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7 Photoprotective responses in a brown macroalgae *Cystoseira*
8 *tamariscifolia* to increases in CO_2 and temperature.

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10 Paula S. M. Celis-Plá[1,2*], Brezo Martínez[3], Nathalie Korbee[2], Jason M. Hall-Spencer[4,5],
11 and Félix L. Figueroa[2].

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13 *Corresponding author: paulacelispla@upla.cl
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19 [1]Laboratory of Costal Environmental Research, Centre of Advanced Studies, University
20 of Playa Ancha, Calle Traslaviña 450, 2581782 Viña del Mar, Chile
21 [2]Department of Ecology, Faculty of Sciences, University of Malaga, 29071 Malaga,
22 Spain
23 [3]Biodiversity and Conservation Unit, Rey Juan Carlos University, 28933 Mostoles, Spain
24 [4]Marine Biology and Ecology Research Centre, Plymouth University, UK
25 [5]Shimoda Marine Research Centre, Tsukuba University, Japan

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ABSTRACT

Global warming and ocean acidification are increasingly affecting coastal ecosystems, with impacts that vary regionally depending upon local biogeography. Ocean acidification drives shifts in seaweed community dominance that depend on interactions with other factors such as light and nutrients. In this study, we investigated the photophysiological responses in the brown macroalgae species *Cystoseira tamariscifolia* (Hudson) Papenfuss with important structural role in the coastal Mediterranean communities. These algae were collected in the Cabo de Gata-Nijar Natural Park in ultraoligotrophic waters (algae exposed under high irradiance and less nutrient conditions) *vs.* those collected in the La Araña beach in oligotrophic waters (algae exposed at middle nutrient and irradiance conditions) in the Mediterranean Sea. They were incubated in mesocosms, under two levels of CO_2; ambient (400-500 ppm) and high CO_2 (1200-1300 ppm), combined with two temperatures (ambient temperature; 20ºC and ambient temperature + 4ºC; 24ºC) and the same nutrient conditions of the waters of the origin of macroalgae. Thalli from two sites on the Spanish Mediterranean coast were significantly affected by increases in $p$$CO_2$ and temperature. The carotenoids (fucoxanthin, violaxanthin and β-carotene) contents were higher in algae from oligotrophic than that from ultraoligotrophic water, i.e., algae collected under higher nutrient conditions respect to less conditions, increase photoprotective pigments content. Thalli from both locations upregulated photosynthesis (as F_v/F_m) at increased $p$$CO_2$ levels. Our study shows that ongoing ocean acidification and warming can increase photoprotection and photosynthesis in intertidal macroalgae.

INTRODUCTION

Atmospheric CO_2 levels have increased seawater temperatures by 0.13°C per decade over the last 50 years (IPCC 2104), causing a dieback in seaweeds at their warmest biogeographic limits (Harley et al., 2006, 2012; Wernberg et al., 2016). The increased concentration CO_2 atmospheric and their uptake is causing ocean acidification which increases the amount of carbon available to algae, and can stimulate their photosynthesis and growth (Johnson et al., 2015; Pajusalu et al., 2016), but it also lowers CO_3^{2-} levels which can cause dissolution of calcified algae (Newcomb et al., 2015).

Investigations into how global change will affect kelp forests and fucoid canopies are a priority as these habitats are of major ecological importance in temperate and cold-water regions of the planet (Brodie et al., 2014). Canopy-forming brown algae often proliferate in areas with naturally high levels of pCO_2 (Porzio et al., 2011; Roleda et al., 2012; Johnson et al., 2012; Linares et al., 2015). However, they have geographic range shifts in abundance over the past 50 years due to anthropogenic perturbations such as siltation, warming and increased nutrients levels (Díez et al., 2012; Strain et al., 2014; Yesson et al., 2015; Krumhansl et al., 2016; Wernberg et al., 2016). In the Mediterranean, the effect of ocean acidification on seaweed community composition is influenced by other factors such as light, nutrients and herbivory (Baggini et al., 2015; Celis-Plá el at., 2015, 2017). Phaeophytes such as *Cystoseira* spp., *Dictyota* spp., *Laminaria rodriguezii*, *Sargassum vulgare* and *Padina pavonica* increase in abundance near CO_2 seeps, where they may benefit from increased carbon availability (Porzio et al., 2011; Johnson et al., 2012; Baggini et al., 2014; Celis-Plá et al., 2015; Linares et al., 2015; Celis-Plá et al., 2017).

Cystoseira spp. are fucoid seaweeds that help maintain the structure and function of coastal ecosystems – they are used as indicators of high water quality in the Mediterranean (Bermejo et al., 2016; Celis-Plá et al., 2016). In this region, low nutrient availability limits algal photoprotection, photosynthesis and growth (Celis-Plá et al., 2016). High irradiance can stimulate an increase in photoprotective compounds, but only if the algae have sufficient nutrients (Abdala-Díaz et al., 2006). Intertidal macroalgae need to cope with large variations in light intensity and use photophysiological responses as photosynthesis activity, photoprotective compounds as carotenoids (violaxanthin, antheraxanthin and zeaxanthin) to help prevent damage to their photosystems (Goss and Jakob, 2010). And provide information about the damage, e.g., the maximum quantum yield of PSII (F_v/F_m), that use to determine photoinhibition and the physiological status of the fucoid macroalga (Figueroa et al., 2014, Celis-Plá et al. 2017).

97 Here, we investigated the interactive effects of pCO_2 (*ca.* 400-500 and *ca.* 1200-1300

98 ppm) and temperature (20ºC and 24ºC) predicted future temperature for the year 2100

99 (IPCC 2014), on *Cystoseira tamariscifolia* (Hudson) Papenfuss (Phaeophyceae, Fucales).

100 The macroalgae were collected from Cabo de Gata-Nijar Natural Park (ultraoligotrophic

101 waters) and La Araña beach, with less limited nutrient parts (oligotrophic waters) of the

102 coast and maintained in mesocosms system with the same origin conditions to assess the

103 projected effects of ocean acidification and warming. The Alboran Sea on Mediterranean

104 coast of Spain is ultraoligotrophic in the southeast part with lower concentrations of

105 nutrient and oligotrophic in the southwest, with increased nutrient levels due to local

106 upwelling's (Ramírez et al., 2005; Mercado et al., 2007, 2012). We compared

107 photophysiological responses of C. tamariscifolia collected from these two regions; our

108 hypothesis was that the alga from oligotrophic waters would benefit in photoprotective

109 compounds under elevated pCO_2 at ambient temperature when nutrient levels were

110 sufficient, but that 4ºC warming would be detrimental.

