Ecosystems (2018) 21: 1269–1282
https://doi.org/10.1007/s10021-017-0212-2

Does Nutrient Availability Regulate Seagrass Response to Elevated CO_2?

Justin E. Campbell[1]* and James W. Fourqurean[2]

[1]Smithsonian Marine Station, 701 Seaway Drive, Fort Pierce, Florida 34949, USA; [2]Department of Biological Sciences and Marine Education and Research Center, Florida International University, 11200 SW 8th Street, Miami, Florida 33199, USA

Abstract

Future increases in oceanic carbon dioxide concentrations ($CO_{2(aq)}$) may provide a benefit to submerged plants by alleviating photosynthetic carbon limitation. However, other environmental factors (for example, nutrient availability) may alter how seagrasses respond to $CO_{2(aq)}$ by regulating the supply of additional resources required to support growth. Thus, questions remain in regard to how other factors influence $CO_{2(aq)}$ effects on submerged vegetation. This study factorially manipulated $CO_{2(aq)}$ and nutrient availability, in situ, within a subtropical seagrass bed for 350 days, and examined treatment effects on leaf productivity, shoot density, above- and belowground biomass, nutrient content, carbohydrate storage, and sediment organic carbon (C_{org}). Clear, open-top chambers were used to replicate $CO_{2(aq)}$ forecasts for the year 2100, whereas nutrient availability was manipulated via sediment amendments of nitrogen (N) and phosphorus (P) fertilizer. We provide modest evidence of a CO_2 effect, which increased seagrass aboveground biomass. $CO_{2(aq)}$ enrichment had no effect on nutrient content, carbohydrate storage, or sediment C_{org} content. Nutrient addition increased leaf productivity and leaf N content, however did not alter above- or belowground biomass, shoot density, carbohydrate storage, or C_{org} content. Treatment interactions were not significant, and thus NP availability did not influence seagrass responses to elevated $CO_{2(aq)}$. This study demonstrates that long-term carbon enrichment may alter the structure of shallow seagrass meadows, even in relatively nutrient-poor, oligotrophic systems.

Key words: ocean acidification; reduced pH; carbon dioxide; climate change; carbon storage; *Thalassia testudinum*.

Introduction

Climate change stands to alter the ecological functioning of many coastal habitats. Increasing carbon dioxide (CO_2) concentrations will influence the carbonate chemistry of seawater via increases in dissolved $CO_{2(aq)}$ and bicarbonate ion (HCO_3^-) concentrations, and declines in carbonate ion (CO_3^{2-}) concentrations. Associated with these shifts is a forecasted 0.3–0.5 unit reduction in seawater pH (Caldeira and Wickett 2003), with negative (albeit variable) consequences for a variety of calcified invertebrates (Kroeker and others 2010). Recently, a growing body of research has identified prominent climate change responses from a broad group of marine plants (seagrasses), which form a substantial component of coastal ecosystems around the world. Unlike calcified organisms, many seagrasses may benefit from forecasted increases in $CO_{2(aq)}$ concentrations because photo-

Received 12 July 2017; accepted 4 December 2017;
published online 22 December 2017

Electronic supplementary material: The online version of this article (https://doi.org/10.1007/s10021-017-0212-2) contains supplementary material, which is available to authorized users.

Authors contributions JEC and JWF designed the study. JEC collected and analyzed data. JEC and JWF wrote the paper.

*Corresponding author; e-mail: campbellju@si.edu

synthesis is limited by $CO_{2(aq)}$ availability at the pH of modern seawater (Beer and Koch 1996). Short-term, physiological experiments demonstrate photosynthetic gains for a number of seagrasses in $CO_{2(aq)}$-rich environments (reviewed in Koch and others 2013). However, long-term studies which document $CO_{2(aq)}$ effects on seagrasses (and the ecosystems they support) remain rare. As the understanding of the effects of atmospheric CO_2 on terrestrial ecosystems has advanced due to studies conducted over expanded spatial and temporal scales (that is, Free Air Carbon Enrichment or FACE), experiments of similar duration, particularly those that encompass multiple seasons, are strongly warranted within marine systems (Gattuso and others 2014). Addressing these topics is critical toward developing a holistic assessment of coastal CO_2 effects, as seagrasses play prominent roles in both carbon cycling (Duarte and Chiscano 1999) and carbon sequestration (Fourqurean and others 2012).

