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Understanding the impact of a complex environmental matrix associated with climate change on the European marshes engineer species *Spartina maritima* --Manuscript Draft--

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Abstract:	<p>A challenge exists in the need to understand plant responses in complex environmental matrixes, such as those predicted by climate change models, being this information essential for species that support important ecosystem functions. A factorial climatic chamber experiment was designed to evaluate the impact of atmospheric CO₂ concentration (400 ppm and 700 ppm CO₂) in combination with two maximum and minimum temperature ranges (28/13°C and 32/17°C) and salinity concentrations (0 and 171 mM NaCl) on the growth and photosynthetic responses of the ecosystem engineer species <i>Spartina maritima</i>. Plants grown at 32/17°C showed a reduction ~39% on relative growth rate (RGR) and this was more drastic (i.e. 64%) in those exposed to 700 ppm CO₂, which also showed an increment in the percentage of dead tillers regardless of salinity. These reductions were explained by the negative impact on net photosynthetic rate (A - N), which decreased with temperature increment, being this reduction more acute at 700 ppm CO₂. This response was associated with an augmentation in CO₂ diffusion limitations, as indicated the lower stomatal conductance (g s⁻¹), together with a down-regulation photochemical apparatus efficiency, as indicated the lower electron transport rate (ETR) and energy fluxes derived from Kautsky curves. In addition, the greatest g s⁻¹ drop at 700 ppm CO₂, would limit plant ability to cope with temperature excess through evapotranspiration, a fact that could have boosted temperature-triggered damage and, consequently, leaf senescence. Therefore, we can conclude that temperature and atmospheric CO₂ increments would compromise the development of <i>S. maritima</i> and consequently the maintaining of its ecosystem functions.</p>
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Response to Reviewers:	

Highlights

- Impact of a complex environmental matrix on *S. maritima* performance was evaluated.
- Temperature and atmospheric CO₂ increments had synergic injury effects.
- These conditions drastically reduced plant growth and increased its senescence.
- Carbon assimilation, light-harvesting and photoprotective impacts were found.
- Atmospheric CO₂ enrichment would limit stomata ability to cope with heat excess.

1 **Understanding the impact of a complex environmental matrix associated with**
2 **climate change on the European marshes engineer species *Spartina maritima***

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23 ABSTRACT

24 A challenge exists in the need to understand plant responses in complex environmental
25 matrixes, such as those predicted by climate change models, being this information
26 essential for species that support important ecosystem functions. A factorial climatic
27 chamber experiment was designed to evaluate the impact of atmospheric CO₂
28 concentration (400 ppm and 700 ppm CO₂) in combination with two maximum and
29 minimum temperature ranges (28/13 °C and 32/17 °C) and salinity concentrations (0 and
30 171 mM NaCl) on the growth and photosynthetic responses of the ecosystem engineer
31 species *Spartina maritima*. Plants grown at 32/17 °C showed a reduction ~39% on
32 relative growth rate (RGR) and this was more drastic (i.e. 64%) in those exposed to 700
33 ppm CO₂, which also showed an increment in the percentage of dead tillers regardless
34 of salinity. These reductions were explained by the negative impact on net
35 photosynthetic rate (A_N), which decreased with temperature increment, being this
36 reduction more acute at 700 ppm CO₂. This response was associated with an
37 augmentation in CO₂ diffusion limitations, as indicated the lower stomatal conductance
38 (g_s), together with a down-regulation photochemical apparatus efficiency, as indicated
39 the lower electron transport rate (ETR) and energy fluxes derived from Kautsky curves.
40 In addition, the greatest g_s drop at 700 ppm CO₂, would limit plant ability to cope with
41 temperature excess through evapotranspiration, a fact that could have boosted
42 temperature-triggered damage and, consequently, leaf senescence. Therefore, we can
43 conclude that temperature and atmospheric CO₂ increments would compromise the
44 development of *S. maritima* and consequently the maintaining of its ecosystem
45 functions.

46 **Keywords:** CO₂ enrichment; Climate change; Chlorophyll fluorescence; Gas exchange;
47 Halophyte; Temperature stress.

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1. Introduction

Climate change arises as one of the greatest challenges for worldwide ecosystems conservation. Thus, along with recognized atmospheric CO₂ enrichment to values ~700 ppm by the end of the century (IPCC, 2014), climate models predict a parallel increase in daily temperature range (between 1.8 - 6.0 °C, depending on the region) and rainfalls pattern alteration which would lead to a decrease in water availability and soil salinization (IPCC, 2014). An important part of the effect of climate change on ecosystems will be related to those variations triggered by environmental conditions in the structure and composition of plant species (Allen and Ort, 2001; Hooper et al., 2005; Short et al., 2016; Zhao et al., 2018), being this impact greater in those ecosystems dominated by few species, which also sustain key functions (such as the control of the fluxes of energy and matter) and contribute to functional diversity and species assemblages (Hooper et al., 2005).

The genus *Spartina* is integrated by grass species widely distributed on every continent except for Antarctica, being many of them frequently found in coastal salt marshes (Bortolus et al., 2019). It has been highlighted the importance of these species in salt marshes ecosystem functions, with a relevant role on coastal accretion and marsh creation, as well as the maintenance of ecosystem primary and secondary production (Bortolus et al., 2019). Among *Spartina* genus species, the European *Spartina maritima* (Curtis) Fernald is an important pioneer and ecosystem engineer in European salt marshes (Castellanos et al., 1994), playing also an important role for the maintenance and conservation of marsh ecosystem biodiversity (Curado et al., 2018). This species develops its populations mainly in the lowest parts of the marshes, acting as a primary colonist contributing to facilitate succession in marsh ecosystems (Castellano et al.,

1994, 1998; Castillo et al., 2008) and playing a key role in shoreline stabilization (Duarte et al. 2014). In addition, this species has demonstrated a high biotechnological potential as a tool for monitoring and phytoremediating metal polluted areas (Padinha et al., 2000; Mesa et al., 2015). Furthermore, many studies have been developed to understand the impact of environmental factors associated with future global change scenarios on the development of this important salt marsh species (Mateos-Naranjo et al., 2010a; Couto et al., 2014; Duarte et al., 2014). Thus, a certain effort has been made to assess the effect of atmospheric CO₂ enrichment on *S. maritima* development, having observed an improvement on plant growth and physiological performance under optimal and suboptimal salinity conditions, despite being a C₄ species (Mateos-Naranjo et al., 2010a). Besides, *S. maritima* has demonstrated the ability to maintain its photosynthetic activity even during prolonged submersion periods associated with the sea level rise due to global warming (Duarte et al., 2014). In addition, a rising temperature model has demonstrated an enhancement in this salt marsh species aboveground biomass (Couto et al., 2014), but it has been identified that an increase in the frequency and duration of high temperature events will lead to a decrease of its photo-biological fitness (Duarte et al., 2016). Despite these efforts, there is great uncertainty about the real effects of climate change on the conservation of this species, since most of those studies only have evaluated plant responses to one or two environmental stressors in combination. Therefore, a challenge exists in determining plant responses in a complex environmental matrix, such as the one predicted by climate models. Consequently, this study was designed and carried out to understand the influence of a complex environmental matrix, characterized by atmospheric CO₂ concentration, air temperature and medium saline concentration variations on *S. maritima* growth and development as well as key photosynthetic parameters. We

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98 hypothesized that, although a direct positive physiological impact of increased
99 atmospheric CO₂ concentration on *S. maritima* has been previously identified (Mateos-
100 Naranjo et al., 2010a), the co-occurrence of other stress factors such as temperature
101 pattern variation and medium salinization could trigger metabolic responses that could
102 jeopardize the development of this important salt marsh species and, consequently, the
103 maintenance of the ecosystem functions in which this plant is involved.

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105 **2. Material and Methods**

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107 *2.1. Plant material*

108 Clumps of *S. maritima* of 20 cm diameter with between 25-30 mature tillers were
109 collected in June of 2019 from a well-established population in a low-marsh site located
110 in the Odiel salt marshes (37°15'N, 6°58'O; SW Spain). Clumps were planted in
111 individual plastic pots (15 cm high × 18 cm diameter) using its own soil as a potting
112 substrate, and placed in a greenhouse under the follow controlled conditions: maximum
113 temperature between 21/25 °C, minimum temperature between 13/11 °C, 40-60%
114 relative humidity and natural daylight of 200 μmol m⁻² s⁻¹ as minimum and 1000 μmol
115 m⁻² s⁻¹ as maximum light flux). Pots were irrigated with tap water, and plants were kept
116 for a stabilization period of 7 days under the previously described conditions before the
117 experiment's onset.

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119 *2.2. Experimental design*

120 At the beginning of the experiment, the number of tillers in each pot was
121 homogenized to 20 tillers completely developed and healthy in order to standardize our

122 samples before the experiment onset and to avoid any effect of tiller health and age in
123 our results. Then, pots were randomly assigned to eight different experimental blocks
124 with ten plants in each one, as follows: two concentrations of atmospheric CO₂ (400
125 ppm and 700 ppm CO₂) in combination with two ranges of ambient maximum and
126 minimum temperature (28/13 °C and 32/17 °C) and irrigation with two salinity
127 concentrations (0 and 171 mM NaCl) for 40 days. For the atmospheric CO₂
128 concentration and temperature range treatments, pots were placed in controlled-
129 environment chambers (Aralab/Fitoclima 18.000EH, Lisbon, Portugal), which were
130 programmed with alternating diurnal regime of 14 h of light and 10 h of darkness with
131 the specific maximum and minimum temperature range, light intensity of 300 μmol m⁻²
132 s⁻¹, 40–60% relative humidity and the specific atmospheric CO₂ concentration.
133 Atmospheric CO₂ concentrations in chambers were continuously monitored by CO₂
134 sensors and maintained by supplying pure CO₂ from a compressed gas cylinder (Air
135 liquide, B50 35K). Finally, NaCl concentrations were established by combining tap
136 water with appropriate amounts of NaCl. At the beginning of the experiment, the pots
137 were placed in plastic trays containing the appropriate NaCl solutions for each specific
138 salinity concentration treatment to a depth of 1 cm. In order to avoid changes of the
139 NaCl concentration caused by water evaporation, levels in the trays were monitored
140 continuously throughout the experimental period.

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142 *2.3. Plant growth analysis*

143 At the beginning and at the end of the experiment, three and seven plants from each
144 specific treatment were harvested and divided into roots and tillers. Then, these biomass
145 fractions were oven dried at 60 °C for 48 h and weighed to obtain both initial and final

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146 dry biomass estimates. In addition, the number of dead tillers was recorded at the end of
147 the experiment.

148 The relative growth rate (RGR) of whole plants was calculated using the formula:

$$149 \text{ RGR} = (\ln B_f - \ln B_i) \cdot D^{-1} \text{ (g g}^{-1} \text{ day}^{-1}\text{)}$$

150 where B_f = final dry mass (the mean of the seven plants from each treatment at the
151 end of the experiment), B_i = initial dry mass (the mean of the three plants from each
152 treatment at the beginning of the experiment) and D = duration of experiment (days).

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154 *2.4. Leaf gas exchange analysis*

155 At the end of the experiment, instantaneous leaf gas exchange measurements were
156 taken on fully developed expanded leaves ($n = 10$) using an infrared gas analyzer in an
157 open system (LI-6400-XT, Li-COR Inc., NE., USA) equipped with a light leaf chamber
158 (Li-6400-02B, Li-Cor Inc.). Net photosynthesis rate (A_N), stomatal conductance (g_s) and
159 intercellular CO_2 concentration (C_i) were recorded under the following leaf chamber
160 settings: a photosynthetic photon flux density (PPFD) of $1000 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$
161 (with 15% blue light to maximize stomatal aperture), vapour pressure deficit of 2.0–3.0
162 kPa, $50 \pm 5\%$ relative humidity, CO_2 concentration surrounding the leaf (C_a) of 400 or
163 $700 \mu\text{mol CO}_2 \text{ mol}^{-1}$ air depending on the atmospheric CO_2 concentration treatment and
164 air temperature of $28 \text{ }^\circ\text{C}$ or $32 \text{ }^\circ\text{C}$ for plants grown at low and high temperature range
165 treatments, respectively. All measurements were made between 10:00 and 13:00 h
166 inserting the order between the different treatments to standardize samples, and before
167 to record each measurement, gas exchange was allowed to equilibrate for 120 s. Finally,

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168 intrinsic water use efficiency ($iWUE$) was calculated as the ratio between A_N and g_s
169 [mmol (CO₂ assimilated) mol⁻¹ (H₂O transpired)].

