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

Running page head: Ocean acidification effects on seagrasses in NGoM

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Abstract:

Ocean acidification is thought to benefit seagrasses because of increased carbon dioxide (CO_2) availability for photosynthesis. However, in order to truly assess ecological responses, effects of ocean acidification need to be investigated in a variety of coastal environments. We tested the hypothesis that ocean acidification would benefit seagrasses in the northern Gulf of Mexico, where the seagrasses *Halodule wrightii* and *Ruppia maritima* coexist in a fluctuating environment. To evaluate if benefits of ocean acidification could alter seagrass bed composition, cores of *H. wrightii* and *R. maritima* were placed alone or in combination into aquaria and maintained in an outdoor mesocosm. Half of the aquaria were exposed to either ambient (mean pH of 8.1 ± 0.04 SD on total scale) or high CO_2 (mean pH 7.7 ± 0.05 SD on total scale) conditions. After 54 days of experimental exposure, the $\delta^{13}C$ values were significantly lower in seagrass tissue in the high CO_2 condition. This integration of a different carbon source (either: preferential use of CO_2, gas from cylinder, or both) indicates that plants were not solely relying on stored energy reserves for growth. Yet, after 41 to 54 days, seagrass morphology, biomass, photo-physiology, metabolism, and carbon and nitrogen content in the high CO_2 condition did not differ from those at ambient. There was also no indication of differences in traits between the homo- or hetero- specific beds. Findings support two plausible conclusions: 1) these seagrasses rely heavily on bicarbonate use and growth will not be stimulated by near future acidification conditions or 2) the mesohaline environment limited the beneficial impacts of increased CO_2 availability.

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

Introduction

The increase in atmospheric CO_2 since the industrial revolution has altered the equilibrium of inorganic carbon compounds in the ocean, increasing the concentrations of bicarbonate (HCO_3^-), carbonic acid (H_2CO_3), and hydrogen ions (H^+) (Elderfield et al. 2005). These changes, referred to as ocean acidification, have caused the average sea surface pH to drop by 0.1 units, and the pH is projected to further decline by 0.06-0.32 units by the end of this century (IPCC, 2013). Ocean acidification is known to impact species physiologies and lead to cascading effects at the ecosystem level (Hall-Spencer et al. 2008).

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

Coastal environments, however, are highly dynamic in terms of fluctuating light, nutrients, and salinity, particularly in mesohaline estuaries. Estuaries commonly receive freshwater inputs that change the chemical and physical properties of the seawater (Aufdenkampe et al. 2011). High biological activity, often fueled by nutrient inputs and hydrodynamic processes in shallow areas, can result in highly variable pH and CO_2 environments. Many estuarine 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 (pCO_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

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

Halodule wrightii Asch. and *Ruppia maritima* L. are widespread seagrasses that co-exist in heterospecific beds in mesohaline estuaries of the north-central Gulf of Mexico. These species have short growth cycles and different seasonal peaks in biomass. *Halodule wrightii* grows throughout the year and typically reaches maximum biomass in late summer-early fall. *Halodule wrightii* also allocates a larger fraction of total biomass to roots and rhizomes compared to *R. maritima* (Dunton 1990; Anton et al. 2009). *Ruppia maritima* grows during cool temperatures and undergoes senescence after flowering in spring (Pulich 1985; Cho and Poirrier 2005; Anton et al. 2009). Even though *H. wrightii* and *R. maritima* provide similar ecosystem services (Christiaen et al. 2016), elevated pCO_2 conditions may stimulate production to change the services they provide (e.g. refuge ability, production). Furthermore, acidification could act to alter the ability for them to coexist. Under environmental stress, *R. maritima* can outcompete *H. wrightii* (Christiaen et al. 2016). Both seagrasses may increase their productivity under elevated pCO_2, but *R. maritima* production is known to be carbon saturated in some settings (Sand-Jensen and Gordon 1984; Koch et al. 2013; Campbell and Fourqurean 2013a). Due to higher richness of species, mixed seagrass beds are expected to attract more associated fauna, to be more productive and to have a broader range of tolerance to environmental conditions than monospecific beds (Duffy et al. 2006; Gustafsson and Boström 2011, 2013). Despite so, it has been little examined how elevated pCO_2 can alter the biomass of *H. wrightii* and *R. maritima* in heterospecific

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

The objectives of this study are to (1) evaluate the effects of ocean acidification on the productivity and vegetative growth of seagrasses in the mesohaline waters of the northern Gulf of Mexico and to (2) test for potential shifts in composition of *H. wrightii* and *R. maritima* resulting from an increase in CO_2 availability. To do this, cores of *H. wrightii* and *R. maritima* were placed alone (homospecific beds) or side by side, in combination (heterospecific beds), into aquaria and maintained in an outdoor mesocosm under ambient and elevated pCO_2 (low pH) conditions for up to five weeks. Afterwards, the morphology and biomass, photo-physiology, chemical composition, and metabolism of the seagrasses were measured. We hypothesized that enhanced CO_2 availability would stimulate photosynthesis and benefit growth and production. We also hypothesized that the stimulation of seagrass productivity would alter the composition of *H. wrightii* and *R. maritima* beds. It is important to note that we were not directly testing competition between seagrass species per se, albeit competition may be happening at the fringing interface between patches, but rather we are testing whether any differences in CO_2 stimulated growth cause densities or biomass to shift through stimulating the productivity of one species more than the other, or through differences in their carbon allocation. Additionally, *Halodule wrightii* and *R. maritima* were not replanted to form a mixed interspersed bed, with presumably more interspecific interactions, because this distribution pattern would not represent the ecology

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

Methods

Seagrass bed collection

Sixty rectangular cores of seagrass beds (10 x 4 cm; 4 cm deep) were collected from single species patches of *H. wrightii* and *R. maritima* from approximately 1 m depth in Point-aux-Pins, Bayou la Batre (30°23'4.26"N, 88°18'42.73"W northern Gulf of Mexico, Alabama, USA) on 27th February 2017. In the field, cores were introduced into 30 aquaria (21 x 13 x 13 cm) in pairs, such that there were 10 aquaria with two cores of *H. wrightii*, 10 aquaria with two cores of *R. maritima*, and 10 aquaria with a core of *H. wrightii* and a core of *R. maritima*. We butted the cores against each other to simulate homospecific beds of either species as well as the fringing area between adjacent beds of *H. wrightii* and *R. maritima*. The aquaria filled with cores were immediately brought back to Dauphin Island Sea Lab and kept in an outdoor experimental setup for seventy days (16 days of acclimation, 54 days of experimental manipulation, with final measures taken after at least 4.9 weeks of different CO_2 exposure, Fig. 1). The experiment was concluded on May 8, 2017, after 54 days of CO_2 exposure. This period of time, from Feb. 27 May 8, was selected because these seagrass species have short shoot turnovers (few months) and increase their growth in spring (Pulich 1985; Dunton 1990; Hemminga and Duarte 2000; Kilminster et al. 2015).

