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Responses of three tropical seagrass species to CO_2 enrichment

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Abstract Increased atmospheric carbon dioxide leads to ocean acidification and carbon dioxide (CO_2) enrichment of seawater. Given the important ecological functions of seagrass meadows, understanding their responses to CO_2 will be critical for the management of coastal ecosystems. This study examined the physiological responses of three tropical seagrasses to a range of seawater pCO_2 levels in a laboratory. *Cymodocea serrulata*, *Halodule uninervis* and *Thalassia hemprichii* were exposed to four different pCO_2 treatments (442–1204 μatm) for 2 weeks, approximating the range of end-of-century emission scenarios. Photosynthetic responses were quantified using optode-based oxygen flux measurements. Across all three species, net productivity and energetic surplus (P_G:R) significantly increased with a rise in pCO_2 (linear models, $P < 0.05$). Photosynthesis–irradiance curve-derived photosynthetic parameters—maximum photosynthetic rates (P_{max}) and efficiency (α)—also increased as pCO_2 increased (linear models, $P < 0.05$). The response for productivity measures was similar across species, i.e. similar slopes in linear models. A decrease in compensation light requirement (E_c) with increasing pCO_2 was evident in *C. serrulata* and *H. uninervis*, but not in *T. hemprichii*. Despite higher productivity with pCO_2 enrichment, leaf growth rates in *C. serrulata* did not increase, while those in *H. uninervis* and *T. hemprichii* significantly increased with increasing pCO_2 levels. While seagrasses can be carbon-limited and productivity can respond positively to CO_2 enrichment, varying carbon allocation strategies amongst species suggest differential growth response between species. Thus, future increase in seawater CO_2 concentration may lead to an overall increase in seagrass biomass and productivity, as well as community changes in seagrass meadows.

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Introduction

Anthropogenic carbon emissions have led to atmospheric carbon dioxide (CO_2) rising by 40 % since pre-industrial times (Raven et al. 2005). By the end of this century, atmospheric CO_2 is predicted to double from current levels (Meehl et al. 2007; Collins et al. 2013). The rise in oceanic CO_2 concentration that follows is projected to decrease seawater pH by 0.3–0.4 units (Caldeira and Wickett 2003; Feely et al. 2004). This reduction can alter the carbonate chemistry of seawater in terms of the relative proportion of the dissolved inorganic carbon (DIC) species. Current concentrations of CO_2 and HCO_3^- in seawater are 8 and 1650 μmol kg^{-1} seawater, respectively (Koch et al. 2013). Under the projected decrease in seawater pH, the proportion of CO_2 will have greater proportional increase (>250 %) than the other DIC constituents (HCO_3^-: 24 % and CO_3^{2-}: −61 %) (Koch et al. 2013). The higher concentration of utilisable carbon for photosynthesis (CO_2 and HCO_3^-) in acidified seawater may benefit marine macrophytes that are limited by the DIC concentration under current conditions (Beer et al. 2002).

Seagrasses can be carbon-limited at the seawater DIC composition under current CO_2 concentrations, given other conditions, such as light, nutrient availability and water temperature are non-limiting (Beer and Koch 1996; Thom 1996; Zimmerman et al. 1997; Invers et al. 2001). Most seagrasses utilise the C3 metabolism for carbon fixation (Koch et al. 2013). Elevated partial pressure of CO_2 (pCO_2) can increase carboxylation rates while reducing oxygenation rates of ribulose-1,5-bisphosphate carboxylase–oxygenase (Rubisco), the initial carboxylating enzyme in C3 plants (Bowes and Ogren 1972; Koch et al. 2013). Furthermore, the predominant DIC species, HCO_3^-, appears to be less efficiently utilised in seagrasses—the increase in photosynthetic rates was much higher when seagrasses were enriched with CO_2 than with HCO_3^- (Sand-Jensen and Gordon 1984; Durako 1993; Beer and Koch 1996; Invers et al. 2001). Although seagrasses have been shown to possess carbon-concentrating mechanisms (CCMs) to more efficiently utilise HCO_3^-, whether these CCMs could effectively saturate the seagrasses to meet their DIC requirements under natural conditions remains to be seen (Beer et al. 2002; Koch et al. 2013). Overall, it is thought that higher pCO_2 not only increases passive diffusion of CO_2 for carbon fixation, but also lowers the loss of fixed carbon through photorespiration (Long et al. 2004). Laboratory and mesocosm experiments conducted over the short and medium term have shown an optimisation of photosynthetic performance, such as light requirements, photosynthetic efficiency and pigment content in response to CO_2 (Zimmerman et al. 1997; Jiang et al. 2010; Campbell and Fourqurean 2013b). This can result in higher rates of carbon fixation with flow-on effects to growth rate, carbohydrate content, biomass and reproductive output (Zimmerman et al. 1997; Jiang et al. 2010; Campbell and Fourqurean 2013b). In the field, higher seagrass productivity and biomass have been observed near natural CO_2 vents, suggesting that acidification of seawater would benefit seagrass meadow productivity over the long term (Hall-Spencer et al. 2008; Fabricius et al. 2011; Russell et al. 2013).

Different seagrass species might vary in the manner and extent to which they respond to CO_2 enrichment. No previous studies have directly compared species responses to CO_2 enrichment, but responses to CO_2 depletion indicate that species are not affected uniformly by changing pCO_2 (Invers et al. 1997; Beer et al. 2006). This makes it difficult to determine whether findings are related to species or methodological differences. Most studies had focussed on temperate species, such as *Zostera marina* (Thom 1996; Zimmerman et al. 1997; Palacios and Zimmerman 2007), *Zostera noltii* (Alexandre et al. 2012) and *Posidonia oceanica* (Invers et al. 2002). Amongst temperate species, Invers et al. (2001) demonstrated that pCO_2 enhancement of photosynthesis was higher in Pacific species (*Z. marina* and *Phyllospadix torreyi*) than in Mediterranean species (*P. oceanica* and *Cymodocea nodosa*). The few studies on tropical seagrasses yielded mixed results. For example, Jiang et al. (2010) showed increased growth and productivity in *T. hemprichii*, while *T. testudinum* showed little change in biomass and productivity to increased pCO_2 (Durako and Sackett 1993; Campbell and Fourqurean 2013a). Hence, differential response to CO_2 enrichment might exist between and within multi-species tropical seagrass meadows.

Differences in carbon utilisation and allocation strategies exist amongst tropical seagrass species (Hemminga and Duarte 2000; Uku et al. 2005). Species-specific differences in DIC uptake mechanisms would result in varying abilities amongst species to utilise the extra DIC (Invers et al. 2001; Uku et al. 2005; Campbell and Fourqurean 2013b). Species-specific carbon allocation strategies could affect how responses to CO_2 enrichment manifest at the plant scale. For example, in species that invest a greater proportion of biomass to belowground tissue, such as *Halodule uninervis* and *Thalassia hemprichii*, there would be a higher metabolic demand on aboveground tissue for photosynthetic carbon fixation (Terrados et al. 1999; Hemminga and Duarte 2000; Tanaka and Nakaoka 2007). Increased availability of CO_2 in seawater could allow for increasing photosynthetic capacity (e.g. more chlorophyll pigments, enhanced shoot growth) and/or increased storage of carbohydrates to support respiratory demands (Zimmerman et al. 1997; Jiang et al. 2010). In addition, small-bodied ephemeral species, such as *Halodule uninervis*, exhibit short turnover of leaves, while bigger and more persistent species such as *Cymodocea serrulata* and *Thalassia hemprichii* have longer shoot plastochrone intervals (Hemminga and Duarte 2000). Turnover rates of assimilated carbon could influence carbon demand (Arp 1991; Hemminga and Duarte 2000). Thus, various measures of productivity, such as tissue growth rates, carbohydrates storage or shoot production could vary amongst co-occurring species in response to CO_2 over different timescales.