111

112 **MATERIALS AND METHODS**

113 *Sampling*

114 *Cystoseira tamariscifolia* (Hudson) Papenfuss (Phaeophyceae, Fucales) specimens

115 (Gómez-Garreta et al., 2001) were collected haphazardly from the low shore on 25

116 September 2013 in the Cabo de Gata-Nijar Natural Park (36º51'N; 2º6'W) and at La

117 Araña Beach (36º42'N; 4º19'W) in the Mediterranean Sea. The Natural Park site is

118 ultraoligotrophic, with lower concentrations of nitrate and phosphate than that in the La

119 Araña site, (Table S1) that is classified as oligotrophic (Ramírez et al., 2005; Mercado et

120 al., 2007, 2012).

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122 *Experimental conditions*

123 After collection, 96 thalli in total (48 individuals from Natural Park and 48

124 individuals from La Araña) were transported to Malaga University where they were

125 incubated for 28 days (after 48 hours of acclimation), in mesocosms with original

126 conditions for ultraoligotrophic and oligotrophic macroalgae. The experimental system

127 comprised 24 open tanks (14 L), with groups of three tanks connected in parallel to a 102

128 L tank. The mesocosms were held in 1000 L water baths (following the experimental set

129 up described by Stengel et al. 2014). The thalli were incubated in four treatments: (1)

130 ambient temperature (20ºC) x ambient CO_2 (*ca.* 400-500 ppm) (ATxACO₂), (2) ambient

131   temperature (20ºC) x high CO_2 (*ca.*1200-1300 ppm) (ATxHCO$_2$), (3) high temperature

132   (24ºC) x ambient CO_2 (*ca.* 400-500 ppm) (HTxACO$_2$) and (4) high temperature (24ºC) x

133   high CO_2 (*ca.*1200-1300 ppm) (HTxHCO$_2$), using 24 tanks in total with three replicate

134   tanks for *C. tamariscifolia* from ultraoligotrophic and oligotrophic waters, respectively.

135   Temperature and DIC levels were controlled using a computer-operated control

136   system (Aqua Medic T2001HC) in each header tank. The system automatically recorded

137   one measurement every 15 min and was programmed to supply pure CO_2 via a solenoid

138   valve as soon as the pH exceeded a threshold of 7.88 ± 0.01 in the header tanks

139   (corresponding to *ca.* 1200-1300 ppm CO_2). The seawater carbonate system was

140   monitored twice a week, taking water samples to measure the salinity, pH$_{NBS}$ and total

141   alkalinity (following methods given by Celis-Plá et al., 2017). The outdoor mesocosms

142   were shaded using a mesh that reduced photosynthetically active radiation (PAR; 400-

143   700 nm) by 35%, and UVA (320-400 nm) and UVB (280-320 nm) by 39%. Incident solar

144   radiation was measured continuously in air using a UV-PAR Multifilter radiometer

145   NILU-6 (Geminali). Levels of UVA and UVB radiation were calculated from the data of

146   the different UV filters according to (Høiskar et al. 2003). Seawater was enriched with 2

147   µM nitrate (KNO$_3$) and 0.1 µM phosphate (KH$_2$PO$_4$) giving an N: P ratio of 20:1 for

148   oligotrophic waters, and with 0.5 µM nitrate (KNO$_3$) and 0.1 µM phosphate (KH$_2$PO$_4$)

149   giving an N:P ratio of 5:1 for ultraoligotrophic waters according to (Ramírez et al., 2005;

150   Mercado et al., 2007, 2012) (Table S1).

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Chlorophyll and carotenoid concentration and composition

152   *Chlorophyll and carotenoid concentration and composition*

153   Carotenoids and chlorophylls pigments content for fucoid macroalgae were

154   determined to evaluate the capacity for acclimation, photoinhibition, photoprotection and

155   vulnerability respect to the different irradiances and abiotic variables.

156   Pigments were extracted each week during the experimental period, 20 mg fresh weight

157   from the apical parts of the algae, using 2 mL of 100% acetone and analysed using an

158   ultra-high-performance liquid chromatographer (Shimadzu Corp., Kyoto, Japan)

159   equipped with a photodiode array detector to measure peaks in the range 350-800 nm.

160   After extraction, samples were centrifuged at 16200 g for 5 min (Sorvall Legend Micro

161   17, Thermo Scientific, Langenselbold, Germany) and then the extracts were filtered (0.22

162   µM nylon filters). The separation, was achieved with one column C-18 reversed phase

163   (Shim-pack XR-ODS column; 3.0×75 mm, i.e.; 2.2 µm particle size; Shimadzu, Kyoto,

164   Japan) protected by a guard column TR-C-160 K1 (Teknokroma, Barcelona, Spain). The

165   carotenoid composition was determined according to (García-Plazaola and Becerril

166   1999), with some modifications (Celis-Plá et al., 2015), using commercial standards (DHI

167   LAB Products).

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Photosynthetic activity as in vivo chlorophyll a fluorescence

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170   *In vivo* chlorophyll *a* fluorescence associated with Photosystem II was determined

171   using a portable pulse amplitude modulated (PAM) fluorometer (Diving-PAM, Walz

172   GmbH, Germany). Thalli of the *C. tamariscifolia* were collected from natural populations

173   (initial time) and monitored on day 7, 14, 21 and 28. In order to obtain rapid light curves

174   (RLC) for each treatment in each week, apical parts of the macroalgae were put into 10

175   mL incubation chambers. The F_o (basal florescence) and F_m (maximal florescence) were

176   measured after 15 minutes in darkness to obtain the maximum quantum yield (F_v/F_m) as

177   a photoinhibition indicator being $F_v=F_m-F_o$, F_o the basal fluorescence of 15 min dark

178   adapted thalli and F_m maximal fluorescence after a saturation light pulse of > 4000 µmol

179   m^{-2} s^{-1} (Schreiber et al. 1995). According Celis-Plá et al. (2014a) were found no

180   significant differences among the tested times (5, 15 and 30 min) for dark adapted in

181   *Cystoseira tamariscifolia* and was selected 15 min for dark adapted as it is the most

182   common dark exposure time found in the literature (Schreiber et al. 1995, Figueroa et al.

183   2014). The maximal quantum yield was used indicator of photoinhibition (F_v/F_m),

184   The Non-photochemical quenching (NPQ) was calculated according to Schreiber et

185   al. (1995), as $NPQ = (F_m - F_{m'})/F_{m'}$. The maximal NPQ ($NPQ_{max}$) was obtained from the

186   tangential function of NPQ versus irradiance function according to (Eilers and Peeters,

187   1998). NPQ was used as estimator of the photoprotection capacity.

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Statistical analysis

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190   Differences between physiological parameters in *C. tamariscifolia* were explored

191   using a multivariate approach. A Principal Coordinates Analysis was performed for this

192   purpose based on Euclidean distance using PERMANOVA+ for PRIMER6 package. This

193   procedure calculates the percentage variation explained by each of the axes in the

194   multidimensional scale. The overlay of the vectors onto the PCA was performed using

195   Spearman correlation (Anderson, 2008).

196   The effects of the combined treatments on the photosynthetic activity and pigment

197   contents of *C. tamariscifolia* were assessed using analysis of variance (ANOVA). This

198   test was performed for *C. tamariscifolia* including time, temperature, pCO_2 and origin of

199   the population (locations) as categorical factors. Student Newman-Keuls tests (SNK)

200   were performed after significant ANOVA interactions (Underwood, 1997). The

201   homogeneity of variance of all data was confirmed by using Cochran tests and by visual

202   inspection of the residuals (Underwood, 1997). Analyses were carried out using SPSS

203   v.23 (IBM, USA).

