012a; Hoppe et al., 2015) in cells that had acclimated to elevated CO2 over 20 generations. In the present study and previous work, this diatom showed enhanced growth rate under PAR of 50-150 μmol m-2s-1, and inhibited growth rates under daytime mean solar PAR >220 μmol m-2s-1, after the cells had acclimated to OA over 10 generations (Wu et al., 2010; Gao et al. 2012a; Li et al. 2014). In the present work, shifting from indoor light of 130 μmol m-2s-1 to outdoor fluctuating sunlight (daytime mean PAR of 305 μmol m-2s-1) did not alter the growth response to OA, probably due to the lack of acclimation. Enhanced growth rates of diatoms grown under elevated CO2 concentrations could be attributed to energy savings from down-regulation of CO2-concentrating mechanisms (CCM) (Wu et al., 2010; Hopkinson et al., 2011; Ravenet al., 2011), though others have found insignificant effects on growth when the CCM was down-regulated (Chen and Gao, 2003; Rost et al., 2002), showing species-specific responses. In the present study, we did observe some evidence for CCM down-regulation under OA as reflected in the suppressed genetic expression of *β-ca*, though the increase in K1/2DIC and K1/2CO2 was not significant. The discrepancy between reports might be accredited to the different growth light levels used, which are known to modulate the efficiency of CCMs (Raven et al., 2011; Reinfelder, 2011). OA treatment stimulated expression of *rbcl* (from hour 28 to 40 and hour 172 to 184)and *nir* (during all the culture period), which could in turn have stimulated C and N assimilation and growth. It is worth noting that the up-regulation of the *rbcl* gene under the OA condition took a time span longer than 6 generations in this species and was most obvious under high light levels, though RbcL protein levels have been shown to decrease in *Thalassiosira weissflogii* and *Emiliania huxleyi* (Losh et al., 2013), to increase in *T. pseudonana* (coastal strain) and *E. huxleyi* (McCarthy et al., 2012) or to be unaffected in *Trichodesmium* IMS101 (Levitan et al., 2010) and *T. pseudonana* (offshore strain) (McCarthy et al., 2012), when grown under projected future CO2 levels, showing the multifarious and species-specific nature of the effects. Enhanced N assimilation could be responsible for the observed increases of cellular organic N under elevated CO2 in *P. tricornutum* (Li et al., 2012), *Skeletonema costatum* (Burkhardt et al., 1999), *E. huxleyi* (Fiorini et al., 2011; McCarthy et al., 2012), *Gephyrocapsa oceanica* (Jin et al., 2013) and *Coccolithus pelagicus* (Rickaby et al., 2010), though POC/PON ratios changed species-specifically under OA (Riebesell and Tortell, 2011 and literatures therein). Additionally, the urea cycle may be integrated into nitrogen metabolism through its connection to glutamine and in the eventual production of urea in *P. tricornutum* and *T. pseudonana* (Allen et al., 2011; Bender et al., 2013). Chautonet al. (2013) studied carbon fixation, storage and utilization in *P. tricornutum* acclimated to light/dark cycles and found that genes related to nitrogen metabolism were up-regulated when the cells were shifted from light to dark, which might be related to the pH drop during the dark period as cells respire and produce CO2. In the present study, however, the expression of the *nir* gene showed maximal expression during the middle of the light period and the diel expression pattern was amplified by elevated CO2, especially under the low light conditions after the cells were acclimated to elevated CO2 for 10 generations (Fig. 5c). Obviously, the OA treatment stimulated expression of this gene. It appears that metabolic pathways involving nitrate uptake or nitrogen assimilation respond to OA for the cells to maintain homeostasis while suffering from acidification stress from the milieu. Shi et al. (2015) showed down-regulated protein expression (but without significant changes for gene expression) and activity of the nitrate reductase in *T. pseudonana* under high CO2 conditions regardless of light levels. Although both nitrate reductase and nitrite reductase are parts of N assimilation pathway, they are different genes and may show different responses to changes in pH. Additionally, there are a number of genomic differences between *T. pseudonan*a and *P. tricornutum* (Armbrust et al., 2004; Bowler et al., 2008). The different roles of the C4 pathway (Reinfelder et al., 2000; Tachibana et al., 2011; Haimovich-Dayan et al., 2013; Samukawa et al., 2014) and differential responses to environmental changes were also reported (Yang et al., 2011; Gao et al., 2012a; Nymark et al., 2013). Therefore, species-specific responses and/or culture conditions could be responsible for the difference. Additionally, Hofmann et al. (2013) reported that the activity of nitrate reductase was stimulated by elevated CO2 in the calcifying rhodophyte *Corallina officinalis*.