Productivity of seagrass meadows is central to their ecological functions as a food source, including for megafauna such as dugongs and turtles, in bio-sequestration ("blue carbon"), and substrate stabilisation (Duarte and Chiscano 1999; Gacia and Duarte 2001; Fourqurean et al. 2012; Vafeiadou et al. 2013). Understanding how productivity responses to CO_2 enrichment vary amongst species is vital for predicting future ecological change. In the present study, we quantified the photosynthetic and growth responses of three tropical seagrass species to increasing pCO_2 levels, bracketing the range of different end-of-century emission scenarios as predicted by IPCC (2013). This allows for the quantification of the response to pCO_2 levels in seagrass productivity and growth. The three species

Table 1 Measured and calculated parameters, and average nutrient concentrations for control and three enriched pCO_2 treatments

pCO_2 treatment	Measured parameters				Calculated parameters					Nutrient concentrations		
	pH range (NBS scale)	Temp (°C)	DIC (μmol kg^{-1} SW)	TA (μmol kg^{-1} SW)	pH (NBS scale)	pCO_2 (μatm)	HCO_3^- (μmol kg^{-1} SW)	CO_3^{2-} (μmol kg^{-1} SW)	CO_2 (μmol kg^{-1} SW)	NH_4^+ (μmol L^{-1})	PO_4^{3-} (μmol L^{-1})	NO_3^- (μmol L^{-1})
High	7.69–7.85	23.9 (0.2)	2146 (7)	2296 (3)	7.76 (0.02)	1204 (59)	2024 (4)	86.2 (4.0)	35.3 (1.8)	0.217 (0.02)	0.206 (0.05)	0.968 (0.70)
Intermediate	7.81–7.98	24.0 (0.2)	2120 (10)	2290 (2)	7.89 (0.02)	884 (52)	1980 (7)	113.4 (6.2)	25.9 (1.5)	0.210 (0.03)	0.167 (0.04)	0.700 (0.50)
Low	7.91–8.01	23.7 (0.2)	2084 (7)	2289 (2)	7.98 (0.01)	694 (20)	1930 (7)	133.2 (3.4)	20.5 (0.6)	0.223 (0.03)	0.211 (0.06)	0.914 (0.61)
Control	8.12–8.16	23.7 (0.2)	2012 (8)	2281 (1)	8.14 (0.01)	442 (6)	1814 (6)	184.5 (2.6)	13.0 (0.2)	0.210 (0.03)	0.184 (0.03)	0.923 (0.64)

Water samples for DIC and nutrients (in duplicates) were taken every 5 days from each tank. Results were pooled and averaged over sampling times and tanks for each treatment ($n = 4$). Standard errors are given in brackets

examined, *Halodule uninervis*, *Cymodocea serrulata* and *Thalassia hemprichii*, are common seagrasses found in the tropical Indo-Pacific region with contrasting growth strategies, ranging from rapid growth in *H. uninervis* to slow growth in *C. serrulata* and *T. hemprichii* (Hemminga and Duarte 2000). It was hypothesised that pCO_2 enrichment would increase photosynthetic and growth rates, but rate of responses may vary between species due to varying carbon uptake and allocation strategies (Campbell and Fourqurean 2013b).

Materials and methods

Experimental species

Seagrasses were collected two to four weeks prior to the start of the experiment. Seagrass species *Cymodocea serrulata* and *Halodule uninervis* were collected from the intertidal meadow at Cockle Bay, Magnetic Island, Northern Great Barrier Reef (19°10.88′S, 146°50.63′E) in March 2013. Average daily and average maximum photosynthetically active radiations (PAR) at this site are 385 and 961 μmol m^{-2} s^{-1}, respectively (Collier, unpublished). Intact plugs of *H. uninervis* and sediment were collected with a trowel and placed into a plastic pot lined with a plastic bag. The bag was pulled up and secured over the seagrass to prevent moisture loss during transport. *C. serrulata* was collected by excavating intact shoots with connected horizontal rhizomes from the sediment before placing into seawater-filled containers for transport to aquaria. *Thalassia hemprichii* was collected from Green Island in the Northern Great Barrier Reef (16°45.37′S, 145°58.19′E), using a similar method to *C. serrulata*. At this site, average daily PAR was 344 μmol m^{-2} s^{-1} and average maximum PAR was 841 μmol m^{-2} s^{-1}, respectively (Collier C, unpublished). Average water temperatures at Cockle Bay (2005–2012) and Green Island (2003–2012) were 26.2 and 26.6 °C, respectively (McKenzie et al. 2014). Seagrasses were planted into orchid pots lined with a pool filter sock, in a mud and sand (roughly 20:80) mixture, within 2 days of collection. For acclimation, all species were kept in an outdoor flow-through aquarium prior to the experiment, under average light levels of 350 μmol m^{-2} s^{-1}, average seawater temperature of 25 °C and salinity at 35 ppt.

Experimental set-up

Seagrasses were exposed to four different seawater pCO_2 concentrations in a flow-through system for two weeks (Table 1). The experiment was conducted in an indoor flow-through aquarium system at the Australian Institute of Marine Sciences, Townsville. Sixteen glass aquaria with four

replicates for each treatment (working volume 18 l) were supplied with fresh filtered seawater from four header tanks. Each aquarium contained all three species. Two sub-replicate pots of each species were placed in each aquarium. pH levels in the header tanks were monitored, as a proxy to control for CO_2 input, with eight potentiometric sensors (±0.01 pH unit) calibrated on the NBS scale. The sensors are connected to a feedback control system that regulates pH levels via a CO_2 gas injection system (AquaMedic, Germany). Pumps and diffusers installed in mixing tanks and experimental aquaria ensured thorough mixing of CO_2. Additional pH readings were taken regularly with a hand held pH probe (pH probe: Eutech, USA; console: Oakton, USA) and compared to Tris seawater standards (Batch 10, Supplied by A. Dixon, Scripps Institute of Oceanography). Water temperature remained constant throughout the experiment around 24 °C (Table 1). Water samples, taken every 5 days, were analysed for dissolved inorganic carbon (DIC) and total alkalinity (AT) concentrations using a Vindta 3C analyser. Carbonate system parameters (Table 1) were calculated by measured values of AT, DIC, temperature and salinity by USGS CO2calc software (Robbins et al. 2010). Illumination was provided with LED lamps (Aqua Illumination) mounted about 40 cm above the aquaria, providing 400 µmol m^{-2} s^{-1} of light set on a 12-h light/dark photoperiod. Duplicate water samples collected from each individual aquaria every 5 days were filtered (0.45 µm pore size) before they were analysed for dissolved inorganic nitrogen and phosphorus concentration according to Ryle et al. (1981).

Photosynthetic response

Photosynthetic rates and respiration of the second youngest leaf (rank 2) of a haphazardly chosen shoot from each pot were measured using optical oxygen sensors ("optode", PreSens, Sensor spots-Pst3) and a PreSens Oxy 4 four-channel fibre-optic oxygen meter after two weeks. While the authors acknowledge that seagrasses could be sensitive to physical manipulations such as removing leaves (Schwarz et al. 2000), care was taken to reduce the impact on leaves such as using the whole leaf and gently rubbing epiphytes off with fingers instead of scrapping with a blade. Small transparent acrylic chambers (200 mL) were set in an array of four (i.e. four separate chambers allowing four parallel measures) and incubated at 25 °C water temperature using a flow-through water system connected to a water bath (Lauda, Ecoline RE 106). Stirrer bars placed within the chambers provided even stirring. The leaves were held upright in the chamber to mimic natural orientation. Oxygen consumption (dark respiration) was measured over a 20-min period in the dark. Photosynthetic rates were then measured on the same leaf over a series of light steps (10, 30, 70, 110, 220, 400, 510 µmol m^{-2} s^{-1}) (Aqua Illumination LED), with each light step lasting 20 min. Seawater within the chambers was replaced with fresh media every two to three steps. Oxygen concentration data in the chambers were logged every 5 s, and respiration and production rates were calculated by fitting a linear regression. Rates were normalised to the dry weight of the leaf. Leaves were dried at 60 °C for 48 h before weighing. Initial periods of incubation (~5 min) prior to stabilisation of photosynthetic rates were omitted from regressions. Each optode was calibrated according to Collier et al. (2011).

Net productivity (NP) was taken to be the photosynthetic rate measured at 400 µmol m^{-2} s^{-1}, which was the experimental light level. Energetic surplus (P_G:R) was calculated as the ratio of gross productivity (sum of net photosynthetic rate and dark respiration rate) to dark respiration rate (Zimmerman et al. 1997). To determine photosynthetic parameters, photosynthesis versus irradiance (P–E) data plots were fitted to the adapted hyperbolic tangent model equation of (Jassby and Platt 1976):

$$P = P_{\max} \times \tanh\left(\frac{\alpha P_{\max}}{E}\right)$$

where P_{max} is the maximal photosynthetic rate (mg O_2 g^{-1} DW h^{-1}), E is irradiance (µmol m^{-2} s^{-1}), and α described photosynthetic efficiency via the gradient of the curve at limiting irradiances (mg O_2 µmol^{-1}). Saturating irradiance (E_k) is the light level at which photosynthesis initially reaches the maximum rate, and compensation irradiance (E_c) is the light level when photosynthetic rate is equal to respiration rate.