204

205   **RESULTS**

206   The average daily-integrated irradiance for the experimental period was 4238 kJ m^{-2}

207   for PAR, 329 kJ m^{-2} for UVA and 22 kJ m^{-2} for UVB. The seawater temperature was

208   19.95 ± 0.15 ℃ in ambient temperature treatments and 23.91 ± 0.01℃ in the high

209   temperature treatments (mean $\pm$ SE, n = 2232) (Table 1). The mean pH during the

210   experimental period were 8.23 ± 0.01 in ATxACO_2, and 7.88 ± 0.01 in ATxHCO_2, 8.22

211   ± 0.01 in HTxACO_2 and 7.88 ± 0.01 in HTxHCO_2 treatments (Table 1), (calculated

212   following methods given by Celis-Plá et al., 2015).

213   Principal coordinates analysis (Fig. 1) shows that at the end of the experiment, there

214   was a positive correlation of the first axis (76.2% of total variation) with maximal

215   quantum yield (F_v/F_m) being highest in samples from ultraoligotrophic waters for high

216   temperature treatments under ambient and elevated pCO_2 (Cabo de Gata-Nijar Natural

217   Park, L1). As well as, in oligotrophic waters samples (La Araña, L2), that were cultured

218   at elevated temperature and ambient pCO_2. The chlorophylls a (as Chl$a$), violaxanthin

219   (Viola), fucoxanthin (Fuco) and β-Carotene (β-Caro) were highest in samples collected

220   in oligotrophic waters (L2) and grown at high temperature with ambient and high pCO_2

221   conditions. In contrast, maximal non-photochemical quenching (NPQ$_{max}$) and

222   chlorophylls c (as Chl$c$) were higher in ambient temperature samples (Fig. 1)

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224   *Chlorophylls and Carotenoids*

225   Chlorophyll a and c in all treatments had a significant interaction between time,

226   temperature and CO_2 ($p<0.01$) (Fig. 2 and Table S2). The Student Newman-Keuls tests

227   (SNK) revealed no clear differences between treatments but an overall trend of decline

228   with time. Significant quantities of the fucoxanthin, violaxanthin and β-carotene (Tables

229   2 and S2) were detected in all treatments but only traces of antheraxanthin, lutein and

230   zeaxanthin were found (data not shown).

231   Fucoxanthin content was affected by time, temperature and location, whereas

232   violaxanthin was affected by the interaction between time, temperature and pCO_2 levels

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(Tables 2 and S2). Fucoxanthin was higher after the experimental period for both locations, but in algae collected from oligotrophic waters, this pigment increased in respect to the ultraoligotrophic waters. Violaxanthin content was higher at the end the experimental period, in oligotrophic and ultraoligotrophic waters, but in oligotrophic waters, the violaxanthin was higher in respect to the other location. β-carotene content was significantly affected by time x pCO_2 conditions (Tables 2 and S2), it increased under increased pCO_2 conditions in thalli collected from oligotrophic waters (Tables 2 and S2).

Photosynthetic responses

Maximal quantum yield (F_v/F_m) as an indicator of photoinhibition varied significantly depending on time, temperature and CO_2 ($p<0.01$) (Table S3). F_v/F_m increased in samples collected in ultraoligotrophic waters under ambient pCO_2 conditions with ambient temperature, in addition in high temperature, the F_v/F_m increases in both pCO_2 levels (Fig. 4). Maximal non-photochemical quenching (NPQ_{max}), had interactive effects between time and temperature ($p<0.01$) (Table S3). The NPQ_{max} increased under high temperature in all thalli, irrespective of collection site and was highest at ambient temperature conditions independent of the pCO_2 levels (Fig. 5).

DISCUSSION

In this study, we show benefits of increased of the levels of dissolved inorganic carbon (DIC). Elevated CO_2 produces an increase in the photosynthetic yield and photoprotective compounds in *Cystoseira tamariscifolia* in the mesocosms system after several day incubation, confirming expected benefits of ocean acidification already reported for brown macroalgae (Cornwall et al. 2012; Bender et al. 2014; Celis-Plá et al. 2017). We show that these benefits in photophysiological responses were more rapid in fucoid *Cystoseira tamariscifolia* collected and grown in oligotrophic waters than ultraoligotrophic conditions. This highlights the fact that the effects of climate change and acidification on canopy-forming brown algae can be expected to differ depending on coastal water type. In ultraoligotrophic waters, levels of nutrients such as nitrate and orthophosphate are much lower than in oligotrophic waters, and this is coupled with the fact that irradiance is higher than in oligotrophic waters due to the high water transparency because the low phytoplankton productivity (Ramírez et al., 2005; Mercado et al., 2007, 2012). Increases in pCO_2 can boost algal growth in carbon-limited taxa (Cornwall et al. submitted) but only if sufficient nutrients and light are available to do so (Celis-Plá et al., 2015, 2017).

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The carotenoid responses and other pigment contents were lowest in thalli that had been collected from ultraoligotrophic waters, suggesting that they were less able to invest in accumulation of biocompounds or other photoprotection system than thalli collected from a site where more nutrients were available (Stengel et al., 2014; Celis-Plá et al., 2014b, 2016). The carotenoid contents as; fucoxanthin, violaxanthin and β-carotene contents increased in those elevated pCO_2 treatments and temperature ambient under oligotrophic waters, i.e., waters with more nutrients contents. This corroborates the findings of Celis-Plá et al. (2015), who showed that *Cystoseira compressa* had higher concentrations of Chl*a*, photoprotectors compounds as phenols and fucoxanthin in nutrient enriched waters under high CO_2 conditions. Here we show that ambient temperature and elevated pCO_2 conditions can benefit algae collected in ultraoligotrophic waters. Many reviews concur that non-calcareous macroalgae production may increase due to beneficial effects of ocean acidification on photosynthesis, as long as the effects of warming and other stressors are not limiting (Harley et al., 2012; Koch et al., 2013; Kroeker et al., 2013). After one month, photoprotective contents, as carotenoids were higher in thalli collected from oligotrophic waters than those collected from ultraoligotrophic waters under high pCO_2 with ambient temperature.