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171 *2.5. Chlorophyll fluorescence analysis*

172 Two different fluorescence protocols were developed at the end of the experiment
173 in the same leaves of gas exchange analysis in order to test how the different
174 combinations of atmospheric CO₂ concentration, air temperature and medium salinity
175 concentration used in this study affect photosystem II (PSII) energy use efficiency.
176 Thus, the saturation pulse method was used to determine the maximum quantum
177 efficiency of PSII photochemistry (F_v/F_m) and quantum efficiency of PSII (Φ_{PSII} ; Genty
178 et al., 1989). As described by Schreiber et al. (1986), a 0.8 s saturating actinic light
179 pulse of 10000 $\mu\text{mol m}^{-2} \text{s}^{-1}$ was applied at midday (1400 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$) in
180 previously dark- and light-adapted leaves ($n = 10$) for 30 min using a modulated
181 fluorimeter (FMS-2; Hansatech Instruments Ltd., UK). Using this information, the
182 electron transport rate (ETR) was calculated according to Krall and Edwards (1992).

183 On the other hand, the chlorophyll *a* fast kinetics, by the OJIP-test (or Kautsky
184 curves), which depicts the rate of reduction kinetics of various components of PSII, was
185 also measured in 30 min dark-adapted leaves ($n = 7$), using the pre-programmed OJIP
186 protocols of the FluorPen FP100 (Photo System Instruments, Czech Republic).

187 Moreover, absorbed (ABS/RC), trapped (TR/RC), electron transport (ET/RC) and
188 dissipated (DI/RC) energy fluxes per reaction center derived from OJIP were calculated
189 according to Strasser et al. (2004).

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191 *2.6. Statistical analysis*

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192 R software ver. 4.0.0 (R Core Team, 2020) was used to perform the statistical
193 analyses. Firstly, a multivariate statistical approach using a principal component
194 analysis (PCA) was performed to get an overview of the plant growth and physiological
195 performance in response to the different experimental treatments. Missing values in the
196 PCA dataset appeared as a result of the different number of measurements in each
197 variable. They were handled using the expectation-maximization (EM) algorithm in the
198 ‘missMDA’ R package (Josse and Husson, 2016). Secondly, a downscaling assessment
199 was carried out through generalized linear models (GLMs) to analyze the main and/or
200 interaction effects of atmospheric CO₂ concentration, maximum and minimum
201 temperature range and low and high NaCl concentration (as categorical factors) on the
202 growth and physiological parameters (as dependent variables) of *S. maritima* plants. In
203 case of significant results, multiple comparisons were analyzed by *post hoc* LSD test
204 (i.e. Fisher's Least Significant Difference). Before statistical analysis, Kolmogorov-
205 Smirnov and Levene tests were used to verify the assumptions of normality and
206 homogeneity of variances, respectively.

208 **3. Results**

209 *3.1. Multivariate approach: global overview of S. maritima growth and physiological* 210 *status*

211 Growth and physiological performance variations of *S. maritima* during the
212 experimental setup were mainly represented in the first two PCA axes. They explained,
213 respectively, 36.0% and 18.4% of the total variation in the recorded data, being both
214 temperature range and atmospheric CO₂ enrichment the experimental factors which led
215 the main groupings (Fig. 1A, B; Table 1). Hence, the PC1 axis reflected a clear
216 divergence of plants grown at 32/17 °C of maximum and minimum temperature range,

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217 which were located in the left part of the plot (especially those grown at 700 ppm CO₂).
218 This separation was mainly explained by the lower RGR, A_N, F_v/F_m, Φ_{PSII} and ETR
219 values, together with an increment in the percentage of dead tillers and DI/RC in those
220 plants compared to the rest of the treatments (Fig. 1B; Table 1). In addition, the PCA
221 revealed a certain divergence between both atmospheric CO₂ concentration treatments
222 along the PC2 axis, with most of non-CO₂ enriched plants located in the upper part,
223 being this response pattern mainly linked with higher g_s and lower C_i values of plants
224 grown at 400 ppm CO₂ (Fig. 1B; Table 1).

225

226 *3.2. Effects of atmospheric CO₂ enrichment, temperature augmentation and NaCl* 227 *concentration on S. maritima growth*

228 A summary of generalized linear model (GLM) results is made available as
229 supplementary material, including fitting tests and confidence intervals (Table A.1).
230 There was a significant effect of temperature range treatment on the RGR of *S.*
231 *maritima* after 40 days of treatment (GLM: Temp., p < 0.01; Table A.1). Additionally,
232 this effect was, to some extent, modulated by atmospheric CO₂ concentration treatment
233 (GLM: [CO₂] x Temp., p < 0.05; Table A.1). Thus, RGR decreased considerably in
234 plants grown under the highest maximum and minimum temperature range treatment
235 (i.e. 32/17 °C), and this effect was more acute in those exposed to 700 ppm CO₂
236 regardless of saline irrigation treatment (Fig. 2A).

237 Similarly, the percentage of dead tillers augmented with temperature and
238 atmospheric CO₂ enrichment regardless of saline irrigation treatment (GLM: Temp., p <
239 0.01; [CO₂], p < 0.05; Table A.1), being this increase more pronounced in plants grown

240 at 700 ppm CO₂ and 32/17 °C compared to the rest of treatments, although no
241 significant differences were found (Fig. 2B).

242

243 3.3. Effects of atmospheric CO₂ enrichment, temperature augmentation and NaCl 244 concentration on photosynthetic apparatus performance

245 There was a significant effect of the environmental factors tested on leaf gas
246 exchange characteristics and PSII photochemical efficiency of *S. maritima* after 40 days
247 of exposure (GLM, $p < 0.05$). Thus, A_N values decreased in plants grown at 32/17 °C
248 (GLM: Temp., $p < 0.01$; Fig. 3A; Table A.1), and these values were overall lower at
249 700 ppm CO₂ although without significant differences. A very similar trend was
250 recorded for g_s in relation to temperature increment (GLM: Temp., $p < 0.01$) but, in
251 addition, g_s values were significantly lower in plants grown at 700 ppm CO₂ (GLM:
252 [CO₂], $p < 0.01$; Fig. 3B; Table A.1). Contrarily, C_i and iWUE values were significantly
253 higher in plants grown at 700 ppm CO₂ compared to their non-CO₂ enriched
254 counterparts, regardless of temperature and saline irrigation treatments (GLM: [CO₂], p
255 < 0.01 ; Fig. 3C, D; Table A.1).

256 Regarding photochemical parameters, F_v/F_m values were lower in plants subjected
257 to the highest minimum and maximum temperature range treatment at both salinity
258 irrigation conditions (GLM: Temp., $p < 0.01$), being this reduction also more
259 pronounced in plants grown at 700 ppm CO₂ (GLM: [CO₂] x Temp., $p < 0.05$; Table
260 A.1), and especially in those plants irrigated with 171 mM NaCl (Fig. 4A). Likewise,
261 minimum and maximum temperature augmentation led to a marked reduction in *S.*
262 *maritima* Φ_{PSII} values, although this overall effect was much more marked in those
263 plants exposed to high atmospheric CO₂ concentration and saline irrigation (GLM:
264 [CO₂] x Temp., $p < 0.05$; Fig. 4B; Table A.1). A very similar response was detected for

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265 ETR, with the lowest values recorded in plants subjected to 700 ppm CO₂, 32/17 °C and
266 irrigation with 171 mM NaCl (Fig. 4C).

267 Focusing on the chlorophyll *a* fast kinetics results, there were remarkable
268 differences in the shape of Kautsky curves between plants grown under the different
269 enviromental factors after 40 days of treatments. Thus, overall fluorescence kinetics
270 values were lower in plants subjected to 700 ppm CO₂ and 32/17 °C, being this trend
271 also more pronounced in plants irrigated with 171 mM NaCl (Fig. 5). These Kautsky
272 curve shape divergence was also accompanied by marked differences in energy fluxes
273 through photosystems. Thus, ABS/RC was lower in plants grown at 700 ppm CO₂ and
274 28/13 °C, compared to those exposed to 400 ppm CO₂ (GLM: [CO₂] x Temp., $p < 0.01$;
275 Table A.1). Moreover, ABS/RC showed an increase in plants subjected to 700 ppm,
276 32/17 °C and irrigation with 171 mM NaCl compared to the rest of treatments (GLM:
277 [CO₂] x Temp. x [NaCl], $p < 0.05$; Fig. 6A; Table A.1). A very similar trend was
278 recorded for DI/RC (Fig. 6D). Opposately, both ET/CS and TR/CS tended to decrease in
279 plants exposed to 700 ppm CO₂ compared with their non-CO₂ enriched counterparts
280 (GLM: [CO₂] , $p < 0.05$; Fig. 6B, C; Table A.1). However, this effect was more
281 pronounced for TR/CS in plants grown at 28/13 °C compared to those grown in a higher
282 temperature range (GLM: [CO₂] x Temp., $p < 0.01$; Fig. 6C; Table A.1).

283

284 **4. Discussion**

285 Gaining a mechanistic understanding of the effect of the main coexisting factors
286 linked with climate change, such as atmospheric CO₂ enrichment, temperature pattern
287 variations and medium salinization, on the growth and physiological performance of
288 key plant species in ecosystem functionality is crucial to obtain a more realistic view on

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289 how this global phenomenon influences ecosystems conservation and the development
290 of these plant communities (Bernacchi et al., 2013).

291 Our results demonstrated that atmospheric CO₂ enrichment, as a consequence of
292 climatic change, would not cause a substantial improvement on growth and
293 development of the important pioneer and salt marsh ecosystem engineer species *S.*
294 *maritima*. However, an increase up to 4 °C in the maximum and minimum daily
295 temperature range, as expected by the end of the century (IPCC, 2007), would entail a
296 negative effect on the development of this species, being this impact even more acute in
297 a CO₂ enriched atmosphere. Thus, our results revealed that *S. maritima* plants grown at
298 400 ppm atmospheric CO₂ concentration and 32/17 °C of maximum and minimum daily
299 temperature range showed a drastic growth reduction, measured as RGR, of ~39%
300 compared to their counterparts exposed to the colder temperature range. This reduction
301 was up to 64% in those plants also exposed to 700 ppm CO₂, regardless of the saline
302 irrigation treatment. The lack of salinity effects on plant growth, as well as for most of
303 the studied parameters, was somewhat expected since *S. maritima* is a halophyte species
304 that has demonstrated a high tolerance to a wide range of salinities (Adams and Bate,
305 1995; Castillo et al., 2008; Mateos-Naranjo et al., 2010a). However, it should be
306 highlighted that the absence of beneficial effects of atmospheric CO₂ enrichment on
307 plant development contrasts with the previous results obtained by Mateos-Naranjo et al.
308 (2010a) for this plant species, who observed that an increment of atmospheric CO₂
309 concentration to 700 ppm stimulated plant RGR ~40% through a salinity range between
310 0 and 510 mM NaCl. Similarly, our results also varied with respect to those shown by
311 other *Spartina* species, such as *S. densiflora*, which experienced an increase in biomass
312 production of 35% and 20%, respectively at 0 and 171 mM NaCl, compared to plants
313 grown at 400 ppm CO₂ (Mateos-Naranjo et al., 2010b). These differences could be

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314 related to the variation in the experimental conditions of each specific study. In this
315 study, plants were subjected to different environmental synergies growing in natural soil
316 whilst, in the aforementioned experiments, plants were grown in perlite. The use of
317 this substrate would allow the previous studies to assess the direct effect of atmospheric
318 CO₂ enrichment on plant performance avoiding the influence of other factors associated
319 to soil physicochemical properties, which could alter plant direct responses to
320 atmospheric CO₂ enrichment. However, we believe that the use of a natural soil-plant
321 complex, as we have done in this study, would allow a more realistic view of the real
322 impact of climate change factors on plant development. In fact, according to our results,
323 Curtis et al. (1989), in an experiment developed under field conditions using OTC
324 chambers, were unable to detect a response to atmospheric CO₂ enrichment in *Spartina*
325 *patens*. Furthermore, Rozema et al. (1991) obtained different results regarding the effect
326 of atmospheric CO₂ enrichment on *S. patens* performance, depending on growth
327 medium aeration level in plants grown in hydroponic conditions. To some degree, this
328 lack of response to atmospheric CO₂ enrichment is consistent with multitude of CO₂
329 enrichment experiments, which indicated that plants with C₄ photosynthetic
330 metabolism, as *S. maritima*, would not be as benefited by the air CO₂ fertilization effect
331 (Ghannoum et al., 2000). However, this overall response could be modified by
332 interactions with other co-occurring stress factors related to climate change, such as
333 temperature pattern variation or medium salinization (IPPC, 2014). Therefore, it has
334 been described that the effect of atmospheric CO₂ enrichment on plant development
335 could be neutral, positive through the improvement of plant tolerance against
336 environmental stress or negative by enhancing environmental stress deleterious effects,
337 being also these responses species-specific (Balfagón et al., 2019). In this respect, as we
338 previously indicated, the increment in temperature together with an atmospheric CO₂

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339 concentration enrichment would substantially reduce *S. maritima* growth. Besides, it is
340 important to emphasize that this lower plant development was accompanied by an
341 increase in senescence level, as reflected by the higher percentage of dead stems at the
342 end of the experiment.

