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Does Ocean Acidification Benefit Seagrasses in a Mesohaline Environment? A Mesocosm Experiment in the Northern Gulf of Mexico

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1 **Title:** Does ocean acidification benefit seagrasses in a mesohaline environment? A mesocosm
2 experiment in the northern Gulf of Mexico.

3 **Running page head:** Ocean acidification effects on seagrasses in NGoM

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22 **Abstract:**

23 Ocean acidification is thought to benefit seagrasses because of increased carbon dioxide
24 (CO₂) availability for photosynthesis. However, in order to truly assess ecological responses,
25 effects of ocean acidification need to be investigated in a variety of coastal environments. We
26 tested the hypothesis that ocean acidification would benefit seagrasses in the northern Gulf of
27 Mexico, where the seagrasses *Halodule wrightii* and *Ruppia maritima* coexist in a fluctuating
28 environment. To evaluate if benefits of ocean acidification could alter seagrass bed composition,
29 cores of *H. wrightii* and *R. maritima* were placed alone or in combination into aquaria and
30 maintained in an outdoor mesocosm. Half of the aquaria were exposed to either ambient (mean
31 pH of 8.1 ± 0.04 SD on total scale) or high CO₂ (mean pH 7.7 ± 0.05 SD on total scale)
32 conditions. After 54 days of experimental exposure, the $\delta^{13}\text{C}$ values were significantly lower in
33 seagrass tissue in the high CO₂ condition. This integration of a different carbon source (either:
34 preferential use of CO₂, gas from cylinder, or both) indicates that plants were not solely relying
35 on stored energy reserves for growth. Yet, after 41 to 54 days, seagrass morphology, biomass,
36 photo-physiology, metabolism, and carbon and nitrogen content in the high CO₂ condition did
37 not differ from those at ambient. There was also no indication of differences in traits between the
38 homo- or hetero- specific beds. Findings support two plausible conclusions: 1) these seagrasses
39 rely heavily on bicarbonate use and growth will not be stimulated by near future acidification
40 conditions or 2) the mesohaline environment limited the beneficial impacts of increased CO₂
41 availability.

42 **Key words:** Carbon dioxide, pH, productivity, seagrass species interactions

43 **Introduction**

44 The increase in atmospheric CO₂ since the industrial revolution has altered the
45 equilibrium of inorganic carbon compounds in the ocean, increasing the concentrations of
46 bicarbonate (HCO₃⁻), carbonic acid (H₂CO₃), and hydrogen ions (H⁺) (Elderfield et al. 2005).
47 These changes, referred to as ocean acidification, have caused the average sea surface pH to drop
48 by 0.1 units, and the pH is projected to further decline by 0.06-0.32 units by the end of this
49 century (IPCC, 2013). Ocean acidification is known to impact species physiologies and lead to
50 cascading effects at the ecosystem level (Hall-Spencer et al. 2008).

51 Seagrass beds are highly productive (Duarte and Cebrián 1996) and they provide refuge
52 for many marine organisms (Hemminga and Duarte 2000). In addition, seagrasses play an
53 important ecological role in coastal waters as carbon sinks (Duarte et al. 2010; Russell et al.
54 2013). Seagrass are expected to benefit from ocean acidification because they are carbon limited
55 at present dissolved inorganic carbon (DIC) levels (Koch et al. 2013). Indeed, previous reports
56 have shown increases in seagrass productivity (Durako 1993; Zimmerman et al. 1997; Invers et
57 al. 2002), vegetative growth (Jiang et al. 2010; Russell et al. 2013; Martínez-Crego et al. 2014;
58 Campbell and Fourqurean 2018), carbohydrate storage (Campbell and Fourqurean 2013b) and
59 flowering frequency (Palacios and Zimmerman 2007) under lowered pH conditions.

60 Coastal environments, however, are highly dynamic in terms of fluctuating light,
61 nutrients, and salinity, particularly in mesohaline estuaries. Estuaries commonly receive
62 freshwater inputs that change the chemical and physical properties of the seawater (Aufdenkampe
63 et al. 2011). High biological activity, often fueled by nutrient inputs and hydrodynamic processes
64 in shallow areas, can result in highly variable pH and CO₂ environments. Many estuarine
65 organisms already experience diurnal incremental changes in pH outside of those predicted for

66 the open ocean within the next century (Duarte et al. 2013). As a result, the decrease in pH by
67 ocean acidification could be similar to which naturally occurs in these estuarine habitats and
68 subsequently it may not alter the usual development of estuarine organisms (Frieder et al. 2014;
69 Pacella et al. 2018). On the other hand, future climate conditions will intensify changes in pH and
70 this may act on organism physiology (Hofmann et al. 2011; Waldbusser and Salisbury 2014).

71 Ocean acidification also has the potential to shift interactions, such as competitive
72 strengths, between species (Connell et al. 2013; Russell et al. 2013; Takeshita et al. 2015). Due to
73 inter-specific differences in HCO_3^- utilization efficiency, the response to lowered pH levels varies
74 considerably among seagrass species (Invers et al. 2001; Campbell and Fourqurean 2013a).
75 Species which rely less on CO_2 and have efficient HCO_3^- use should be less sensitive to altered
76 future carbonate chemistry and thus benefit less from ocean acidification (Koch et al. 2013).
77 Seagrasses also have different carbon allocation strategies, which further suggests differential
78 growth responses to elevated partial pressure of CO_2 ($p\text{CO}_2$; Ow et al. 2015). Some seagrass
79 species invest more in below ground tissue (i.e. *Enhalus acoroides*; Duarte and Chiscano 1999),
80 other ephemeral seagrasses have short leaf turnover (i.e. *Halodule wrightii* and *Ruppia maritima*;
81 Gallegos et al. 1994; Dunton 1990), while other long-lived species such as *Posidonia oceanica*
82 have longer shoot plastochrone intervals (Duarte and Chiscano 1999; Kilminster et al. 2015).
83 These differences in turnover of carbon could alter their carbon demand (see discussion in Ow et
84 al. 2015). Additionally, in terrestrial communities, the direct positive effects of elevated CO_2 for
85 plant species are at times outweighed by negative effects due to stimulation of the growth of
86 other plant competitors (Poorter and Navas 2003). Indeed, differences in seagrass species
87 composition have been observed near a CO_2 volcanic vent; species with large blade-like leaves

88 dominated and presumably kept the smaller successional species from benefitting (Takeshita et
89 al. 2015). Despite these observations, there have been few investigations on the differential
90 impacts of ocean acidification on co-habiting seagrass species, and how such impacts affect
91 species composition and structure.

92 *Halodule wrightii* Asch. and *Ruppia maritima* L. are widespread seagrasses that co-exist
93 in heterospecific beds in mesohaline estuaries of the north-central Gulf of Mexico. These species
94 have short growth cycles and different seasonal peaks in biomass. *Halodule wrightii* grows
95 throughout the year and typically reaches maximum biomass in late summer-early fall. *Halodule*
96 *wrightii* also allocates a larger fraction of total biomass to roots and rhizomes compared to *R.*
97 *maritima* (Dunton 1990; Anton et al. 2009). *Ruppia maritima* grows during cool temperatures
98 and undergoes senescence after flowering in spring (Pulich 1985; Cho and Poirrier 2005; Anton
99 et al. 2009). Even though *H. wrightii* and *R. maritima* provide similar ecosystem services
100 (Christiaen et al. 2016), elevated $p\text{CO}_2$ conditions may stimulate production to change the
101 services they provide (e.g. refuge ability, production). Furthermore, acidification could act to
102 alter the ability for them to coexist. Under environmental stress, *R. maritima* can outcompete *H.*
103 *wrightii* (Christiaen et al. 2016). Both seagrasses may increase their productivity under elevated
104 $p\text{CO}_2$, but *R. maritima* production is known to be carbon saturated in some settings (Sand-Jensen
105 and Gordon 1984; Koch et al. 2013; Campbell and Fourqurean 2013a). Due to higher richness of
106 species, mixed seagrass beds are expected to attract more associated fauna, to be more productive
107 and to have a broader range of tolerance to environmental conditions than monospecific beds
108 (Duffy et al. 2006; Gustafsson and Boström 2011, 2013). Despite so, it has been little examined
109 how elevated $p\text{CO}_2$ can alter the biomass of *H. wrightii* and *R. maritima* in heterospecific

110 seagrass beds formed in the Gulf of Mexico. Since these seagrasses can alter their cycle of
111 development with changes in environmental condition (Cho et al. 2008), this knowledge is
112 essential for the persistence of mixed seagrass beds and any ecological benefits heterospecific
113 beds may provide.

114 The objectives of this study are to (1) evaluate the effects of ocean acidification on the
115 productivity and vegetative growth of seagrasses in the mesohaline waters of the northern Gulf of
116 Mexico and to (2) test for potential shifts in composition of *H. wrightii* and *R. maritima* resulting
117 from an increase in CO₂ availability. To do this, cores of *H. wrightii* and *R. maritima* were placed
118 alone (homospecific beds) or side by side, in combination (heterospecific beds), into aquaria and
119 maintained in an outdoor mesocosm under ambient and elevated *p*CO₂ (low pH) conditions for
120 up to five weeks. Afterwards, the morphology and biomass, photo-physiology, chemical
121 composition, and metabolism of the seagrasses were measured. We hypothesized that enhanced
122 CO₂ availability would stimulate photosynthesis and benefit growth and production. We also
123 hypothesized that the stimulation of seagrass productivity would alter the composition of *H.*
124 *wrightii* and *R. maritima* beds. It is important to note that we were not directly testing
125 competition between seagrass species per se, albeit competition may be happening at the fringing
126 interface between patches, but rather we are testing whether any differences in CO₂ stimulated
127 growth cause densities or biomass to shift through stimulating the productivity of one species
128 more than the other, or through differences in their carbon allocation. Additionally, *Halodule*
129 *wrightii* and *R. maritima* were not replanted to form a mixed interspersed bed, with presumably
130 more interspecific interactions, because this distribution pattern would not represent the ecology

131 observed in the area. Seagrasses were observed growing in discrete bordering patches in the
132 natural setting.

133 **Methods**

134 **Seagrass bed collection**

135 Sixty rectangular cores of seagrass beds (10 x 4 cm; 4 cm deep) were collected from
136 single species patches of *H. wrightii* and *R. maritima* from approximately 1 m depth in Point-
137 aux-Pins, Bayou la Batre (30°23'4.26"N, 88°18'42.73"W northern Gulf of Mexico, Alabama,
138 USA) on 27th February 2017. In the field, cores were introduced into 30 aquaria (21 x 13 x 13
139 cm) in pairs, such that there were 10 aquaria with two cores of *H. wrightii*, 10 aquaria with two
140 cores of *R. maritima*, and 10 aquaria with a core of *H. wrightii* and a core of *R. maritima*. We
141 butted the cores against each other to simulate homospecific beds of either species as well as the
142 fringing area between adjacent beds of *H. wrightii* and *R. maritima*. The aquaria filled with cores
143 were immediately brought back to Dauphin Island Sea Lab and kept in an outdoor experimental
144 setup for seventy days (16 days of acclimation, 54 days of experimental manipulation, with final
145 measures taken after at least 4.9 weeks of different CO₂ exposure, Fig. 1). The experiment was
146 concluded on May 8, 2017, after 54 days of CO₂ exposure. This period of time, from Feb. 27
147 May 8, was selected because these seagrass species have short shoot turnovers (few months) and
148 increase their growth in spring (Pulich 1985; Dunton 1990; Hemminga and Duarte 2000;
149 Kilminster et al. 2015).

150 **Experimental setup**

151 Two aquaria of each seagrass bed type (*Halodule-Halodule*, HH; *Ruppia-Ruppia*, RR;
152 and heterospecific, *Halodule-Ruppia*, HR) were randomly assigned to five experimental blocks in

153 an outdoor flow through system (Fig. 1). Then, one of the two aquaria for each type within the
154 block was assigned to the ambient CO₂ treatment (natural *p*CO₂/ pH), and the other to the high
155 CO₂ treatment (high *p*CO₂/ low pH). Aquaria were arranged randomly within each block and
156 covered with screen to prevent excess light stress (Fig.1; Cebrian et al. 2013). Seawater was
157 pumped from the bay (1 m depth) into header tanks, from where it was channeled into the aquaria
158 to overflow into surrounding water bath and released back into the bay. There were two header
159 tanks per block, one for the ambient CO₂ aquaria and another for the high CO₂ aquaria, for 10
160 headers tanks in total and each tank feeding three aquaria (Fig. 1). The residence time of the
161 seawater in each aquarium was approximately 30 minutes. The experiment had six treatments
162 resulting from the crossing between seagrass beds types and CO₂ levels (i.e. HH/ambient;
163 HH/high; RR/ambient; RR/high; HR/ambient; and HR/high), with five replicates per treatment.
164 However, due to system failure and human error, replicate aquaria were reduced for some
165 treatments.