Goss and Jakob (2010) indicated that the xanthophyll cycle related to NPQ represents an important photoprotection mechanism in plant cells. Demmig-Adams and Adams (2006) and García-Mendoza and Colombo-Pallota (2007) suggest more photoprotection, when increased the violaxanthin content, which is involved in photoprotection via the xanthophyll cycle (Demmig-Adams and Adams, 2006; García-Mendoza and Colombo-Pallota, 2007). In this study, showed in *C. tamariscifolia* important significant quantities of violaxanthin showed a differences in algae's from la Araña *vs* Cabo de Gata -Nijar, as responses of the photoprotection, in addition, a higher non-photochemical quenching, in the alga from La Araña in ambient temperature with ambient and higher CO_2 conditions. Responses of the xanthophyll cycle could reflect a regulatory and photoprotective response that down-regulates the delivery of excitation energy into the electron-transport chain to match the rates at which products of electron transport can be consumed in these algae (Demmig-Adams and Adams, 2006). García-Mendoza and Colombo-Pallota (2007) showed in brown algae *Macrocystis pyrifera* important ecophysiological responses of the photoprotection, a higher non-photochemical quenching, in the surface of the blades when the macroalgae were exposed to saturating light conditions. This thermal dissipation is measured as non-photochemical PSII fluorescence quenching (NPQ) is triggered by

the trans-thylakoidal proton gradient (ΔpH) along the thylakoid membrane that provides energy for the synthesis of ATP by the ATP-synthase complex and zeaxanthin (ZEA) synthesized through the xanthophyll cycle (Gilmore and Björkman, 1994; García-Plazaola et al., 2012).

In this study, we found significant quantities of the violaxanthin was detected as carotenoids involved xanthophyll cycle, or cycle with the corresponding formation of the zeaxanthin (Z), but only traces of zeaxanthin. The activity of the xanthophyll or violaxanthin (V-), cycle with the corresponding formation of zeaxanthin (Z) (Demmig-Adams and Adams, 2006). Maximal non-photochemical quenching (NPQ_{max}) decreased in enriched pCO_2 and high temperature conditions for algae collected in both localities Elevated carbon content helps explain the dominance of these brown algae at a variety of shallow water carbon dioxide seeps around Mediterranean coasts (Johnson et al., 2012; Connell et al., 2013). Increased carbon availability is a direct stimulus for photosynthesis (Mercado et al., 1998; Raven and Hurd, 2012) and can be used to make photoprotective compounds that help algae dissipate excess thermal energy (Demmig-Adams and Adams, 2006; García-Plazaola et al., 2012).

At initial time, fucoxanthin content was about 8% higher in algae harvested from ultraoligotrophic than that oligotrophic waters whereas the ratio between the main carotenoid and chlorophyll (fucoxanthin: Chl*a*) was still higher (about 33.0%). After 28 days, however, the highest increase was produced in oligotrophic collected algae except in high temperature with high CO_2 levels. However, experimental period submitted to different pCO_2 and temperature treatments, the fucoxanthin/Chl*a* ratio was similar in algae collected from both locations, i.e., 0.38-0.39 except in high temperature with high pCO_2, i.e., 0.41 in ultraoligotrophic and 0.35 oligotrophic waters. Thus, the fucoxanthin/Chl*a* ratio were favourable under the increase of both pCO_2 levels and temperature in ultraoligotrophic than that oligotrophic harvested macroalgae. The high proportion of photoprotective carotenoid (Goss and Jakob 2010) respect to chlorophyll was expected since the penetration of both PAR and UVR (Figueroa and Gómez 2001) is higher in ultraoligotrophic compared to oligotrophic waters due to its lowest turbidity and high transparency (Mercado et al., 1998; Figueroa and Gómez, 2001). The high proportion of the pigment content in *C. tamariscifolia* showed a high photoacclimation in algae collected from ultraoligotrophic waters. We suggest that the decrease of the carotenoids in ultraoligotrophic waters could be compensated by the other photoprotectors, such as phenolic compounds. Celis-Plá et al. (2017) showed higher

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335 concentration of phenolic compounds and antioxidant activity in algae collected from
336 ultraoligotrophic waters, under high pCO$_2$ with ambient temperature, these suggest the
337 increase of phenolic compounds under elevated pCO$_2$ could increase the photoprotection
338 of *C. tamariscifolia* in future scenario of ocean acidification. The polyphenolic
339 compounds are not only UV screen photoprotectors but also they have antioxidant
340 capacity too (Celis-Plá et al., 2016). Thus, they can effective photoprotectors in waters
341 with high UV penetration as Abdala-Díaz et al. (2006) showed previously in a yearly
342 study in Cabo de Gata-Nijar Natural Park, ultraoligotrophic waters.

343

344 **CONCLUSIONS**
345 Elevated pCO$_2$ allowed *C. tamariscifolia* to up-regulate both photosynthetic yields
346 and the production of photoprotective compounds. Our study shows that ocean
347 acidification can interact with temperature and have beneficial effects on the
348 accumulation of photoprotective carotenoids, as well as stimulating algal photosynthesis.
349 We show that *C. tamariscifolia* is able to benefit from an increase in pCO$_2$ levels, rapidly
350 changing their photoprotective composition and photophysiological responses, but the
351 effects will depends upon interactions with other physicochemical parameters such as
352 nutrient availability. Long-term experiments monitoring the effects of climate change on
353 seaweeds in both tanks and *in situ* are necessary to know the vulnerability and adaptation
354 capacity of primary producers in coastal habitats. *In vivo* chlorophyll *a* fluorescence is
355 proving to be a useful tool in evaluations of the physiological status of algae under
356 different climate change scenarios, of ocean acidification. The benefits of the ocean
357 acidification for fucoids in the Spanish coast will be depend on there being enough
358 nutrients and light in the intertidal communities. As well as, are not exceeded the thermal
359 tolerances of their distribution limits.

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536 Table 1 Temperature, pH, $p\mathrm{CO_2}$ (ppm) and Total Alkalinity (μmol kg^{-1}) in mesocosms
537 system in four treatments. ATxACO$_2$ (ambient temperature, 20ºC x ambient CO$_2$, *ca.*
538 400-500 ppm), ATxHCO$_2$ (ambient temperature, 20ºC x high CO$_2$, *ca.*1200-1300 ppm),
539 HTxACO$_2$ (high temperature, 24ºC x ambient CO$_2$, *ca.* 400-500 ppm) and HTxHCO$_2$
540 (high temperature, 24ºC x high CO$_2$, *ca.*1200-1300 ppm) (mean values ± SE).

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	ATxACO$_2$	ATxHCO$_2$	HTxACO$_2$	HTxHCO$_2$
Temperature (°C)	19.8 ± 0.01	20.1 ± 0.01	23.9 ± 0.02	23.9 ± 0.01
pH$_{NBS}$	8.34 ± 0.01	7.88 ± 0.01	8.22 ± 0.01	7.88 ± 0.01
$p\mathrm{CO_2}$ (μatm)	455.6 ± 11.9	1264.1 ± 30.2	509.8 ± 7.8	1274.8 ± 17.9
Total Alkalinity (μmol kg^{-1})	2431 ± 11.99	3585 ± 14.16	3059 ± 13.45	3793 ± 5.31

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556 Table 2 Fucoxanthin, violaxanthin and β-carotene (µg g^{-1} DW) of *Cystoseira tamariscifolia* at the start of the experiment (*It*) and after 7, 14, 21

557 and 28 days of incubation, for ultraoligotrophic waters (Cabo de Gata-Nijar Natural Park), oligotrophic waters (La Araña beach) and 4 treatments.