343 The deleterious effect of increased temperatures and, to a greater extent, CO₂-
344 enriched atmosphere conditions on *S. maritima* development was partly explained by
345 the negative impact of these factors on some essential steps of plant photosynthetic
346 apparatus performance. Thus, our results showed a substantial reduction in plant carbon
347 fixation capacity, measured as A_N, at elevated temperature range treatment being this
348 reduction in certain degree more pronounced at elevated atmospheric CO₂
349 concentration. This effect was associated with an augmentation in CO₂ diffusion
350 limitations through stoma in plants grown at 32/17 °C, as indicated their lower g_s values.
351 Temperature is one of the most variable environmental factors and it can affect many
352 plant physiological processes, including plant gas exchange characteristics, but little is
353 known about its effect on g_s, especially at high temperatures (Teskey et al., 2015). In
354 fact, several studies have recorded a wide range of plant responses to temperature
355 increment, including stomal opening (Lu et al., 2000; Mott and Peak, 2010) and closure
356 (Weston and Bauerle, 2007; Lahr et al., 2015) but also non-significant responses
357 (Cerasoli et al., 2014; von Caemmerer and Evans, 2015). Moreover, stomatal behaviour
358 to temperature could be modified by interactions with other environmental factors
359 (Flexas et al., 2012). In this sense, there is a need to highlight that g_s reduction with
360 temperature increment was even more acute in those plants exposed to 700 ppm CO₂. In
361 addition, this reduction was greater than the one reported in A_N, contributing to overall
362 higher \dot{V} WUE values under rising atmospheric CO₂ conditions. Thus, this decoupling
363 between g_s and A_N in plants grown at 700 ppm CO₂ and 32/17 °C would reflect the

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364 higher stomatal sensitivity to CO₂ variations. In fact, different studies have shown that
365 one of the most consistent responses of C₄ plant species to atmospheric CO₂
366 concentration enrichment is a decrease in g_s (Ainsworth and Rogers, 2007). Robredo et
367 al. (2007) explained that C_i increase caused by the high CO₂ concentration could
368 promote partial closure of the stoma, as it was recorded in this study. However, the
369 higher stomatal sensitivity to elevated CO₂ regardless of temperature, could have had
370 also important consequences for plant tolerance to heat stress. Thus, although stomatal
371 behaviour is a key factor to preserve the trade-off between CO₂ acquisition for
372 photosynthetic processes and water losses, it is necessary to emphasize its role for plant
373 adaptation to thermal stress through the regulation of heat excess by increasing leaf
374 evapotranspiration (Feller, 2006). Therefore, the importance of stoma in leaf cooling has
375 been identified, and while the stomata remains open, the evaporative cooling can
376 mitigate the negative effect of temperature excess, positively affecting plant
377 photosynthesis, yield and survival (Lu et al., 1994; Ameye et al., 2012). Therefore, the
378 higher g_s drop in *S. maritima* plants under elevated atmospheric CO₂ concentration
379 could have been limiting their ability to modulate leaf temperature compared to plants
380 grown at 400 ppm, which could also contribute to explain the greater injury effect of
381 temperature increment at these experimental conditions.

382 On the other hand, the impact of temperature increase, alone or in combination with
383 atmospheric CO₂ enrichment, had a great impact on *S. maritima* photochemical
384 apparatus functionality. According to our results, one of the best recognized effects of
385 high temperature on plant photosynthetic apparatus is the destruction of PSII
386 components (Flexas et al., 2012; Pérez-Romero et al., 2019a; López-Jurado et al.,
387 2020). Likewise, in concordance with this study, Mateos-Naranjo et al. (2010b) and
388 Duarte et al. (2014) also found that CO₂ enrichment affected PSII photochemistry in the

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389 halophytes *S. densiflora* and *S. maritima*, respectively. However, this is the first study
390 that corroborates that this effect would be exacerbated with an increase up to 4 °C in the
391 maximum and minimum daily temperature range and, to some extent, with the
392 augmentation in medium salinity concentration. Thus, our results showed that F_v/F_m and
393 Φ_{PSII} values were affected by the elevated temperature range and this effect was more
394 acute in plants grown at 700 ppm CO₂ and irrigated with 171 mM NaCl, suggesting that
395 the combination of these environmental conditions would increase photoinhibition
396 (Werner et al., 2002). Likewise, we attributed the negative impact of these
397 environmental factors on *S. maritima* photochemical apparatus to the down-regulation
398 of its electron transport chain functionality, as indicated by the lower ETR values
399 recorded in plants grown at 700 ppm CO₂, 32/17 °C and irrigated with 171 mM NaCl.
400 This fact indicated that these plants would have had more difficulties to transform the
401 captured energy in their photosystems, suggesting the synergistic impact of elevated
402 temperature and atmospheric CO₂ enrichment on the functionality of reaction centers.
403 Thus, this idea was also supported by the assessment of energy fluxes per reaction
404 center derived from Kautsky curves, which showed that, although plants were exposed
405 to elevated atmospheric CO₂ and air temperature range, they increased their ABS/RC
406 values compared to the rest of treatments. This would mean a greater number of active
407 reaction centers functioning as a heat radiator, hence protecting plant photosystems
408 against high temperature and light intensities (Strasser et al., 2004; Pérez-Romero et al.,
409 2018, 2019a,b). This elevated absorption rate would exceed the capacity of
410 photosystems to metabolize energy, as was corroborated by the decoupling with TR/RC
411 and ET/RC values. Moreover, the high ABS/RC would unbalance the trade-off between
412 the energy absorbed and metabolized, being necessary the activation of some defense
413 mechanisms to dissipate the energy excess as heat, as indicated also higher DI/RC

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414 values recorded. As a consequence, much of the absorbed energy would not take the
415 photochemical pathway (Flexas et al., 2012), a circumstance that would affect
416 photosynthetic productivity and, consequently, plant growth, a fact which would
417 contribute to explain the observed growth pattern in response to tested environmental
418 factors. Therefore, our results would suggest that temperature and atmospheric CO₂
419 increments related to climate change would jeopardize the development and
420 maintenance of natural populations of *S. maritima*. This circumstance would cause
421 significant structural and functional changes in the drainage system of the marshes
422 systems dominated by this grass species, due to its importance in shoreline stabilization
423 and salt marsh accretion during the first phases of ecological succession, with the
424 consequent alteration of sediment, mineral elements and nutrient cycles, as well as the
425 fluxes of energy necessary for other levels of the trophic chain in the ecosystem.

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428 **5. Conclusion**

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This study reveals the high vulnerability of the salt marsh key species *S. maritima* to increases up to 4 °C in the maximum and minimum daily temperature, as predicted for future scenarios by global change models, being this susceptibility even higher under CO₂ enriched atmosphere conditions. This idea would be motivated by drastic plant growth decrease and leaf senescence increment recorded at 700 ppm CO₂ and 32/17 °C of maximum and minimum daily temperature range. This response was associated with the negative effect of temperature and atmospheric CO₂ concentration on plant carbon fixation machinery through increased stomatal limitations to CO₂

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438 diffusion and the unbalance between the energy absorbed and metabolized in *S.*
439 *maritima* photosystems, linked with a down-regulation of the electron transport chain
440 functionality. The higher g_s drop at 700 ppm CO₂ would also limit plant ability to
441 regulate heat excess through leaf evapotranspiration, a fact that could have boosted
442 temperature-triggered damage and, consequently, leaf senescence compared to plants
443 grown at 400 ppm. Finally, from a conservational perspective, these results suggest that
444 temperature increment and atmospheric CO₂ enrichment associated with future climate
445 change scenarios would jeopardize the development of *S. maritima* populations and,
446 consequently, the maintenance of ecosystem functions derived from the presence of this
447 important pioneer and ecosystem engineer salt marshes plant species.
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457 **CRedit authorship contribution statement**

458 **Enrique Mateos-Naranjo:** Conceptualization, Methodology, Formal analysis, Funding
459 acquisition, Writing - original draft, Writing - review & editing. **Javier López Jurado:**
460 Methodology, Formal analysis, Writing - review & editing. **Jennifer Mesa Marín:**
461 Methodology, Writing - review & editing. **Carlos Javier Luque:** Conceptualization,
462 Methodology, Writing - review & editing. **Eloy Manuel Castellanos:**
463 Conceptualization, Methodology, Writing - review & editing. **Jesús Alberto Pérez-**
464 **Romero:** Methodology. **Susana Redondo-Gómez:** Conceptualization, Methodology,
465 Funding acquisition, Writing - review & editing.

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468 **Declaration of Competing Interest**

469 The authors declare that they have no known competing financial interests or personal
470 relationships that could have appeared to influence the work reported in this paper.

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482 **Figure legends**

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3 483 **Fig. 1.** Representation of the principal component analysis (PCA) biplot obtained for
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5 484 the growth and physiological data of *Spartina maritima* plants in the experimental set-
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8 485 up. Panel A represents the distribution of the selected variables loading on these axes
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10 486 and the percentage of explained variance for these axes (see Table 1 for a description of
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12 487 these variables and their contribution to the two main axes of PCA). Panel B shows the
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14 488 ellipses (95% confidence level) which encompass occurrence points in the two main
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16 489 axes for the environmental factors tested.

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21 490 **Fig. 2.** Relative growth rate, RGR (A) and percentage of dead tillers (B) in *Spartina*
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23 491 *maritima* plants in response to treatment with two atmospheric CO₂ concentrations (400
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25 492 ppm and 700 ppm) in combination with two temperature ranges (28/13 °C and 32/17 °C)
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27 493 and irrigation with 0 mM NaCl (-) or 171 mM NaCl (+) for 40 days. Values represent
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29 494 mean ± SE, n = 7. [CO₂], Temp., NaCl or their interactions in the corner of the panels
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31 495 indicate main or interaction significant effects (GLM test, *P < 0.05, **P < 0.01).

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36 496 **Fig. 3.** Net photosynthetic rate, A_N (A), stomatal conductance, g_s (B), intercellular CO₂
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38 497 concentration, C_i (C) and intrinsic water use efficiency (iWUE) in randomly selected
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40 498 fully developed expanded leaves of *Spartina maritima* in response to treatment with two
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42 499 atmospheric CO₂ concentrations (400 ppm and 700 ppm) in combination with two
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44 500 temperature ranges (28/13 °C and 32/17 °C) and irrigation with 0 mM NaCl (-) or 171
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46 501 mM NaCl (+) for 40 days. Values represent mean ± SE, n = 10. [CO₂], Temp., NaCl or
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48 502 their interactions in the corner of the panels indicate main or interaction significant
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50 503 effects (GLM test, *P < 0.05, **P < 0.01).

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56 504 **Fig. 4.** Maximum quantum efficiency of PSII photochemistry, F_v/F_m (A), quantum
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58 505 efficiency of PSII, Φ_{PSII} (B) and electron transport rate, ETR (C), at midday in randomly
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506 selected fully developed expanded leaves of *Spartina maritima* in response to treatment
507 with two atmospheric CO₂ concentrations (400 ppm and 700 ppm) in combination with
508 two temperature ranges (28/13 °C and 32/17 °C) and irrigation with 0 mM NaCl (-) or
509 171 mM NaCl (+) for 40 days. Values represent mean ± SE, n = 10. [CO₂], Temp.,
510 NaCl or their interactions in the corner of the panels indicate main or interaction
511 significant effects (GLM test, *P < 0.05, **P < 0.01).

512 **Fig. 5.** Transient fluorescence (Kautsky curves) of dark-adapted leaves of *Spartina*
513 *maritima* plants grown at 400 ppm (A) and 700 ppm (B) atmospheric CO₂ in
514 combination with two temperature ranges (28/13 °C and 32/17 °C) and irrigation with 0
515 mM NaCl (-) or 171 mM NaCl (+) for 40 days. Values represent mean of seven
516 measurements per treatment combination.

517 **Fig. 6.** Absorbed energy flux, ABS/RC (A), electron transport energy flux, ET/RC (B),
518 trapped energy flux, TR/RC (C) and dissipated energy flux, DI/RC (D), per reaction
519 centre in randomly selected fully developed expanded dark-adapted leaves of *Spartina*
520 *maritima* in response to treatment with two atmospheric CO₂ concentrations (400 ppm
521 and 700 ppm) in combination with two temperature ranges (28/13 °C and 32/17 °C) and
522 irrigation with 0 mM NaCl (-) or 171 mM NaCl (+) for 40 days. Values represent mean
523 ± SE, n = 7. [CO₂], Temp., NaCl or their interactions in the corner of the panels indicate
524 main or interaction significant effects (GLM test, *P < 0.05, **P < 0.01).