Experimental setup

Two aquaria of each seagrass bed type (*Halodule-Halodule*, HH; *Ruppia-Ruppia*, RR; 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 CO_2 treatment (natural pCO_2/ pH), and the other to the high
155 CO_2 treatment (high pCO_2/ 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 CO_2 aquaria and another for the high CO_2 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 CO_2 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 CO_2 from a
167 gas cylinder into the header tanks for the high CO_2 aquaria. For each block, the header tank
168 bubbled with CO_2 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

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

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

The pCO_2 in the ambient treatment was ~350 µatm, which corresponded to the value found in local coastal waters. In the "high CO_2" treatment was applied a pCO_2 of ~1244 µatm or a pH offset of approximately -0.3 to -0.4 to mimic the maximum pH decrease expected by the end of this century based on IPCC scenario for 2100 (IPCC, 2013).

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 CO_2 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 CO_2, 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 CO_2. 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 CO_2 centered in the production of new leaves.
212 Plant biomass was only measured at the end of the study (54 days of CO_2 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 60ºC, and the dry weight (DW) determined. Above-ground biomass contained

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

Photo-physiology

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

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

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

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

Metabolism

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

Chemical composition

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

period varied from 8 – 8.4 in the ambient treatment and of 7.2 – 8.0 in the high CO_2 treatment (Table 1). In ambient header tanks, pCO_2 and total DIC (C_T) ranged from 118.8 to 426.6 µatm and from 1268 to 1686 µmol kg^{-1}, respectively; while in the high CO_2 header tanks values ranged from 342.4 to 2910.4 µatm and from 1504 to 2001 µmol kg^{-1}. Levels of A_T in the header tanks did not differ between treatments but, they significantly fluctuated during the experimental period (Table 1, Supplementary Table 3). In the ambient treatment header tanks, A_T ranged from 1443.7 to 1835.9 µmol kg^{-1} and from 1543.7 to 2069.9 µmol kg^{-1} in the high CO_2 treatment header tanks (Table 1). The fluctuation was related to changes in salinity. As salinity decreased the levels of A_T also decreased in a linear manner; perhaps this relationship is due to the dilution of weathering products. Salinity and temperature in the header tanks significantly varied through time, but not between treatments (Supplementary Table 3). The seawater in the ambient treatment was saturated with respect to both aragonite and calcite. In the high CO_2 treatment calcite and aragonite were under saturation most of the time, except after the storms on the 20th of March and 28th of April (Table 1). Furthermore, levels of seawater saturation also differed between treatments.

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

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

Morphology and biomass

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

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

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

Photo-physiology

The parameters derived from the rapid light curves of *H. wrightii* did not differ between ambient and elevated pCO_2 exposure and did not differ with bed type (Table 2, Fig. 5). For example, the derived α for *H. wrightii* was 0.29 ± 0.01 and 0.30 ± 0.01 electrons/photons in homo-specific aquaria and 0.32 ± 0.01 and 0.32 ± 0.01 electrons/photons in hetero-specific aquaria after exposure to ambient and elevated pCO_2 conditions, respectively. Furthermore, mean $rETR_{max}$, E_K, and β values did not significantly differ (Table 2) among bed type and pCO_2 condition for *H. wrightii* (mean ± SD: $rETR_{max}$ from 99.1 ± 10.9 to 108.3± 21.6 μmol electrons m^{-2} s^{-1}, E_K from 308.8 ± 34.5 to 356.4 ± 54.5 μmol photon $m^{-2}s^{-1}$, and β from 98.5± 8.4 to 105.9 ± 5.6 electrons/photons).

After 43 days of pH manipulation, the parameters derived from the rapid light curves of *R. maritima* also did not differ between ambient and elevated pCO_2 exposure and did not differ with bed type (Table 2, Fig. 5). This result is evident in the curves (Fig. 5) with the similar range of derived values of α, $rETR_{max,}$ and E_K regardless of growing condition (mean ± SD: α from 0.29 ± 0.02 to 0.32 ± 0.02 electrons/photons, $rETR_{max}$ from 103.8 ± 23.4 to 111.9 ± 11.1μmol electrons m^{-2} s^{-1}, E_K from 325.2 ± 89.6 to 377.5 ± 84.7 μmol photon m^{-2} s^{-1}). Similar to observations for *H. wrightii*, there was a trend of greater photoinhibition for *R. maritima* plants within the ambient CO_2, heterospecific bed condition when compared to the other treatments. 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 pCO_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 pCO_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 mg O_2 m^2 h^{-1}) did not statistically differ between
377 ambient and elevated pCO_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 pCO_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 $\delta^{13}C$ values in above- and below- ground biomass of *H. wrightii* differed between the
385 high and ambient CO_2 treatment. The $\delta^{13}C$ 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

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

Discussion

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

Response of seagrass morphology and biomass

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

carbohydrates (Beer et al. 1977; Durako 1993; Hall-Spencer et al. 2008; Jiang et al. 2010; Campbell and Fourqurean 2013b; Cox et al. 2015; Zimmerman et al. 2017). In contrast, other studies support our findings and have found a neutral effect of ocean acidification on productivity and/or biomass of some seagrass species (Burnell et al. 2014; Apostolaki et al. 2014; Cox et al. 2016; Campbell and Fourqurean 2018). This 'lack of effect' is often attributed to other limitations or stressors in the seagrass environment. For example, the increased pCO_2 availability for seagrass species did not counteract negative impacts of warming temperatures (Collier et al. 2018), limiting light (Hendriks et al. 2017), or heavy metals (Olivé et al. 2017). Other researchers have underscored CO_2 availability as one abiotic factor of several limiting seagrass physiology (Burnell et al. 2014; Cox et al. 2016; Schneider et al. 2017; Pajusalu et al. 2018). Furthermore, outcomes may differ when the producer is held under constant or fluctuating pH (Britton et al. 2016).

Efficient users of bicarbonate

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

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

Duration of study

Discounting acclimation and adaptation, it is unlikely that there is a long-term benefit from the high CO_2 condition on vegetative growth for these species that was not captured by our experimental duration. *H. wrightii* and *R. maritima* have relatively short shoot turnover rates where growth can be 2 to 4 mm per day (Dunton 1990). For instance, *Halodule wrightii* is able translocate 14% of carbon from the leaves to the rhizome and roots in few hours (Moriarty et al. 1986). The short turnovers of these species appear to be specially marked in the estuarine waters of the Gulf of Mexico where *R.* maritima, completes its growth cycle in four months after flowering (Pulich 1985; Cho and Poirrier 2005). The experimental duration was during the period of peak biomass for *R. maritima* and a portion of the growth period for *H. wrightii*. Initiation of flowering by *R. maritima* and early flower stages were noted in homospecific and heterospecific beds under ambient and high CO_2 conditions but, unlike effects reported for *Zostera marina* (Palacios and Zimmerman 2007), the onset of flowering was not more frequent at either pCO_2 condition. *Halodule wrightii*, on the other hand, allocates more carbon in below ground tissue (Anton et al. 2011), yet we did not find any statistically significant differences in biomass allocation and we did not detect changes in nitrogen storage in the leaves or roots which could indicate an early positive response to the high CO_2 levels.