In terrestrial ecosystems, increasing CO_2 generally elicits a fertilization effect, whereby vegetation responds to increases in CO_2 supply via elevated rates of carbon fixation and net primary production (Long and others 2004). Although individual, physiological responses to elevated CO_2 in terrestrial plants are well documented, ecosystem level effects can be increasingly nuanced. Increased aboveground plant biomass with elevated CO_2 is widely noted, yet the magnitude of this effect is variable and potentially dependent upon nutrient availability (Reich and others 2006; Reich and others 2014). Due to the nutritional balance of plant vegetation, CO_2-stimulated production of additional biomass may require an increase in the supply rate of mineral resources (Rastetter and others 1997); thus, it has been suggested that plant responses may become constrained by nutrient limitation (Luo and others 2004). Some nutrient-poor ecosystems display minor responses to CO_2 (Oren and others 2001; Ellsworth and others 2017), although other systems show declines in plant nutrient content (Norby and others 2010). As an instructive example, Reich and others (2006) demonstrate that low nitrogen (N) availability suppresses the positive responses of plant biomass to CO_2 in a perennial grassland. Thus, although elevated CO_2 can alter plant architecture (Pritchard and others 1999), these responses may be either enhanced or limited by N supply. Central to these studies is the ultimate question of whether elevated CO_2 enhances ecosystem C storage and C sequestration, which pertains to shifts in both plant biomass and belowground C stocks (soil organic matter). Although under certain conditions, elevated $CO_{2(aq)}$ increases soil C stocks (Jastrow and others 2005), general conclusions suggest that this response may be similarly limited without the addition of N fertilizers (Hungate and others 2009). Comparatively, in marine systems, many of these carbon–nutrient interactions have yet to receive adequate attention.

Seagrass studies document short-term shifts in leaf nutrient content with elevated $CO_{2(aq)}$, suggesting that elemental resources may similarly play an important role in plant responses to climate change. Jiang and others (2010) report declines in leaf N content (%N) under elevated $CO_{2(aq)}$ for the tropical seagrass *Thalassia hemprichii*. In situ manipulations of $CO_{2(aq)}$ also show declines in N and phosphorus (P) content (%N and %P) for another tropical species, *Thalassia testudinum* (Campbell and Fourqurean 2013). Although it has been proposed that non-structural carbohydrate (NSC) dilution is likely responsible for these relatively rapid declines, the true mechanism behind this effect has yet to be detailed. In terrestrial plants, CO_2-mediated declines in nutrient content might only be partially attributable to NSC dilution (Stitt and Krapp 1999), with other factors, such as the inability of nutrient uptake to meet increased nutrient demand playing a strong role. Ultimately, experimental timescale might serve as the distinguishing factor, as short-term declines in nutrient content are likely attributable to NSC dilution, while long-term declines may be attributable to mismatches between environmental supply and plant demand.

It is important to understand the relationship between $CO_{2(aq)}$ and nutrient availability, as the high light requirement of seagrasses (Duarte 1991) restricts them to environments of relatively low nutrient availability that cannot support high micro- and macro-algal biomass. Thus, ecosystem responses to $CO_{2(aq)}$ could be muted because of insufficient nutrient supply (as suggested in Alexandre and others 2012; Apostolaki and others 2014). Several in situ studies have documented limited seagrass responses to CO_2 enrichment (Apostolaki and others 2014; Olivé and others 2017; Cox and others 2016), potentially attributable to interactions from other environmental variables. Increases in leaf growth and NSC concentrations under elevated $CO_{2(aq)}$ have been noted (Jiang and others 2010; Campbell and Fourqurean 2013; Ow and others 2015), yet these studies document shorter responses (weeks to months) and do not test the interactive effects of other resources (yet note, Ow and others 2016;

Martinez-Crego and others 2014). The objective of this current study was to assess whether nutrient availability influences the long-term responses of the seagrass *T. testudinum* to elevated $CO_{2(aq)}$. Using an aquatic analog of terrestrial FACE experiments, in situ carbon enrichment was used to mimic year 2100 $CO_{2(aq)}$ concentrations in a subtropical seagrass bed (Campbell and Fourqurean 2011, 2013, 2014). Both $CO_{2(aq)}$ and NP availability were factorially manipulated for an extended duration (350 days), whereas seagrass productivity and seagrass leaf NP content were periodically monitored. Measurements of above- and belowground biomass, shoot density, and non-structural carbohydrates were taken at the end of the experiment to examine shifts in canopy structure and plant C storage. Sediment properties (organic matter, organic carbon, and inorganic carbon) were additionally measured in all plots to examine whether elevated $CO_{2(aq)}$ influences long-term C sequestration. Determining the role that nutrient availability plays in governing seagrass $CO_{2(aq)}$ responses will provide a broader outlook on future shifts in the functioning of these widely distributed systems.