In diatoms, the fucoxanthin-chlorophyll protein (FCP) is bound to chlorophylls *a*, *c* and the carotenoid fucoxanthin (Fuco), as the major complex in light-harvesting centers. About six FCP genes have been reported in *P. tricornutum* (Bhaya and Grossman, 1993), and are known to show differential responses when exposed to different light levels (Park et al., 2010). In the present study, although the genes encoding FCP and mtATP synthase were slightly suppressed during the initial phase of OA (at the initial 2 days), they increased their expression levels under the OA treatment after the cells had acclimated for 6 generations, indicating a homeostatic response to an acidic perturbation to the cells (Giordano, 2013). The diel changes in the gene expression of the Lhcf 3 protein, being the lowest in the dark and the highest at the late light period, reflects a light dependency of this gene. Such a diel pattern of this gene’s function was maintained even when the cells were grown under fluctuating sunlight, but was weakened by the elevated CO2 under high sunlight (Fig. 4b; Table 1). While phytoplankton cells usually expend extra energy to cope with fluctuating light (Brunet and Lavaud, 2010), we found lowered photosynthetic rates when the cells were transferred to fluctuating sunlight conditions (Fig. 3c) with enhanced NPQ (Fig. 2c; Table 1), though such changes were similar in HC and LC-grown cells.

In the present study, when the cells were transferred from constant low light level to fluctuating sunlight level, the genetic expression of *β-ca*, facilitating the interconversion of HCO3- and CO2, increased remarkably in the low CO2 grown cells (Fig. 4a), reflecting an induction of this enzyme with the combination of elevated light and reduced CO2 availability (Harada et al., 2005; Wu and Gao, 2009). Expressions of *lhcf3*, *mtATP*, *pmp* genes were also stimulated by high light; however, such enhancement was muted by the OA treatment (Fig.4b, 5a and b). While elevated light levels down-regulated the expressions of genes for *pbcl* and *nir*, OA treatment offset the decrease (Fig.4c and 5c). Such genetic changes must link to changes in enhanced photo-protection under OA (Gao et al. 2012a) and balanced energy demands among diverse metabolic pathways. On the other hand, the effects of light/dark periods, circadian rhythms and the connection between carbon fixation and nitrogen metabolism or the urea cycle, which has been found in the diatom *P. tricornutum* (Allen et al., 2011) and is a key pathway for anaplerotic carbon fixation into nitrogenous compounds, need to be examined in the context of OA effects.

In brief, physiological responses of the diatom *P. tricornutum* to OA and changing levels or regimes of light were found to be linked with Ci acquisition, nitrite reduction, respiration, photosynthetic and photoprotective processes, with expressions of the related genes up- or down-regulated. This work is the first attempt to elucidate the time-dependence of molecular responses underlying observed physiological changes in diatoms grown under OA, which could be mediated by the growth light.

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**Author contributions**

On the basis of an original idea from K. G. and K. W., the data were obtained by Y. L. and S. Z., who contributed equally. All the authors contributed to data analysis and the writing of the paper.