558 ATxACO$_2$ (ambient temperature, 20ºC x ambient CO$_2$, *ca.* 400-500 ppm), ATxHCO$_2$ (ambient temperature, 20ºC x high CO$_2$, *ca.*1200-1300 ppm),

559 HT*ACO$_2$ (high temperature, 24ºC x ambient CO$_2$, *ca.* 400-500 ppm) and HTxHCO$_2$ (high temperature, 24ºC x high CO$_2$, *ca.*1200-1300 ppm)

560 (mean values ± SE).

		Cystoseira tamariscifolia									
		Ultraoligotrophic waters					Oligotrophic waters				
		It	*7 d*	*14 d*	*21 d*	*28 d*	*It*	*7 d*	*14 d*	*21 d*	*28 d*
Fucoxanthin	ATºCxACO$_2$	206.3 ± 78.1	316.8 ± 102.5	386.7 ± 52.7	386.5 ± 71.3	426.5 ± 14.7	191.2 ± 31.5	352.8 ± 37.7	527.8 ± 112.1	536.1 ± 101.5	904.1 ± 82.2
	ATºCxHCO$_2$		247.3 ± 30.1	429.2 ± 17.3	417.6 ± 14.1	321.5 ± 34.9		386.1 ± 89.1	443.7 ± 61.3	547.8 ± 84.3	801.1 ± 159.5
	HTºCxACO$_2$		230.5 ± 37.5	246.9 ± 36.8	380.2 ± 77.1	434.1 ± 43.6		443.2 ± 81.1	573.8 ± 44.1	477.3 ± 89.9	830.1 ± 131.8
	HTºCxHCO$_2$		361.8 ± 65.2	437.7 ± 73.7	276.7 ± 33.4	437.3 ± 56.1		558.6 ± 110.4	626.1 ± 99.6	478.6 ± 58.3	401.9 ± 93.9
Violaxanthin	ATºCxACO$_2$	29.5 ± 6.5	65.3 ± 4.5	49.9 ± 10.5	57.2 ± 5.7	64.6 ± 0.7	46.4 ± 13.4	44.3 ± 15.7	68.9 ± 11.2	81.7 ± 14.1	118.2 ± 6.1
	ATºCxHCO$_2$		23.8 ± 8.1	52.3 ± 7.3	57.7 ± 2.1	45.1 ± 5.1		33.3 ± 2.7	69.5 ± 8.7	82.6 ± 11.2	113.2 ± 21.6
	HTºCxACO$_2$		26.7 ± 3.4	38.1 ± 4.8	52.4 ± 9.5	63.2 ± 5.1		22.7 ± 4.7	69.1 ± 3.1	65.1 ± 8.5	111.1 ± 13.6
	HTºCxHCO$_2$		51.4 ± 9.7	58.1 ± 6.1	38.7 ± 4.3	60.7 ± 7.6		90.4 ± 23.7	82.5 ± 20.8	72.5 ± 11.3	68.1 ± 12.6
β- carotene	ATºCxACO$_2$	21.5 ± 3.4	53.9 ± 3.7	56.4 ± 11.3	70.5 ± 3.2	74.6 ± 3.4	61.7 ± 15.6	63.4 ± 16.3	103.1 ± 13.5	113.1 ± 15.1	107.4 ± 7.8
	ATºCxHCO$_2$		47.1 ± 15.9	83.2 ± 17.7	83.9 ± 10.1	60.1 ± 8.6		119.1 ± 23.8	94.1 ± 15.5	96.3 ± 7.6	107.6 ± 11.5
	HTºCxACO$_2$		48.2 ± 10.1	69.5 ± 15.9	69.1 ± 2.1	91.1 ± 15.1		64.1 ± 5.8	88.8 ± 4.2	81.6 ± 2.4	113.8 ± 13.4
	HTºCxHCO$_2$		43.1 ± 4.1	63.9 ± 3.7	156.4 ± 7.6	73.6 ± 0.7		76.7 ± 12.1	92.2 ± 11.9	77.3 ± 11.5	78.4 ± 4.3
Fucoxanthin /Chla	ATºCxACO$_2$	0.14 ± 0.03	0.11 ± 0.03	0.43 ± 0.01	0.40 ± 0.01	0.38 ± 0.01	0.10 ± 0.01	0.11 ± 0.01	0.36 ± 0.01	0.37 ± 0.01	0.39 ± 0.02
	ATºCxHCO$_2$		0.14 ± 0.01	0.42 ± 0.01	0.40 ± 0.01	0.39 ± 0.01		0.12 ± 0.01	0.34 ± 0.01	0.37 ± 0.01	0.38 ± 0.01
	HTºCxACO$_2$		0.13 ± 0.01	0.39 ± 0.01	0.42 ± 0.01	0.39 ± 0.01		0.20 ± 0.02	0.40 ± 0.02	0.40 ± 0.03	0.39 ± 0.01
	HTºCxHCO$_2$		0.13 ± 0.01	0.40 ± 0.01	0.44 ± 0.02	0.41 ± 0.02		0.16 ± 0.03	0.40 ± 0.02	0.39 ± 0.01	0.35 ± 0.01

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563 **Figure Captions**

564 Figure 1 Principal component analysis of *Cystoseira tamariscifolia* respect to variables;

565 maximal quantum yield (F_v/F_m), maximal non-photochemical quenching (NPQ_{max}),

566 Chlorophylls *a* and *c* (Chl*a* and Chl*c*), carotenoids pigments; fucoxanthin (Fuco),

567 violaxanthin (Violo) and β-carotene (β-Caro). For ultraoligotrophic (L1) and oligotrophic

568 (L2) waters, after exposure to four treatments, ATxACO₂ (ambient temperature, 20ºC x

569 ambient CO₂, *ca.* 400-500 ppm), ATxHCO₂ (ambient temperature, 20ºC x high CO₂,

570 *ca.*1200-1300 ppm), HTxACO₂ (high temperature, 24ºC x ambient CO₂, *ca.* 400-500

571 ppm) and HTxHCO₂ (high temperature, 24ºC x high CO₂, *ca.*1200-1300 ppm).

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573 Figure 2 Chlorophyll *a* (mg g⁻¹ DW), a) *Cystoseira tamariscifolia* from ultraoligotrophic

574 waters and b) oligotrophic waters, after exposure to four treatments. Ambient TºC (20ºC)

575 x ambient CO₂ (*ca.* 400-500 ppm), ambient TºC (20ºC) x high CO₂ (*ca.*1200-1300 ppm),

576 high TºC (24ºC) x ambient CO₂ (*ca.* 400-500 ppm) and high TºC (24ºC) x high CO₂

577 (*ca.*1200-1300 ppm). Lower-case letters denote significant differences after SNK test

578 (*p*<0.05).