166 A pH stat system (*IKS Aquastar Germany*) was used to control bubbling of CO₂ from a
167 gas cylinder into the header tanks for the high CO₂ aquaria. For each block, the header tank
168 bubbled with CO₂ was chosen at random from the two.

169 Environmental conditions in the aquaria were constantly monitored. Water temperature
170 was logged by HOBO pendants using 1 logger per block (HOBO Onset Computer Corporation,
171 Bourne, MA, USA). Surface photosynthetic active radiation (PAR) was downloaded from an
172 environmental station maintained by the Dauphin Island Sea Lab (30°15.075'N, 88°04.670'W
173 Dauphin Island, Alabama, USA; <http://cf.disl.org/mondata/mainmenu.cfm>) located within 0.1
174 miles from the outdoor flow-through system. Point measurements of salinity were obtained

175 throughout the study duration using a hand-held YSI-85 conductivity probe (YSI, Yellow
176 Springs, Ohio, USA).

177 pH was monitored in aquarium and header tanks with an inLab Routine Pro calibrated
178 glass electrode (Mettler Toledo, Ohio, USA). The pH was measured on the total scale (pH_T)
179 using certified reference material provided by A. Dickson (Batch 30). Using this method, pH_T
180 was measured in aquaria approximately every three days. In addition to measuring pH_T and total
181 alkalinity (A_T) in header tanks, water samples (120 ml) were collected approximately once per
182 week and at the same hour of the morning. These water samples were collected from one of the
183 ambient and high CO_2 treatment header tanks chosen at random. pH was also 'spot' checked
184 (data not reported) with loggers and discrete measures at different hours in header tanks and
185 aquaria to make certain that the offsets between experimental treatments were maintained.
186 Samples for A_T were filtered on combusted glass microfiber filters membranes and immediately
187 inoculated with 72 μl of 33% saturated mercuric chloride solution (HgCl_2) and stored until
188 analyzed. A standard provided by A. Dickson (Batch 157) was used to check precision and
189 accuracy (A_T , 3.9 and 0.1 $\mu\text{mol kg}^{-1}$, respectively; $n = 7$). The carbonate chemistry was assessed
190 using pH_T , A_T , salinity and temperature using the R package "seacarb" (Gattuso et al. 2018).

191 The $p\text{CO}_2$ in the ambient treatment was $\sim 350 \mu\text{atm}$, which corresponded to the value
192 found in local coastal waters. In the "high CO_2 " treatment was applied a $p\text{CO}_2$ of $\sim 1244 \mu\text{atm}$ or a
193 pH offset of approximately -0.3 to -0.4 to mimic the maximum pH decrease expected by the end
194 of this century based on IPCC scenario for 2100 (IPCC, 2013).

195 **Morphology and biomass**

196 Shoot density was determined during the acclimation period (day 2), and after 54 days of
197 exposure to experimental conditions. Shoot density was measured for each core, and the two
198 cores representing the same species were averaged for homospecific aquaria. On day 2 of the
199 acclimation period and after 54 days of CO₂ perturbation, we haphazardly selected five shoots
200 from each core in each aquarium. We counted the leaves on the shoots and measured the length
201 of each leaf on the shoot. With these measurements, we calculated shoot height (average leaf
202 length per shoot), leaf number per shoot, and summed the length of the leaf material per shoot.
203 Then we calculated the average for the ten shoots in homospecific aquaria or the average of five
204 shoots of each species in heterospecific aquaria. In combination, these measurements allowed us
205 to infer whether, as a response to enhanced CO₂, shoots grew existing leaves longer, produced
206 shorter and younger leaves, or a combination of both. For instance, the average number of leaves
207 per shoot may not change, but shoots may show longer leaves (increased shoot height) and larger
208 total leaf material, indicating shoots elongate their existing leaves, but do not produce more new
209 leaves under enhanced CO₂. In contrast, higher number of leaves per shoot in combination with
210 shorter shoot height and larger total leaf material per shoot would indicate a response to enhanced
211 CO₂ centered in the production of new leaves.

212 Plant biomass was only measured at the end of the study (54 days of CO₂ exposure) due
213 to destructive sampling. Sediment was carefully rinsed off above-ground (leaves and vertical
214 rhizomes) and below-ground materials (roots and horizontal rhizomes) in distilled water and
215 epiphytes were carefully scraped off their surfaces. Above- and below-ground materials were
216 separated, dried at 60°C, and the dry weight (DW) determined. Above-ground biomass contained

217 parts of the plant exposed to light and the below ground biomass contained parts of the plant that
218 were buried in the sediment.

219 **Photo-physiology**

220 Photo-physiological measurements (dark- and light- adapted yield and rapid light curves)
221 were done with a diving-pulse amplitude modulated fluorometer (diving-PAM, Waltz, Germany)
222 11 days into the acclimation period and after 43 days of exposure to experimental conditions. To
223 take the measurements, the leaves were placed side by side on the Waltz dark-adapted fiberoptic
224 clip, so that the initial F' value would read above 400. For dark-adapted yield measurements,
225 leaves were placed in the dark for five minutes prior to exposure to a saturating light pulse. The
226 same leaf location was used for light-adapted measures which were collected after allowing the
227 leaves to acclimate to light conditions for 10 minutes. We used the same leaf location for both
228 measures to minimize stress or damage to leaves. All measures were collected in one day, from
229 mid-morning to late afternoon. To account for the changing environmental conditions over this
230 time period, all fluorescence measures were collected randomly within a block (1 replicate of
231 each condition in a block) before proceeding to the next block. Fluorescence measures for each
232 block were completed within a 1.5 – 2 h window. Because all replicates in both experimental
233 conditions were handled similarly and given the same period of relaxation and excitation, we
234 were able to make direct comparisons of results.

235 The intensity and width of the saturation pulse was adjusted to ensure a distinct plateau of
236 maximum quantum yield at a set distance from the blade. Namely, for all samples a saturation
237 intensity setting of 1 with a width of 0.8 was used in the initial measurements, and an intensity of
238 2 and a width of 0.8 in the final measurements (Genty et al. 1989).

239 The irradiances for rapid light curves (RLCs) were each applied for 10 s followed by a
240 saturating pulse of 0.8. Irradiances ranged between 0 to 1700 $\mu\text{mol m}^{-2} \text{s}^{-1}$ and were corrected for
241 battery decline using the standard function in the WinControl software. Thus, irradiances at each
242 increasing light step from 0 were as follows: 11 –14, 49 – 67, 134 – 178, 255 – 332, 411 – 539,
243 593 – 786, 924 – 1227 and 855 – 1563 $\mu\text{mol m}^{-2} \text{s}^{-1}$. The absorption factor needed to calculate
244 RLCs parameters was determined using the methods described in Beer and Björk (2000) and
245 averaged to 0.84. The $r\text{ETR}$ values were plotted against the light irradiances to produce a curve
246 fitting the exponential model proposed by Platt et al. (1980). Derived parameters of RLCs include
247 photosynthetic efficiency (α), dynamic photoinhibition parameter (β), relative electron transport
248 rate maximum ($r\text{ETR}_{\text{max}}$) and the minimum saturation irradiance (E_K), which were all calculated
249 following Ralph and Gademann (2005).

250 To better interpret the photo-physiological experiments, we also measured leaf
251 chlorophyll *a* (Chl *a*) content, but only at the end of the experiment (54 days of exposure to
252 experimental conditions) due to the destructive nature of this sampling. To do this, we
253 haphazardly selected one shoot from each core (two shoots of the same species in the
254 homospecific aquaria, and one shoot of each species in the heterospecific aquaria) and clipped the
255 upper 5 cm section of the middle leaf on the shoot. Chlorophyll was extracted from that section in
256 the dark in 90% acetone for 24 h, and the extract measured in a fluorometer (Model TD-700
257 Turner Designs, California, USA, Welschmeyer 1994). The two values of Chl *a* content from the
258 same species in homospecific aquaria were averaged to avoid pseudo-replication.

259 **Metabolism**

260 Net community productivity (NCP), and respiration rates were determined from the
261 change in dissolved oxygen content during two-hour incubations using clear (for NCP) or dark
262 (for respiration) chambers (10.2 x 5.7 x 5 cm) placed onto both cores in each aquarium.
263 Measurements were done 7 days after collection and after 48 days of experimental exposure. At
264 each sampling time, one clear and one dark chamber were placed at the exact same location on
265 the core (i.e. the location of the chambers was marked in the first deployment and repeated for the
266 second). Incubations were performed on clear days (mean PAR of 880 $\mu\text{mol photons m}^2 \text{s}^{-1}$ in the
267 first incubation and 1150 $\mu\text{mol photons m}^2 \text{s}^{-1}$ in the final incubation). Dissolved oxygen content
268 was measured with a Portable Meter Hach connected to probe with an optical sensor (HQ30d,
269 Hach, Loveland, Colorado, USA; accuracy of 0.1 mg/L over a range of 0 to 8 mg/L and precision
270 $\pm 0.5\%$ of accuracy range). Rates of NCP and respiration were derived, and rates of gross primary
271 productivity (GCP) from those rates, as explained in Cebrian et al. 2009. The two values of GCP
272 were averaged in the homospecific aquaria to avoid pseudo-replication.

273 **Chemical composition**

274 At the end of the experiment (after 54 days of experimental conditions), $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$
275 values and carbon (C) and nitrogen (N) content were analyzed in the below- and above-ground
276 tissue. Dried plant tissue (previously prepared for biomass determination) was ground, weighed,
277 and subsequently measured at the stable isotope facility at the University of California, Davis
278 using an elemental analyzer (Elementar Analysensysteme GmbH, Hanau, Germany) interfaced to
279 a continuous flow isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK). Isotope values
280 are reported in standard d-notation relative to an international standard (V-PDB and air for

281 carbon and N, respectively). Glycine reference compounds with well-characterized isotopic
282 compositions were used to ensure accuracy of all isotope measurements.

283 **Data analysis**

284 Two-way ANOVAs were used to test for differences in environmental variables in the
285 header tanks (T, S, pH_T , A_T , pCO_2 , C_T , Ω_A and Ω_C); “ph treatment” and “time” were used as fixed
286 factors. The parameters measured on seagrasses were also analyzed with two-way ANOVA
287 separately for each species with seagrass bed type and pH treatment as fixed factors for data
288 obtained at the end of the experiment to test for CO_2 effects. Tukey’s multiple comparison tests
289 were used to examine pairwise differences. Comparisons were additionally done for data
290 obtained during the acclimation period to ensure homogeneous conditions among treatments
291 before starting the CO_2 application (Supplementary Table 1). Prior to analyses, data were tested
292 for normality using the Shapiro test and for homogeneity of variance using the Bartlett’s test, and
293 transformed when necessary to comply with the assumptions of ANOVA. The statistical α was
294 adjusted to < 0.01 in order to account for the many comparisons and avoid false positives
295 (Benjamini and Hochberg 1995). For the same reason, the statistical α was adjusted to < 0.005 for
296 four parameters which could not be transformed to meet parametric requirements (Underwood
297 1997). All results are expressed as mean \pm standard error (SE) throughout this manuscript unless
298 otherwise stated.

299 **Results**

300 **Environmental conditions**

301 pH_T , pCO_2 , and total DIC (C_T) significantly differed between the ambient and high CO_2
302 header tanks (Supplementary Table 3). The pH_T in the header tanks during the experimental

303 period varied from 8 – 8.4 in the ambient treatment and of 7.2 – 8.0 in the high CO₂ treatment
304 (Table 1). In ambient header tanks, *p*CO₂ and total DIC (*C*_T) ranged from 118.8 to 426.6 μatm
305 and from 1268 to 1686 μmol kg⁻¹, respectively; while in the high CO₂ header tanks values ranged
306 from 342.4 to 2910.4 μatm and from 1504 to 2001 μmol kg⁻¹. Levels of *A*_T in the header tanks
307 did not differ between treatments but, they significantly fluctuated during the experimental period
308 (Table 1, Supplementary Table 3). In the ambient treatment header tanks, *A*_T ranged from 1443.7
309 to 1835.9 μmol kg⁻¹ and from 1543.7 to 2069.9 μmol kg⁻¹ in the high CO₂ treatment header tanks
310 (Table 1). The fluctuation was related to changes in salinity. As salinity decreased the levels of
311 *A*_T also decreased in a linear manner; perhaps this relationship is due to the dilution of weathering
312 products. Salinity and temperature in the header tanks significantly varied through time, but not
313 between treatments (Supplementary Table 3). The seawater in the ambient treatment was
314 saturated with respect to both aragonite and calcite. In the high CO₂ treatment calcite and
315 aragonite were under saturation most of the time, except after the storms on the 20th of March and
316 28th of April (Table 1). Furthermore, levels of seawater saturation also differed between
317 treatments.