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1 **Understanding the impact of a complex environmental matrix associated with**
2 **climate change on the European marshes engineer species *Spartina maritima***

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23 ABSTRACT

24 A challenge exists in the need to understand plant responses in complex environmental
25 matrixes, such as those predicted by climate change models, being this information
26 essential for species that support important ecosystem functions. A factorial climatic
27 chamber experiment was designed to evaluate the impact of atmospheric CO₂
28 concentration (400 ppm and 700 ppm CO₂) in combination with two maximum and
29 minimum temperature ranges (28/13 °C and 32/17 °C) and salinity concentrations (0 and
30 171 mM NaCl) on the growth and photosynthetic responses of the ecosystem engineer
31 species *Spartina maritima*. Plants grown at 32/17 °C showed a reduction ~39% on
32 relative growth rate (RGR) and this was more drastic (i.e. 64%) in those exposed to 700
33 ppm CO₂, which also showed an increment in the percentage of dead tillers regardless
34 of salinity. These reductions were explained by the negative impact on net
35 photosynthetic rate (A_N), which decreased with temperature increment, being this
36 reduction more acute at 700 ppm CO₂. This response was associated with an
37 augmentation in CO₂ diffusion limitations, as indicated the lower stomatal conductance
38 (g_s), together with a down-regulation photochemical apparatus efficiency, as indicated
39 the lower electron transport rate (ETR) and energy fluxes derived from Kautsky curves.
40 In addition, the greatest g_s drop at 700 ppm CO₂, would limit plant ability to cope with
41 temperature excess through evapotranspiration, a fact that could have boosted
42 temperature-triggered damage and, consequently, leaf senescence. Therefore, we can
43 conclude that temperature and atmospheric CO₂ increments would compromise the
44 development of *S. maritima* and consequently the maintaining of its ecosystem
45 functions.

46 **Keywords:** CO₂ enrichment; Climate change; Chlorophyll fluorescence; Gas exchange;
47 Halophyte; Temperature stress.

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1. Introduction

Climate change arises as one of the greatest challenges for worldwide ecosystems conservation. Thus, along with recognized atmospheric CO₂ enrichment to values ~700 ppm by the end of the century (IPCC, 2014), climate models predict a parallel increase in daily temperature range (between 1.8 - 6.0 °C, depending on the region) and rainfalls pattern alteration which would lead to a decrease in water availability and soil salinization (IPCC, 2014). An important part of the effect of climate change on ecosystems will be related to those variations triggered by environmental conditions in the structure and composition of plant species (Allen and Ort, 2001; Hooper et al., 2005; Short et al., 2016; Zhao et al., 2018), being this impact greater in those ecosystems dominated by few species, which also sustain key functions (such as the control of the fluxes of energy and matter) and contribute to functional diversity and species assemblages (Hooper et al., 2005).

The genus *Spartina* is integrated by grass species widely distributed on every continent except for Antarctica, being many of them frequently found in coastal salt marshes (Bortolus et al., 2019). It has been highlighted the importance of these species in salt marshes ecosystem functions, with a relevant role on coastal accretion and marsh creation, as well as the maintenance of ecosystem primary and secondary production (Bortolus et al., 2019). Among *Spartina* genus species, the European *Spartina maritima* (Curtis) Fernald is an important pioneer and ecosystem engineer in European salt marshes (Castellanos et al., 1994), playing also an important role for the maintenance and conservation of marsh ecosystem biodiversity (Curado et al., 2018). This species develops its populations mainly in the lowest parts of the marshes, acting as a primary colonist contributing to facilitate succession in marsh ecosystems (Castellano et al.,

1994, 1998; Castillo et al., 2008) and playing a key role in shoreline stabilization (Duarte et al. 2014). In addition, this species has demonstrated a high biotechnological potential as a tool for monitoring and phytoremediating metal polluted areas (Padinha et al., 2000; Mesa et al., 2015). Furthermore, many studies have been developed to understand the impact of environmental factors associated with future global change scenarios on the development of this important salt marsh species (Mateos-Naranjo et al., 2010a; Couto et al., 2014; Duarte et al., 2014). Thus, a certain effort has been made to assess the effect of atmospheric CO₂ enrichment on *S. maritima* development, having observed an improvement on plant growth and physiological performance under optimal and suboptimal salinity conditions, despite being a C₄ species (Mateos-Naranjo et al., 2010a). Besides, *S. maritima* has demonstrated the ability to maintain its photosynthetic activity even during prolonged submersion periods associated with the sea level rise due to global warming (Duarte et al., 2014). In addition, a rising temperature model has demonstrated an enhancement in this salt marsh species aboveground biomass (Couto et al., 2014), but it has been identified that an increase in the frequency and duration of high temperature events will lead to a decrease of its photo-biological fitness (Duarte et al., 2016). Despite these efforts, there is great uncertainty about the real effects of climate change on the conservation of this species, since most of those studies only have evaluated plant responses to one or two environmental stressors in combination. Therefore, a challenge exists in determining plant responses in a complex environmental matrix, such as the one predicted by climate models. Consequently, this study was designed and carried out to understand the influence of a complex environmental matrix, characterized by atmospheric CO₂ concentration, air temperature and medium saline concentration variations on *S. maritima* growth and development as well as key photosynthetic parameters. We

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98 hypothesized that, although a direct positive physiological impact of increased
99 atmospheric CO₂ concentration on *S. maritima* has been previously identified (Mateos-
100 Naranjo et al., 2010a), the co-occurrence of other stress factors such as temperature
101 pattern variation and medium salinization could trigger metabolic responses that could
102 jeopardize the development of this important salt marsh species and, consequently, the
103 maintenance of the ecosystem functions in which this plant is involved.

104

105 **2. Material and Methods**

106

107 *2.1. Plant material*

108 Clumps of *S. maritima* of 20 cm diameter with between 25-30 mature tillers were
109 collected in June of 2019 from a well-established population in a low-marsh site located
110 in the Odiel salt marshes (37°15'N, 6°58'O; SW Spain). Clumps were planted in
111 individual plastic pots (15 cm high × 18 cm diameter) using its own soil as a potting
112 substrate, and placed in a greenhouse under the follow controlled conditions: maximum
113 temperature between 21/25 °C, minimum temperature between 13/11 °C, 40-60%
114 relative humidity and natural daylight of 200 μmol m⁻² s⁻¹ as minimum and 1000 μmol
115 m⁻² s⁻¹ as maximum light flux). Pots were irrigated with tap water, and plants were kept
116 for a stabilization period of 7 days under the previously described conditions before the
117 experiment's onset.

118

119 *2.2. Experimental design*

120 At the beginning of the experiment, the number of tillers in each pot was
121 homogenized to 20 tillers completely developed and healthy in order to standardize our

122 samples before the experiment onset and to avoid any effect of tiller health and age in
123 our results. Then, pots were randomly assigned to eight different experimental blocks
124 with ten plants in each one, as follows: two concentrations of atmospheric CO₂ (400
125 ppm and 700 ppm CO₂) in combination with two ranges of ambient maximum and
126 minimum temperature (28/13 °C and 32/17 °C) and irrigation with two salinity
127 concentrations (0 and 171 mM NaCl) for 40 days. For the atmospheric CO₂
128 concentration and temperature range treatments, pots were placed in controlled-
129 environment chambers (Aralab/Fitoclima 18.000EH, Lisbon, Portugal), which were
130 programmed with alternating diurnal regime of 14 h of light and 10 h of darkness with
131 the specific maximum and minimum temperature range, light intensity of 300 μmol m⁻²
132 s⁻¹, 40–60% relative humidity and the specific atmospheric CO₂ concentration.
133 Atmospheric CO₂ concentrations in chambers were continuously monitored by CO₂
134 sensors and maintained by supplying pure CO₂ from a compressed gas cylinder (Air
135 liquide, B50 35K). Finally, NaCl concentrations were established by combining tap
136 water with appropriate amounts of NaCl. At the beginning of the experiment, the pots
137 were placed in plastic trays containing the appropriate NaCl solutions for each specific
138 salinity concentration treatment to a depth of 1 cm. In order to avoid changes of the
139 NaCl concentration caused by water evaporation, levels in the trays were monitored
140 continuously throughout the experimental period.

141

142 *2.3. Plant growth analysis*

143 At the beginning and at the end of the experiment, three and seven plants from each
144 specific treatment were harvested and divided into roots and tillers. Then, these biomass
145 fractions were oven dried at 60 °C for 48 h and weighed to obtain both initial and final

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146 dry biomass estimates. In addition, the number of dead tillers was recorded at the end of
147 the experiment.

148 The relative growth rate (RGR) of whole plants was calculated using the formula:

$$149 \text{ RGR} = (\ln B_f - \ln B_i) \cdot D^{-1} \text{ (g g}^{-1} \text{ day}^{-1}\text{)}$$

150 where B_f = final dry mass (the mean of the seven plants from each treatment at the
151 end of the experiment), B_i = initial dry mass (the mean of the three plants from each
152 treatment at the beginning of the experiment) and D = duration of experiment (days).

153

154 *2.4. Leaf gas exchange analysis*

155 At the end of the experiment, instantaneous leaf gas exchange measurements were
156 taken on fully developed expanded leaves ($n = 10$, ~~one in each plant per treatment and~~
157 ~~three extra randomly selected for each treatment~~) using an infrared gas analyzer in an
158 open system (LI-6400-XT, Li-COR Inc., NE., USA) equipped with a light leaf chamber
159 (Li-6400-02B, Li-Cor Inc.). Net photosynthesis rate (A_N), stomatal conductance (g_s) and
160 intercellular CO_2 concentration (C_i) were recorded under the following leaf chamber
161 settings: a photosynthetic photon flux density (PPFD) of $1000 \mu\text{mol photons m}^{-2} \text{s}^{-1}$
162 (with 15% blue light to maximize stomatal aperture), vapour pressure deficit of 2.0–3.0
163 kPa, $50 \pm 5\%$ relative humidity, CO_2 concentration surrounding the leaf (C_a) of 400 or
164 $700 \mu\text{mol CO}_2 \text{ mol}^{-1}$ air depending on the atmospheric CO_2 concentration treatment and
165 air temperature of $28 \text{ }^\circ\text{C}$ or $32 \text{ }^\circ\text{C}$ for plants grown at low and high temperature range
166 treatments, respectively. All measurements were made between 10:00 and 13:00 h
167 inserting the order between the different treatments to standardize samples, and before
168 to record each measurement, gas exchange was allowed to equilibrate for 120 s. Finally,

169 intrinsic water use efficiency ($iWUE$) was calculated as the ratio between A_N and g_s
170 [mmol (CO₂ assimilated) mol⁻¹ (H₂O transpired)].

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172 2.5. Chlorophyll fluorescence analysis

173 Two different fluorescence protocols were developed at the end of the experiment
174 in the same leaves of gas exchange analysis in order to test how the different
175 combinations of atmospheric CO₂ concentration, air temperature and medium salinity
176 concentration used in this study affect photosystem II (PSII) energy use efficiency.
177 Thus, the saturation pulse method was used to determine the maximum quantum
178 efficiency of PSII photochemistry (F_v/F_m) and quantum efficiency of PSII (Φ_{PSII} ; Genty
179 et al., 1989). As described by Schreiber et al. (1986), a 0.8 s saturating actinic light
180 pulse of 10000 $\mu\text{mol m}^{-2} \text{s}^{-1}$ was applied at midday (1400 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$) in
181 previously dark- and light-adapted leaves (n = 10, ~~one in each plant per treatment and~~
182 ~~three extra randomly selected for each treatment~~) for 30 min using a modulated
183 fluorimeter (FMS-2; Hansatech Instruments Ltd., UK). Using this information, the
184 electron transport rate (ETR) was calculated according to Krall and Edwards (1992).

185 On the other hand, the chlorophyll *a* fast kinetics, by the OJIP-test (or Kautsky
186 curves), which depicts the rate of reduction kinetics of various components of PSII, was
187 also measured in 30 min dark-adapted leaves (n = 7, one in each plant per treatment),
188 using the pre-programmed OJIP protocols of the FluorPen FP100 (Photo System
189 Instruments, Czech Republic). Moreover, absorbed (ABS/RC), trapped (TR/RC),
190 electron transport (ET/RC) and dissipated (DI/RC) energy fluxes per reaction center
191 derived from OJIP were calculated according to Strasser et al. (2004).