Determination of growth rates

Growth was measured following Short and Duarte (2001). All shoots from each pot were marked at the top of the sheath with a needle at the start of the experiment. At the end of the experiment, the shoots were harvested. The length of new tissue growth was excised, dried at 60 °C for 48 h and weighed for determination of weight of new leaf growth. Leaf tissue growth was normalised to the above-ground biomass of its respective pot to derive relative leaf growth rates (RGR).

Specific leaf area (SLA) was calculated from biomass and areal measurements of leaves. Specific leaf area refers to the total leaf area normalised by the total biomass of the leaves and could be used to infer whole-plant changes in leaf biomass and area in response to pCO_2 enrichment (Chiariello et al. 1989). Leaves were separated from shoots and placed on a flat surface. Areal measurement of leaves was then carried out by capturing a clear image of all the leaves and analysing with CPCe software (version 3.6) (Kohler and Gill 2006). Finally, the leaves were dried at 60 °C for 48 h and weighed to obtain biomass measurements.

Chlorophyll content

A young mature leaf (rank 2) from each pot was collected and stored immediately at −20 °C at the end of the experiment. To determine chlorophyll concentration, a 10- to 15-mm section of leaf was cut from the middle of a fully mature leaf and the width of the leaf segment was measured using a pair of callipers. The leaves were blotted dry and weighed before they were ground in a chilled mortar. Depending on the species and the weight of the leaf segment, 5–6 mL of cold (4 °C) 90 % acetone was added to extract chlorophyll from the sample. The solution was gently shaken, left in the dark to extract for 24 h at 4 °C and then centrifuged at 2680 g for 4 min to settle the pellet. The extract was measured for chlorophyll concentration according to Granger and Izumi (2002).

Non-structural carbohydrate (NSC) content

Roots and rhizomes were dried at 60 °C for 48 h, before being finely ground in a bead beater (Daintree Scientific). Four replicate samples per treatment and species were sent to the Agriculture and Food Sciences laboratory in University of Queensland for non-structural carbohydrate content analysis. Briefly, soluble carbohydrates were extracted twice with 80 % ethanol at 80 °C for 10 min from 200 mg of ground plant material. Extracts were then passed through a de-colourising column to remove phenolic compounds. After acid hydrolysis, the amount of soluble carbohydrates was assayed with ferricyanide reagent and absorbance measured on a UV–Vis spectrophotometer at 420 nm (McCleary and Codd 1991).

Starch content was analysed according to Karkalas (1985). Residue from the soluble carbohydrate extraction was solubilised in boiling water. After cooling to room temperature, samples underwent enzyme digestion where amylase and amyloglucosidase were added. After incubation, the concentration of glucose is measured using a commercially available glucose oxidase/peroxidase (GOPOD) testing reagent (Megazyme). Absorbance was then measured at 510 nm.

Total non-structural carbohydrate (NSC) content, which was the sum of the amount of soluble carbohydrate and starch content, was expressed as milligrams dry weight^{-1} of tissue.

Statistical analyses

All statistical analyses were carried out with R software (R Development Core Team 2011). Changes in photosynthetic and growth responses were tested using linear models with average pCO_2 levels for each treatment as explanatory variable. Data from sub-replicate pots from each tank were averaged for the analysis. Assumptions of homogeneity of variances and normality were checked using box plots and residual plots. To satisfy the assumptions, photosynthetic efficiency (α) and compensation irradiance (E_c) for *T. hemprichii* were square-root-transformed prior to analysis. One data point was identified as an outlier (>2 SD from mean of remaining replicates) in each of the P_G:*R* and α dataset and was subsequently removed. To examine species differences in productivity and growth responses to increasing pCO_2, confidence intervals (CI) of the slopes (degree of response per 100 µatm rise in pCO_2) from linear models were calculated and compared.

Results

Experimental parameters

Water temperature (23.7–24.0 °C) and salinity (35 ppt) in the experimental tanks were near-constant throughout the experiment (Table 1). Carbonate system parameters of the enriched pCO_2 treatments remained well within the target range (control pCO_2 = 442 ± 6 µatm; low pCO_2 = 694 ± 20 µatm; intermediate pCO_2 = 884 ± 52 µatm; high pCO_2 = 1204 ± 59 µatm) (Table 1). Inorganic nutrient concentrations were similar between tanks and averaged to an ammonium concentration of 0.22 ± 0.01 µM, nitrate concentration of 0.88 ± 0.3 µM and phosphate concentration of 0.19 ± 0.02 µM.

Photosynthetic performance

Carbon dioxide enrichment increased seagrass net productivity (NP). Under the chosen light level (400 µmol m^{-2} s^{-1}), NP significantly increased with increasing pCO_2 levels for all species (Fig. 1; Table 2). Across species, the increase in NP ranged from 0.757 to 1.040 mg O_2 g^{-1} DW h^{-1} for every 100 µatm increase in pCO_2; however, no species difference in the slope was detected (based on overlapping confidence intervals) (Table 2).

Energetic surplus, or gross photosynthetic to respiration ratios (P_G:*R*), significantly increased with increasing pCO_2 for all three species (Fig. 1; Table 2). No distinct differences in the slopes (0.32–0.47 units as pCO_2 increased by 100 µatm, Table 2) indicated that P_G:*R* responses in the different species were similar.

Photosynthetic rates in all three species exhibited typical *P–E* (PAR) response curves. Photosynthetic rates increased linearly (initial slope, α) with light under limiting irradiances, before levelling off at the maximum photosynthetic rate (P_{max}) past saturating irradiance (E_k). Photosynthesis–irradiance (*P–E*) curves demonstrated a good fit ($R^2 > 0.85$; $P < 0.05$) to the adapted hyperbolic tangent model.

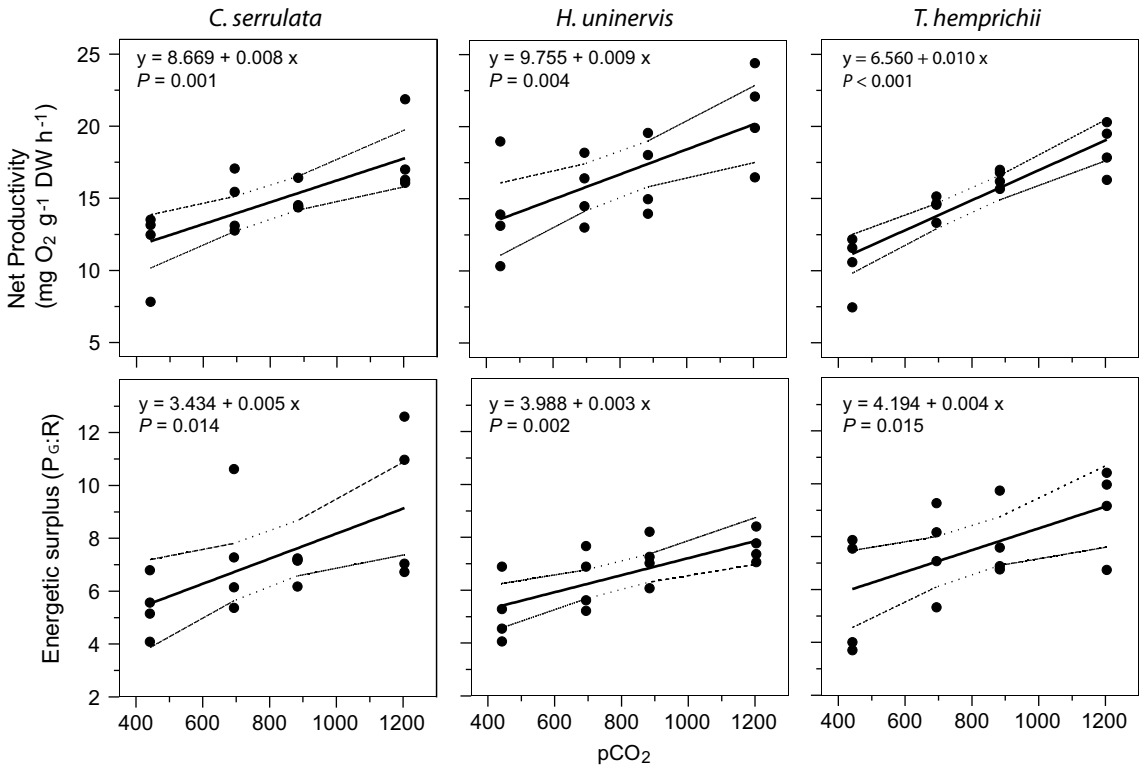

Fig. 1 Linear model fits (*dotted lines* indicate 95 % confidence intervals) for net productivity and energetic surplus (P_G:R) of *C. serrulata*, *H. uninervis* and *T. hemprichii* in response to pCO_2 enrichment. $N = 4$