318 The environment variables in the aquaria reflected those of the header tanks (Fig. 2). The
319 mean (± SD) temperature logged by HOBO pendants was 23.0 ± 0.6°C, ranging from 13.6 to
320 31.8°C (Supplementary Table 2). Salinity in aquaria over the duration of the study ranged from
321 4.3 to 30.7 (Fig. 2, Supplementary Table 1). During daylight hours of the study, mean PAR (±
322 SD) was 774.3 ± 3.4 μmol photons m⁻²s⁻¹ and ranged from 10.0 as a minimum in morning and in
323 twilight hours to a maximum of 2123.3 μmol photons m⁻² s⁻¹ at the peak of a sunny day.

324 The pH_T in aquaria was variable in both, ambient and high CO_2 treatment, but the range
325 of pH_T difference between the treatments was maintained between -0.29 to -0.44 along the
326 experimental period (Fig. 2). Under the ambient treatment the pH_T in aquaria averaged (\pm SD)
327 8.09 ± 0.04 , while in the high CO_2 treatment it was 7.70 ± 0.05 (Fig. 2). The pH_T offset from
328 ambient was similar between the three seagrass habitat types (HH, HR and RR), showing an
329 average pH_T offset of -0.39 ± 0.08 (Fig. 2).

330 **Morphology and biomass**

331 After 54 days of pH manipulation, shoot and leaf development of *H. wrightii* and *R.*
332 *maritima* did not appear to be affected by elevated pCO_2 and plants also did not differ in
333 morphology when grown in homo- or hetero-specific beds (Table 2, Figs. 3 and 4). Over the
334 course of the experiment, in *H. wrightii* cores means (\pm SE) of: shoot density per core (from 27.6
335 ± 2.0 to $35.1 \pm 2.$), leaf number per shoot (from 2.4 ± 0.1 to 2.8 ± 0.1), and total leaf material
336 (from 13.0 ± 0.7 to 20.9 ± 1.3 cm) increased. The mean (\pm SE) shoot density of *R. maritima* per
337 core was 34.9 ± 3.1 at the initial assessment and was 31.3 ± 3.6 at the final assessment. Over the
338 course of the experiment, the means (\pm SE) of: leaves per shoot (from 2.8 ± 0.1 to 3.3 ± 0.1), total
339 leaf material (from 12.4 ± 0.5 to 22.2 ± 1.0 cm) and average shoot height (from 4.6 ± 0.2 to $6.5 \pm$
340 0.22 cm) increased.

341 The above-ground biomass was not significantly affected by pCO_2 and nor by co-
342 occurrence of other seagrass species (Table 2). Above-ground biomass was 0.38 ± 0.04 g DW in
343 *H. wrightii* and 0.21 ± 0.04 g DW in *R. maritima*. The allocation of biomass to below-ground also
344 did not differ for seagrasses grown in homo- or hetero- specific beds and for seagrasses at the two

345 pH treatments (Table 2). The below-ground biomass for *H. wrightii* and *R. maritima* at the end of
346 the experiment was 0.34 ± 0.08 and 0.16 ± 0.07 g DW, respectively (Table 2, Figs. 3 and 4).

347 **Photo-physiology**

348 The parameters derived from the rapid light curves of *H. wrightii* did not differ between
349 ambient and elevated $p\text{CO}_2$ exposure and did not differ with bed type (Table 2, Fig. 5). For
350 example, the derived α for *H. wrightii* was 0.29 ± 0.01 and 0.30 ± 0.01 electrons/photons in
351 homo-specific aquaria and 0.32 ± 0.01 and 0.32 ± 0.01 electrons/photons in hetero-specific
352 aquaria after exposure to ambient and elevated $p\text{CO}_2$ conditions, respectively. Furthermore, mean
353 $r\text{ETR}_{\text{max}}$, E_K , and β values did not significantly differ (Table 2) among bed type and $p\text{CO}_2$
354 condition for *H. wrightii* (mean \pm SD: $r\text{ETR}_{\text{max}}$ from 99.1 ± 10.9 to 108.3 ± 21.6 $\mu\text{mol electrons}$
355 $\text{m}^{-2} \text{s}^{-1}$, E_K from 308.8 ± 34.5 to 356.4 ± 54.5 $\mu\text{mol photon m}^{-2}\text{s}^{-1}$, and β from 98.5 ± 8.4 to 105.9
356 ± 5.6 electrons/photons).

357 After 43 days of pH manipulation, the parameters derived from the rapid light curves of
358 *R. maritima* also did not differ between ambient and elevated $p\text{CO}_2$ exposure and did not differ
359 with bed type (Table 2, Fig. 5). This result is evident in the curves (Fig. 5) with the similar range
360 of derived values of α , $r\text{ETR}_{\text{max}}$, and E_K regardless of growing condition (mean \pm SD: α from 0.29
361 ± 0.02 to 0.32 ± 0.02 electrons/photons, $r\text{ETR}_{\text{max}}$ from 103.8 ± 23.4 to 111.9 ± 11.1 μmol
362 $\text{electrons m}^{-2} \text{s}^{-1}$, E_K from 325.2 ± 89.6 to 377.5 ± 84.7 $\mu\text{mol photon m}^{-2} \text{s}^{-1}$). Similar to
363 observations for *H. wrightii*, there was a trend of greater photoinhibition for *R. maritima* plants
364 within the ambient CO_2 , heterospecific bed condition when compared to the other treatments.
365 This trend also occurred in the initial period (Supplementary Figure 2d), but it was not

366 statistically significant at $\alpha < 0.05$ nor when the statistical α was adjusted to 0.01 for the many
367 comparisons (Table 2, β ranged from 95.9 ± 11.9 to 103.7 ± 14.0 electrons/photons).

368 For both species, dark- and light-adapted yields did not differ with bed type nor $p\text{CO}_2$
369 condition. *H. wrightii* plants yielded 0.74 ± 0.01 after the dark acclimation and 0.70 ± 0.03 in the
370 light. *R. maritima* plants yielded 0.76 ± 0.02 and 0.69 ± 0.02 after dark and light acclimation,
371 respectively.

372 Leaf Chl *a* content was not affected by $p\text{CO}_2$ nor by seagrass bed type (Table 2). The
373 average of leaf Chl *a* content was 0.011 ± 0.002 and 0.010 ± 0.002 mg cm^{-2} per leaf for *H.*
374 *wrightii* and *R. maritima*, respectively.

375 **Metabolism**

376 NCP, GCP, and respiration (in units of $\text{mg O}_2 \text{ m}^{-2} \text{ h}^{-1}$) did not statistically differ between
377 ambient and elevated $p\text{CO}_2$ condition for either species, and rates did not differ when plants were
378 grown in homo- or hetero- specific beds (Table 2, Fig. 6). It was noted that there was a lot of
379 variation in some metabolic measures at the end of the study, particularly for *H. wrightii* beds in
380 hetero-specific aquaria maintained under elevated $p\text{CO}_2$ conditions. In *H. wrightii* beds, the NCP
381 was 1.15 ± 0.24 , respiration was -0.86 ± 0.14 , and GCP was 2.01 ± 0.21 . In *R. maritima* beds the
382 NCP was 0.76 ± 0.19 , respiration was -1.08 ± 0.12 , and GCP was 1.84 ± 0.19 .

383 **Chemical composition**

384 The $\delta^{13}\text{C}$ values in above- and below- ground biomass of *H. wrightii* differed between the
385 high and ambient CO_2 treatment. The $\delta^{13}\text{C}$ values were significantly decreased in the plants
386 grown in the high CO_2 condition (-4.02 ± 0.07 ‰ in leaf and -2.59 ± 0.04 ‰ in root) than in the
387 plants which developed in the ambient treatment (-3.29 ± 0.07 ‰ in leaf and -2.29 ± 0.03 ‰ in

388 root; Table 2, Fig. 6). The $\delta^{13}\text{C}$ value of *R. maritima* above- and below- ground biomass were
389 also significantly decreased in the CO_2 than in the ambient treatment, showing $-3.87 \pm 0.06 \text{ ‰}$
390 (above-ground) and $-2.77 \pm 0.04 \text{ ‰}$ (below-ground) in the CO_2 treatment and $-3.32 \pm 0.05 \text{ ‰}$
391 (above-ground) and $-2.35 \pm 0.03 \text{ ‰}$ (below-ground) in the ambient treatment (Table 2, Fig. 6).
392 The $\delta^{15}\text{N}$ value and C and N contents in above- and below-ground biomass did not differ between
393 treatments, indicating a similar carbon and nitrogen investment regardless of pH treatment and
394 seagrass bed type (Table 2, Fig. 6).

395 **Discussion**

396 Seagrasses did not benefit from ocean acidification conditions and there were no observed
397 changes in seagrass bed composition during this study. This experimental duration (54 days in
398 March to May) captured a large portion of the peak growth period. The lower $\delta^{13}\text{C}$ values in
399 above and below ground tissues within the high CO_2 condition indicates plants were integrating a
400 different carbon source into their tissues and thus, they not solely relying on stored energy
401 reserves for growth. Nevertheless, we did not observe a difference in seagrass traits for plants
402 grown under high CO_2 conditions. Furthermore, there was no evidence of increased production
403 (using oxygen evolution, fluorescence, carbon content) needed for long-term carbon gains. This
404 outcome indicates that there is some complexity in seagrass response to increased CO_2 predicted
405 in the coming decades (Fig. 7).

406 **Response of seagrass morphology and biomass**

407 The absence of a response in seagrass morphology and biomass to ocean acidification
408 conditions are in contrast with those obtained in others studies where stimulation has resulted in
409 seagrass gains in productivity, above-ground development, root biomass, and non-structural

410 carbohydrates (Beer et al. 1977; Durako 1993; Hall-Spencer et al. 2008; Jiang et al. 2010;
411 Campbell and Fourqurean 2013b; Cox et al. 2015; Zimmerman et al. 2017). In contrast, other
412 studies support our findings and have found a neutral effect of ocean acidification on productivity
413 and/or biomass of some seagrass species (Burnell et al. 2014; Apostolaki et al. 2014; Cox et al.
414 2016; Campbell and Fourqurean 2018). This ‘lack of effect’ is often attributed to other
415 limitations or stressors in the seagrass environment. For example, the increased $p\text{CO}_2$ availability
416 for seagrass species did not counteract negative impacts of warming temperatures (Collier et al.
417 2018), limiting light (Hendriks et al. 2017), or heavy metals (Olivé et al. 2017). Other researchers
418 have underscored CO_2 availability as one abiotic factor of several limiting seagrass physiology
419 (Burnell et al. 2014; Cox et al. 2016; Schneider et al. 2017; Pajusalu et al. 2018). Furthermore,
420 outcomes may differ when the producer is held under constant or fluctuating pH (Britton et al.
421 2016).

422 **Efficient users of bicarbonate**

423 Another highly plausible reason for the lack of ocean acidification stimulation could be
424 related to the physiologies of *Halodule wrightii* and *Ruppia maritima*. Both species have
425 physiologies that rely heavily on bicarbonate use. For example, seagrass species of the genus
426 *Halodule* sp. was shown to be less sensitive to the increases of DIC than other tropical species
427 such as *Cymodocea serrulata* under high light conditions (Ow et al. 2015). Campbell and
428 Fourqurean (2013b) additionally showed that *Thalassia testudinum* increased photosynthesis by
429 100% from a pH of 8.2 to 7.4 while *H. wrightii* relied more on bicarbonate use with an increase
430 of 20% over the same pH range. In addition, the internal inorganic carbon concentrations of *R.*
431 *maritima* was close to saturation under natural conditions when the ratio of DIC to oxygen was

432 low and photorespiration occurred (Buapet et al. 2013; Koch et al. 2013). Lastly, in culture, *R.*
433 *maritima* had adequate growth on a bicarbonate media (Bird et al. 2016). Therefore, it appears
434 that these two species are not as sensitive to pH changes as some other seagrass species.