192

193 2.6. Statistical analysis

194 R software ver. 4.0.0 (R Core Team, 2020) was used to perform the statistical
195 analyses. Firstly, a multivariate statistical approach using a principal component
196 analysis (PCA) was performed to get an overview of the plant growth and physiological
197 performance in response to the different experimental treatments. Missing values in the
198 PCA dataset appeared as a result of the different number of measurements in each
199 variable. They were handled using the expectation-maximization (EM) algorithm in the
200 ‘missMDA’ R package (Josse and Husson, 2016). Secondly, a downscaling assessment
201 was carried out through generalized linear models (GLMs) to analyze the main and/or
202 [interactive-interaction](#) effects of atmospheric CO₂ concentration, maximum and
203 minimum temperature range and low and high NaCl concentration (as categorical
204 factors) on the growth and physiological parameters (as dependent variables) of *S.*
205 *maritima* plants. In case of significant results, multiple comparisons were analyzed by
206 *post hoc* LSD test (i.e. Fisher's Least Significant Difference). Before statistical analysis,
207 Kolmogorov-Smirnov and Levene tests were used to verify the assumptions of
208 normality and homogeneity of variances, respectively. [A summary of generalized linear
209 model \(GLM\) results is made available as supplementary material, including fitting
210 tests and confidence intervals \(Table A.1\).](#)

212 3. Results

213 3.1. Multivariate approach: global overview of *S. maritima* growth and physiological 214 status

215 Growth and physiological performance variations of *S. maritima* during the
216 experimental setup were mainly represented in the first two PCA axes. They explained,
217 respectively, 36.0% and 18.4% of the total variation in the recorded data, being both
218 temperature range and atmospheric CO₂ enrichment the experimental factors which led

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219 the main groupings (Fig. 1A, B; Table 1). Hence, the PC1 axis reflected a clear
220 divergence of plants grown at 32/17 °C of maximum and minimum temperature range,
221 which were located in the left part of the plot (especially those grown at 700 ppm CO₂).
222 This separation was mainly explained by the lower RGR, A_N, F_v/F_m, Φ_{PSII} and ETR
223 values, together with an increment in the percentage of dead tillers and DI/RC in those
224 plants compared to the rest of the treatments (Fig. 1B; Table 1). In addition, the PCA
225 revealed a certain divergence between both atmospheric CO₂ concentration treatments
226 along the PC2 axis, with most of non-CO₂ enriched plants located in the upper part,
227 being this response pattern mainly linked with higher g_s and lower C_i values of plants
228 grown at 400 ppm CO₂ (Fig. 1B; Table 1).

229
230 *3.2. Effects of atmospheric CO₂ enrichment, temperature augmentation and NaCl*
231 *concentration on S. maritima growth*

232 [A summary of generalized linear model \(GLM\) results is made available as](#)
233 [supplementary material, including fitting tests and confidence intervals \(Table A.1\).](#)

234 There was a significant effect of temperature range treatment on the RGR of *S.*
235 *maritima* after 40 days of treatment (GLM: Temp., $p < 0.01$; Table A.1). Additionally,
236 this effect was, to some extent, modulated by atmospheric CO₂ concentration treatment
237 (GLM: [CO₂] x Temp., $p < 0.05$; Table A.1). Thus, RGR decreased considerably in
238 plants grown under the highest maximum and minimum temperature range treatment
239 (i.e. 32/17 °C), and this effect was more acute in those exposed to 700 ppm CO₂
240 regardless of saline irrigation treatment (Fig. 2A).

241 Similarly, the percentage of dead tillers augmented with temperature and
242 atmospheric CO₂ enrichment regardless of saline irrigation treatment (GLM: Temp., $p <$

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243 0.01; [CO₂], $p < 0.05$; Table A.1), being this increase more pronounced in plants grown
244 at 700 ppm CO₂ and 32/17 °C compared to the rest of treatments, although no
245 significant differences were found (Fig. 2B).

247 3.3. Effects of atmospheric CO₂ enrichment, temperature augmentation and NaCl 248 concentration on photosynthetic apparatus performance

249 There was a significant effect of the environmental factors tested on leaf gas
250 exchange characteristics and PSII photochemical efficiency of *S. maritima* after 40 days
251 of exposure (GLM, $p < 0.05$). Thus, A_N values decreased in plants grown at 32/17 °C
252 (GLM: Temp., $p < 0.01$; Fig. 3A; Table A.1), and these values were overall lower at
253 700 ppm CO₂ although without significant differences. A very similar trend was
254 recorded for g_s in relation to temperature increment (GLM: Temp., $p < 0.01$) but, in
255 addition, g_s values were significantly lower in plants grown at 700 ppm CO₂ (GLM:
256 [CO₂], $p < 0.01$; Fig. 3B; Table A.1). Contrarily, C_i and iWUE values were significantly
257 higher in plants grown at 700 ppm CO₂ compared to their non-CO₂ enriched
258 counterparts, regardless of temperature and saline irrigation treatments (GLM: [CO₂], p
259 < 0.01 ; Fig. 3C, D; Table A.1).

260 Regarding photochemical parameters, F_v/F_m values were lower in plants subjected
261 to the highest minimum and maximum temperature range treatment at both salinity
262 irrigation conditions (GLM: Temp., $p < 0.01$), being this reduction also more
263 pronounced in plants grown at 700 ppm CO₂ (GLM: [CO₂] x Temp., $p < 0.05$; Table
264 A.1), and especially in those plants irrigated with 171 mM NaCl (Fig. 4A). Likewise,
265 minimum and maximum temperature augmentation led to a marked reduction in *S.*
266 *maritima* Φ_{PSII} values, although this overall effect was much more marked in those
267 plants exposed to high atmospheric CO₂ concentration and saline irrigation (GLM:

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268 [CO₂] x Temp., $p < 0.05$; Fig. 4B; Table A.1). A very similar response was detected for
269 ETR, with the lowest values recorded in plants subjected to 700 ppm CO₂, 32/17 °C and
270 irrigation with 171 mM NaCl (Fig. 4C).

271 Focusing on the chlorophyll *a* fast kinetics results, there were remarkable
272 differences in the shape of Kautsky curves between plants grown under the different
273 environmental factors after 40 days of treatments. Thus, overall fluorescence kinetics
274 values were lower in plants subjected to 700 ppm CO₂ and 32/17 °C, being this trend
275 also more pronounced in plants irrigated with 171 mM NaCl (Fig. 5). These Kautsky
276 curve shape divergence was also accompanied by marked differences in energy fluxes
277 through photosystems. Thus, ABS/RC was lower in plants grown at 700 ppm CO₂ and
278 28/13 °C, compared to those exposed to 400 ppm CO₂ (GLM: [CO₂] x Temp., $p < 0.01$;
279 Table A.1). Moreover, ABS/RC showed an increase in plants subjected to 700 ppm,
280 32/17 °C and irrigation with 171 mM NaCl compared to the rest of treatments (GLM:
281 [CO₂] x Temp. x [NaCl], $p < 0.05$; Fig. 6A; Table A.1). A very similar trend was
282 recorded for DI/RC (Fig. 6D). Oppositely, both ET/CS and TR/CS tended to decrease in
283 plants exposed to 700 ppm CO₂ compared with their non-CO₂ enriched counterparts
284 (GLM: [CO₂] , $p < 0.05$; Fig. 6B, C; Table A.1). However, this effect was more
285 pronounced for TR/CS in plants grown at 28/13 °C compared to those grown in a higher
286 temperature range (GLM: [CO₂] x Temp., $p < 0.01$; Fig. 6C; Table A.1).

287 288 **4. Discussion**

289 Gaining a mechanistic understanding of the effect of the main coexisting factors
290 linked with climate change, such as atmospheric CO₂ enrichment, temperature pattern
291 variations and medium salinization, on the growth and physiological performance of
292 key plant species in ecosystem functionality is crucial to obtain a more realistic view on

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293 how this global phenomenon influences ecosystems conservation and the development
294 of these plant communities (Bernacchi et al., 2013).

295 Our results demonstrated that atmospheric CO₂ enrichment, as a consequence of
296 climatic change, would not cause a substantial improvement on growth and
297 development of the important pioneer and salt marsh ecosystem engineer species *S.*
298 *maritima*. However, an increase up to 4 °C in the maximum and minimum daily
299 temperature range, as expected by the end of the century (IPCC, 2007), would entail a
300 negative effect on the development of this species, being this impact even more acute in
301 a CO₂ enriched atmosphere. Thus, our results revealed that *S. maritima* plants grown at
302 400 ppm atmospheric CO₂ concentration and 32/17 °C of maximum and minimum daily
303 temperature range showed a drastic growth reduction, measured as RGR, of ~39%
304 compared to their counterparts exposed to the colder temperature range. This reduction
305 was up to 64% in those plants also exposed to 700 ppm CO₂, regardless of the saline
306 irrigation treatment. The lack of salinity effects on plant growth, as well as for most of
307 the studied parameters, was somewhat expected since *S. maritima* is a halophyte species
308 that has demonstrated a high tolerance to a wide range of salinities (Adams and Bate,
309 1995; Castillo et al., 2008; Mateos-Naranjo et al., 2010a). However, it should be
310 highlighted that the absence of beneficial effects of atmospheric CO₂ enrichment on
311 plant development contrasts with the previous results obtained by Mateos-Naranjo et al.
312 (2010a) for this plant species, who observed that an increment of atmospheric CO₂
313 concentration to 700 ppm stimulated plant RGR ~40% through a salinity range between
314 0 and 510 mM NaCl. Similarly, our results also varied with respect to those shown by
315 other *Spartina* species, such as *S. densiflora*, which experienced an increase in biomass
316 production of 35% and 20%, respectively at 0 and 171 mM NaCl, compared to plants
317 grown at 400 ppm CO₂ (Mateos-Naranjo et al., 2010b). These differences could be

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318 related to the variation in the experimental conditions of each specific study. In this
319 study, plants were subjected to different environmental synergies growing in natural soil
320 whilst, in the aforementioned experiments, plants were grown in perlite. The use of
321 this substrate would allow the previous studies to assess the direct effect of atmospheric
322 CO₂ enrichment on plant performance avoiding the influence of other factors associated
323 to soil physicochemical properties, which could alter plant direct responses to
324 atmospheric CO₂ enrichment. However, we believe that the use of a natural soil-plant
325 complex, as we have done in this study, would allow a more realistic view of the real
326 impact of climate change factors on plant development. In fact, according to our results,
327 Curtis et al. (1989), in an experiment developed under field conditions using OTC
328 chambers, were unable to detect a response to atmospheric CO₂ enrichment in *Spartina*
329 *patens*. Furthermore, Rozema et al. (1991) obtained different results regarding the effect
330 of atmospheric CO₂ enrichment on *S. patens* performance, depending on growth
331 medium aeration level in plants grown in hydroponic conditions. To some degree, this
332 lack of response to atmospheric CO₂ enrichment is consistent with multitude of CO₂
333 enrichment experiments, which indicated that plants with C₄ photosynthetic
334 metabolism, as *S. maritima*, would not be as benefited by the air CO₂ fertilization effect
335 (Ghannoum et al., 2000). However, this overall response could be modified by
336 interactions with other co-occurring stress factors related to climate change, such as
337 temperature pattern variation or medium salinization (IPPC, 2014). Therefore, it has
338 been described that the effect of atmospheric CO₂ enrichment on plant development
339 could be neutral, positive through the improvement of plant tolerance against
340 environmental stress or negative by enhancing environmental stress deleterious effects,
341 being also these responses species-specific (Balfagón et al., 2019). In this respect, as we
342 previously indicated, the increment in temperature together with an atmospheric CO₂

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343 concentration enrichment would substantially reduce *S. maritima* growth. Besides, it is
344 important to emphasize that this lower plant development was accompanied by an
345 increase in senescence level, as reflected by the higher percentage of dead stems at the
346 end of the experiment.