579

580 Figure 3 Chlorophyll *c* (μg g⁻¹ DW), a) *Cystoseira tamariscifolia* from ultraoligotrophic

581 waters and b) oligotrophic waters, after experimental period and four treatments. Ambient

582 TºC (20ºC) x ambient CO₂ (*ca.* 400-500 ppm), ambient TºC (20ºC) x high CO₂ (*ca.*1200-

583 1300 ppm), high TºC (24ºC) x ambient CO₂ (*ca.* 400-500 ppm) and high TºC (24ºC) x

584 high CO₂ (*ca.*1200-1300 ppm). Lower-case letters denote significant differences after

585 SNK test (*p*<0.05).

586

587 Figure 4 Maximal quantum yield (F_v/F_m), a) *Cystoseira tamariscifolia* from

588 ultraoligotrophic waters and b) oligotrophic waters (La Araña beach), after experimental

589 period and four treatments. Ambient TºC (20ºC) x ambient CO₂ (*ca.* 400-500 ppm),

590 ambient TºC (20ºC) x high CO₂ (*ca.*1200-1300 ppm), high TºC (24ºC) x ambient CO₂

591 (*ca.* 400-500 ppm) and high TºC (24ºC) x high CO₂ (*ca.*1200-1300 ppm). Lower-case

592 letters denote significant differences after SNK test (*p*<0.05).

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594 Figure 5 Maximal non-photochemical quenching (NPQ_{max}), a) *Cystoseira tamariscifolia*

595 from ultraoligotrophic waters and b) oligotrophic waters after experimental period and

596 four treatments. Ambient TºC (20ºC) x ambient CO₂ (*ca.* 400-500 ppm), ambient TºC

597 (20ºC) x high CO_2 (*ca.*1200-1300 ppm), high TºC (24ºC) x ambient CO_2 (*ca.* 400-500

598 ppm) and high TºC (24ºC) x high CO_2 (*ca.*1200-1300 ppm). Lower-case letters denote

599 significant differences after SNK test ($p<0.05$).

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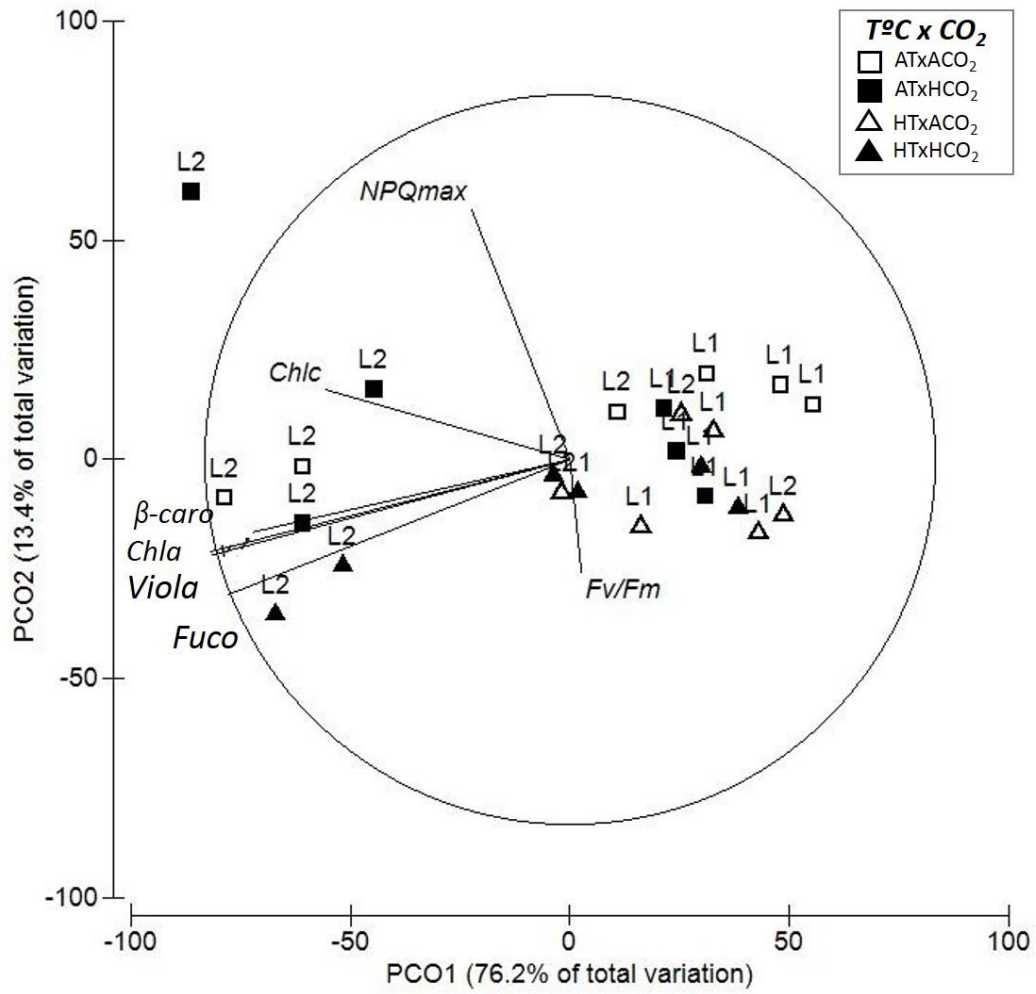615