435 **Duration of study**

436 Discounting acclimation and adaptation, it is unlikely that there is a long-term benefit
437 from the high CO₂ condition on vegetative growth for these species that was not captured by our
438 experimental duration. *H. wrightii* and *R. maritima* have relatively short shoot turnover rates
439 where growth can be 2 to 4 mm per day (Dunton 1990). For instance, *Halodule wrightii* is able
440 translocate 14% of carbon from the leaves to the rhizome and roots in few hours (Moriarty et al.
441 1986). The short turnovers of these species appear to be specially marked in the estuarine waters
442 of the Gulf of Mexico where *R. maritima*, completes its growth cycle in four months after
443 flowering (Pulich 1985; Cho and Poirrier 2005). The experimental duration was during the period
444 of peak biomass for *R. maritima* and a portion of the growth period for *H. wrightii*. Initiation of
445 flowering by *R. maritima* and early flower stages were noted in homospecific and heterospecific
446 beds under ambient and high CO₂ conditions but, unlike effects reported for *Zostera marina*
447 (Palacios and Zimmerman 2007), the onset of flowering was not more frequent at either *p*CO₂
448 condition. *Halodule wrightii*, on the other hand, allocates more carbon in below ground tissue
449 (Anton et al. 2011), yet we did not find any statistically significant differences in biomass
450 allocation and we did not detect changes in nitrogen storage in the leaves or roots which could
451 indicate an early positive response to the high CO₂ levels.

452 The analysis of the stable carbon isotope composition of plants is a useful tool in
453 understanding physiological processes and the response of plants to varying environmental

454 conditions (Hemminga and Mateo, 1996). In our study, there was low $\delta^{13}\text{C}$ values measured in
455 above and below ground tissues for plants grown in the high CO_2 condition. Seagrasses
456 preferentially use CO_2 over HCO_3^- and atmospheric CO_2 is more depleted in ^{13}C (-9 ‰, Kroopnick
457 et al. 1985). Therefore, under ocean acidification conditions (higher $p\text{CO}_2$), we would expect
458 seagrasses to have lower $\delta^{13}\text{C}$ values. However, the isotope value of the gas from the cylinders (-
459 4.9 ‰ median measured from cylinders by Campbell and Fourqurean 2011) is also depleted in ^{13}C
460 and background measures of DIC were not measured. Therefore, we cannot rule out the influence
461 of the gas from the cylinder on $\delta^{13}\text{C}$ values. Nevertheless, the integration of a different carbon
462 source in tissues (i.e. different $\delta^{13}\text{C}$ from ambient) and the observed increase in mean biomass in
463 both conditions over the study duration allows us to conclude that the absence of positive effects
464 in the high $p\text{CO}_2$ condition are not likely due to reliance and growth resulting from stored
465 reserves.

466 **Other limitations and potential stressors**

467 Other limitations or stressors in the environment could be a factor contributing to our
468 results. The seagrass beds of *H. wrightii* and *R. maritima* were grown under highly variable
469 environmental conditions (See Fig. 2), which are typical of mesohaline estuarine habitats (Pulich
470 1985; Cho and Poirrier 2005; Anton et al. 2009). The northern central Gulf of Mexico has six
471 rivers that drain into it, thus it could be less suited for seagrass growth than in other estuarine
472 waters in determinate moments, especially after periods of heavy storms. For instance, during the
473 second month of the experiment, heavy rainfall in the study area resulted in seagrasses
474 experiencing a mean salinity of 16 with low salinity events persisting for several days. These
475 storms also increased water turbidity and caused the average salinity in the Bay to decrease from

476 17 to 7 psu, reaching a minimum of 3.8 (see Fig. 2). *Ruppia maritima* and *H. wrightii* are eury- to
477 mixo-haline species and thus, low salinity water outside their preferred range can slow
478 productivity and seawater below 6 can be lethal (Adair et al. 1994; Doering et al. 2002). These
479 seagrasses also seem to be negatively affected by high turbidity (Kantrud 1991; Dunton 1996;
480 Cho and Poirrier 2005). Therefore, the environmental changes in salinity and turbidity during our
481 experiment could limit the productivity and development of *H. wrightii* and *R. maritima*, counter
482 acting any positive effects of ocean acidification.

483 **Conclusions**

484 The outcome of this study (Fig. 7), in context with literature, leads to the speculation that
485 acidification in the next decade will not stimulate the vegetative growth of *H. wrightii* and *R.*
486 *maritima* to alter seagrass bed structure. The absence of positive effects on physiology and
487 growth may be related to the variable environmental conditions and, albeit not measured by this
488 study, the efficiency of these seagrasses to use HCO_3^- .

489 Although we did not find the increase in $p\text{CO}_2$ to stimulate vegetative growth for
490 seagrasses in the northern Gulf of Mexico, ocean acidification is known to positively affect the
491 physiology or growth of other seagrass species. Therefore, the responses of seagrass meadows to
492 ocean acidification appear to vary with seagrass species and their capacity to tolerate changes in
493 the environment. As climate change continues, it is necessary to integrate the influence of
494 environmental variability, as well as species interactions, for seagrass ecosystems to determine
495 their susceptibility to anthropogenic perturbations.

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738

739 **Tables**

740

Table 1. Environmental data and carbonate chemistry (as calculated from pH_T , A_T) in the header tanks of the ambient and high CO_2 treatments during the experimental period. Temperature (T, °C); salinity (S); pH on the total scale (pH_T); total alkalinity (A_T , $\mu\text{mol kg}^{-1}$); partial pressure of CO_2 ($p\text{CO}_2$, μatm); dissolved inorganic carbon (C_T , $\mu\text{mol kg}^{-1}$) and saturation states with respect to aragonite (Ω_A) and calcite (Ω_C). Error estimates can be found in Supplementary Table 4 and were generated using the precision around the standard (for A_T precision was $3.9 \mu\text{mol kg}^{-1}$, $n = 7$) together with the error for probes ($\text{pH} = 0.01$, $T = 0.1$, and $S = 0.01$) in seacarb.

Day	Date	Ambient								High CO_2							
		T	S	A_T	pH_T	$p\text{CO}_2$	C_T	Ω_A	Ω_C	T	S	A_T	pH_T	$p\text{CO}_2$	C_T	Ω_A	Ω_C
1	17 March 2017	15.6	15.2	1443.7	8.4	118.8	1268	2.1	3.5	15.6	15.3	1586.4	8.0	342.4	1504	1.1	1.9
3	20 March 2017	19.0	12.9	1525.3	8.4	131.3	1342	2.3	4.0	18.9	12.7	1543.7	7.4	1824.8	1591	0.3	0.5
11	28 March 2017	23.3	20.5	1835.9	8.1	344.9	1670	2.1	3.4	23.8	20.3	1835.5	7.7	825.7	1771	1.1	1.7
15	1 April 2017	21.2	17.2	1829.2	8.1	337.5	1686	1.9	3.2	21.2	17.4	1905.5	7.7	954.4	1867	0.9	1.5
33	19 April 2017	24.6	21.4	1801.6	8.1	315.7	1617	2.3	3.7	24.5	22.3	2069.9	7.7	1010.7	2001	1.2	1.9
42	28 April 2017	26.0	16.9	1692.7	8.0	409.5	1568	1.7	2.8	26.1	16.9	1751.9	7.2	2910.4	1813	0.3	0.6
45	5 May 2017	25.2	17.2	1642.0	8.0	426.6	1530	1.5	2.5	25.5	17.3	1672.0	7.7	839.8	1623	0.9	1.5
Mean		22.1	17.3	1681.5	8.2	297.8	1526	2.0	3.3	22.2	17.5	1766.4	7.6	1244.0	1739	0.8	1.4
SD		3.8	2.9	154.2	0.2	124.5	162	0.3	0.5	3.9	3.1	185.9	0.3	856.7	174	0.4	0.6

Table 2. Summary of two-way ANOVA results testing for the effects of ambient and elevated $p\text{CO}_2$ on the morphology, photo-physiology, and metabolism of *H. wrightii* and *R. maritima* in homospecific and heterospecific aquaria (n= 4 to 5). Degrees freedoms were 1 for all analyses. Significant effects are marked in bold. Asterisks above variables indicate that data did not meet parametric assumptions and a statistical $\alpha < 0.005$ was used.

Response variable	Species	Factors	F-stat	<i>P</i> -value
Morphology and biomass				
Shoot density	<i>H. wrightii</i>	<i>pH</i>	0.997	0.335
		<i>Bed type</i>	0.589	0.456
		<i>pH x Bed type</i>	0.913	0.355
	<i>R. maritima</i>	<i>pH</i>	0.126	0.728
		<i>Bed type</i>	0.129	0.725
		<i>pH x Bed type</i>	6.005	0.028
Number of leaves per shoot	<i>H. wrightii</i>	<i>pH</i>	0.037	0.850
		<i>Bed type</i>	1.771	0.206
		<i>pH x Bed type</i>	0.359	0.559
	<i>R. maritima</i>	<i>pH</i>	1.213	0.291
		<i>Bed type</i>	0.153	0.702
		<i>pH x Bed type</i>	0.032	0.862
Total leaf material	<i>H. wrightii</i>	<i>pH</i>	0.184	0.675
		<i>Bed type</i>	8.418	0.012
		<i>pH x Bed type</i>	0.173	0.685
	<i>R. maritima</i>	<i>pH</i>	1.466	0.248
		<i>Bed type</i>	2.656	0.127
		<i>pH x Bed type</i>	3.163	0.099
Average shoot height	<i>H. wrightii</i>	<i>pH</i>	0.380	0.548
		<i>Bed type</i>	6.349	0.026
		<i>pH x Bed type</i>	0.154	0.701
	<i>R. maritima</i>	<i>pH</i>	0.392	0.542

		<i>Bed type</i>	1.146	0.304
		<i>pH x Bed type</i>	3.689	0.077
Above-ground biomass	<i>H. wrightii</i>	<i>pH</i>	0.445	0.516
		<i>Bed type</i>	0.546	0.472
		<i>pH x Bed type</i>	0.456	0.511
	<i>R. maritima</i>	<i>pH</i>	0.440	0.518
	(<i>Ln(x)</i> transformed)	<i>Bed type</i>	0.006	0.937
		<i>pH x Bed type</i>	1.086	0.315
Below-ground biomass	<i>H. wrightii</i> *	<i>pH</i>	4.065	0.063
		<i>Bed type</i>	0.483	0.498
		<i>pH x Bed type</i>	0.496	0.493
	<i>R. maritima</i>	<i>pH</i>	0.001	0.976
	(<i>Ln(x)</i> transformed)	<i>Bed type</i>	0.544	0.473
		<i>pH x Bed type</i>	0.815	0.382
Photo-physiology				
Chlorophyll <i>a</i>	<i>H. wrightii</i>	<i>pH</i>	0.226	0.642
		<i>Bed type</i>	0.558	0.467
		<i>pH x Bed type</i>	0.186	0.673
	<i>R. maritima</i>	<i>pH</i>	0.379	0.548
		<i>Bed type</i>	0.776	0.393
		<i>pH x Bed type</i>	0.493	0.494
α	<i>H. wrightii</i>	<i>pH</i>	0.135	0.721
		<i>Bed type</i>	2.600	0.135
		<i>pH x Bed type</i>	0.575	0.464
	<i>R. maritima</i>	<i>pH</i>	1.942	0.189
		<i>Bed type</i>	0.282	0.605
		<i>pH x Bed type</i>	0.250	0.626
β	<i>H. wrightii</i>	<i>pH</i>	0.091	0.768
		<i>Bed type</i>	3.666	0.082
		<i>pH x Bed type</i>	0.734	0.410
	<i>R. maritima</i>	<i>pH</i>	0.377	0.551

		<i>Bed type</i>	0.173	0.685
		<i>pH x Bed type</i>	0.648	0.436
$rETR_{max}$	<i>H. wrightii</i>	<i>pH</i>	0.031	0.864
		<i>Bed type</i>	1.478	0.249
		<i>pH x Bed type</i>	0.276	0.610
	<i>R. maritima</i>	<i>pH</i>	0.001	0.986
		<i>Bed type</i>	0.068	0.798
		<i>pH x Bed type</i>	0.518	0.485
E_K	<i>H. wrightii</i>	<i>pH</i>	0.026	0.874
		<i>Bed type</i>	3.528	0.087
		<i>pH x Bed type</i>	0.739	0.408
	<i>R. maritima</i>	<i>pH</i>	0.425	0.527
		<i>Bed type</i>	0.140	0.714
		<i>pH x Bed type</i>	0.510	0.489
Light-adapted yield	<i>H. wrightii</i>	<i>pH</i>	0.484	0.498
		<i>Bed type</i>	2.190	0.161
		<i>pH x Bed type</i>	0.073	0.791
	<i>R. maritima*</i>	<i>pH</i>	0.001	0.980
		<i>Bed type</i>	0.143	0.711
		<i>pH x Bed type</i>	0.216	0.649
Dark-adapted yield	<i>H. wrightii*</i>	<i>pH</i>	0.226	0.642
		<i>Bed type</i>	0.558	0.467
		<i>pH x Bed type</i>	0.186	0.673
	<i>R. maritima*</i>	<i>pH</i>	0.379	0.548
		<i>Bed type</i>	0.776	0.393
		<i>pH x Bed type</i>	0.493	0.494
Metabolism				
GCP	<i>H. wrightii</i>	<i>pH</i>	0.001	0.972
	(Square root transformed)	<i>Bed type</i>	0.001	0.981
		<i>pH x Bed type</i>	0.484	0.498
	<i>R. maritima</i>	<i>pH</i>	0.798	0.388