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

conditions (Hemminga and Mateo, 1996). In our study, there was low $\delta^{13}C$ values measured in above and below ground tissues for plants grown in the high CO_2 condition. Seagrasses preferentially use CO_2 over HCO_3^- and atmospheric CO_2 is more deplete in ^{13}C (-9 ‰, Kroopnick et al. 1985). Therefore, under ocean acidification conditions (higher pCO_2), we would expect seagrasses to have lower $\delta^{13}C$ values. However, the isotope value of the gas from the cylinders (-4.9 ‰ median measured from cylinders by Campbell and Fourqurean 2011) is also deplete in ^{13}C and background measures of DIC were not measured. Therefore, we cannot rule out the influence of the gas from the cylinder on $\delta^{13}C$ values. Nevertheless, the integration of a different carbon source in tissues (i.e. different $\delta^{13}C$ from ambient) and the observed increase in mean biomass in both conditions over the study duration allows us to conclude that the absence of positive affects in the high pCO_2 condition are not likely due to reliance and growth resulting from stored reserves.

Other limitations and potential stressors

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

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

Conclusions

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

Although we did not find the increase in pCO_2 to stimulate vegetative growth for seagrasses in the northern Gulf of Mexico, ocean acidification is known to positively affect the physiology or growth of other seagrass species. Therefore, the responses of seagrass meadows to ocean acidification appear to vary with seagrass species and their capacity to tolerate changes in the environment. As climate change continues, it is necessary to integrate the influence of environmental variability, as well as species interactions, for seagrass ecosystems to determine their susceptibility to anthropogenic perturbations.

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Tables

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, ºC); salinity (S); pH on the total scale (pH_T); total alkalinity (A_T, µmolkg^{-1}); partial pressure of CO_2 (pCO_2, µatm); dissolved inorganic carbon (C_T, µmol 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 µmol kg^{-1}, n = 7) together with the error for probes (pH = 0.01, T = 0.1, and S = 0.01) in seacarb.

		Ambient								**High CO_2**							
Day	**Date**	**T**	**S**	**A_T**	**pH_T**	**pCO_2**	**C_T**	**Ω_A**	**Ω_C**	**T**	**S**	**A_T**	**pH_T**	**pCO_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 pCO_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	*p*-value
Morphology and biomass				
Shoot density	*H. wrightii*	*pH*	0.997	0.335
		Bed type	0.589	0.456
		pH x Bed type	0.913	0.355
	R. maritima	*pH*	0.126	0.728
		Bed type	0.129	0.725
		pH x Bed type	6.005	0.028
Number of leaves per shoot	*H. wrightii*	*pH*	0.037	0.850
		Bed type	1.771	0.206
		pH x Bed type	0.359	0.559
	R. maritima	*pH*	1.213	0.291
		Bed type	0.153	0.702
		pH x Bed type	0.032	0.862
Total leaf material	*H. wrightii*	*pH*	0.184	0.675
		Bed type	8.418	0.012
		pH x Bed type	0.173	0.685
	R. maritima	*pH*	1.466	0.248
		Bed type	2.656	0.127
		pH x Bed type	3.163	0.099
Average shoot height	*H. wrightii*	*pH*	0.380	0.548
		Bed type	6.349	0.026
		pH x Bed type	0.154	0.701
	R. maritima	*pH*	0.392	0.542

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

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

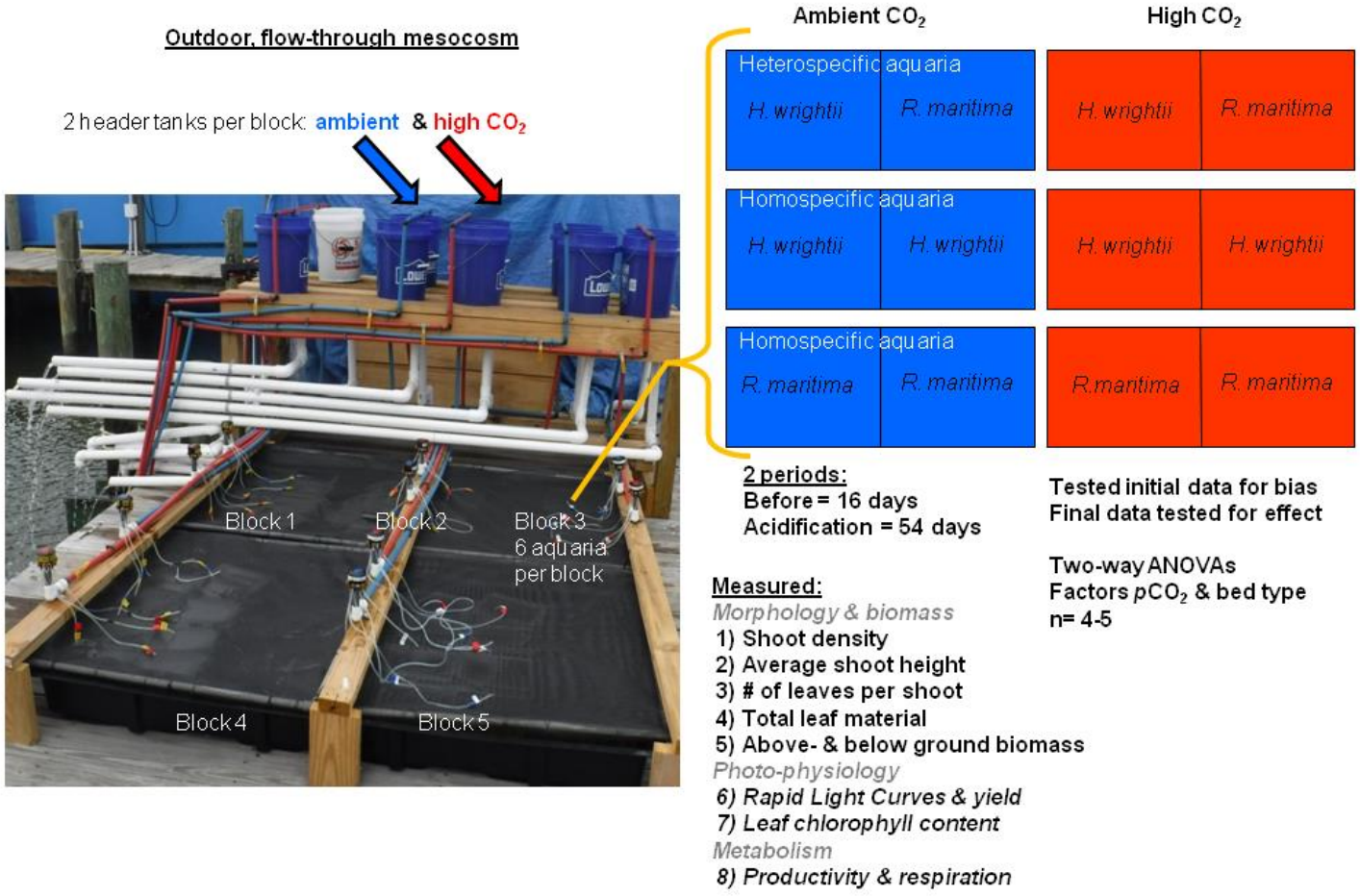
			Bed type	0.308	0.588
			pH x Bed type	1.662	0.220
NCP		*H. wrightii (Square root transformed)*	*pH*	0.034	0.856
			Bed type	0.973	0.341
			pH x Bed type	3.509	0.082
		R. maritima	*pH*	2.643	0.128
			Bed type	0.005	0.943
			pH x Bed type	0.408	0.534
Respiration		*H. wrightii*	*pH*	0.141	0.713
			Bed type	0.823	0.380
			pH x Bed type	3.155	0.097
		R. maritima	*pH*	1.537	0.237
			Bed type	0.614	0.447
			pH x Bed type	1.088	0.316
$\delta^{13}C$	*H. wrightii*	*Leaf* *	*pH*	64.400	**<0.001**
			Bed type	0.390	0.543
			pH x Bed type	4.599	0.051
		Root	*pH*	59.890	**<0.001**
			Bed type	0.336	0.572
			pH x Bed type	6.709	0.022
	R. maritima	*Leaf*	*pH*	36.680	**<0.001**
			Bed type	0.006	0.940
			pH x Bed type	0.828	0.384
		Root	*pH*	51.880	**<0.001**
			Bed type	0.014	0.908
			pH x Bed type	0.159	0.699
$\delta^{15}N$	*H. wrightii*	*Leaf*	*pH*	0.431	0.523
			Bed type	0.080	0.782
			pH x Bed	0.004	0.948