347 The deleterious effect of increased temperatures and, to a greater extent, CO₂-
348 enriched atmosphere conditions on *S. maritima* development was partly explained by
349 the negative impact of these factors on some essential steps of plant photosynthetic
350 apparatus performance. Thus, our results showed a substantial reduction in plant carbon
351 fixation capacity, measured as A_N, at elevated temperature range treatment being this
352 reduction in certain degree more pronounced at elevated atmospheric CO₂
353 concentration. This effect was associated with an augmentation in CO₂ diffusion
354 limitations through stoma in plants grown at 32/17 °C, as indicated their lower g_s values.
355 Temperature is one of the most variable environmental factors and it can affect many
356 plant physiological processes, including plant gas exchange characteristics, but little is
357 known about its effect on g_s, especially at high temperatures (Teskey et al., 2015). In
358 fact, several studies have recorded a wide range of plant responses to temperature
359 increment, including stomal opening (Lu et al., 2000; Mott and Peak, 2010) and closure
360 (Weston and Bauerle, 2007; Lahr et al., 2015) but also non-significant responses
361 (Cerasoli et al., 2014; von Caemmerer and Evans, 2015). Moreover, stomatal behaviour
362 to temperature could be modified by interactions with other environmental factors
363 (Flexas et al., 2012). In this sense, there is a need to highlight that g_s reduction with
364 temperature increment was even more acute in those plants exposed to 700 ppm CO₂. In
365 addition, this reduction was greater than the one reported in A_N, contributing to overall
366 higher \dot{V} WUE values under rising atmospheric CO₂ conditions. Thus, this decoupling
367 between g_s and A_N in plants grown at 700 ppm CO₂ and 32/17 °C would reflect the

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368 higher stomatal sensitivity to CO₂ variations. In fact, different studies have shown that
369 one of the most consistent responses of C₄ plant species to atmospheric CO₂
370 concentration enrichment is a decrease in g_s (Ainsworth and Rogers, 2007). Robredo et
371 al. (2007) explained that C_i increase caused by the high CO₂ concentration could
372 promote partial closure of the stoma, as it was recorded in this study. However, the
373 higher stomatal sensitivity to elevated CO₂ regardless of temperature, could have had
374 also important consequences for plant tolerance to heat stress. Thus, although stomatal
375 behaviour is a key factor to preserve the trade-off between CO₂ acquisition for
376 photosynthetic processes and water losses, it is necessary to emphasize its role for plant
377 adaptation to thermal stress through the regulation of heat excess by increasing leaf
378 evapotranspiration (Feller, 2006). Therefore, the importance of stoma in leaf cooling has
379 been identified, and while the stomata remains open, the evaporative cooling can
380 mitigate the negative effect of temperature excess, positively affecting plant
381 photosynthesis, yield and survival (Lu et al., 1994; Ameye et al., 2012). Therefore, the
382 higher g_s drop in *S. maritima* plants under elevated atmospheric CO₂ concentration
383 could have been limiting their ability to modulate leaf temperature compared to plants
384 grown at 400 ppm, which could also contribute to explain the greater injury effect of
385 temperature increment at these experimental conditions.

386 On the other hand, the impact of temperature increase, alone or in combination with
387 atmospheric CO₂ enrichment, had a great impact on *S. maritima* photochemical
388 apparatus functionality. According to our results, one of the best recognized effects of
389 high temperature on plant photosynthetic apparatus is the destruction of PSII
390 components (Flexas et al., 2012; Pérez-Romero et al., 2019a; López-Jurado et al.,
391 2020). Likewise, in concordance with this study, Mateos-Naranjo et al. (2010b) and
392 Duarte et al. (2014) also found that CO₂ enrichment affected PSII photochemistry in the

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393 halophytes *S. densiflora* and *S. maritima*, respectively. However, this is the first study
394 that corroborates that this effect would be exacerbated with an increase up to 4 °C in the
395 maximum and minimum daily temperature range and, to some extent, with the
396 augmentation in medium salinity concentration. Thus, our results showed that F_v/F_m and
397 Φ_{PSII} values were affected by the elevated temperature range and this effect was more
398 acute in plants grown at 700 ppm CO₂ and irrigated with 171 mM NaCl, suggesting that
399 the combination of these environmental conditions would increase photoinhibition
400 (Werner et al., 2002). Likewise, we attributed the negative impact of these
401 environmental factors on *S. maritima* photochemical apparatus to the down-regulation
402 of its electron transport chain functionality, as indicated by the lower ETR values
403 recorded in plants grown at 700 ppm CO₂, 32/17 °C and irrigated with 171 mM NaCl.
404 This fact indicated that these plants would have had more difficulties to transform the
405 captured energy in their photosystems, suggesting the synergistic impact of elevated
406 temperature and atmospheric CO₂ enrichment on the functionality of reaction centers.
407 Thus, this idea was also supported by the assessment of energy fluxes per reaction
408 center derived from Kautsky curves, which showed that, although plants were exposed
409 to elevated atmospheric CO₂ and air temperature range, they increased their ABS/RC
410 values compared to the rest of treatments. This would mean a greater number of active
411 reaction centers functioning as a heat radiator, hence protecting plant photosystems
412 against high temperature and light intensities (Strasser et al., 2004; Pérez-Romero et al.,
413 2018, 2019a,b). This elevated absorption rate would exceed the capacity of
414 photosystems to metabolize energy, as was corroborated by the decoupling with TR/RC
415 and ET/RC values. Moreover, the high ABS/RC would unbalance the trade-off between
416 the energy absorbed and metabolized, being necessary the activation of some defense
417 mechanisms to dissipate the energy excess as heat, as indicated also higher DI/RC

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418 values recorded. As a consequence, much of the absorbed energy would not take the
419 photochemical pathway (Flexas et al., 2012), a circumstance that would affect
420 photosynthetic productivity and, consequently, plant growth, a fact which would
421 contribute to explain the observed growth pattern in response to tested environmental
422 factors. Therefore, our results would suggest that temperature and atmospheric CO₂
423 increments related to climate change would jeopardize the development and
424 maintenance of natural populations of *S. maritima*. This circumstance would cause
425 significant structural and functional changes in the drainage system of the marshes
426 systems dominated by this grass species, due to its importance in shoreline stabilization
427 and salt marsh accretion during the first phases of ecological succession, with the
428 consequent alteration of sediment, mineral elements and nutrient cycles, as well as the
429 fluxes of energy necessary for other levels of the trophic chain in the ecosystem.

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432 **5. Conclusion**

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434 This study reveals the high vulnerability of the salt marsh key species *S. maritima*
435 to increases up to 4 °C in the maximum and minimum daily temperature, as predicted
436 for future scenarios by global change models, being this susceptibility even higher
437 under CO₂ enriched atmosphere conditions. This idea would be motivated by drastic
438 plant growth decrease and leaf senescence increment recorded at 700 ppm CO₂ and
439 32/17 °C of maximum and minimum daily temperature range. This response was
440 associated with the negative effect of temperature and atmospheric CO₂ concentration
441 on plant carbon fixation machinery through increased stomatal limitations to CO₂

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442 diffusion and the unbalance between the energy absorbed and metabolized in *S.*
443 *maritima* photosystems, linked with a down-regulation of the electron transport chain
444 functionality. The higher g_s drop at 700 ppm CO₂ would also limit plant ability to
445 regulate heat excess through leaf evapotranspiration, a fact that could have boosted
446 temperature-triggered damage and, consequently, leaf senescence compared to plants
447 grown at 400 ppm. Finally, from a conservational perspective, these results suggest that
448 temperature increment and atmospheric CO₂ enrichment associated with future climate
449 change scenarios would jeopardize the development of *S. maritima* populations and,
450 consequently, the maintenance of ecosystem functions derived from the presence of this
451 important pioneer and ecosystem engineer salt marshes plant species.
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461 **CRedit authorship contribution statement**

462 **Enrique Mateos-Naranjo:** Conceptualization, Methodology, Formal analysis, Funding
463 acquisition, Writing - original draft, Writing - review & editing. **Javier López Jurado:**
464 Methodology, Formal analysis, Writing - review & editing. **Jennifer Mesa Marín:**
465 Methodology, Writing - review & editing. **Carlos Javier Luque:** Conceptualization,
466 Methodology, Writing - review & editing. **Eloy Manuel Castellanos:**
467 Conceptualization, Methodology, Writing - review & editing. **Jesús Alberto Pérez-**
468 **Romero:** Methodology. **Susana Redondo-Gómez:** Conceptualization, Methodology,
469 Funding acquisition, Writing - review & editing.

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472 **Declaration of Competing Interest**

473 The authors declare that they have no known competing financial interests or personal
474 relationships that could have appeared to influence the work reported in this paper.

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486 **Figure legends**

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3 487 **Fig. 1.** Representation of the principal component analysis (PCA) biplot obtained for
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5 488 the growth and physiological data of *Spartina maritima* plants in the experimental set-
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8 489 up. Panel A represents the distribution of the selected variables loading on these axes
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10 490 and the percentage of explained variance for these axes (see Table 1 for a description of
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12 491 these variables and their contribution to the two main axes of PCA). Panel B shows the
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14 492 ellipses (95% confidence level) which encompass occurrence points in the two main
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16 493 axes for the environmental factors tested.
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21 494 **Fig. 2.** Relative growth rate, RGR (A) and percentage of dead tillers (B) in *Spartina*
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23 495 *maritima* plants in response to treatment with two atmospheric CO₂ concentrations (400
24
25 496 ppm and 700 ppm) in combination with two temperature ranges (28/13 °C and 32/17 °C)
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27 497 and irrigation with 0 mM NaCl (-) or 171 mM NaCl (+) for 40 days. Values represent
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29 498 mean ± SE, n = 7. [CO₂], Temp., NaCl or their interactions in the corner of the panels
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31 499 indicate main or interaction significant effects (GLM test, *P < 0.05, **P < 0.01).
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36 500 **Fig. 3.** Net photosynthetic rate, A_N (A), stomatal conductance, g_s (B), intercellular CO₂
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38 501 concentration, C_i (C) and intrinsic water use efficiency (iWUE) in randomly selected
39
40 502 fully developed expanded leaves of *Spartina maritima* in response to treatment with two
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42 503 atmospheric CO₂ concentrations (400 ppm and 700 ppm) in combination with two
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44 504 temperature ranges (28/13 °C and 32/17 °C) and irrigation with 0 mM NaCl (-) or 171
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46 505 mM NaCl (+) for 40 days. Values represent mean ± SE, n = 10. [CO₂], Temp., NaCl or
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48 506 their interactions in the corner of the panels indicate main or interaction significant
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50 507 effects (GLM test, *P < 0.05, **P < 0.01).
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56 508 **Fig. 4.** Maximum quantum efficiency of PSII photochemistry, F_v/F_m (A), quantum
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58 509 efficiency of PSII, Φ_{PSII} (B) and electron transport rate, ETR (C), at midday in randomly
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510 selected fully developed expanded leaves of *Spartina maritima* in response to treatment
511 with two atmospheric CO₂ concentrations (400 ppm and 700 ppm) in combination with
512 two temperature ranges (28/13 °C and 32/17 °C) and irrigation with 0 mM NaCl (-) or
513 171 mM NaCl (+) for 40 days. Values represent mean ± SE, n = 10. [CO₂], Temp.,
514 NaCl or their interactions in the corner of the panels indicate main or interaction
515 significant effects (GLM test, *P < 0.05, **P < 0.01).

516 **Fig. 5.** Transient fluorescence (Kautsky curves) of dark-adapted leaves of *Spartina*
517 *maritima* plants grown at 400 ppm (A) and 700 ppm (B) atmospheric CO₂ in
518 combination with two temperature ranges (28/13 °C and 32/17 °C) and irrigation with 0
519 mM NaCl (-) or 171 mM NaCl (+) for 40 days. Values represent mean of seven
520 measurements per treatment combination.

521 **Fig. 6.** Absorbed energy flux, ABS/RC (A), electron transport energy flux, ET/RC (B),
522 trapped energy flux, TR/RC (C) and dissipated energy flux, DI/RC (D), per reaction
523 centre in randomly selected fully developed expanded dark-adapted leaves of *Spartina*
524 *maritima* in response to treatment with two atmospheric CO₂ concentrations (400 ppm
525 and 700 ppm) in combination with two temperature ranges (28/13 °C and 32/17 °C) and
526 irrigation with 0 mM NaCl (-) or 171 mM NaCl (+) for 40 days. Values represent mean
527 ± SE, n = 7. [CO₂], Temp., NaCl or their interactions in the corner of the panels indicate
528 main or interaction significant effects (GLM test, *P < 0.05, **P < 0.01).