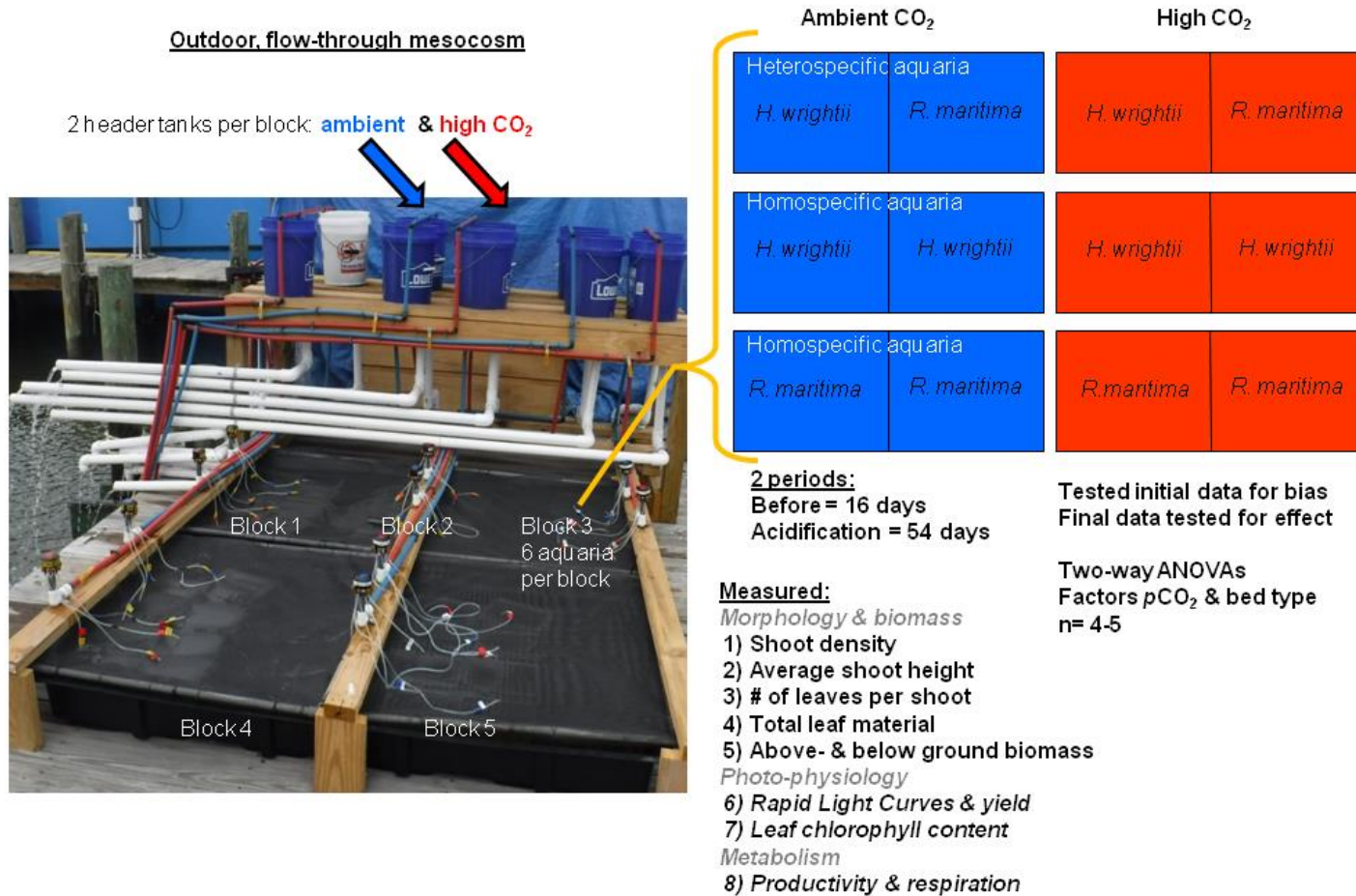
			<i>Bed type</i>	0.308	0.588
			<i>pH x Bed type</i>	1.662	0.220
NCP		<i>H. wrightii</i> (<i>Square root transformed</i>)	<i>pH</i>	0.034	0.856
			<i>Bed type</i>	0.973	0.341
			<i>pH x Bed type</i>	3.509	0.082
		<i>R. maritima</i>	<i>pH</i>	2.643	0.128
			<i>Bed type</i>	0.005	0.943
			<i>pH x Bed type</i>	0.408	0.534
Respiration		<i>H. wrightii</i>	<i>pH</i>	0.141	0.713
			<i>Bed type</i>	0.823	0.380
			<i>pH x Bed type</i>	3.155	0.097
		<i>R. maritima</i>	<i>pH</i>	1.537	0.237
			<i>Bed type</i>	0.614	0.447
			<i>pH x Bed type</i>	1.088	0.316
$\delta^{13}\text{C}$	<i>H. wrightii</i>	<i>Leaf</i> *	<i>pH</i>	64.40 0	<0.001
			<i>Bed type</i>	0.390	0.543
			<i>pH x Bed type</i>	4.599	0.051
		<i>Root</i>	<i>pH</i>	59.89 0	<0.001
			<i>Bed type</i>	0.336	0.572
			<i>pH x Bed type</i>	6.709	0.022
	<i>R. maritima</i>	<i>Leaf</i>	<i>pH</i>	36.68 0	<0.001
			<i>Bed type</i>	0.006	0.940
			<i>pH x Bed type</i>	0.828	0.384
		<i>Root</i>	<i>pH</i>	51.88 0	<0.001
			<i>Bed type</i>	0.014	0.908
			<i>pH x Bed type</i>	0.159	0.699
$\delta^{15}\text{N}$	<i>H. wrightii</i>	<i>Leaf</i>	<i>pH</i>	0.431	0.523
			<i>Bed type</i>	0.080	0.782
			<i>pH x Bed type</i>	0.004	0.948

			<i>type</i>		
		<i>Root</i>	<i>pH</i>	0.287	0.601
			<i>Bed type</i>	0.701	0.418
			<i>pH x Bed type</i>	0.140	0.714
	<i>R. maritima</i>	<i>Leaf</i>	<i>pH</i>	0.863	0.377
			<i>Bed type</i>	0.090	0.770
			<i>pH x Bed type</i>	3.118	0.111
		<i>Root</i>	<i>pH</i>	2.293	0.161
			<i>Bed type</i>	1.123	0.314
			<i>pH x Bed type</i>	0.003	0.954
C content	<i>H. wrightii</i>	<i>Leaf</i>	<i>pH</i>	0.428	0.428
			<i>Bed type</i>	0.069	0.069
			<i>pH x Bed type</i>	0.371	0.371
		<i>Root</i>	<i>pH</i>	0.003	0.956
		<i>(Ln(x) transformed)</i>	<i>Bed type</i>	0.177	0.681
			<i>pH x Bed type</i>	0.835	0.378
	<i>R. maritima</i>	<i>Leaf</i>	<i>pH</i>	0.509	0.492
			<i>Bed type</i>	0.303	0.594
			<i>pH x Bed type</i>	0.680	0.429
		<i>Root</i>	<i>pH</i>	0.868	0.373
			<i>Bed type</i>	0.001	0.972
			<i>pH x Bed type</i>	2.730	0.129
N content	<i>H. wrightii</i>	<i>Leaf</i>	<i>pH</i>	0.668	0.429
			<i>Bed type</i>	0.031	0.864
			<i>pH x Bed type</i>	0.143	0.712
		<i>Root</i>	<i>pH</i>	0.002	0.968
			<i>Bed type</i>	0.006	0.938
			<i>pH x Bed type</i>	0.227	0.642
	<i>R. maritima</i>	<i>Leaf</i>	<i>pH</i>	2.918	0.118
			<i>Bed type</i>	0.303	0.594
			<i>pH x Bed type</i>	0.981	0.345
			<i>type</i>		

<i>Root</i>	<i>pH</i>	5.991	0.034
	<i>Bed type</i>	0.422	0.531
	<i>pH x Bed type</i>	0.002	0.964

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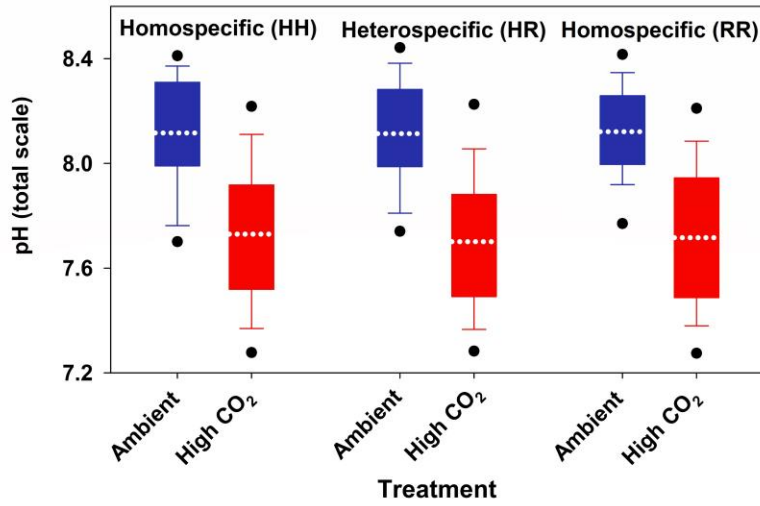
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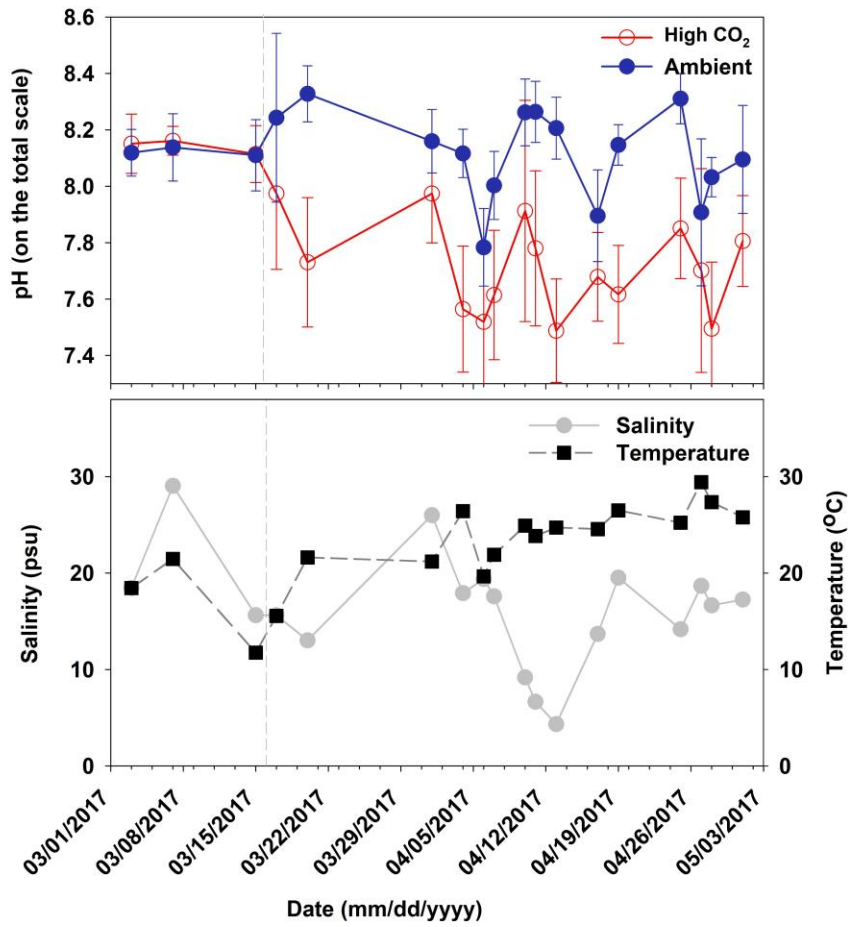
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744 **Fig. 1** Experimental setup applied in this study. See text in methods for description.