Increasing pCO_2 levels significantly increased maximum photosynthetic rates (P_{max}) for all three species (Table 2; Fig. 2). Maximal photosynthetic rates (P_{max}) increased by 0.677–0.929 mg O_2 g^{-1} DW h^{-1} for every 100 µatm rise in seawater pCO_2. Photosynthetic efficiency (α) significantly increased with pCO_2 levels across all species (Table 2; Fig. 2). Photosynthetic efficiency increased by 0.004–0.013 with every 100 µatm rise in pCO_2 level across all species.

Saturating irradiance (E_k) was not significantly altered by the pCO_2 treatments (Table 2; Fig. 2). Increasing pCO_2 enrichment reduced compensation irradiance (E_c) for *C. serrulata* and *H. uninervis* (Table 2; Fig. 2); however, in *T. hemprichii*, E_c was not affected by pCO_2 enrichment (Table 2; Fig. 2).

Overall, most photosynthetic parameters responded significantly to pCO_2 increase. Although some variation exists in the slopes, overlapping CIs indicated that species differences were non-significant (Table 2).

Plant-scale responses (leaf growth and rhizome carbohydrates)

Leaf growth responses to pCO_2 enrichment differed between species. *C. serrulata* did not show differences in growth rates with increasing pCO_2 levels (Fig. 3; Table 2). By contrast, growth rates increased with pCO_2 enrichment for *H. uninveris* (Fig. 3; Table 2) and *T. hemprichii* (Fig. 3; Table 2). Slopes for relative leaf growth rates (RGR) were about 0.001 units for every 100 µatm increase in pCO_2 for both species.

For plant-scale response to pCO_2, amongst the three species only *T. hemprichii* displayed an increase in specific leaf area (SLA; leaf area per unit dry weight) with increasing pCO_2 (Table 2). No significant effects of pCO_2 on chlorophyll content were detected for all three species at the end of the experiment (Table 2). Starch content in *C. serrulata* rhizomes decreased as pCO_2 levels increased from 442 to 1204 µatm (Table 2). There were no significant changes for starch content in *H. uninervis* and *T. hemprichii* rhizomes with pCO_2 enrichment (Table 2). Neither NSC nor soluble carbohydrate content showed significant changes with pCO_2 enrichment for all three species (Table 2).

Discussion

Under predicted future scenarios of ocean acidification, marine macrophytes on coral reefs could be amongst the "winners", because growth and survival will be enhanced

Table 2 Linear models for all response variables measured

Species	Parameter	Intercept	PCO_2	95 % CI	R^2	$F_{(1,14)}$	P
C. serrulata	NP	8.669	0.757	(0.352, 1.162)	0.502	16.094	0.001**
	P_G:R	3.434	0.474	(0.111, 0.838)	0.313	7.845	0.014*
	P_{max}	9.532	0.677	(0.291, 1.062)	0.468	14.179	0.002**
	α	0.052	0.006	(0.002, 0.010)	0.427	12.190	0.004**
	E_k	198.834	−4.748	(−11.141, 1.646)	0.093	2.537	0.134
	E_c	48.540	−2.557	(−4.588, −0.525)	0.295	7.288	0.017*
	RGR	0.030	-2.811×10^{-5}	$(-4.455 \times 10^{-4}, 3.893 \times 10^{-4})$	0.070	0.021	0.887
	SLA	405.793	−0.346	(−5.883, 5.190)	0.070	0.018	0.895
	chl a	0.795	−0.004	(−0.038, 0.031)	0.068	0.049	0.828
	chl b	0.404	−0.004	(−0.020, 0.013)	0.055	0.216	0.649
	NSC	10.450	0.026	(−0.844, 0.895)	0.071	0.004	0.951
	Sol carbs	10.020	0.049	(−0.823, 0.922)	0.070	0.015	0.906
	Starch	0.424	−0.023	(−0.041, −0.004)	0.277	6.741	0.021*
H. uninervis	NP	9.755	0.867	(0.318, 1.416)	0.411	11.459	0.004**
	P_G:R	3.988	0.322	(0.140, 0.505)	0.471	14.328	0.002**
	P_{max}	9.866	0.857	(0.314, 1.401)	0.410	11.434	0.004**
	α	0.090	0.004	(0.001, 0.008)	0.276	6.337	0.026*
	E_k	110.927	2.866	(−2.296, 8.028)	0.027	1.418	0.254
	E_c	37.282	−1.413	(−2.513, −0.312)	0.305	7.572	0.016*
	RGR	0.018	0.001	$(7.240 \times 10^{-4}, 1.890 \times 10^{-3})$	0.596	23.128	0.000***
	SLA	450.908	1.094	(−5.010, 7.199)	0.060	0.148	0.706
	chl a	1.558	−0.009	(−0.081, 0.062)	0.065	0.080	0.782
	chl b	0.776	−0.005	(−0.050, 0.041)	0.068	0.047	0.831
	NSC	17.196	−0.077	(−0.796, 0.641)	0.067	0.054	0.820
	Sol carbs	2.469	0.050	(−0.122, 0.223)	0.042	0.390	0.542
	Starch	14.726	−0.128	(−0.751, 0.495)	0.057	0.194	0.667
T. hemprichii	NP	6.560	1.040	(0.746, 1.334)	0.791	57.586	0.000***
	P_G:R	4.194	0.410	(0.094, 0.730)	0.309	7.712	0.015*
	P_{max}	7.720	0.929	(0.691, 1.168)	0.822	70.109	0.000***
	Sqrt α	0.215	0.013	(0.006, 0.019)	0.518	17.134	0.001**
	E_k	177.312	−2.548	(−8.231, 3.135)	0.005	0.925	0.353
	Sqrt E_c	6.67	−0.191	(−0.387, 0.004)	0.237	4.448	0.054
	RGR	0.025	0.001	$(7.277 \times 10^{-4}, 2.114 \times 10^{-3})$	0.550	19.330	0.001***
	SLA	333.021	6.169	(1.158, 11.182)	0.285	6.971	0.019*
	chl a	0.934	−0.013	(−0.040, 0.014)	0.007	1.110	0.310
	chl b	0.453	−0.008	(−0.022, 0.006)	0.028	1.430	0.252
	NSC	7.477	0.624	(−0.230, 1.478)	0.089	2.457	0.139
	Sol carbs	1.894	0.227	(−0.241, 0.696)	0.005	1.082	0.316
	Starch	5.574	0.398	(−0.221, 1.016)	0.057	1.900	0.190

All parameters were analysed with pCO_2 treatments as explanatory variable and tanks as replicates ($n = 4$). Slopes (pCO_2) and 95 % confidence intervals (CI) are expressed per 100 µatm pCO_2. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. Parameters: NP—net productivity, P_G:R—gross photosynthesis to respiration ratio, P_{max}—maximum photosynthetic rate, α—photosynthetic efficiency, E_k—saturating irradiance, E_c—compensation irradiance, RGR—relative growth rate, SLA—specific leaf area, chl a—chlorophyll a, chl b—chlorophyll b, NSC—total non-structural carbohydrates. α and E_c have been square-root-transformed for *T. hemprichii*

by higher CO_2 availability (Fabricius et al. 2011; Koch et al. 2013). The present study supports this hypothesis as all three species benefitted from higher rates of photosynthesis (i.e. P_{max} increased) and adjusted photosynthetic kinetics in response to pCO_2 enrichment of coastal seawater. Enhanced photosynthetic responses and growth rates were observed after two weeks of exposure to enriched pCO_2. Although photosynthetic responses were very similar