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

	Root	*pH*	5.991	0.034
		Bed type	0.422	0.531
		pH x Bed type	0.002	0.964

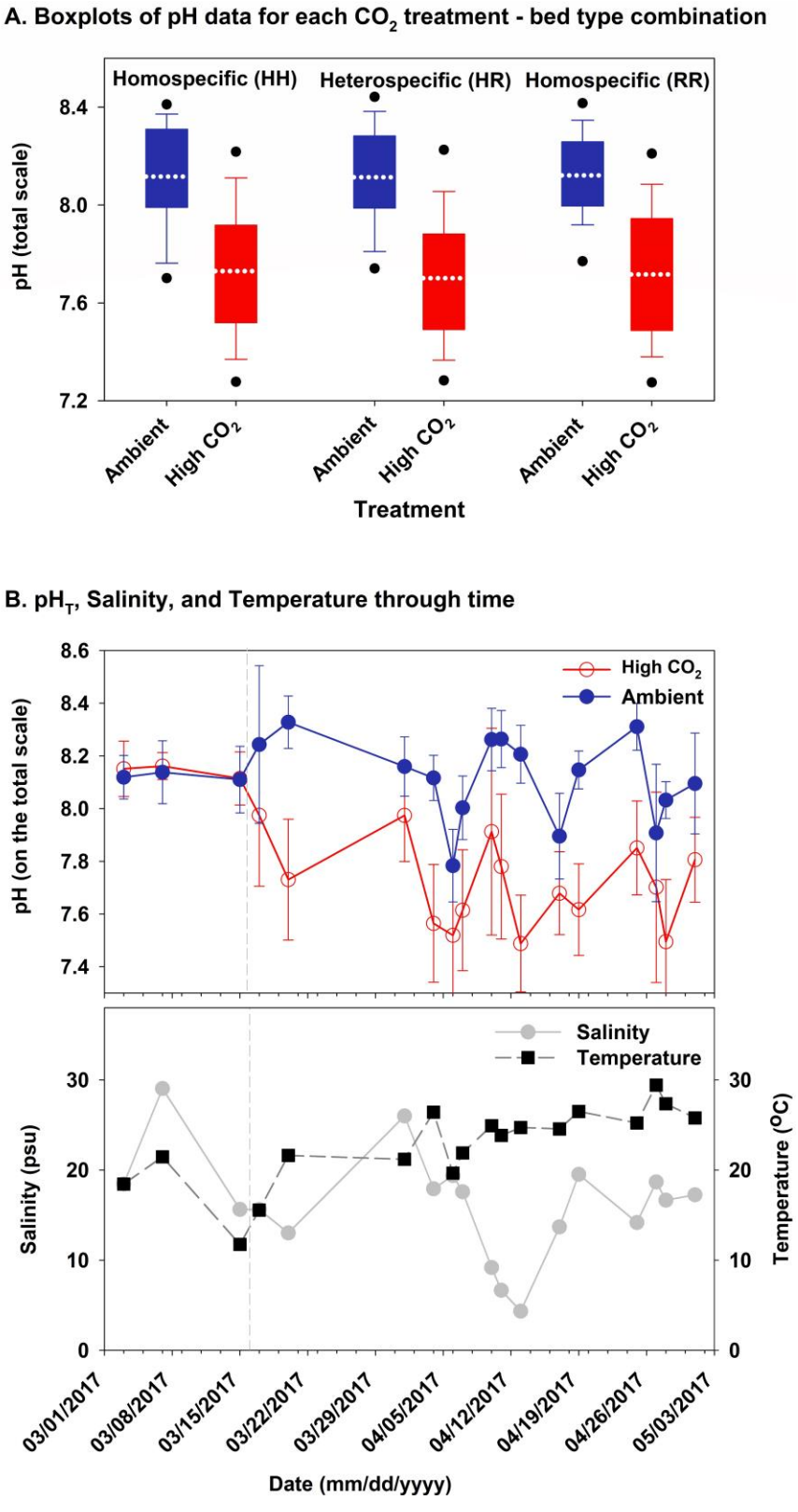
741

742



743

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


A. Boxplots of pH data for each CO_2 treatment - bed type combination
Homospecific (HH)
Heterospecific (HR)
Homospecific (RR)
pH (total scale)
8.4
8.0
7.6
7.2
Ambient
High CO_2
Ambient
High CO_2
Ambient
High CO_2
Treatment
B. pH_T, Salinity, and Temperature through time
pH (on the total scale)
8.6
8.4
8.2
8.0
7.8
7.6
7.4
High CO_2
Ambient
Salinity
Temperature
Salinity (psu)
Temperature (^oC)
30
20
10
0
03/01/2017
03/08/2017
03/15/2017
03/22/2017
03/29/2017
04/05/2017
04/12/2017
04/19/2017
04/26/2017
05/03/2017
Date (mm/dd/yyyy)

745

Fig. 2 The pH_T, salinity and temperature in the ambient and high CO_2 aquaria. Panel A is a boxplot of the all the discrete measures of pH_T presented by bed type (homo- or hetero-specific and CO_2 treatment (ambient or high). The dotted white line within the bar is the mean and the whiskers from the bars capture the 5th and 95th percentile. Panel B top, shows the evolution of pH_T (mean ± SE, n = 27 aquaria) throughout the experiment as a function of (bottom) probed temperature and salinity (n = 5) used to calculate the carbonate chemistry. The dotted lines indicate the beginning of the perturbation.