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**Table 1** Daily dose (µmol m-2), averaged and highest intensity (μmol m-2 s-1) of PAR for *P. tricornutum* cells grown in ambient (390 μatm; LC) or elevated CO2 (1000 μatm; HC) levels under constant (indoor) and fluctuating (outdoor) light conditions.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | | Dose | Mean PAR | Highest PAR |
| Indoor culture | | 5.62×106 | 130 | 130 |
| Outdoor  culture | 1st | 1.70×107 | 305 | 1027 |
| 2nd | 1.67×107 | 299 | 887 |
| 4th | 5.95×106 | 107 | 1200 |

**Figure legends**

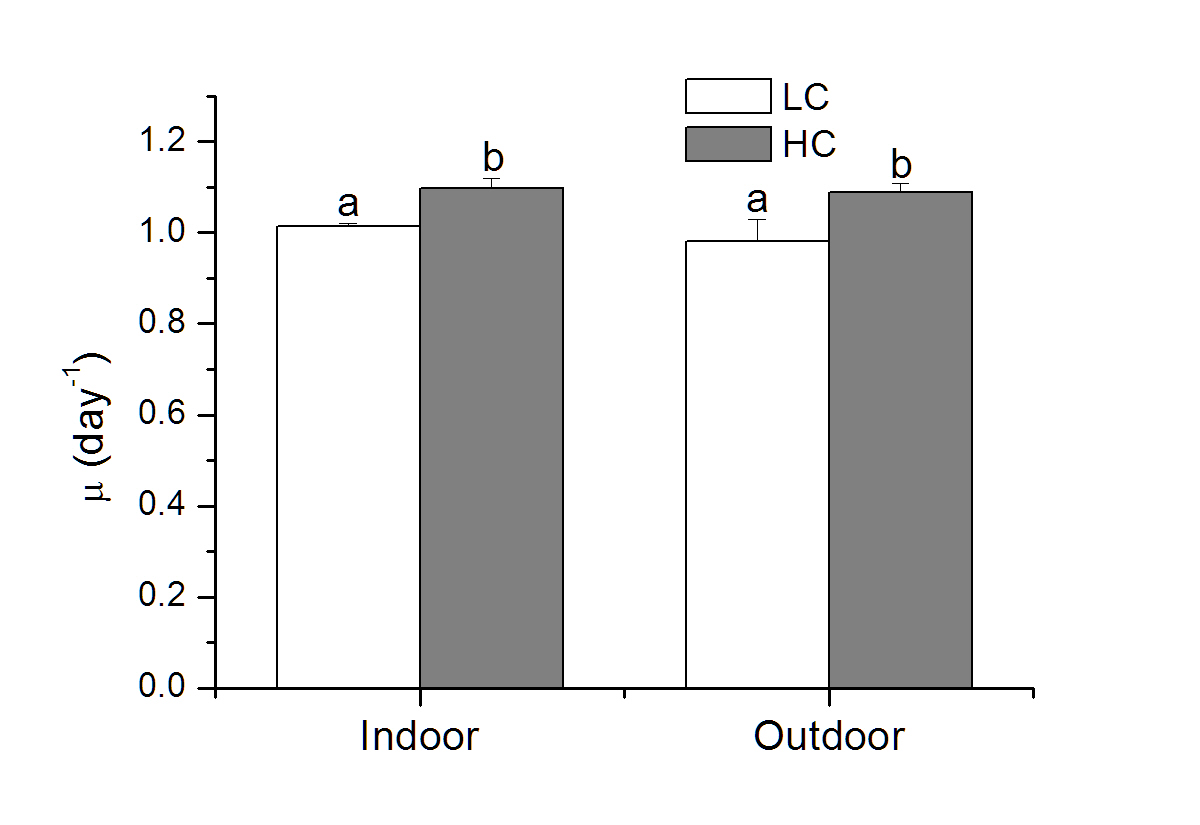
**Fig.1** Specific growth rate (μ) of *P. tricornutum* cells grown at ambient (390 μatm; LC) and elevated CO2 (1000 μatm; HC) levels under different light regimes. “Indoor” and “Outdoor” represent cultures grown in the laboratory (constant light during the light phase) and with solar exposure (fluctuating light, without UVR, during the light phase), respectively. Data are the means ± SD, n = 3 (triplicate cultures). Different letters above the histogram bars indicate significant differences (p<0.05) between different treatments.