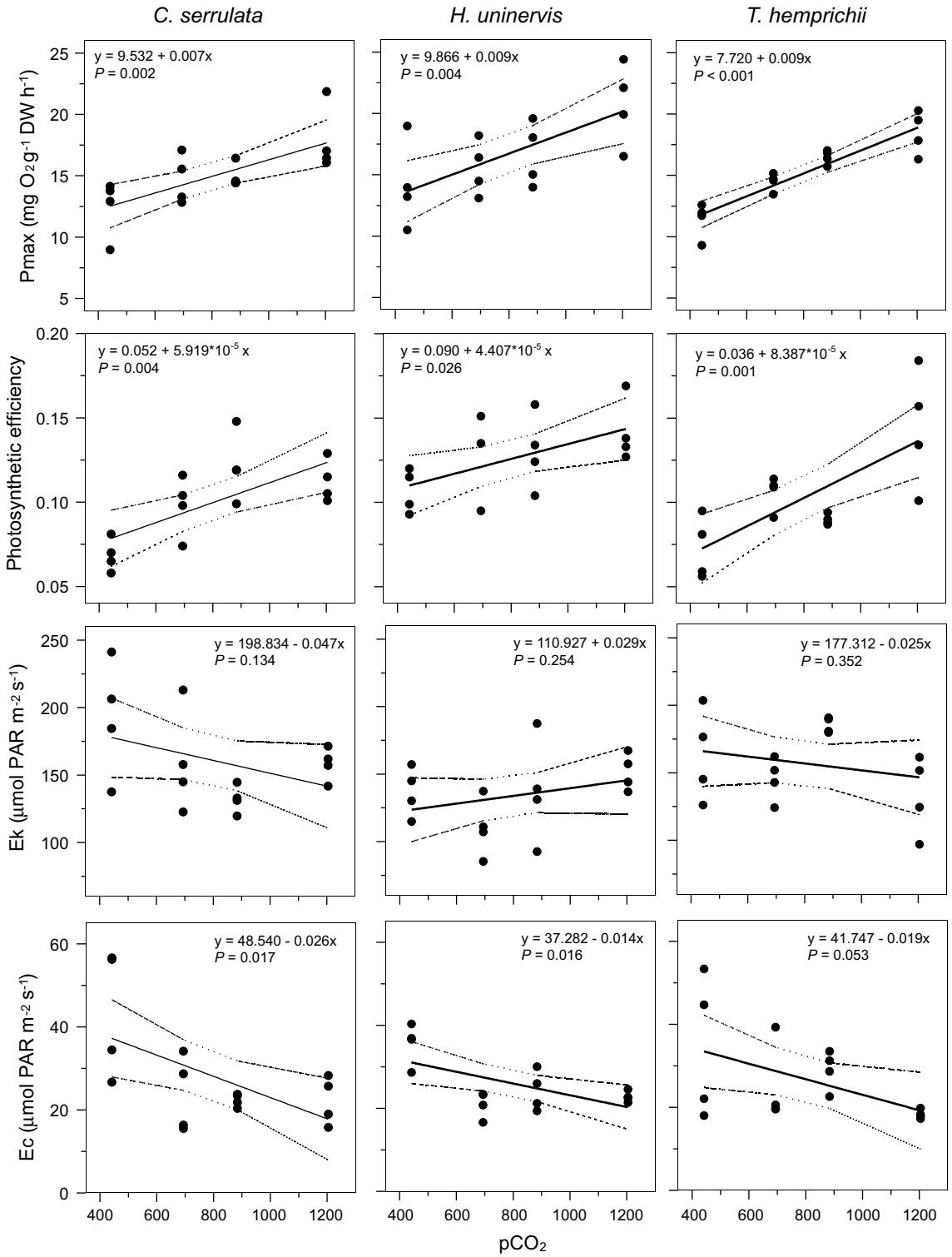

Fig. 2 Parameters derived from *P–E* curves. *Top row*—maximal photosynthetic rates (P_{max}); *second row*—photosynthetic efficiency (α); *third row*—saturating irradiance (E_k); *bottom row*—compensation irradiance (E_c). Data were fitted with linear models (*dotted lines* 95 % confidence intervals). $N = 4$

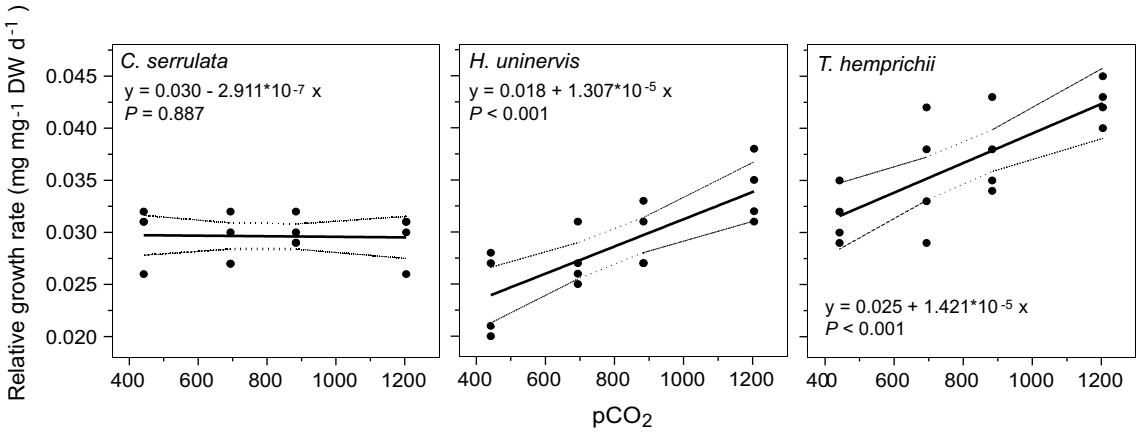

Fig. 3 Linear model fits (*dotted lines* indicate 95 % confidence intervals) for relative growth rates of *C. serrulata*, *H. uninervis* and *T. hemprichii* in response to pCO_2 enrichment (mgDW mg^{-1}DW day^{-1}). $N = 4$

between species, magnitude of plant-scale responses was species specific.

Physiological responses to pCO_2 enrichment

Carbon dioxide enrichment increased net productivity (NP) and energetic surplus (P_G:R) in all three species tested. The increase in NP and P_G:R was quantified as 0.757–1.040 and 0.322–0.474 units per 100 µatm pCO_2, respectively. This response is consistent with previous findings that photosynthetic rates increase with pCO_2 in seagrasses (Thom 1996; Zimmerman et al. 1997; Invers et al. 2002; Alexandre et al. 2012). Having greater energetic surplus could indicate flow-on effects to plant-scale responses, such as growth and shoot production (Invers et al. 2002; Palacios and Zimmerman 2007). Energetic status can affect growth, response to physical disturbances such as grazing (Eklöf et al. 2009), abundance and spatial distribution (Dennison et al. 1993; Zimmerman et al. 1997), and even reproductive output (Palacios and Zimmerman 2007).

Maximum photosynthetic rates (P_{max}) and efficiency (α) in all species were raised at higher pCO_2 levels although chlorophyll content was not affected. Photoacclimation had been reported for several temperate and tropical seagrass species (Invers et al. 1997; Zimmerman et al. 1997; Jiang et al. 2010; Alexandre et al. 2012), but this is the first study to compare short-term responses to CO_2 enrichment amongst three tropical species in one experiment. In general, *P–E* curves did not show differences between species in their photosynthetic response to CO_2 enrichment. The responses over the range of pCO_2 in photosynthetic parameters were similar between species (similar slope, as shown by overlapping confidence intervals). Maximum relative electron transport rate ($rETR_{max}$) could increase with CO_2 enrichment (Jiang et al. 2010). While comparisons between quantum efficiency and O_2 production need to be viewed with caution (Beer et al. 2001), these findings indicate that there was a stronger response of P_{max} in the present study using respirometry per 100 µatm rise in pCO_2 (7.86 % increase at 1204 µatm for 2 weeks) compared with $rETR_{max}$ (3.37 % increase in $rETR_{max}$ at ~807 µatm (pH 7.75) for 3 weeks, Jiang et al. 2010). Temperate species increased P_{max} with pCO_2 enrichment too, but showed much more variable response rates per 100 µatm pCO_2 (*Zostera marina* 0.59 % increase per 100 µatm pCO_2 for 3 weeks, Zimmerman et al. 1997; *Zostera noltii* 9.64 % increase per 100 µatm pCO_2 for 5 months, Alexandre et al. 2012).