Methods

Study Site

In situ $CO_{2(aq)}$ and nutrient manipulation was conducted from August 2010–July 2011 within a shallow seagrass bed (1 m depth) in the Florida Keys, Florida, USA (24.55 N, 81.75 W). The benthic community was dominated by the seagrass *T. testudinum*, with sparse abundances of the seagrasses *Syringodium filiforme* and *Halodule wrightii* (< 5% by biomass). Several species of calcareous green algae (*Halimeda* spp. and *Penicillus* spp.) were additionally present with a patchy distribution (< 10% by biomass). The sediments were comprised of roughly 9% organic matter, with the remaining mineral fraction consisting of fine biogenic carbonates.

Experimental Design

A balanced, 3 × 2 factorial experiment was established to study the interactive effects of $CO_{2(aq)}$ and nutrient enrichment on seagrass structure and productivity (Figure S1). $CO_{2(aq)}$ treatments consisted of 3 levels: elevated $CO_{2(aq)}$ chambers; ambient $CO_{2(aq)}$ chambers; and unchambered open plots. Unchambered plots were included to control for potential 'bottle effects' imposed by the chambers and were demarcated by corner PVC posts. The nutrient treatment consisted of 2 levels: + NP and − NP. Thirty experimental seagrass plots (0.17 m^2) were arranged in a grid (5 rows × 6 columns). Replicates (n = 5) for each treatment were then randomly assigned within each column. Plots were spaced at 1-m intervals throughout the grid.

$CO_{2(aq)}$ Enrichment

Optically clear, open-top acrylic chambers were used to establish the carbon-enriched plots (see Campbell and Fourqurean 2011 for a detailed description). $CO_{2(aq)}$-enriched seawater was generated in the field by bubbling pure CO_2 gas into submerged water pumps, which subsequently delivered $CO_{2(aq)}$-enriched seawater into the carbon-enriched chambers via an underwater PVC network. This technique has been demonstrated as an effective means of confining $CO_{2(aq)}$ enrichment to a localized area of the benthos, with no influence on the surrounding area. As $CO_{2(aq)}$-enriched seawater was pumped into the chambers, it cycled within the seagrass canopy before being flushed out the top of the chamber. This design allowed for moderate constraint of carbonate parameters within the enriched chambers while limiting reductions in light and water motion. Ambient $CO_{2(aq)}$ chambers received unenriched seawater from an independent PVC network connected to a separate series of water pumps. Given the continuous circulation within each chamber (turnover rate ~ 60x/day), shifts in diffusion boundary layers adjacent to the leaf surfaces were limited, as evidenced by similar stable carbon isotope values ($\delta^{13}C$) between seagrasses within the ambient chambers and open plots over a 6-month testing period (Campbell and Fourqurean 2011). Other techniques of free ocean carbon enrichment (FOCE) have recently been developed and generally employ a similar design, whereby a predetermined pH offset is established and maintained over an extended period within a semi-enclosed environment (Gattuso and others 2014; Kline and others 2012). Many of these alternate FOCE designs employ feedback mechanisms, whereby pH is continuously monitored within enclosures and directly controlled via the intermittent injection of $CO_{2(aq)}$-enriched seawater. Due to the high turnover and open-top design of our chambers, CO_2 injection was maintained at a continuous rate and did not require feedback mechanisms to establish a pH offset.

The level of $CO_{2(aq)}$ enrichment was controlled by adjusting the flow of CO_2 gas into the pumps and set to mimic 'business-as-usual' forecasts for

the year 2100, approximately a 0.3-unit reduction in pH (Caldeira and Wickett 2003). All chambers were periodically scrubbed clear of any fouling, and periodic pH measurements (NBS scale, relative accuracy ± 0.002) were taken within all chambers and control plots during the 1200–1500 time period (n = 35 discrete sampling events). The pH electrode was calibrated with fresh buffers for each use. Water samples were collected once every 6 weeks (n = 8 discrete sampling events) to monitor salinity and total alkalinity (TA). During each TA sampling, 40 ml of seawater was collected from twelve randomly selected chambers/open plots (96 water samples total), filtered through a 0.7 μm GFF filter, and stored on ice until further processing. TA was measured via automated, potentiometric open-cell titration with 0.1 N HCL. Salinity was measured with an Orion conductivity meter. Within each sampling event, TA did not significantly differ across CO_2 treatments and was subsequently pooled for carbonate calculations. Carbonate parameters ($[CO_{2(aq)}]$, $[HCO_3^-]$, $[CO_3^{2-}]$, and calcite/aragonite saturation states) were calculated using the observed values of pH, temperature, salinity, and TA with the CO_2SYS Excel Macro (Lewis and Wallace 1998), using the dissociation constants of Mehrbach and others (1973), refit by Dickson and Millero (1987). Seawater temperatures were recorded every 6 h with a HOBO temperature logger, and light levels at the top of the seagrass canopy were periodically measured with planar photosynthetic photon flux density (PPFD) sensor (WALZ, Diving PAM).