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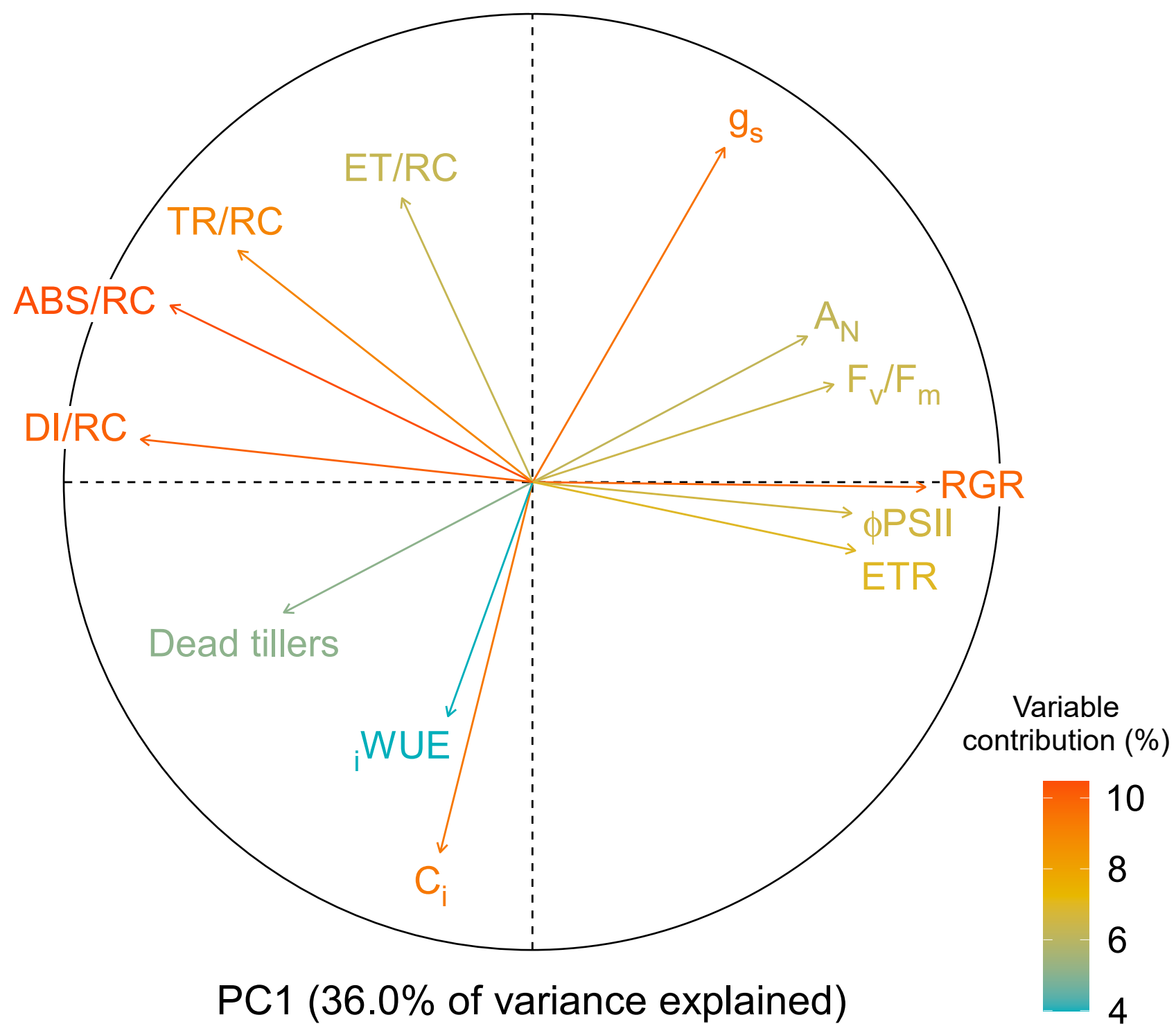
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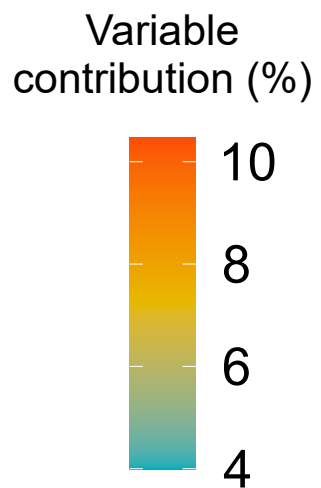
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Figure 1
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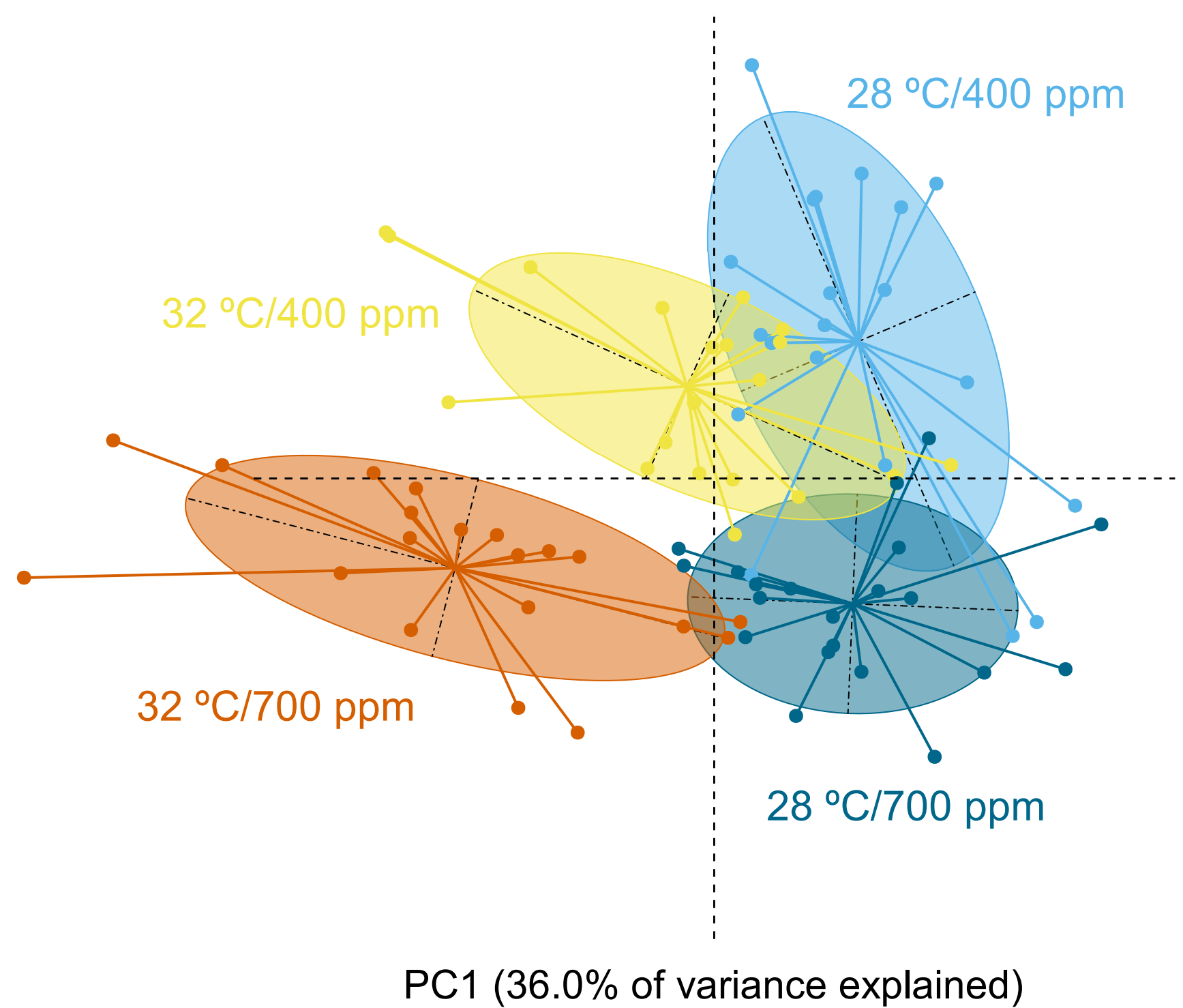
PC2 (18.4% of variance explained)



PC1 (36.0% of variance explained)

**B**

PC2 (18.4% of variance explained)



PC1 (36.0% of variance explained)