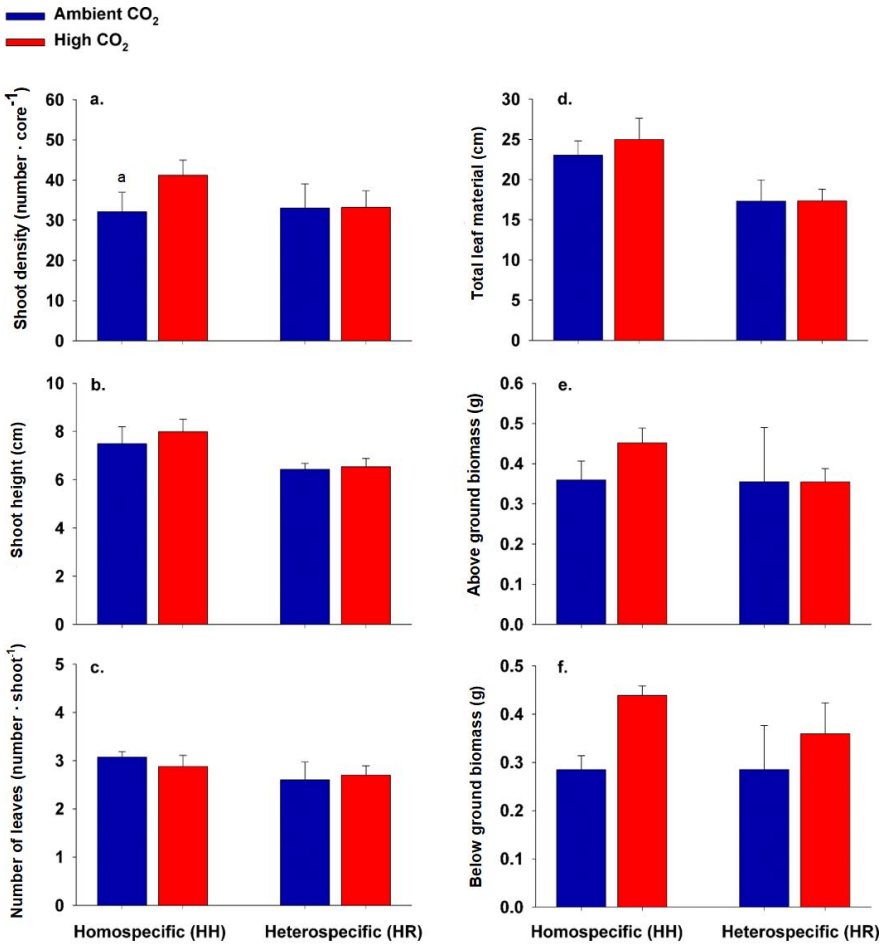
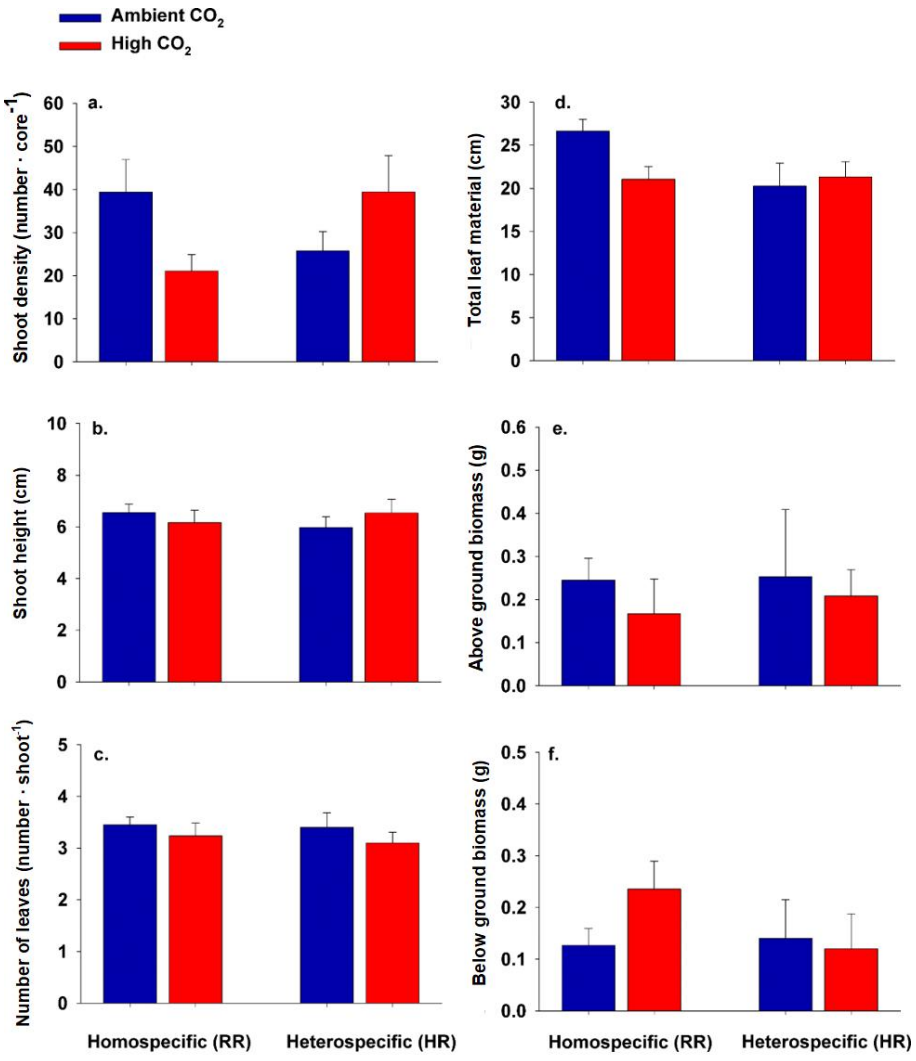
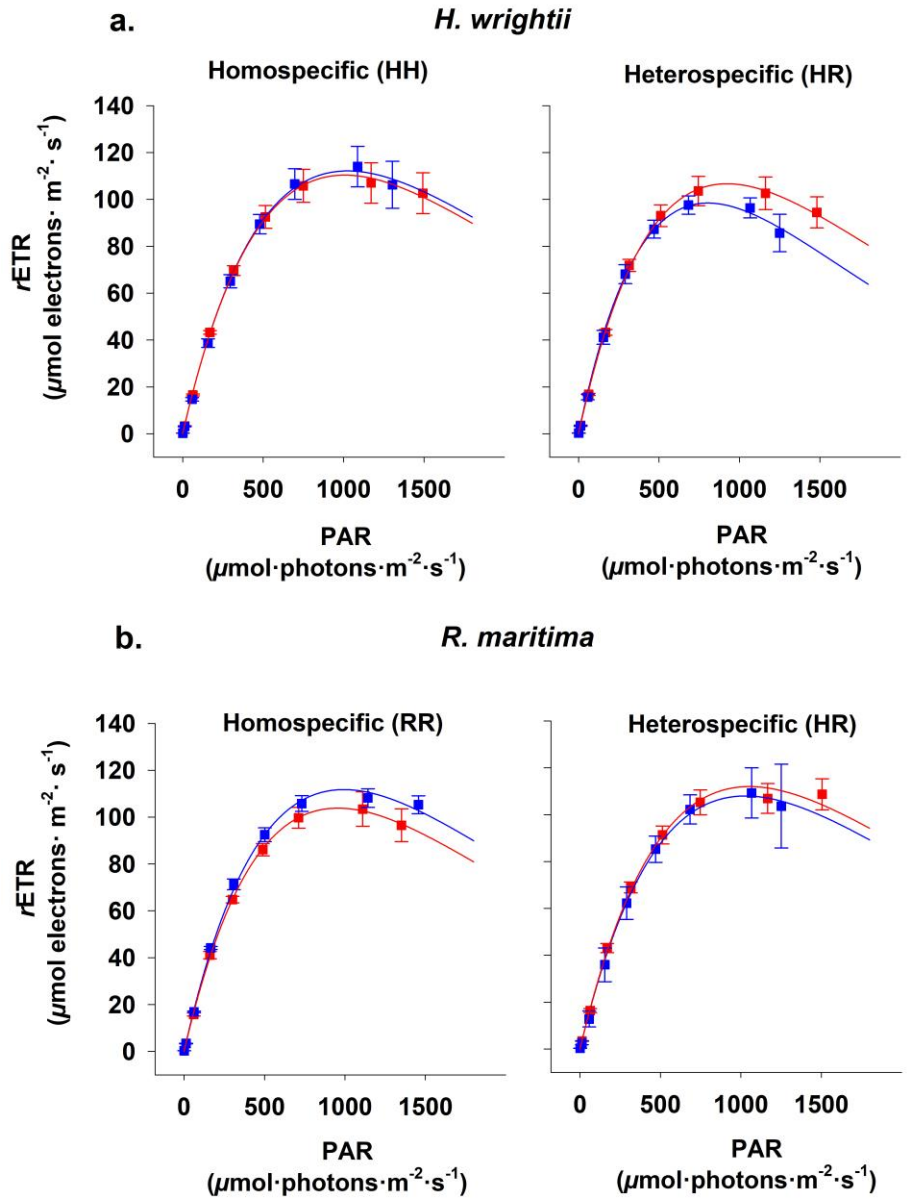