Overall, our study concurs that seagrasses can raise productivity from pCO_2 enrichment, at least in the short term (2 weeks exposure). The response of net productivity and P_G:R to increasing pCO_2 followed a linear trend, indicating that any future change in pCO_2 could have an effect on seagrass productivity. In numerous studies on terrestrial plants, the initial stimulation of photosynthesis and growth in elevated CO_2 can decline over time, as Rubisco is down-regulated, carbohydrates accumulate and nitrogen content decreases (Stitt and Krapp 1999). Observations of high seagrass abundance at CO_2 seep sites indicate seagrass productivity might continually benefit from pCO_2 enrichment over the long term (decades) (Fabricius et al. 2011). However, interaction from other co-occurring influences, such as the lowered competition from photosynthetic calcifiers or intrinsic genetic capacity to respond within the population, should be taken into account too. Whether such longer term acclimatory responses to pCO_2 enrichment would manifest in tropical seagrasses remains unknown. Furthermore, the capacity of seagrasses to respond to increasing pCO_2 is likely to depend on other limiting factors such as nutrient or light availability (Invers et al. 1997).

Light availability is often the primary limiting factor for seagrass productivity. Exposure to low light conditions (such as high turbidity and high epiphyte loads) is a common factor causing seagrass loss (Waycott et al. 2009; Collier et al. 2012). Here, a lowering in the light requirement to meet respiratory demands (E_c) and an increase in light efficiency (α) were observed with increasing pCO_2. This could imply that a lower amount of light energy would be required to meet metabolic balances (Schwarz et al. 2000; Long et al. 2004). Therefore, CO_2 enrichment could potentially increase the tolerance of seagrasses to conditions of low light, for example during flood plume events. In contrast, there was no change in the light level required to reach maximum photosynthetic rates (E_k). pCO_2 enrichment increased E_k in *Z. marina* (Zimmerman et al. 1997), *Z. noltii* (Alexandre et al. 2012) and *T. hemprichii* (Jiang et al. 2010). An increase in P_{max} without a simultaneous rise in light requirement might be explained by the strong upregulation of photosynthetic efficiency (α). pCO_2 enrichment can affect light requirements, and this could be important for how seagrasses will respond to changing environmental conditions—including water quality—in the future.

Seagrasses can utilise the predominant HCO_3^- in seawater via carbon-concentrating mechanisms (CCMs), somewhat alleviating the problem of carbon limitation at higher pH (Durako 1993; Bjork et al. 1997; Uku et al. 2005; Campbell and Fourqurean 2013b). In favourable conditions where other factors are non-limiting, CCMs might cause some seagrasses to be carbon-saturated (Schwarz et al. 2000; Beer et al. 2002). Such mechanisms were thought to be less efficient in *Thalassia* (*T. hemprichii* and *T. testudinum*), rendering this genus less capable of utilising HCO_3^- than other species (Uku et al. 2005; Campbell and Fourqurean 2013b). Hence, an increase in CO_2 availability would be important in raising productivity for *Thalassia*. *Cymodocea* and *Halodule* reportedly possess CCMs that allow them to utilise HCO_3^- under ambient conditions (Schwarz et al. 2000; Uku et al. 2005). Both species were able to increase photosynthetic rates under enriched pCO_2 conditions, where the relative increase in CO_2 was much greater than that in HCO_3^- (Koch et al. 2013). Both species have been observed to become more dominant and have increased biomass around highly enriched volcanic CO_2 seeps (Takahashi et al. under review). All the three species tested responded at similar rates in terms of net productivity. It appears that regardless of whether the species possess CCMs or not, CO_2 enrichment can increase photosynthetic rates for different species to a similar extent.

Sinks for carbon: plant-scale responses

As a result of increased photosynthetic rates and relatively stable dark respiration rates, energetic surplus (P_G:R) was increased at higher pCO_2 for all species. The rate of increase with pCO_2 levels in P_G:R was similar between the three species. There are a number of possible sinks for this additional fixed C. In this short-term study, we measured growth and storage carbohydrates in rhizomes, but other sinks, such as biomass or sexual reproduction, exist.

Response in leaf growth rates to pCO_2 enrichment differed between species. Growth of *H. uninervis* and *T. hemprichii* responded strongly, but not in *C. serrulata*. Specifically, relative growth rate (RGR) increased significantly, and in *T. hemprichii*, an increase in leaf area relative to leaf biomass (SLA) was also observed. Leaf growth response appeared to vary amongst the limited number of studies on tropical seagrass. While Campbell and Fourqurean (2013a) found no differences in leaf growth rates with pCO_2 enrichment in *T. testudinum*, Jiang et al. (2010) showed a 2.63 % rate increase in leaf growth (per 100 µatm pCO_2) at pH 7.76 after 3 weeks of exposure in *T. hemprichii*. This is about half of the 5.62 % rate of increase in leaf growth observed in *T. hemprichii* here. The effect of CO_2 enrichment on growth rate can be influenced by the tissue nutrient requirement of the species and other prevailing environmental conditions (Zimmerman et al. 1997; Palacios and Zimmerman 2007; Jiang et al. 2010; Alexandre et al. 2012; Campbell and Fourqurean 2013a). Under nutrient limitation, seagrasses could direct the fixed carbon towards carbon-rich tissues such as belowground tissues, instead of investing in nitrogen-rich tissue such as leaves (Poorter et al. 1996; Stitt and Krapp 1999). *C. serrulata*, which has a higher proportion of its biomass existing as shoots and leaves (Hemminga and Duarte 2000), might have required a simultaneous increase in nitrogen availability in order to assimilate the carbon into its leaves. Temperature strongly influences carbon and nitrogen metabolism (Touchette and Burkholder 2007) and could also affect the growth response of seagrasses to pCO_2 (Atkin et al. 2005; Collier et al. 2011). Whether these, and other, environmental parameters affected the differences in growth response amongst species warrants further investigation.

Sink strength, or carbon demand, could modulate growth response in seagrasses to CO_2 enrichment, similar to that in terrestrial C3 species (Arp 1991; Poorter et al. 1996). Increased energetic surplus from pCO_2 enrichment indicates extra assimilated carbon available for storage, growth and metabolism. While little change in NSC content was observed in the present study, seagrasses do possess a number of alternative "carbon sinks", with the size of carbon demand for each sink dependent on species-specific growth strategy (Doust 1981; Hemminga and Duarte 2000) (described further below). For example, the shorter time taken for shoot initiation for *H. uninervis* (average 7.9 days), compared to *C. serrulata* (average 21.2 days) and *T. hemprichii* (average 38.5 days), meant a faster turnover of

aboveground biomass for *H. uninervis* (Duarte 1991; Marba and Duarte 1998). Therefore, *H. uninervis* might have a strong carbon demand in leaf growth. The relatively greater proportion of belowground biomass in *H. uninervis* and *T. hemprichii* suggests higher storage potential and metabolic demand (Duarte 1991; Marba and Duarte 1998). In these species, more carbon could be directed to belowground biomass, and/or leaf area could be expanded to increase photosynthetic rates. Extra carbon could also be directed to increased shoot production and flowering, as observed in *Z. marina* after 1 year of CO_2 enrichment (Palacios and Zimmerman 2007). Essentially, the extra carbon assimilated could be directed to a single "sink", such as the growth of new leaves, or it could be spread amongst various metabolic functions and storage organs. The latter makes distinguishing the fate of the extra carbon complicated, especially for short-term experiments such as this study.

In general, our results imply that the availability of higher pCO_2 might alter future interspecific competition amongst co-occurring species. With deteriorating water quality, i.e. low light and high nutrients, species that are able to readily assimilate and mobilise carbon resources with the extra CO_2 might outcompete other species. Under optimal growth conditions, species that are able to rapidly utilise the extra CO_2 to occupy more "space", i.e. either upwards on vertical stems or via horizontal rhizomes, could potentially increase their abundance and distribution.

Seagrasses as "winners"?