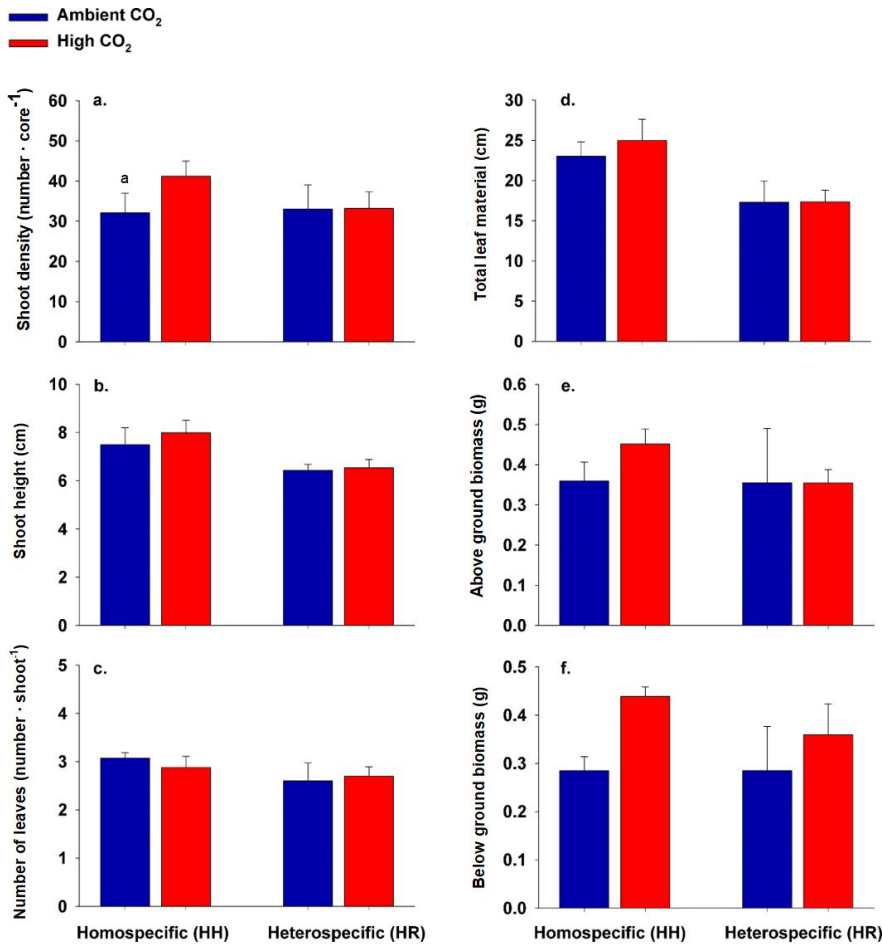
A. Boxplots of pH data for each CO₂ treatment - bed type combination



B. pH_T, Salinity, and Temperature through time



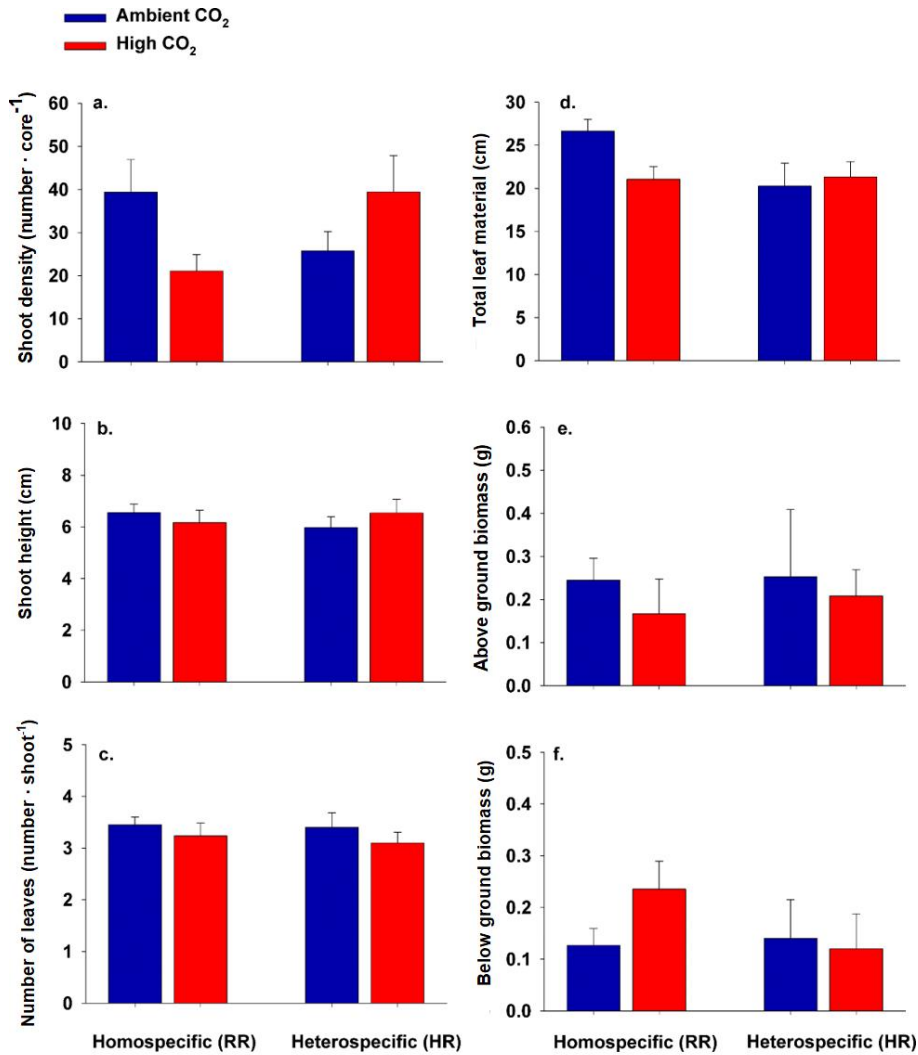
746 **Fig. 2** The pH_T , salinity and temperature in the ambient and high CO_2 aquaria. Panel A is
 747 a boxplot of the all the discrete measures of pH_T presented by bed type (homo- or hetero-
 748 specific and CO_2 treatment (ambient or high). The dotted white line within the bar is the
 749 mean and the whiskers from the bars capture the 5th and 95th percentile. Panel B top,
 750 shows the evolution of pH_T (mean \pm SE, $n = 27$ aquaria) throughout the experiment as a
 751 function of (bottom) probed temperature and salinity ($n = 5$) used to calculate the
 752 carbonate chemistry. The dotted lines indicate the beginning of the perturbation.
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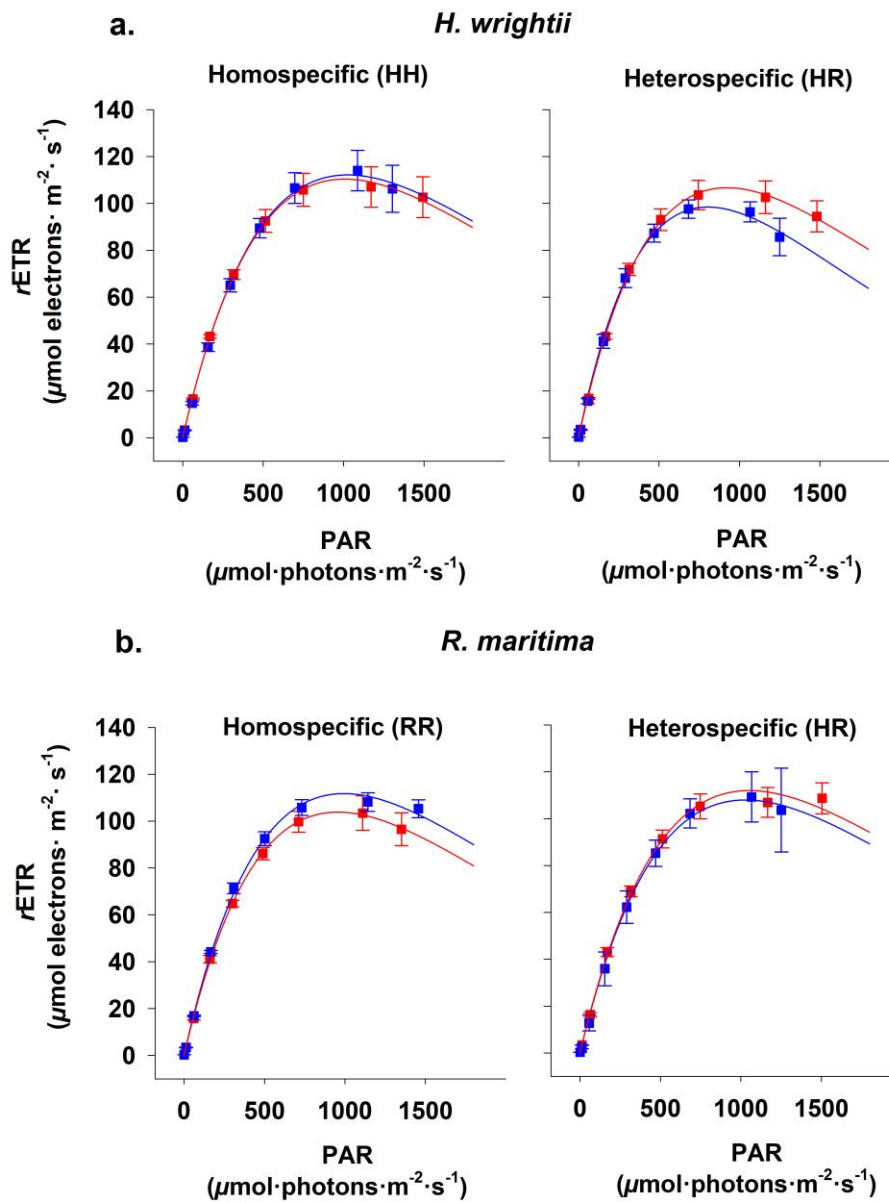
755 **Fig. 3** *Halodule wrightii* (mean \pm SE; $n = 4$ to 5) morphology (shoot density, a; shoot
 756 height, b; leaves per shoot, c; total leaf material, d) and above- and below ground biomass
 757 (e, f) after maintained for 34, 41, or 54 days at ambient (blue) and high CO_2 (red)
 758 treatments. *Halodule wrightii* was grown in homospecific (*H. wrightii* with *H. wrightii*,
 759 HH) and heterospecific (*H. wrightii* with *R. maritima*, HR) beds. Data did not show
 760 significant differences between treatments.

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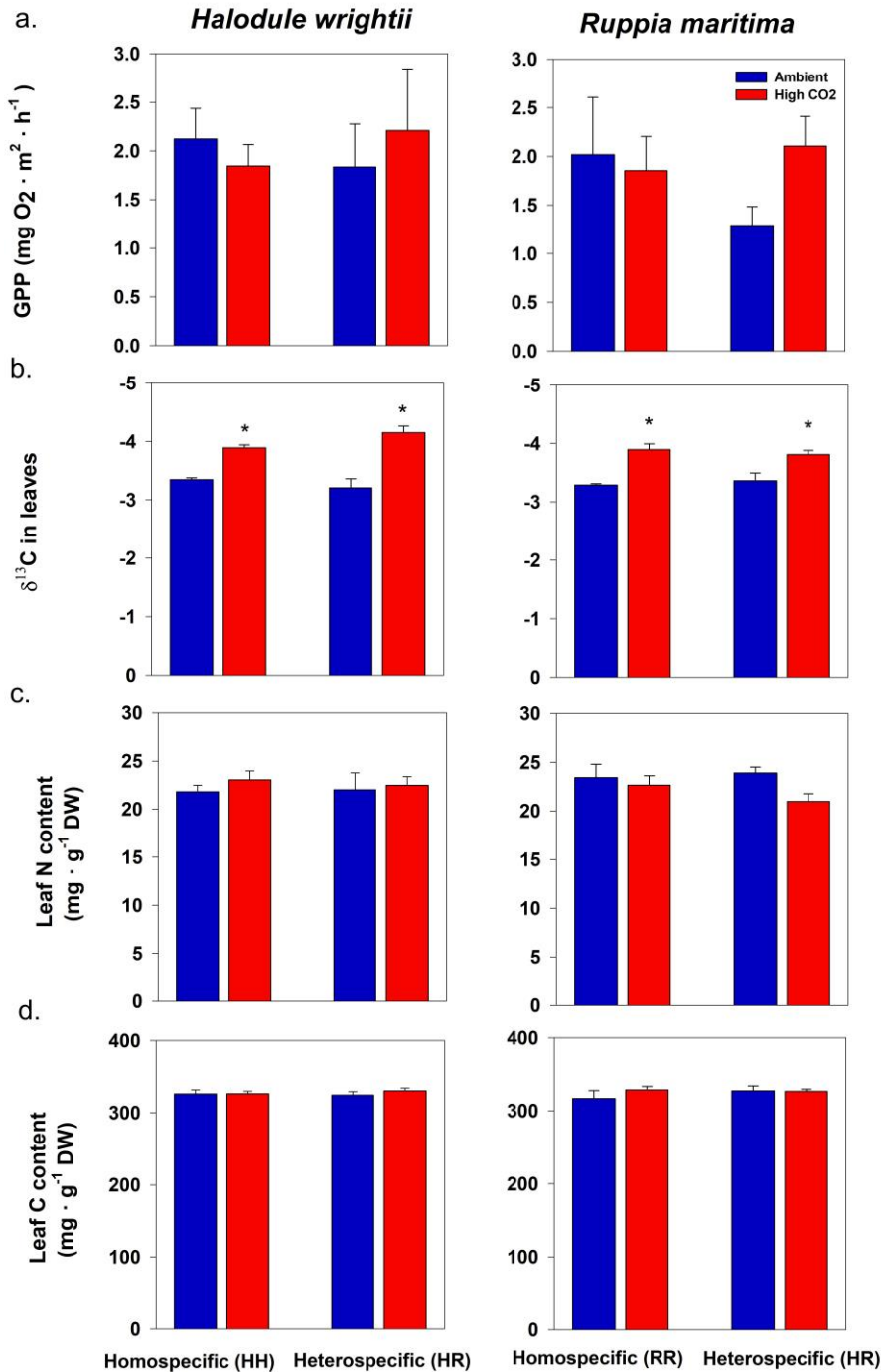
762