Nutrient Enrichment

Fertilizer was evenly distributed by hand over the sediments in each designated + NP chamber or + NP open plot on a monthly basis. Nitrogen was added in the form of urea-coated slow-release N fertilizer (Polyon, Pursell Technologies; 38-0-0, 94% nitrogen as urea), and phosphorus was added as defluorinated granular phosphate rock [Multifos, IMC Phosphates] $Ca_3(PO_4)^2$, 18%P). Final loading rates were 1.54 g N m^{-2} d^{-1} and 0.24 g P m^{-2} d^{-1} and were similar to prior nutrient enrichment studies within this region (Armitage and others 2005; Armitage and others 2011). This method of enrichment is effective at increasing nutrient availability to both the above- and belowground compartments of seagrass communities, as demonstrated by prior work employing ^{14}N-enriched fertilizer (Mutchler and others 2004). Experiments using this technique have further shown that the direct application of fertilizer to the benthos is effective at increasing nutrient availability to the epiphyte community (Armitage and others 2006; Gil and others 2006), and increasing sediment and plant nutrient content, especially over extended periods (12–18 months) (Armitage and others 2005; Ferdie and Fourqurean 2004). We similarly document increased plant N content and declines in plant C/N ratios with fertilizer application (see ''Results'').

Seagrass Productivity

Leaf production was measured 6 times using a modified leaf marking technique (Zieman 1974; Fourqurean and others 2001). Measuring leaf production requires the removal of multiple shoots, and thus the number of sampling events was limited to prevent overharvesting. During each sampling, 3–5 shoots within each chamber and open plot were pierced at the base with a hypodermic needle. These marked shoots were then harvested after 7–10 days of in situ growth. In the laboratory, epiphytes were removed with a razor, and the lengths and widths of all leaves were recorded. Each shoot was divided into new and old leaf material, and dried in a 70°C oven. Relative leaf growth rates (mg new leaf mass * g^{-1} total leaf mass * day^{-1}) and areal productivity (mg new - leaf mass * m^{-2} * day^{-1}) were calculated.

Seagrass Nutrient Content

Seagrass leaf nutrient content was measured on the biomass from the productivity harvest. Newly produced leaf material (leaf rank 1) was analyzed for CNP content (% g dry wt). Dried leaf material was ground into a fine powder with a mortar and pestle, and analyzed for CN content using a CHN analyzer. Phosphorus content of dried leaf material was determined via dry oxidation, acid hydrolysis extraction followed by colorimetric analysis (Fourqurean and others 1992).

Biomass Partitioning

Benthic cores were used to assess seagrass biomass partitioning at the end of the study. Two, 15-cm-diameter cores were taken within each chamber and control plot. Cores were pushed into the sediment until the underlying limestone bedrock was reached, and all aboveground and belowground biomass was collected. Both cores from within each plot were placed in a single mesh bag, rinsed free of sediment, and transported to the laboratory in coolers. All shoots were counted, measured for length, and separated into aboveground leaves and

belowground root and rhizome fractions. The number of active horizontal meristems was also recorded. All biomass fractions were dried in 70°C ovens to obtain dry weights.

Soluble Carbohydrates

Non-structural carbohydrate content (NSC) of seagrass rhizomes was determined utilizing the MBTH (3-methyl-2-benzothiazolinone hydrazone hydrochloride) analysis method (Johnson and others 1981; Pakulski and Benner 1992). All rhizome material collected from the biomass cores was ground into a fine powder and transferred into 20-ml glass scintillation vials. Chemical analysis of aliquots of each sample involved a borohydride reduction of hydrolyzed monosaccharides to sugar alcohols, followed by periodate oxidation to formaldehyde, and colorimetric determination of formaldehyde by MBTH (Lee and Dunton 1997). NSC is reported as mg soluble carbohydrate * g^{-1} DW. Total areal soluble carbohydrate content was calculated by multiplying sample carbohydrate concentration by total rhizome dry weight.

Sediment Properties

Sediment cores were taken at the end of the experiment to measure total organic matter content, organic carbon content, and inorganic carbon content. A single sediment core was taken within each replicate chamber and open plot with a 60-ml syringe (capturing the upper 10 cm of sediment) and dried to a constant weight in a 70°C oven. Sediment samples were passed through a (1 mm) sieve to remove large particles, homogenized with a mortar and pestle, and aliquots were ashed at 500°C for 5 h to determine total organic content (calculated as % wt loss on ignition). Ashed samples were further analyzed for inorganic carbon content (IC) using a CHN analyzer (Fisons NA1500). Additional aliquots of the pre-ashed sediment samples were analyzed for total carbon content (TC) using a CHN analyzer, and organic carbon content (OC) was calculated as the difference between TC and IC.