**Fig.2** Time series of the maximal quantum yield (Fv/Fm; a), the maximal electron transport rate (rETRmax; b) and non-photochemical quenching (NPQ; c) of *P. tricornutum* cells grown at ambient (390 μatm; LC) and elevated CO2 (1000 μatm; HC) levels under constant light (indoor) or fluctuating sunlight levels (outdoor). The shaded bars under the x-coordinate represent the dark period. Data are the means ± SD, n = 3 (triplicate cultures).

**Fig.3** The half-saturation constants for dissolved inorganic carbon (a: K1/2 DIC; μmol L-1) or CO2 (b: K1/2 CO2; μmol L-1) concentrations and the maximal photosynthetic rate (c: Pmax; pg C cell-1 h-1) of *P. tricornutum* cells grown at ambient (390 μatm; LC) and elevated CO2 (1000 μatm; HC) levels under constant light (indoor) or fluctuating sunlight levels (outdoor). Data are the means ± SD, n = 3 (triplicate cultures). Different letters above the histogram bars indicate significant differences (p<0.05) between different treatments.

**Fig.4** Time series of the relative abundances of transcripts for β-carbonic anhydrase (β-CA; a), fucoxanthin chlorophyll a/c protein, lhcf type (FCP, Lhcf 3; b), ribulose-1, 5-bisphosphate carboxylase/oxygenase large subunit gene (RbcL; c) determined by quantitative real-time PCR (qPCR) of *P. tricornutum* cells grown at ambient (390 μatm; LC) and elevated CO2 (1000 μatm; HC) levels under constant light (indoor) or fluctuating sunlight levels (outdoor). The shaded bars under the x-coordinate represent the dark period. Data are presented as means ± SD, n = 3 (triplicate cultures). Two asterisks indicate a significant difference between HC and LC grown cells at *p* < 0.01, and one asterisk represents a significant difference at *p* < 0.05.