763 **Fig. 4** *Ruppia maritima* (mean ± SE; n= 4 to 5) morphology (shoot density, a; shoot
 764 height, b; leaves per shoot, c; total leaf material, d) and above- and below ground biomass
 765 (e, f) after maintained for 34, 41, or 54 days at ambient (blue) and high CO₂ (red)
 766 treatments. *Ruppia maritima* was grown in homospecific (*R. maritima* with *R. maritima*,
 767 RR) and heterospecific (*R. maritima* with *H. wrightii*, HR) beds. Data did not show
 768 significant differences between treatments.
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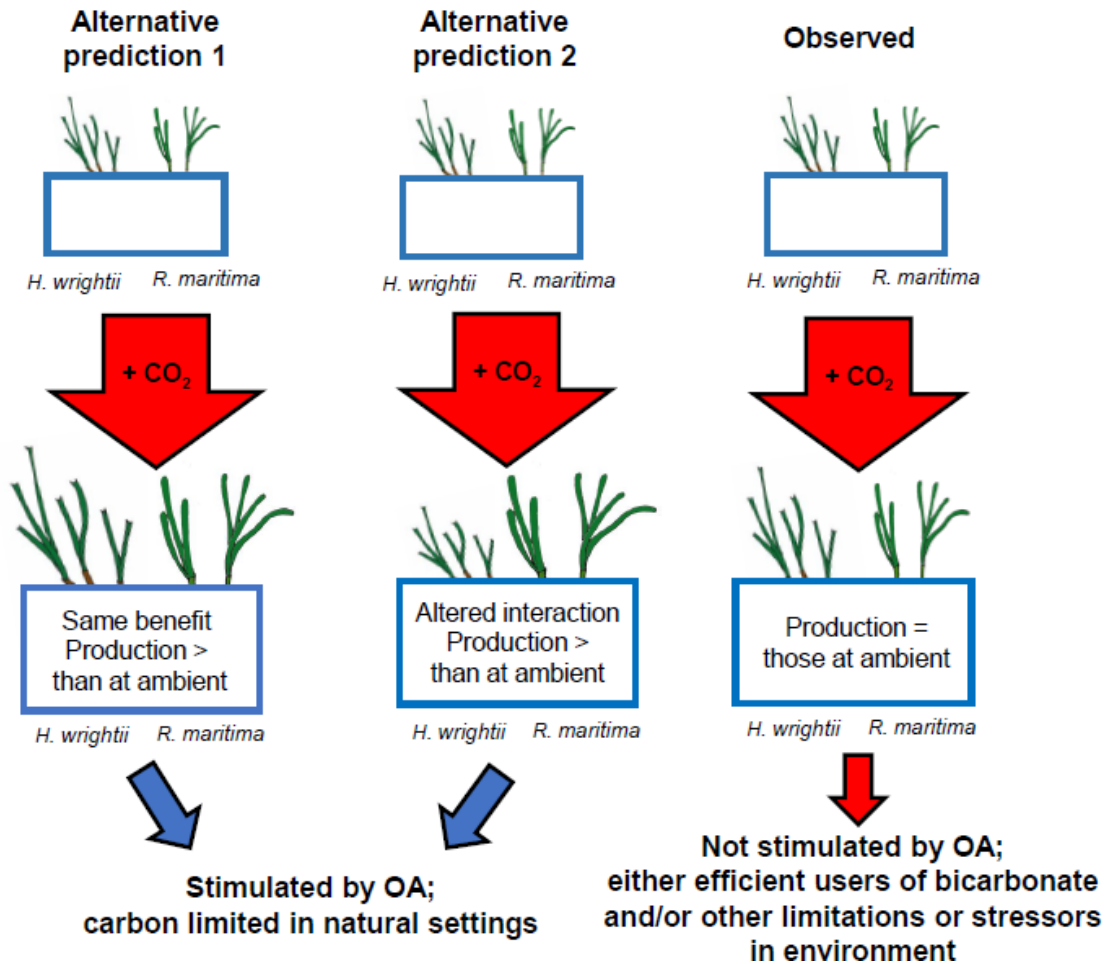
771 **Fig. 5** Rapid light curves from *H. wrightii* (top, a) and *R. maritima* (bottom, b) placed
 772 within homospecific (left) and heterospecific (right) beds (*H. wrightii* with *H. wrightii*,
 773 HH; *R. maritima* with *R. maritima*, RR and *H. wrightii* with *R. maritima*, HR) after
 774 maintained for 43 days under ambient and high CO₂ treatments (continuous modeled
 775 lines). Modeled lines and $rETR$ (mean \pm SE) values are based upon an average from 4 to
 776 5 aquaria. PAR units were $\mu\text{mol photons m}^{-2}\text{s}^{-1}$.
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779 **Fig. 6** Gross community productivity (GCP; a), $\delta^{13}\text{C}$ (b) and carbon (c) and nitrogen (d)
 780 content in leaves obtained in *H. wrightii* (left) and *R. maritima* (right) placed within
 781 homospecific and heterospecific beds (*H. wrightii* with *H. wrightii*, HH; *R. maritima* with
 782 *R. maritima*, RR and *H. wrightii* with *R. maritima*, HR; mean \pm SE; n= 4 to 5) after
 783 maintained for 48 days under ambient (blue) and high CO₂ treatments (red). Asterisks (*)
 784 indicates significant differences between treatment.

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787 **Fig. 7** Graphical summary of the effects of ocean acidification (OA). In a heterospecific
788 bed (represented by aquaria with two species seen in the blue boxes) the increased $p\text{CO}_2$
789 was predicted to increase seagrass growth and production with either little change to bed
790 composition (alternative prediction 1) or with a shifted interaction where one species
791 comes to dominate in abundance (alternative prediction 2). Yet, we observed no effect of
792 increased $p\text{CO}_2$ on seagrass growth and production. Therefore, these species must either
793 be efficient users of bicarbonate and/or other stressors and limitations outweighed any
794 stimulation from increased $p\text{CO}_2$.

Supplementary Table 1. Summary of two-way ANOVA results obtained in the initial measurements of morphology and biomass, photo-physiology, and metabolic parameters for *H. wrightii* and *R. maritima* in homospecific and heterospecific aquaria (n=5). Degrees freedoms were 1 for all analyses.

Response variable	Species	Factors	F-stat	p-value	
Morphology and biomass					
Shoot density	<i>H. wrightii</i>	<i>pH</i>	0.357	0.560	
		<i>Bed type</i>	0.654	0.432	
		<i>pH x Bed type</i>	0.226	0.642	
	<i>R. maritima</i>	<i>pH</i>	0.005	0.946	
		<i>Bed type</i>	0.003	0.960	
		<i>pH x Bed type</i>	1.282	0.277	
	Number of leaves per shoot	<i>H. wrightii</i>	<i>pH</i>	8.401	0.012
			<i>Bed type</i>	0.535	0.477
			<i>pH x Bed type</i>	0.245	0.628
<i>R. maritima</i>		<i>pH</i>	4.826	0.045	
		<i>Bed type</i>	0.180	0.678	
		<i>pH x Bed type</i>	0.356	0.560	
Total leaf material		<i>H. wrightii</i>	<i>pH</i>	5.643	0.032
			<i>Bed type</i>	0.892	0.361
			<i>pH x Bed type</i>	0.014	0.908
	<i>R. maritima</i>	<i>pH</i>	0.011	0.916	
		<i>Bed type</i>	0.270	0.611	
		<i>pH x Bed type</i>	0.096	0.761	
	Shoot height	<i>H. wrightii</i>	<i>pH</i>	0.500	0.491
			<i>Bed type</i>	0.568	0.464
			<i>pH x Bed type</i>	0.907	0.357
<i>R. maritima</i>		<i>pH</i>	3.405	0.086	
		<i>Bed type</i>	0.669	0.427	
		<i>pH x Bed type</i>	0.064	0.804	
Photo-physiology					
α		<i>H. wrightii</i>	<i>pH</i>	0.162	0.694
			<i>Bed type</i>	3.078	0.103
	<i>pH x Bed type</i>		0.088	0.772	
	<i>R. maritima</i>	<i>pH</i>	0.388	0.545	
		<i>Bed type</i>	0.129	0.726	

β	<i>H. wrightii</i>	<i>pH x Bed type</i>	0.388	0.545	
		<i>pH</i>	0.179	0.679	
		<i>Bed type</i>	0.254	0.622	
	<i>R. maritima</i>	<i>pH x Bed type</i>	0.410	0.533	
		<i>pH</i>	0.057	0.816	
		<i>Bed type</i>	0.038	0.848	
$rETR_{max}$	<i>H. wrightii</i>	<i>pH x Bed type</i>	0.766	0.399	
		<i>pH</i>	0.010	0.923	
		<i>Bed type</i>	3.175	0.098	
	<i>R. maritima</i>	<i>pH x Bed type</i>	0.238	0.634	
		<i>pH</i>	0.844	0.376	
		<i>Bed type</i>	0.302	0.593	
E_K	<i>H. wrightii</i>	<i>pH x Bed type</i>	1.834	0.201	
		<i>pH</i>	0.091	0.767	
		<i>Bed type</i>	3.507	0.838	
	<i>R. maritima</i>	<i>pH x Bed type</i>	0.388	0.544	
		<i>pH</i>	0.120	0.735	
		<i>Bed type</i>	0.058	0.813	
Dark-adapted yield	<i>H. wrightii</i>	<i>pH x Bed type</i>	0.377	0.551	
		<i>pH</i>	2.568	0.129	
		<i>Bed type</i>	2.893	0.108	
	<i>R. maritima</i>	<i>pH x Bed type</i>	0.003	0.955	
		<i>pH</i>	0.021	0.888	
		<i>Bed type</i>	1.505	0.238	
Light-adapted yield	<i>H. wrightii</i>	<i>pH x Bed type</i>	0.736	0.403	
		<i>pH</i>	1.622	0.224	
		<i>Bed type</i>	2.672	0.124	
	<i>R. maritima</i>	<i>pH x Bed type</i>	1.987	0.180	
		<i>pH</i>	0.227	0.642	
		<i>Bed type</i>	0.058	0.813	
Metabolism	GCP (squared root transformed)	<i>H. wrightii</i>	<i>pH x Bed type</i>	0.025	0.876
			<i>pH</i>	0.155	0.701
			<i>Bed type</i>	4.713	0.051
		<i>R. maritima</i>	<i>pH x Bed type</i>	0.998	0.338
			<i>pH</i>	0.001	0.988

(squared root transformed)

		Bed type	2.609	0.132
NCP	<i>H. wrightii</i>	<i>pH x Bed type</i>	1.589	0.231
		<i>pH</i>	0.281	0.606
		<i>Bed type</i>	1.074	0.320
	<i>R. maritima</i>	<i>pH x Bed type</i>	0.326	0.579
		<i>pH</i>	0.684	0.424
		<i>Bed type</i>	0.259	0.620
Respiration	<i>H. wrightii</i>	<i>pH x Bed type</i>	2.066	0.176
		<i>pH</i>	1.471	0.249
		<i>Bed type</i>	0.964	0.346
	<i>R. maritima</i>	<i>pH x Bed type</i>	0.100	0.757
		<i>pH</i>	2.199	0.164
		<i>Bed type</i>	4.299	0.060
		<i>pH x Bed type</i>	0.058	0.814

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Supplementary Table 2. Temperature, salinity and pH (mean \pm SD) measured per block during the experiment in the header tanks. Means of pH were separate between treatments (Ambient and high $p\text{CO}_2$).