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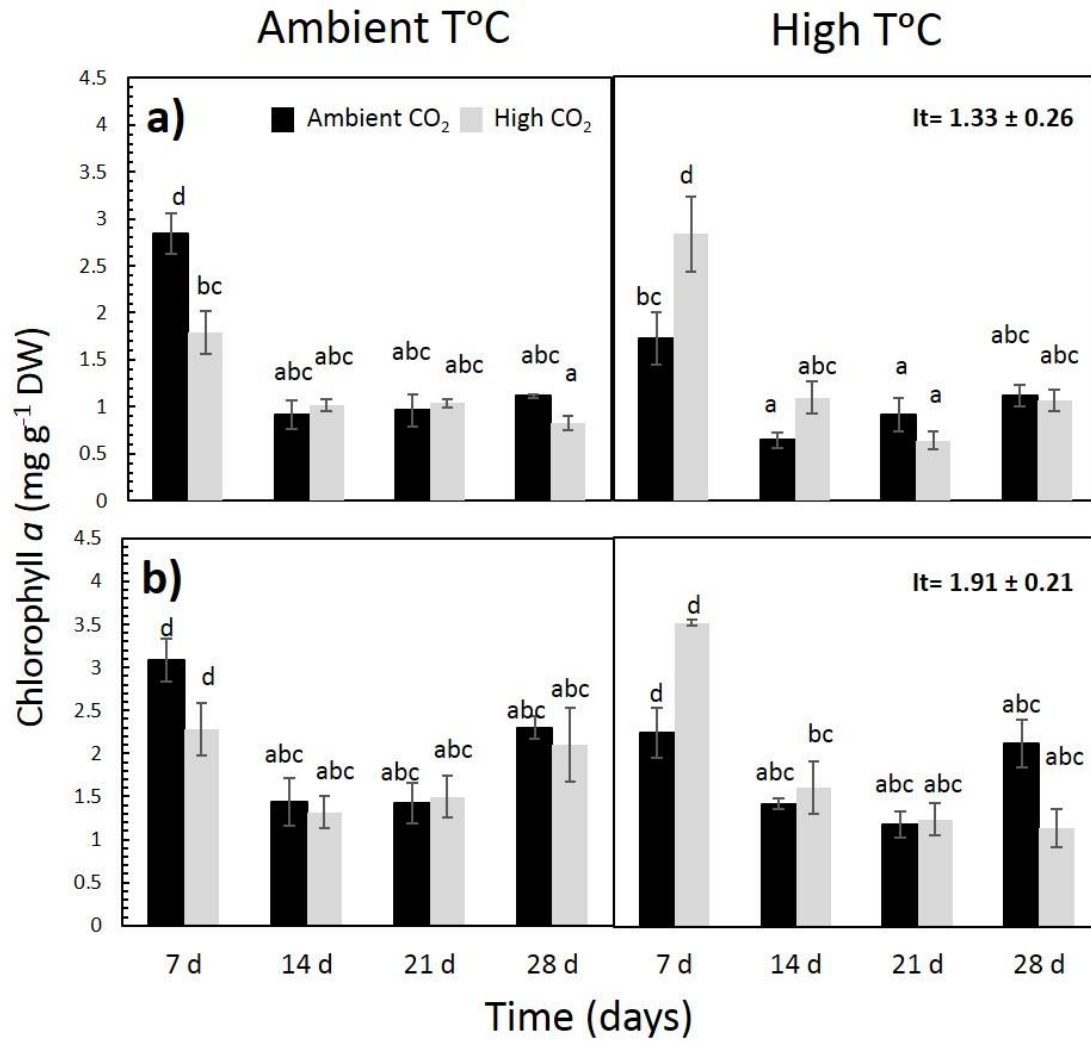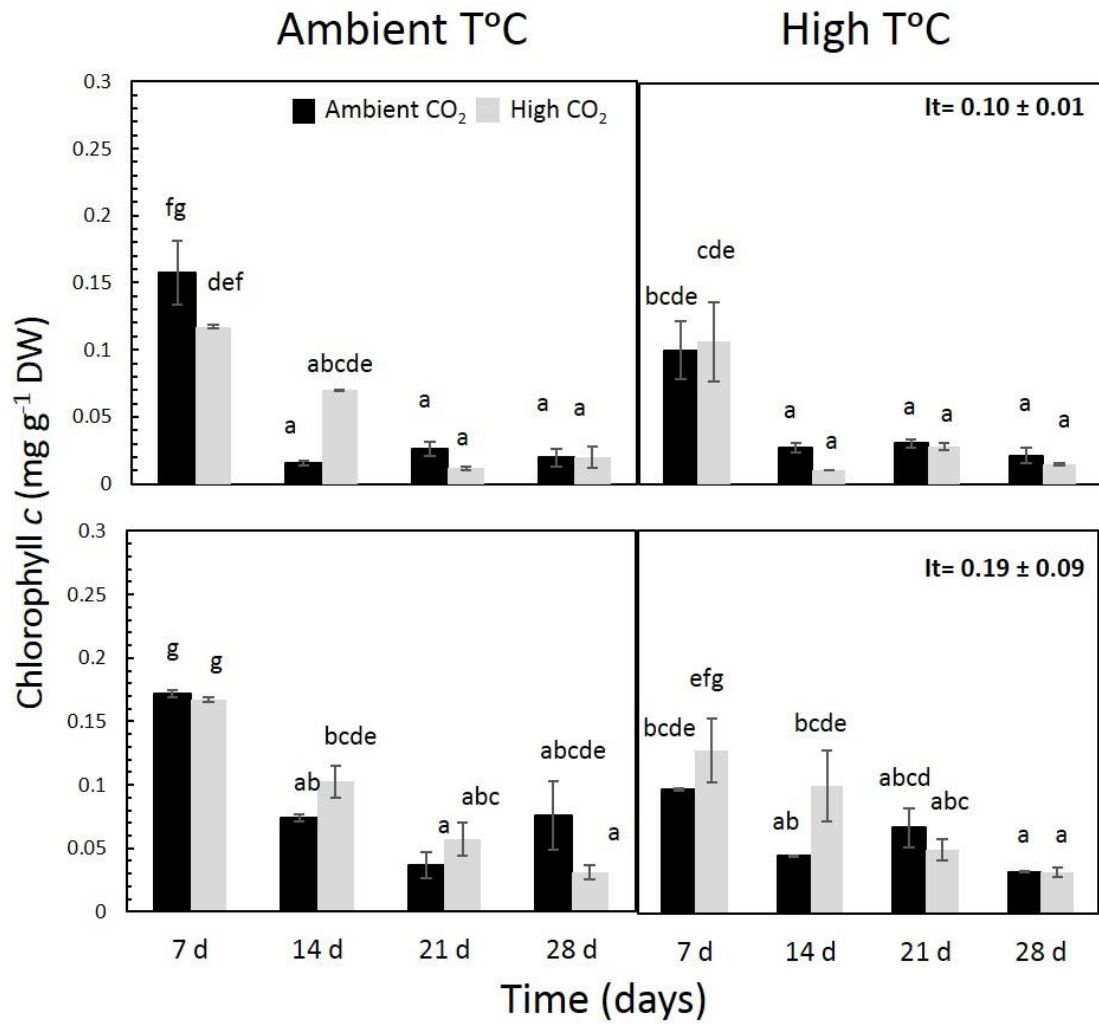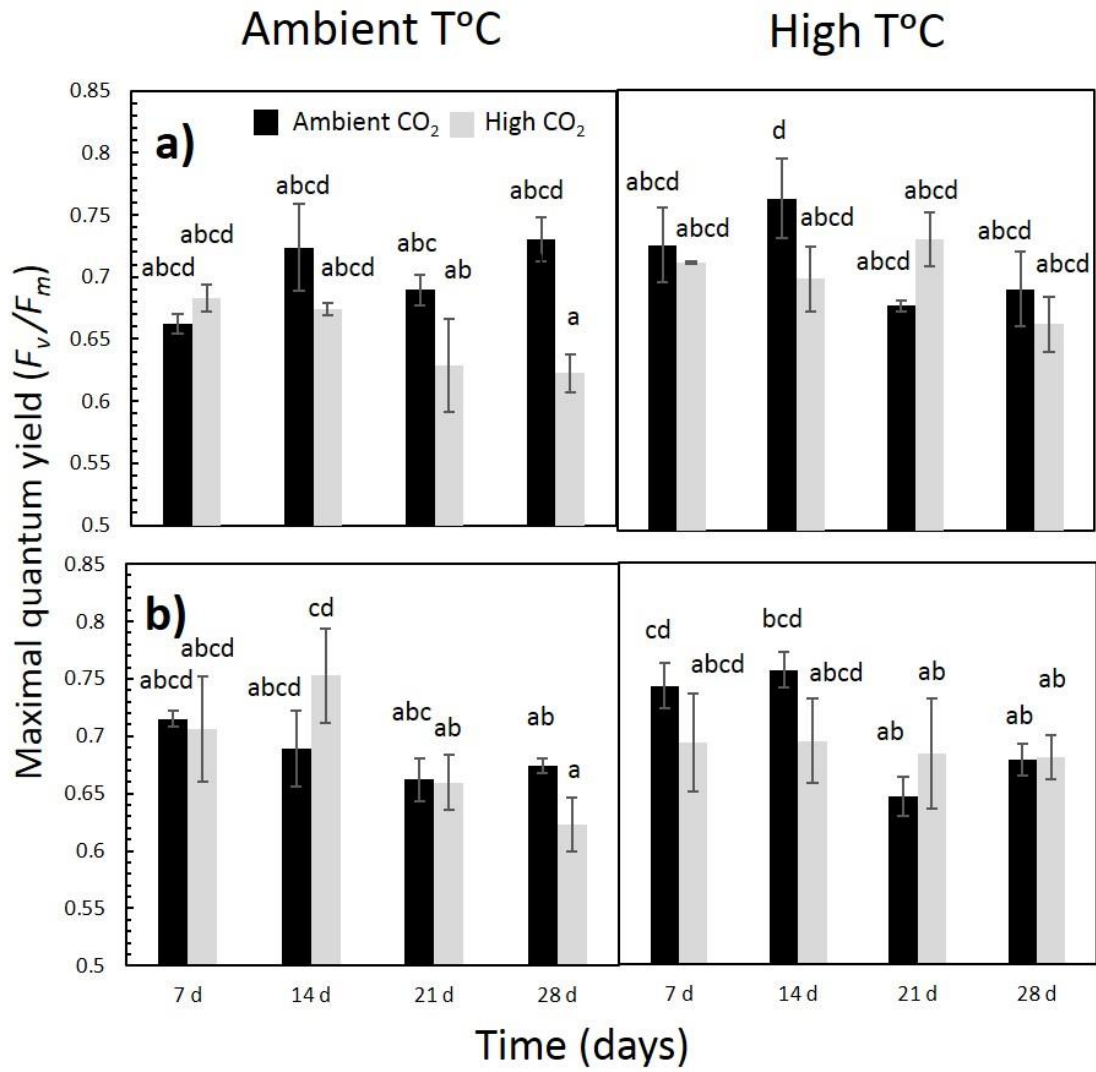621