Fig. 3 *Halodule wrightii* (mean ± SE; n= 4 to 5) morphology (shoot density, a; shoot height, b; leaves per shoot, c; total leaf material, d) and above- and below ground biomass (e, f) after maintained for 34, 41, or 54 days at ambient (blue) and high CO_2 (red) treatments. *Halodule wrightii* was grown in homospecific (*H. wrightii* with *H. wrightii*, HH) and heterospecific (*H. wrightii* with *R. maritima*, HR) beds. Data did not show significant differences between treatments.



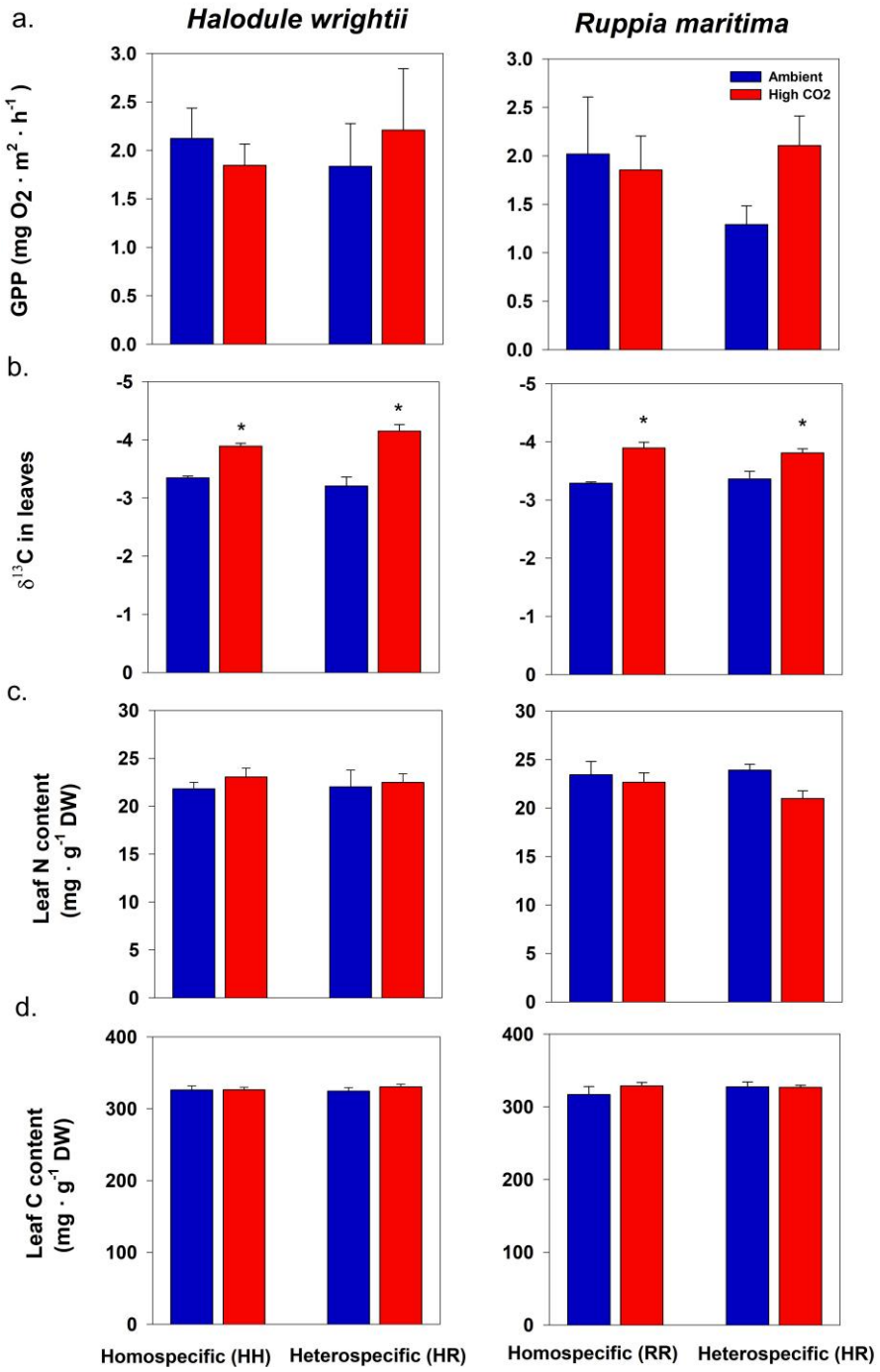
762

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 CO_2 (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.
769



770

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 CO_2 treatments (continuous modeled
775 lines). Modeled lines and *r*ETR (mean ±SE) values are based upon an average from 4 to
776 5 aquaria. PAR units were µmol photons $m^{-2}s^{-1}$.
777



778

779 **Fig. 6** Gross community productivity (GCP; a), $\delta^{13}C$ (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 ± SE; n= 4 to 5) after
783 maintained for 48 days under ambient (blue) and high CO_2 treatments (red). Asterisks (*)
784 indicates significant differences between treatment.