The ability of marine macro-autotrophs to utilise the greater CO_2 availability suggests that they will thrive under future scenarios of climate change (Koch et al. 2013). This present study has built evidence to support this, with increased growth, productivity and biomass from pCO_2 enrichment (Zimmerman et al. 1997; Invers et al. 2002; Palacios and Zimmerman 2007; Jiang et al. 2010; Campbell and Fourqurean 2013a). This study has also quantified the change in physiological parameters with respect to CO_2 enrichment. Surveys at natural CO_2 seeps further attest to this, where greater seagrass cover, shoot density, root biomass and productivity were reported at low pH/high CO_2 sites when compared to adjacent high pH/low CO_2 sites (Hall-Spencer et al. 2008; Fabricius et al. 2011; Russell et al. 2013; Takahashi et al. under review). For calcifying marine autotrophs, such as hard corals, foraminifera and coralline algae, ocean acidification lowers calcification and growth rates and increases rates of bio-erosion (Kuffner et al. 2007; de Putron et al. 2010; Fabricius et al. 2011; Doo et al. 2014; James et al. 2014), and calcifying organisms might be outcompeted (Russell et al. 2011; Short et al. 2014). A shift in the ecological diversity and functions in coastal habitats might result.

This study demonstrated that tropical seagrasses can increase their photosynthetic rates, adjust photosynthetic performance and increase growth rates in response to CO_2 enrichment. Varying plant-scale responses to CO_2 enrichment between species might affect interspecies competition, especially in mixed species meadows (Takahashi et al. under review). Under CO_2 enrichment scenarios, carbon utilisation and allocation traits between seagrass species come into consideration, such as carbon uptake mechanisms, the ability to assimilate additional carbon and the response time of rhizome and shoot elongation to DIC enrichment (Hall-Spencer et al. 2008; Russell et al. 2013; Takahashi et al. under review). Furthermore, environmental conditions such as light and nutrients, which result from water quality changes, could limit species response to CO_2 enrichment in the long term. Changes in species composition and diversity in tropical seagrass meadows could potentially impact the functional diversity offered by these productive ecosystems. Interspecific variation amongst seagrasses in response to ocean acidification, over different temporal scales, deserves further examination.

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References

Alexandre A, Silva J, Buapet P, Bjork M, Santos R (2012) Effects of CO_2 enrichment on photosynthesis, growth, and nitrogen metabolism of the seagrass *Zostera noltii*. Ecol Evol 2:2620–2630

Arp WJ (1991) Effects of source-sink relations on photosynthetic acclimation to elevated CO_2. Plant Cell Environ 14:869–875

Atkin OK, Bruhn D, Hurry VM, Tjoelker MG (2005) The hot and the cold: unravelling the variable response of plant respiration to temperature. Funct Plant Biol 32:87–105

Beer S, Koch E (1996) Photosynthesis of marine macroalgae and seagrasses in globally changing CO_2 environments. Mar Ecol Prog Ser 141:199–204

Beer S, Bjork M, Gademann R, Ralph P (2001) Measurements of photosynthetic rates in seagrasses. In: Short FT, Coles RC (eds) Global seagrass research methods. Elsevier Science B.V., Amsterdam, pp 183–198

Beer S, Bjork M, Hellblom F, Axelsson L (2002) Inorganic carbon utilisation in marine angiosperms (seagrasses). Funct Plant Biol 29:349–354

Beer S, Mtolera M, Lyimo T, Bjork M (2006) The photosynthetic performance of the tropical seagrass *Halophila ovalis* in the upper intertidal. Aqua Bot 84:367–371

Bjork M, Weil A, Semesi S, Beer S (1997) Photosynthetic utilisation of inorganic carbon by seagrasses from Zanzibar, East Africa. Mar Biol 129:363–366

Bowes G, Ogren WL (1972) Oxygen inhibition and other properties of soybean ribulose 1,5-diphosphate carboxylase. J Biol Chem 247:2171–2176

Caldeira K, Wickett ME (2003) Anthropogenic carbon and ocean pH. Nature 425:365

Campbell JE, Fourqurean JW (2013a) Effects of in situ CO_2 enrichment on the structural and chemical characteristics of the seagrass *Thalassia testudinum*. Mar Biol 160:1465–1475

Campbell JE, Fourqurean JW (2013b) Mechanisms of bicarbonate use influence the photosynthetic carbon dioxide sensitivity of tropical seagrasses. Limnol Oceanogr 58:839–848

Chiariello NR, Mooney HA, Williams K (1989) Growth, carbon allocation and cost of plant tissues. In: Percy RW, Ehleringer J, Mooney HA, Rundel PW (eds) Plant physiology ecology: field methods and instrumentation. Chapman and Hall, New York, p 457

Collier CJ, Uthicke S, Waycott M (2011) Thermal tolerance of two seagrass species at contrasting light levels: implications for future distribution in the Great Barrier Reef. Limnol Oceanogr 56:2200–2210

Collier CJ, Waycott M, McKenzie LJ (2012) Light thresholds derived from seagrass loss in the coastal zone of the northern Great Barrier Reef, Australia. Ecol Indic 23:211–219

Collins M, Knutti R, Arblaster J, Dufresne JL, Fichefet T, Friedlingstein P, Gao X, Gutowski WJ, Johns T, Krinner G, Shongwe M, Tebaldi C, Weaver AJ, Wehner M (2013) Long-term Climate change: projections, commitments and irreversibility. In: Stocker TF, Qin D, Plattner GK, Tignor M, Allen SK, Boschung J, Nauels A, Xia Y, Bex V, Midgley PM (eds) Climate change 2013: the physical science basis Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge

de Putron SJ, McCorkle DC, Cohen AI, Dillon AB (2010) The impact of seawater saturation state and bicarbonate ion concentration on calcification by new recruits of two Atlantic corals. Coral Reefs 30:321–328

Dennison WC, Orth RJ, Moore KA, Court Stevenson J, Carter V, Kollar S, Bergstrom PW, Batiuk RA (1993) Assessing water quality with submersed aquatic vegetation. Bioscience 43:86–94

Development Core Team R (2011) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna

Doo SS, Fujita K, Byrne M, Uthicke S (2014) Fate of calcifying tropical symbiont-bearing large benthic foraminifera: living sands in a changing ocean. Biol Bull 226:169–186

Doust LL (1981) Population dynamics and local specialization in a clonal perennial (*Ranunculus repens*). J Ecol 69:743–755

Duarte CM (1991) Allometric scaling of seagrass form and productivity. Mar Ecol Prog Ser 77:289–300

Duarte CM, Chiscano CL (1999) Seagrass biomass and production: a reassessment. Aqua Bot 65:159–174

Durako MJ (1993) Photosynthetic utilization of $CO_{2(aq)}$ and HCO_3^- in *Thalassia testudinum* (Hydrocharitaceae). Mar Biol 115:373–380

Durako MJ, Sackett WM (1993) Effects of $CO_{2\ (aq)}$ on the carbon isotopic composition of the seagrass *Thalassia testudinum* Banks ex Konig (Hydrocharitaceae). J Expt Mar Biol Ecol 169:167–180

Eklöf JS, McMahon K, Lavery PS (2009) Effects of multiple disturbances in seagrass meadows: shading decreases resilience to grazing. Mar Freshw Res 60:1317–1327

Fabricius KE, Langdon C, Uthicke S, Humphrey C, Noonan S, Death G, Okazaki R, Muehllehner N, Glas MS, Logh JM (2011) Losers and winners in coral reefs acclimatized to elevated carbon dioxide concentrations. Nat Clim Change 1:165–169

Feely RA, Sabine CL, Lee K, Berelson W, Kleypas J, Fabry VJ, Millero FJ (2004) Impact of Anthropogenic CO_2 on the $CaCO_3$ system in the oceans. Science 305:362–366

Fourqurean JW, Duarte CM, Kennedy H, Marba N, Holmer M, Mateo MA, Apostolaki ET, Kendrick GA, Krause-Jensen D, McGlathery KJ, Serrano O (2012) Seagrass ecosystems as a globally significant carbon stock. Nat Geosci 5:505–509

Gacia E, Duarte CM (2001) Sediment retention by a Mediterranean *Posidonia oceanica* meadow: the balance between deposition and resuspension. Estuar Coast Shelf Sci 52:505–514

Granger S, Izumi H (2002) Water quality measuement methods for seagrass habitats. Elsevier Science, Amsterdam