Fig. 2

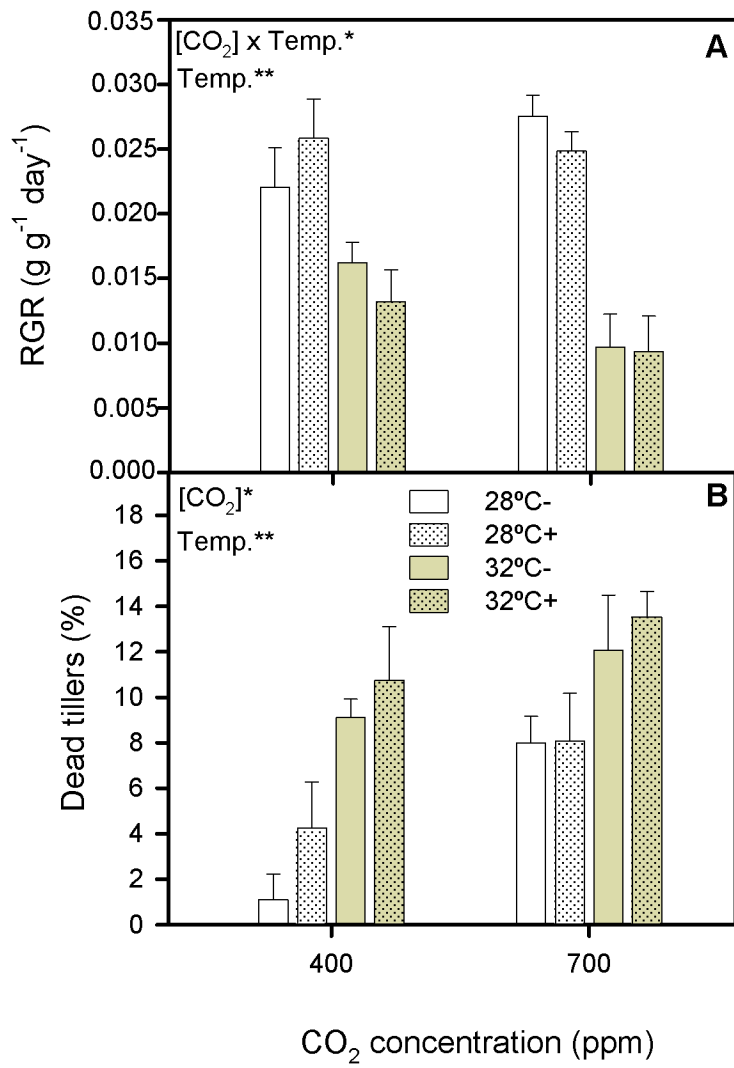


Fig. 3

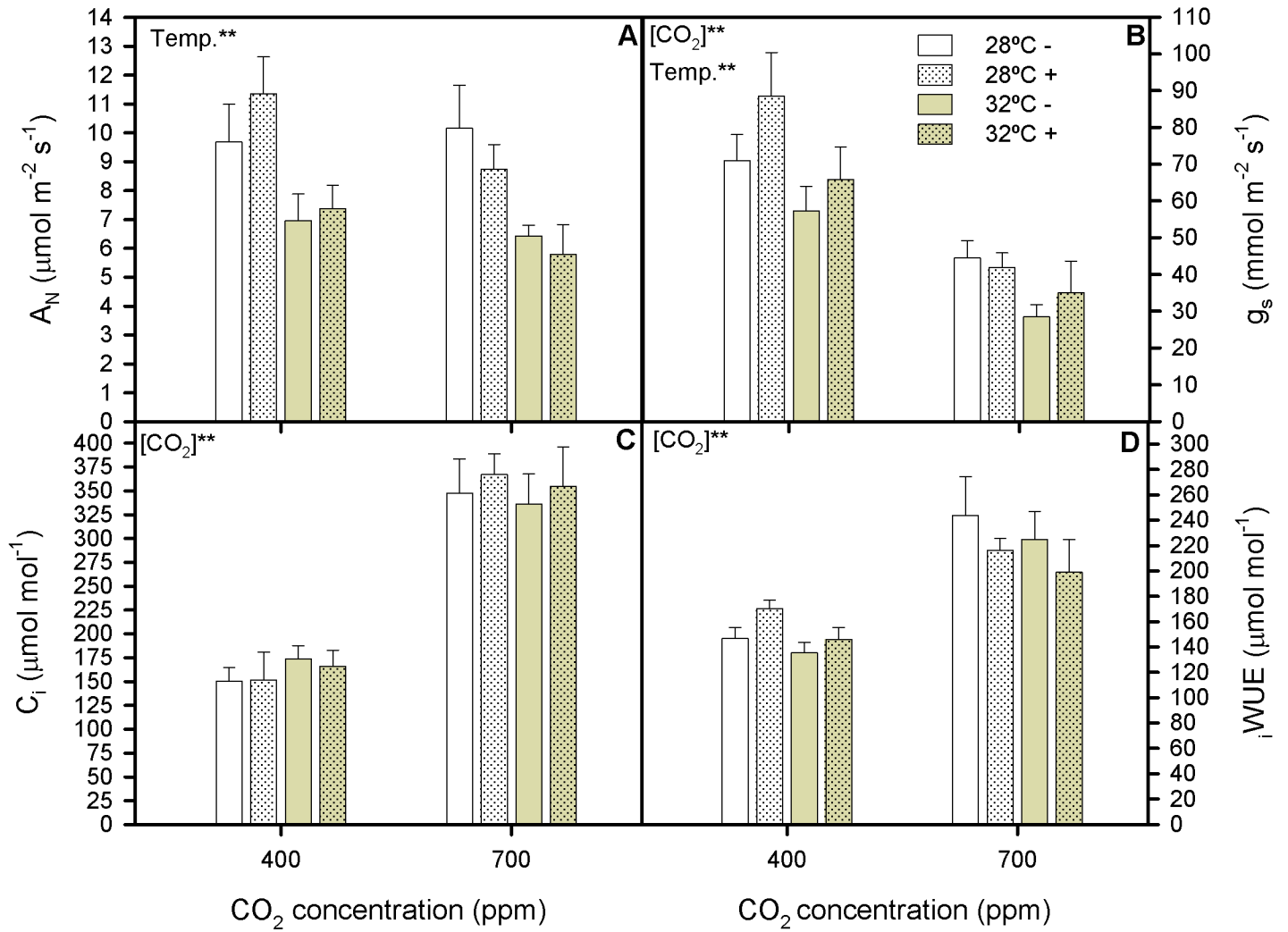


Fig. 4

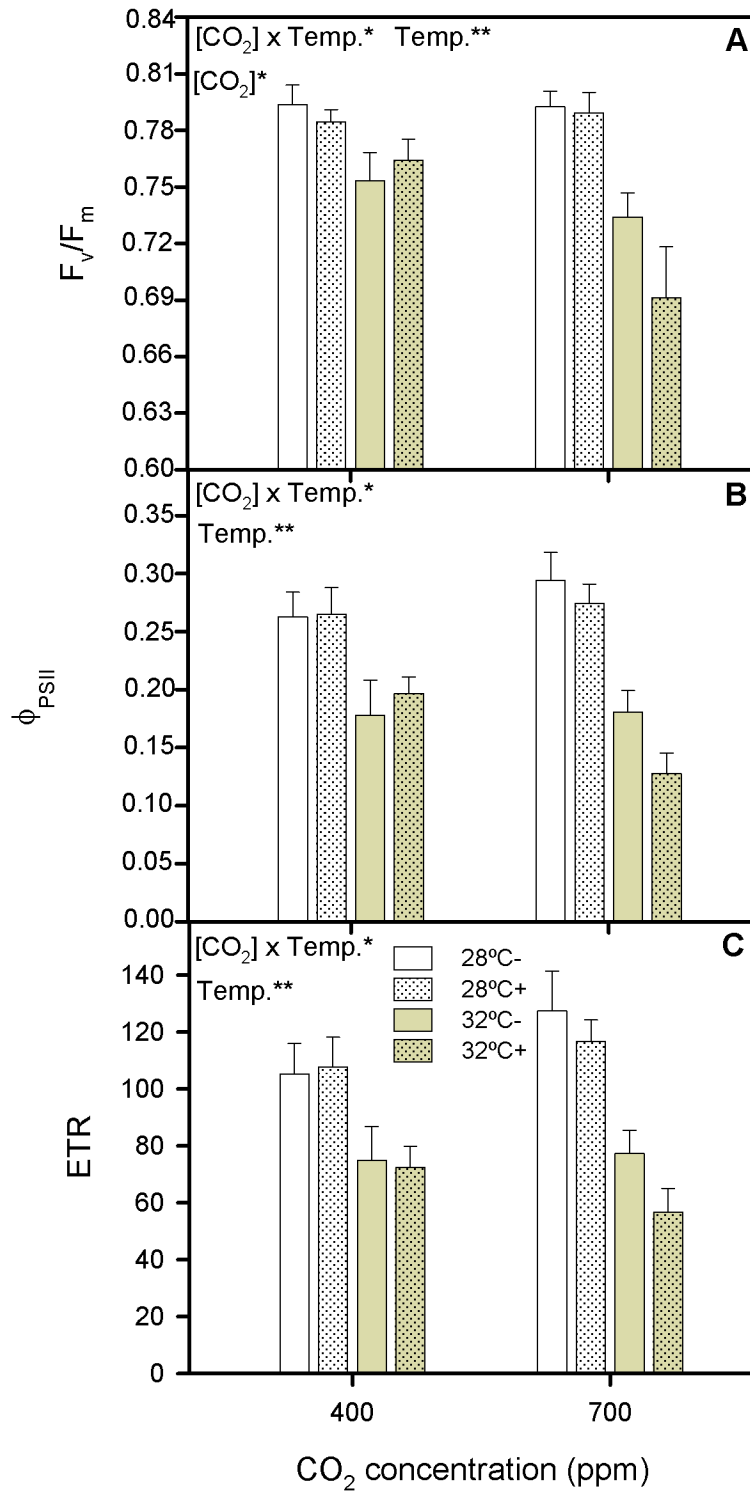


Fig. 5

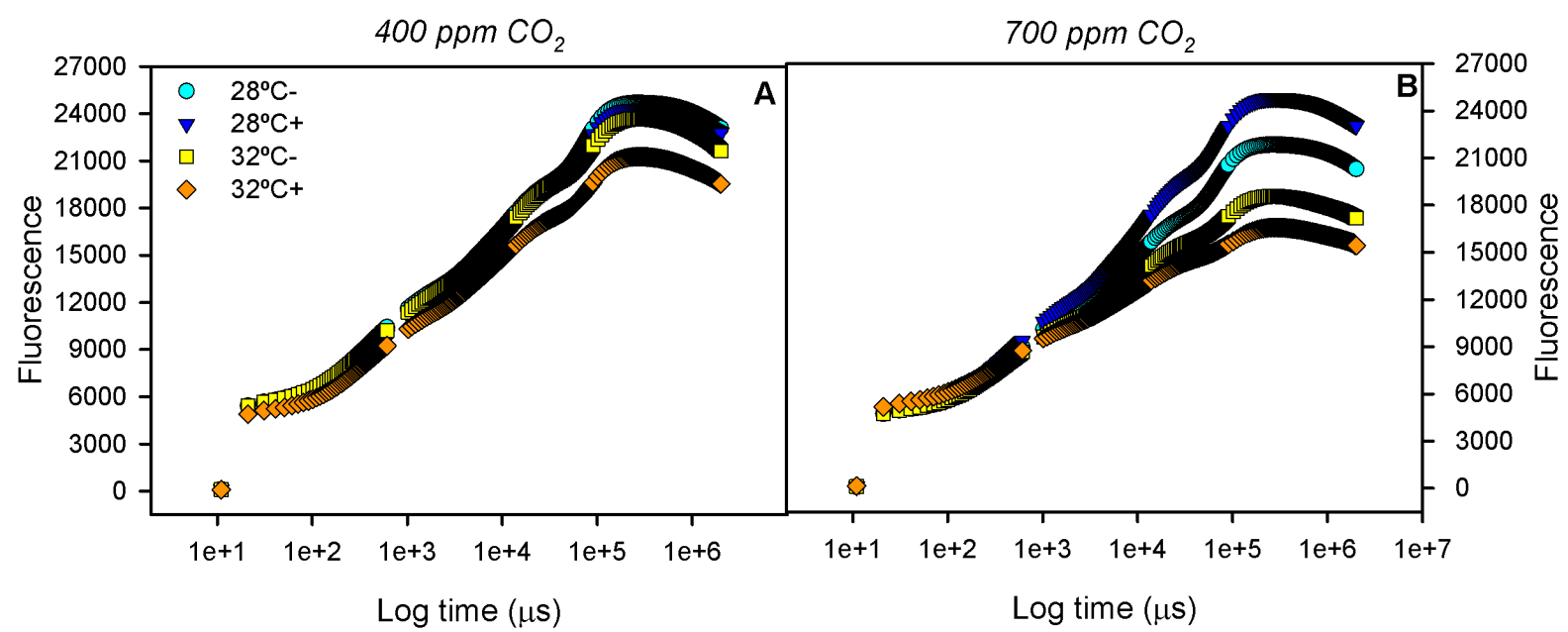
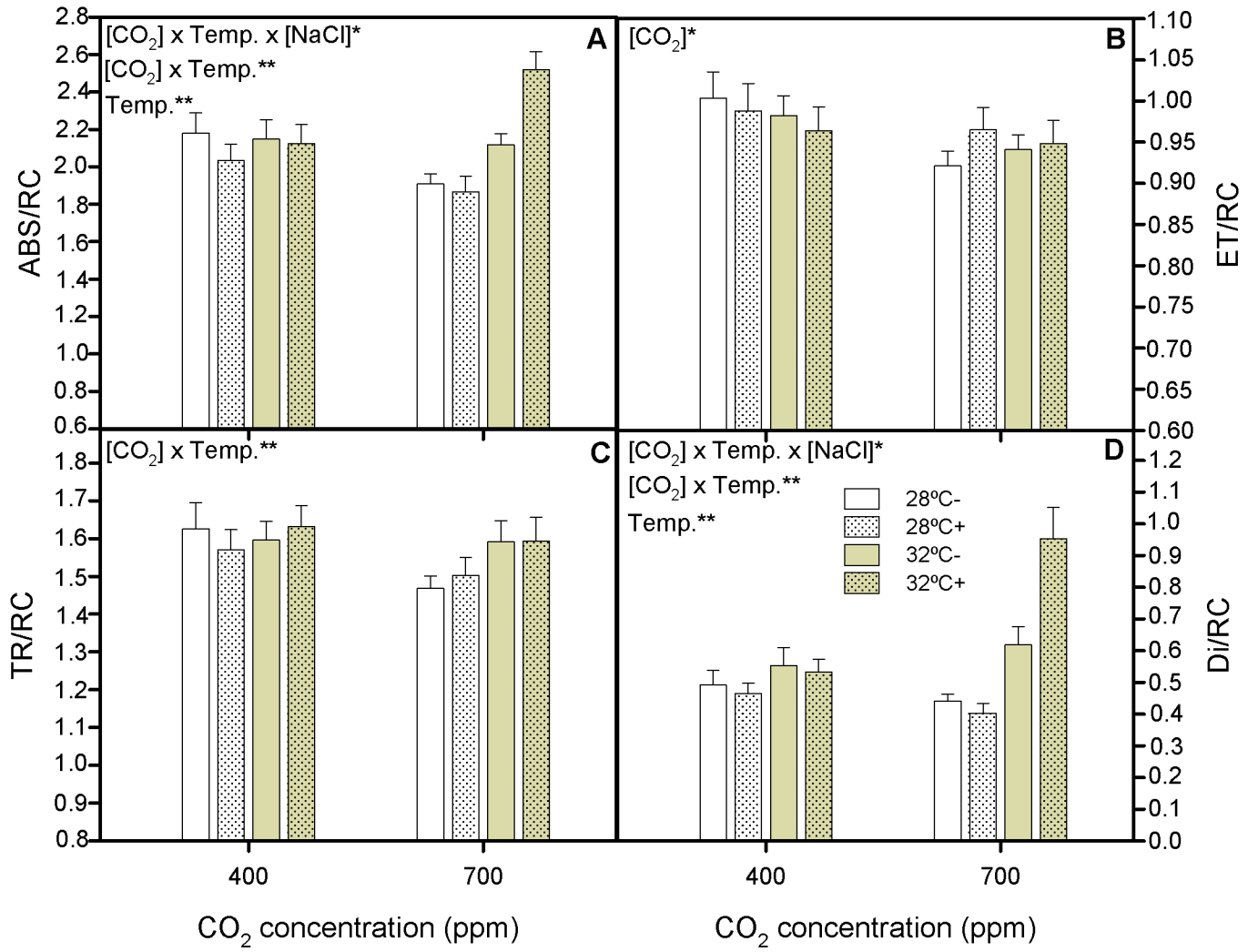


Fig. 6





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Table

Table 1.docx





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Table

Table A.1.docx



Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRedit authorship contribution statement

Enrique Mateos-Naranjo: Conceptualization, Methodology, Formal analysis, Funding acquisition, Writing - original draft, Writing - review & editing. **Javier López Jurado:** Methodology, Formal analysis, Writing - review & editing. **Jennifer Mesa Marín:** Methodology, Writing - review & editing. **Carlos Javier Luque:** Conceptualization, Methodology, Writing - review & editing. **Eloy Manuel Castellanos:** Conceptualization, Methodology, Writing - review & editing. **Jesús Alberto Pérez-Romero:** Methodology. **Susana Redondo-Gómez:** Conceptualization, Methodology, Funding acquisition, Writing - review & editing.