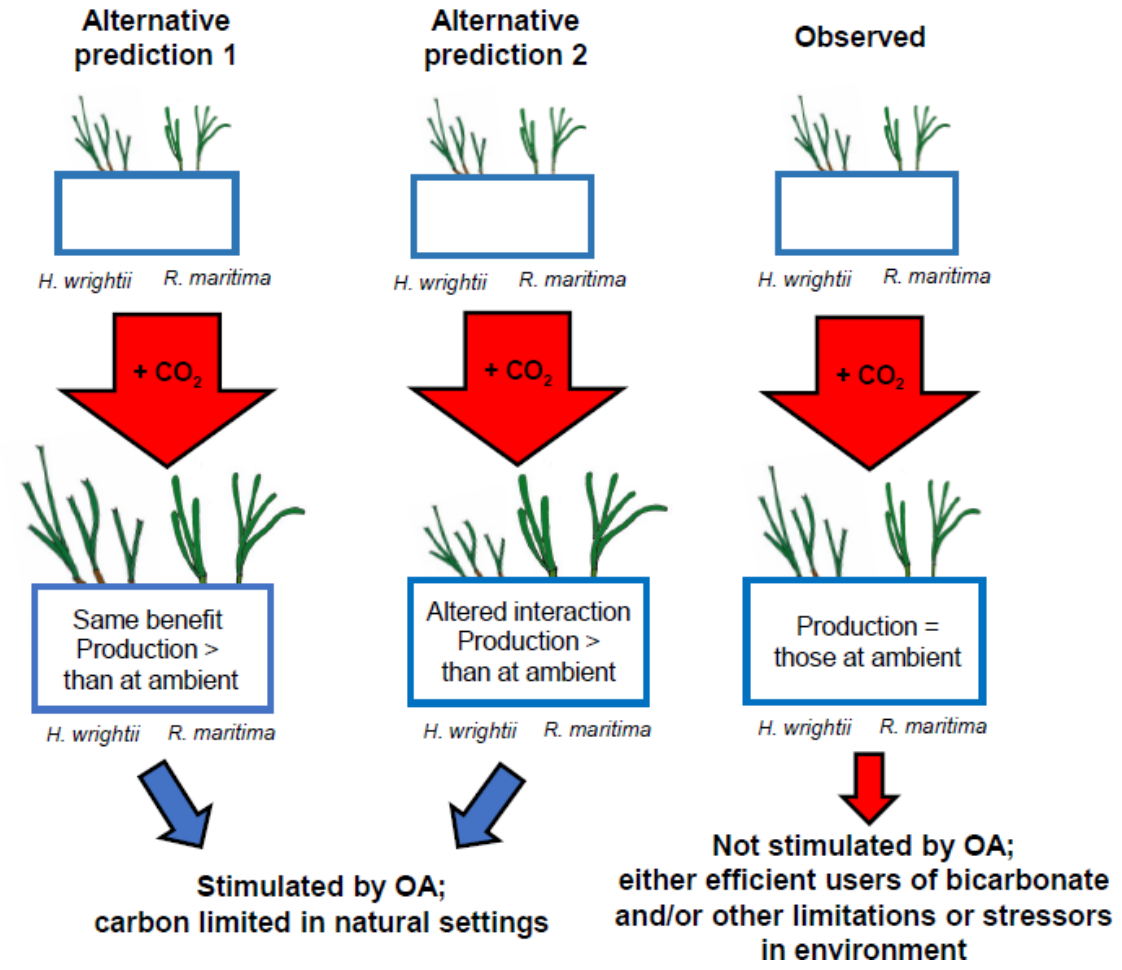


Fig. 7 Graphical summary of the effects of ocean acidification (OA). In a heterospecific bed (represented by aquaria with two species seen in the blue boxes) the increased pCO_2 was predicted to increase seagrass growth and production with either little change to bed composition (alternative prediction 1) or with a shifted interaction where one species comes to dominate in abundance (alternative prediction 2). Yet, we observed no effect of increased pCO_2 on seagrass growth and production. Therefore, these species must either be efficient users of bicarbonate and/or other stressors and limitations outweighed any stimulation from increased pCO_2.

795 **Appendices**

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

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

		(squared root transformed)	*Bed type*	2.609	0.132
			pH x Bed type	1.589	0.231
NCP		*H. wrightii*	*pH*	0.281	0.606
			Bed type	1.074	0.320
			pH x Bed type	0.326	0.579
		R. maritima	*pH*	0.684	0.424
			Bed type	0.259	0.620
			pH x Bed type	2.066	0.176
Respiration		*H. wrightii*	*pH*	1.471	0.249
			Bed type	0.964	0.346
			pH x Bed type	0.100	0.757
		R. maritima	*pH*	2.199	0.164
			Bed type	4.299	0.060
			pH x Bed type	0.058	0.814

Supplementary Table 2. Temperature, salinity and pH (mean ± SD) measured per block during the experiment in the header tanks. Means of pH were separate between treatments (Ambient and high pCO_2).

	Temperature		**Salinity**		**pH**			
					Ambient		**High CO_2**	
	MEAN	±SD	MEAN	±SD	MEAN	±SD	MEAN	±SD
Tank 1	23.31	2.290	15.48	6.201	8.074	0.199	7.754	0.289
Tank 2	23.22	2.248	16.25	5.829	8.108	0.212	7.628	0.376
Tank 3	23.13	2.577	16.43	5.993	8.056	0.228	7.722	0.298
Tank 4	23.28	2.353	16.37	5.892	8.159	0.158	7.668	0.320
Tank 5	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

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 pCO_2) and "Time" (day of sample collection).Temperature (T, ºC); salinity (S); pH on the total scale (pH_T); total alkalinity (A_T,µmolkg-1); partial pressure of CO_2 (pCO_2,µatm); dissolved inorganic carbon (C_T,µmol 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