Hall-Spencer JM, Rodolfa-Metalpa R, Martin S, Ransome E, Fine M, Turner SM, Rowley SJ, Tedesco D, Buia MC (2008) Volcanic carbon dioxide vents show ecosystem effects of ocean acidification. Nature 454:96–99

Hemminga MA, Duarte CM (2000) Seagrass ecology. Cambridge University Press, Cambridge

Invers O, Romero J, Perez M (1997) Effects of pH on seagrass photosynthesis: a laboratory and field assessment. Aqua Bot 59:185–194

Invers O, Zimmerman RC, Alberte RS, Perez M, Romero J (2001) Inorganic carbon sources for seagrass photosynthesis: an experimental evaluation of bicarbonate use in species inhabiting temperate waters. J Exp Mar Biol Ecol 365:203–217

Invers O, Tomas F, Perez M, Romero J (2002) Potential effect of increased global CO_2 availability on the depth distribution of the seagrass *Posidonia oceanica* (L.) Delile: a tentative assessment using a carbon balance model. B Mar Sci 71:1191–1198

IPCC (2013) Climate change 2013: The physical science basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge

James RK, Hepburn CD, Cornwall CE, McGraw CM, Hurd CL (2014) Growth responses of an early successional assemblage of coralline algae and benthic diatoms to ocean acidification. Mar Biol 161:1687–1696

Jassby AD, Platt T (1976) Mathematical formulation of the relationship between photosynthesis and light for phytoplankton. Limnol Oceanogr 21:540–547

Jiang ZJ, Huang X, Zhang J (2010) Effects of CO_2 enrichment on photosynthesis, growth and biochemical composition of seagrass *Thalassia hemprichii* (Ehrenb.) Aschers. J Integr Plant Biol 52:904–913

Karkalas J (1985) An improved enzymic method for the determination of native and modified starch. J Sci Food Agric 36:1019–1027

Koch M, Bowes G, Ross C, Zhang XH (2013) Climate change and ocean acidification effects on seagrasses and marine macroalgae. Glob Change Biol 19:103–132

Kohler KE, Gill SM (2006) Coral Point Count with Excel extensions (CPCe): a Visual Basic program for the determination of coral and substrate coverage using random point count methodology. Comput Geosci 32:1259–1269

Kuffner IB, Andersson AJ, Jokiel PL, Rodgers KS, Mackenzie FT (2007) Decreased abundance of crustose coralline algae due to ocean acidification. Nat Geosci 1:114–117

Long SP, Ainsworth EA, Rogers A, Ort DR (2004) Rising atmospheric carbon dioxide: plants FACE the future. Annu Rev Plant Biol 55:591–628

Marba N, Duarte CM (1998) Rhizone elongation and seagrass clonal growth. Mar Ecol Prog Ser 174:269–280

McCleary BV, Codd R (1991) Measurement of (1→3), (1→4)-ß-D-Glucan in barley and oats: a streamlined enzymic procedure. J Sci Food Agric 55:303–312

McKenzie LJ, Collier CJ, Waycott M (2014) Reef Rescue Marine Monitoring Program—Inshore Seagrass, Annual report for the sampling period 1st July 2–11 = 31st May 2012. James Cook University, Cairns

Meehl GA, Stocker TF, Collins WD, Friedlingstein P, Gaye T, Gregory JM, Kitoh A, Knutti R, Murphy JM, Noda A, Raper SCB, Watterson IG, Weaver AJ, Zhao ZC (2007) Global climate projections. In: Solomon SD, Qin D, Manning M, Chen Z, Marquis M, Averyt KB, Tignor M, Miller HL (eds) IPCC, 2007: Climate Change (2007) The physical science basis contribution of

Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change Cambridge University Press, Cambridge, pp 747–845

Palacios SL, Zimmerman RC (2007) Response of eelgrass *Zostera marina* to CO_2 enrichment: possible impacts of climate change and potential for remediation of coastal habitats. Mar Ecol Prog Ser 344:1–13

Poorter H, Roumet C, Campbell BD (1996) Interspecific variation in the growth response of plants to elevated CO_2: a search for functional types. In: Korner C, Bazzaz FA (eds) Carbon dioxide, populations and communities. Academic Press, California, pp 375–412

Raven JA, Caldeira K, Elderfield H, Hoegh-Guldberg O, Liss P, Riebesell U, Shepherd J, Turkey C, Watson A, Heap R, Banes R, Quinn R (2005) Ocean acidification due to increasing atmospheric carbon dioxide. The Clyvedon Press Ltd, Cardiff

Robbins LL, Hansen ME, Kleypas JA, Meylan SC (2010) CO2 calc—a user-friendly seawater carbon calculator for windows, Mac OS X, and iOS (iPhone) open-file report 2010. Geological Survey U.S, Reston

Russell BD, Passarelli CA, Connell SD (2011) Forecasted CO_2 modifies the influence of light in shaping subtidal habitat. J Phycol 47:744–752

Russell BD, Connell SD, Uthicke S, Muehllehner N, Fabricius K, Hall-Spencer J (2013) Future seagrass beds: can increased productivity lead to increased carbon storage? Mar Poll Bull 73:463–469

Ryle VD, Mueller HR, Gentien P, Science AIoM (1981) Automated analysis of nutrients in tropical sea waters. Australian Institute of Marine Science

Sand-Jensen K, Gordon DM (1984) Differential ability of marine and freshwater macrophytes to utilize HCO_3^- and CO_2. Mar Biol 80:247–253

Schwarz AM, Bjork M, Buluda T, Mtolera M, Beer S (2000) Photosynthetic utilisation of carbon and light by two tropical seagrass species as measured in situ. Mar Biol 137:755–761

Short FT, Duarte CM (2001) Methods for the measurement of seagrass growth and production. In: Short FT, Coles RC (eds) Global Seagrass research methods. Elsevier Science B.V., Amsterdam, pp 155–182

Short J, Kendrick GA, Falter J, McCulloch MT (2014) Interactions between filamentous turf algae and coralline algae are modified under ocean acidification. J Exp Mar Biol Ecol 456:70–77

Stitt M, Krapp A (1999) The interaction between elevated carbon dioxide and nitrogen nutrition: the physiological and molecular background. Plant Cell Environ 22:583–621

Takahashi M, Noonan S, Fabricius K, Collier CJ (under review) The effects of long term *in situ* CO_2 enrichment on tropical seagrass communities: implications for ocean acidification

Tanaka Y, Nakaoka M (2007) Interspecific variation in photosynthesis and respiration balance of three seagrasses in relation to light availability. Mar Ecol Prog Ser 350:63–70

Terrados J, Borum J, Duarte CM, Fortes MD, Kamp-Nielsen L, Agawin NSR, Kenworthy WJ (1999) Nutrient and mass allocation of South-east Asian seagrasses. Aqua Bot 63:203–217

Thom RM (1996) CO_2-enrichment effects on eelgrass (*Zostera marina* L.) and bull kelp (*Nereocystis luetkeana* (Mert.) P. & R.). Water Air Soil Poll 88:383–391

Touchette BW, Burkholder JM (2007) Carbon and nitrogen metabolism in the seagrass, *Zostera marina* L.: environmental control of enzymes involved in carbon allocation and nitrogen assimilation. J Exp Mar Biol Ecol 350:216–233

Uku J, Beer S, Bjork M (2005) Buffer sensitivity of photosynthetic carbon utilisation in eight tropical seagrasses. Mar Biol 147:1085–1090

Vafeiadou A, Materatski P, Adao H, De Troch M, Moens T (2013) Food sources of macrobenthos in an estuarine seagrass habitat (*Zostera noltii*) as revealed by dual stable isotope signatures. Mar Biol 160:2517–2523

Waycott M, Duarte CM, Carruthers TJB, Orth RJ, Dennison WC, Olyamik S, Calladine A, Fourqurean JW, Heck JKL, Hughes AR, Kendrick GA, Kenworthy WJ, Short FT, Williams SL (2009) Accelerating loss of seagrasses across the globe threatens coastal ecosystems. Proc Nat Acad Sci 106:12377–12381

Zimmerman RC, Kohrs DG, Stellar DL, Alberte RS (1997) Impacts of CO_2 enrichment on productivity and light requirements of eelgrass. Plant Physiol 115:599–607