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	Temperature		Salinity		pH			
					Ambient		High CO_2	
	MEAN	\pm SD	MEAN	\pm SD	MEAN	\pm SD	MEAN	\pm SD
<i>Tank 1</i>	23.31	2.290	15.48	6.201	8.074	0.199	7.754	0.289
<i>Tank 2</i>	23.22	2.248	16.25	5.829	8.108	0.212	7.628	0.376
<i>Tank 3</i>	23.13	2.577	16.43	5.993	8.056	0.228	7.722	0.298
<i>Tank 4</i>	23.28	2.353	16.37	5.892	8.159	0.158	7.668	0.320
<i>Tank 5</i>	21.90	3.936	16.36	5.842	8.080	0.228	7.742	0.275
Total	22.97	0.602	16.18	0.395	8.095	0.040	7.703	0.053

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Supplementary Table 3. Summary of the results obtained in the two-way ANOVAs performed in the environmental variables of header tanks for the factors “pH treatment” (ambient vs. high $p\text{CO}_2$) and “Time” (day of sample collection). Temperature (T, $^\circ\text{C}$); salinity (S); pH on the total scale (pH_T); total alkalinity ($A_T, \mu\text{mol kg}^{-1}$); partial pressure of CO_2 ($p\text{CO}_2, \mu\text{atm}$); dissolved inorganic carbon ($C_T, \mu\text{mol kg}^{-1}$) and saturation states with respect to aragonite (Ω_a) and calcite (Ω_c). Significant effects are marked in bold. The bolded text and values in parenthesis next to carbonate chemistry parameters are the range of estimated errors calculated in R with seacarb using the precision around the

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809 standard (for A_T precision was $3.9 \mu\text{mol kg}^{-1}$, $n = 7$) together with the error for probes
 810 (pH = 0.01, T = 0.1, and S = 0.01)
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	Source	d.f.	MS	F-stat	p-value
T					
	pH treatment (P)	1	0.035	1.4	0.281
	Time (T)	6	29.07	1162.79	<0.01
	Residual	6	0.025		
	Total	13	13.43		
S					
	pH treatment (P)	1	0.067	1.124	0.330
	Time (T)	6	18.41	307.818	<0.01
	Residual	6	0.060		
	Total	13	8.527		
A_T					
	pH treatment (P)	1	25241.5	5.78	0.053
	Time (T)	6	53967.5	12.358	<0.01
	Residual	6	4367.0		
	Total	13	28865.2		
pH_T					
	pH treatment (P)	1	0.913	22.036	<0.01
	Time (T)	6	0.064	1.555	0.303
	Residual	6	0.041		
	Total	13	0.119		
pCO₂ (ambient 4.0 - 13.9, high CO₂ 10.6 -88.3)					
	pH treatment (P)	1	3133930.0	9.018	<0.01
	Time (T)	6	401951.2	1.157	0.432
	Residual	6	347500.4		
	Total	13	586972.3		
C_T (all error < 0.00)					
	pH treatment (P)	1	0.001	31.546	<0.01
	Time (T)	6	0.001	10.243	0.006
	Residual	6	0.001		
	Total	13	0.001		

Ω_A (ambient: 0.09 – 0.13, high CO₂: 10.6 -88.3)

pH treatment (P)	1	4.750	45.142	<0.01
Time (T)	6	0.125	1.184	0.421
Residual	6	0.105		
Total	13	0.471		

 Ω_C (ambient: 0.18 – 0.22, high CO₂: 0.03 -0.11)

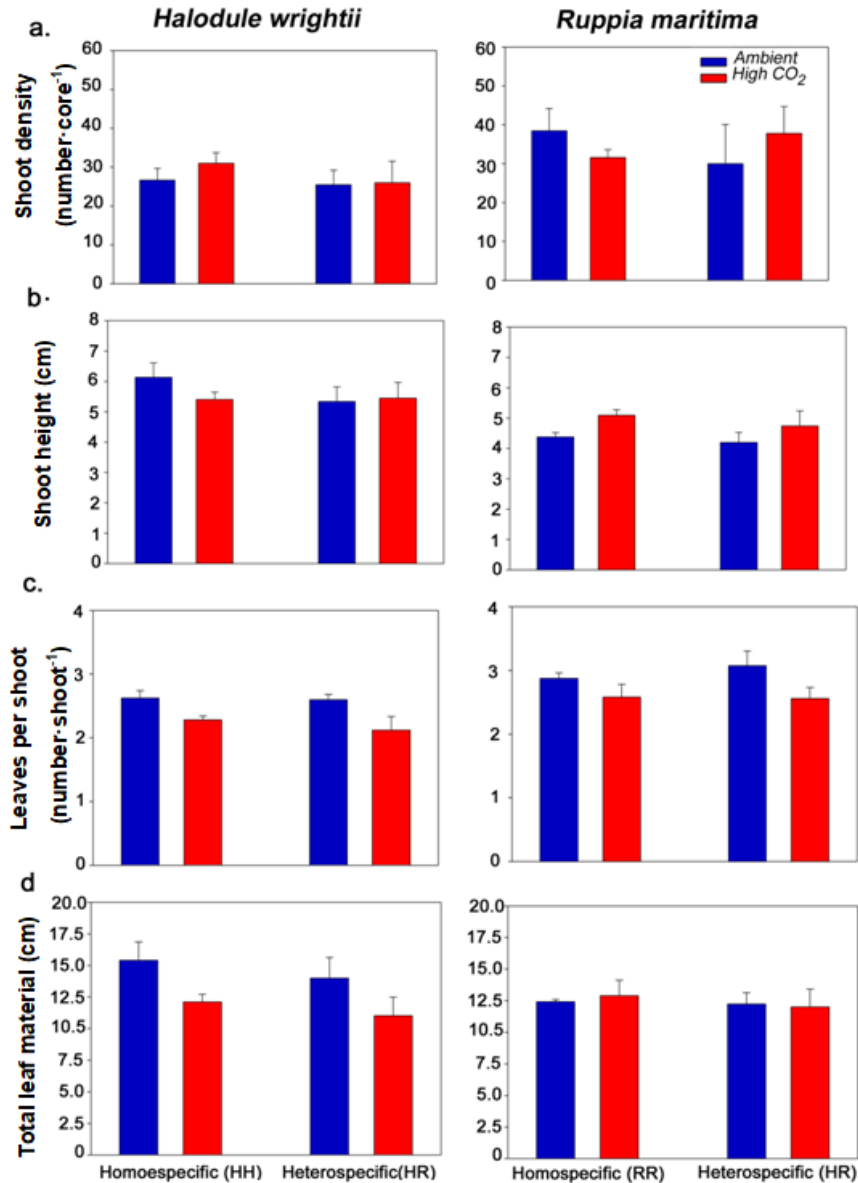
pH treatment (P)	1	13.08	40.954	<0.01
Time (T)	6	0.313	0.98	0.509
Residual	6	0.319		
Total	13	1.298		

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813 **Supplementary Table 4.** Error estimates for the variables $p\text{CO}_2$, C_T , Ω_A and Ω_C using
814 the function “errors” in the “seacarb” package in R software (A_T precision of 3.9 μmol
815 kg^{-1} , $n = 7$; error for probes of $\text{pH} = 0.01$, $T = 0.1$, and $S = 0.01$).

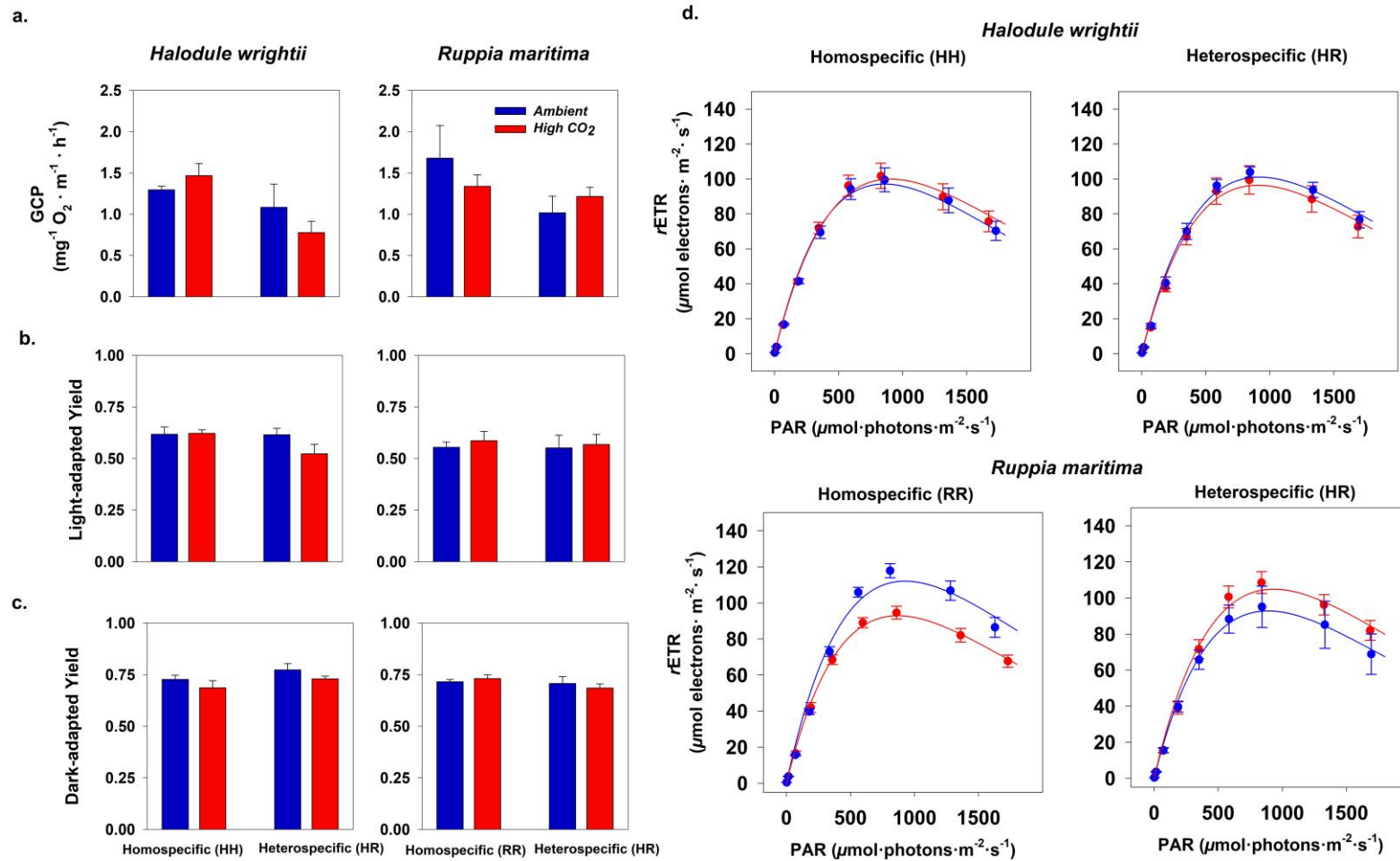
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Day	Date	Ambient				High CO ₂			
		$p\text{CO}_2$	C_T	Ω_A	Ω_C	$p\text{CO}_2$	C_T	Ω_A	Ω_C
1	17 March 2017	4.02	6.28	0.12	0.20	10.55	5.06	0.07	0.11
3	20 March 2017	4.58	6.50	0.13	0.22	55.55	4.77	0.02	0.03
11	28 March 2017	11.29	6.58	0.12	0.20	25.55	5.15	0.07	0.10
15	1 April 2017	11.13	6.14	0.11	0.18	29.63	4.91	0.05	0.09
33	19 April 2017	10.48	6.86	0.13	0.21	31.18	5.44	0.07	0.12
42	28 April 2017	13.46	5.77	0.10	0.16	88.31	5.18	0.02	0.03
45	5 May 2017	13.92	5.52	0.09	0.15	26.39	4.84	0.05	0.09



817

818 **Supplementary Figure 1.** Initial morphology of *H. wrightii* and *R. maritima* (shoot
 819 density, a; shoot height, b; leaves per shoot, c; total leaf material, d; mean \pm SE) before
 820 maintained for 34, 41, and 54 days at ambient (blue) and high CO₂ (red) treatments in
 821 homospecific and heterospecific beds (*H. wrightii* with *H. wrightii*, HH; *R. maritima* with
 822 *R. maritima*, RR and *H. wrightii* with *R. maritima*, HR).



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824 **Supplementary Figure 2.** Initial GCP rates (a), initial dark-adapted and light-adapted yields (b and c) and initial RLCs (d) obtained in
 825 *H. wrightii* (left) and *R. maritima* (right) placed within homospecific and heterospecific beds (*H. wrightii* with *H. wrightii*, HH; *R.*
 826 *maritima* with *R. maritima*, RR and *H. wrightii* with *R. maritima*, HR).