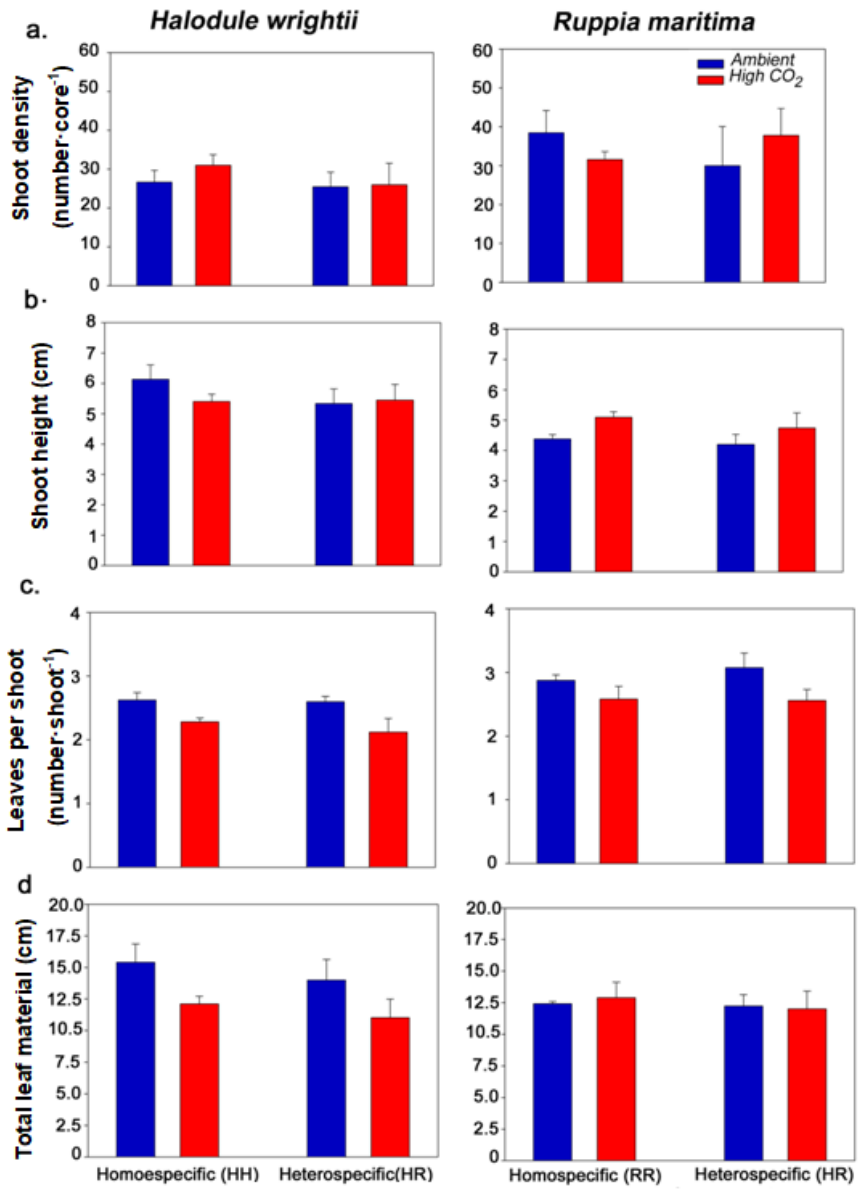
standard (for A_T precision was 3.9 µmol kg^{-1}, n = 7) together with the error for probes (pH = 0.01, T = 0.1, and S = 0.01)

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		
***p*CO2 (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 CO_2: 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 CO_2: 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		

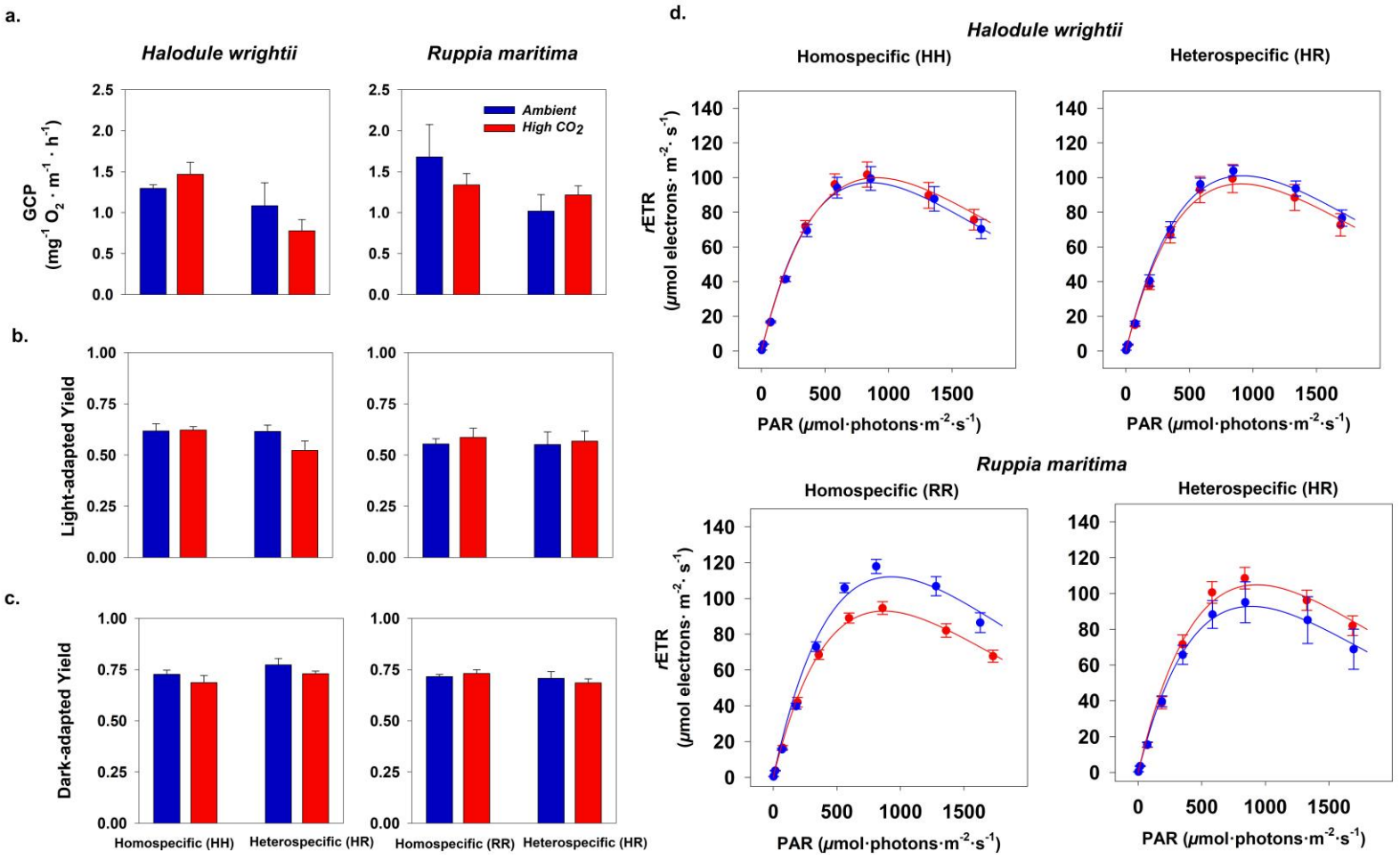
Supplementary Table 4. Error estimates for the variables pCO_2, C_T, Ω_A and Ω_C using the function "errors" in the "seacarb" package in R software (A_T precision of 3.9 µmol kg^{-1}, n = 7; error for probes of pH = 0.01, T = 0.1, and S = 0.01).

		Ambient				**High CO_2**			
Day	**Date**	***p*CO_2**	***C*$_T$**	**Ω_A**	**Ω_C**	***p*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 ± SE) before
820 maintained for 34, 41, and 54days at ambient (blue) and high CO_2 (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).



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