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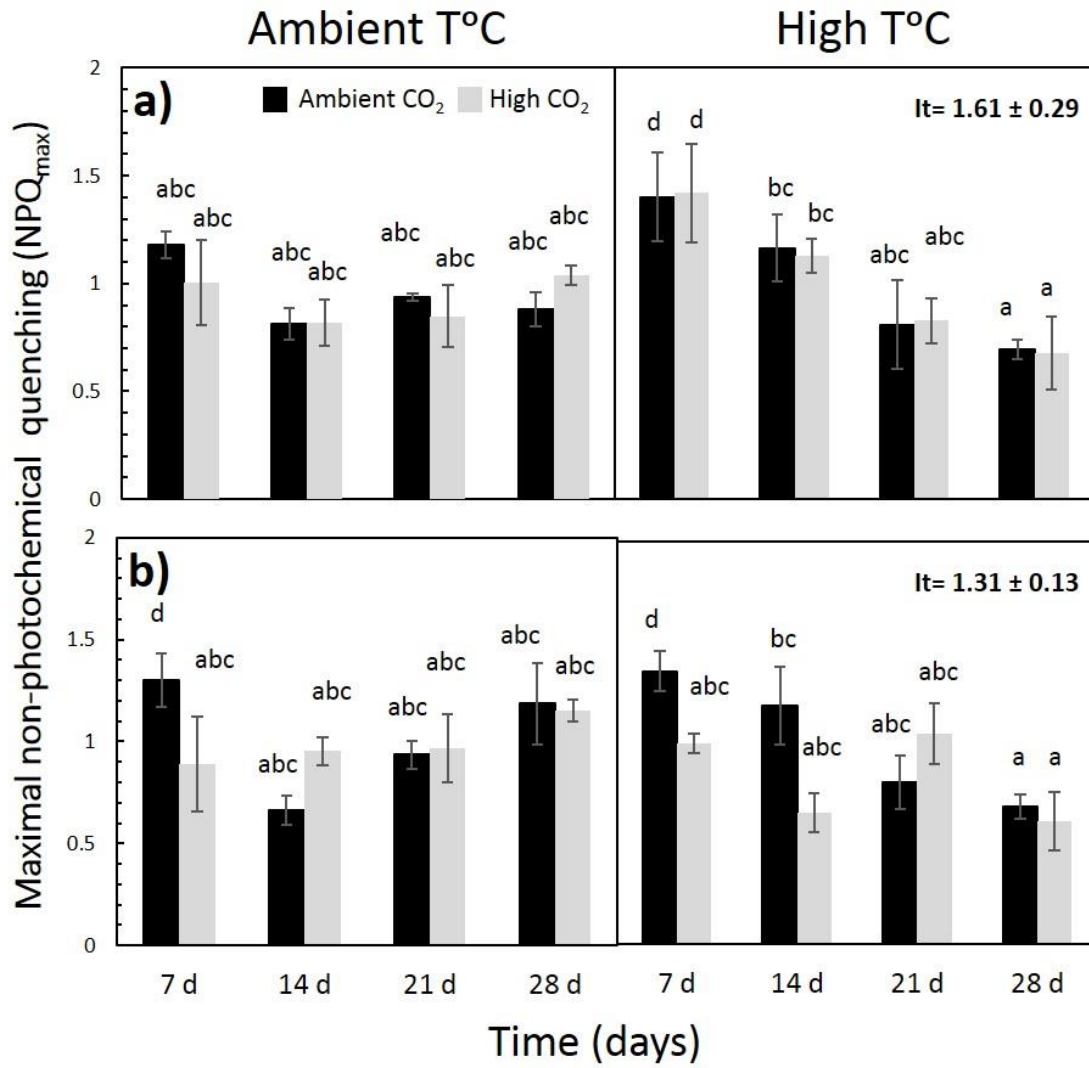644

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