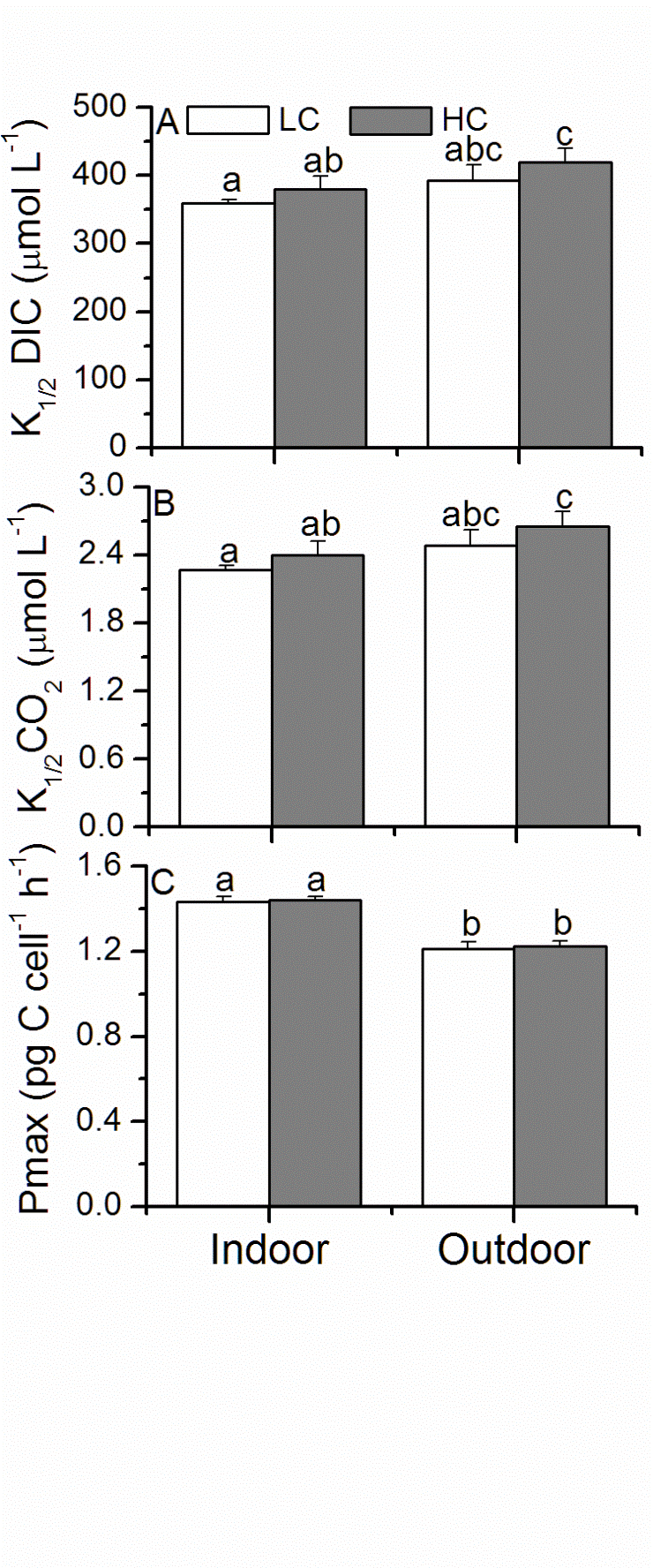
**Fig.5** The time series of the relative abundances of transcripts for mitochondrial ATP synthase (mATP; a), peroxisomal membrane protein-related (PMP; b), nitrite reductase (NiR; c) and NADH dehydrogenase subunit 2 (Ndh2; d) determined by quantitative real-time PCR (qPCR) of *P. tricornutum* cells grown at ambient (390 μatm; LC) and elevated CO2 (1000 μatm; HC) levels under constant light (indoor) or fluctuating sunlight levels (outdoor). The shaded bars under the x-coordinate represent the dark period. Data are presented as means ± SD, n = 3 (triplicate cultures). Two asterisks indicate significant difference between HC and LC grown cells at *p* < 0.01, and one asterisk represents a difference at *p* < 0.05



**Fig.1**

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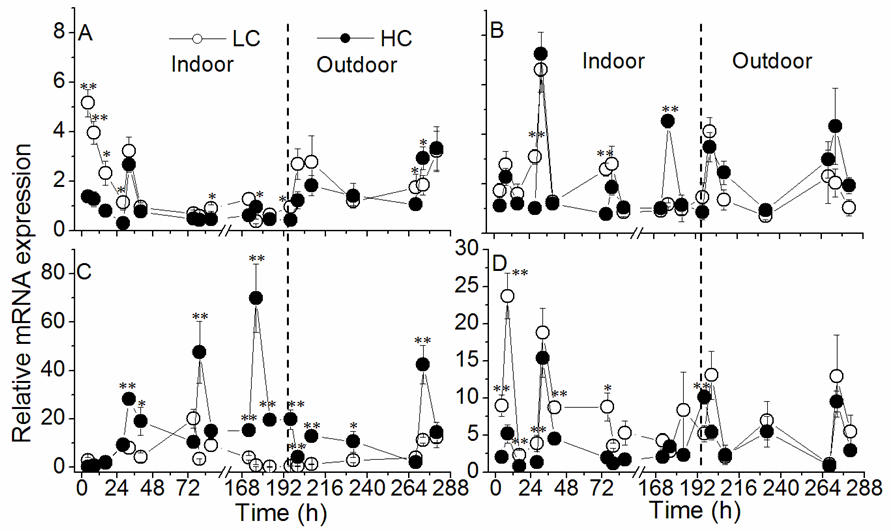
**Fig.2**



**Fig.3**

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**Fig.4**



**Fig.5**