Statistical Analyses

Two-way repeated-measures ANOVA ($\alpha = 0.05$) was used to analyze repeated measurements of seagrass leaf growth and nutrient content, with sampling date as the within-subjects factor and $CO_{2(aq)}$ and nutrient treatment as the between subjects factors. To avoid pseudoreplication, statistical analyses were conducted with the means of replicate subsamples from each chamber or open plot. Biomass partitioning, sediment characteristics, and soluble carbohydrate content collected at the end of the experiment were analyzed with a two-way ANOVA ($\alpha = 0.05$). All data were tested for normality and variance homogeneity. If test assumptions were violated, data were either log-transformed or analyzed by ranked values.

Results

Seawater Chemistry

The elevated $CO_{2(aq)}$ chambers displayed pH values (mean ± SE) of 7.86 ± 0.03 and 7.90 ± 0.03 for the − NP and + NP treatments, respectively (Table S1; Figure S2). The ambient $CO_{2(aq)}$ chambers displayed pH values of 8.20 ± 0.02 and 8.19 ± 0.02 for the − NP and + NP treatments, respectively. The open plots (− NP and + NP) both displayed pH values of 8.19 ± 0.02. The pH differential between the elevated $CO_{2(aq)}$ chambers and the ambient chambers/open plots was 0.32 ± 0.02 units. Calculated seawater parameters followed similar trends, with the elevated $CO_{2(aq)}$ chambers displaying $CO_{2(aq)}$ concentrations of 29.9 ± 2.6 and 27.4 ± 2.6 μmol kg^{-1} SW for the − NP and + NP treatments, respectively. The ambient $CO_{2(aq)}$ chambers displayed $CO_{2(aq)}$ concentrations of 11.3 ± 0.6 and 11.1 ± 0.6 μmol kg^{-1} SW for the − NP and + NP treatments, respectively. The open plots (− NP and + NP) averaged 11.6 ± 0.6 and 11.5 ± 0.6 μmol kg^{-1} SW, respectively. Calculated pCO_2 averaged 429 and 437 μatm for the ambient $CO_{2(aq)}$ chambers (− NP and + NP, respectively) and 1147 and 1054 μatm for the elevated $CO_{2(aq)}$ chambers (− NP and + NP, respectively). Background diel pH variation was evident, and likely the result biological activity, with benthic and water column processes (photosynthesis and respiration) driving CO_2 uptake and release. A temporary malfunction of the CO_2 regulator produced a brief low pH event in February (Figure S2). This issue was promptly remedied, and pH offsets returned to normal.

The elevated $CO_{2(aq)}$ chambers displayed higher pH variation among replicate chambers compared to the ambient chambers and open plots (note errors bars in Figure S2). On average, pH varied by 3.6% across replicate elevated $CO_{2(aq)}$ chambers. Comparatively, ambient $CO_{2(aq)}$ chambers displayed mean pH variation of 0.4% among replicates, while the open plots displayed a pH variation of 0.2%. Averaged over the course of the study, calculated 95% confidence intervals indicate that

pH values were not statistically different between individual chambers within the same treatment, suggesting that no replicate chamber or open plot displayed consistently abnormal pH values for their given treatment.

Total alkalinity (mean ± SE) was 2508.9 ± 41.6 μmol kg^{-1} SW and was not altered by $CO_{2(aq)}$ or the chamber structure. Salinity was 35.5 ± 0.6, ranging from 33.9 in November to 37.8 in May. Seawater temperatures averaged 29.1 ± 0.7°C and ranged from 33.9°C in August to 19.6°C in December. There was no effect of the chamber structure itself on seawater temperatures. Light levels inside the chambers ranged from 600 to 700 μmol photons m^{-2} s^{-1} at the top of the seagrass canopy at noon.

Seagrass Biomass, Canopy Structure, and Soluble Carbohydrates

Elevated $CO_{2(aq)}$ modestly increased seagrass standing crop as compared to the ambient $CO_{2(aq)}$ chambers (Table 1; Figure 1A, $P = 0.049$), whereas nutrient enrichment had no effect on standing crop ($P = 0.678$), and there was no interaction between $CO_{2(aq)}$ and nutrient enrichment on standing crop ($P = 0.809$). Seagrass shoot and horizontal meristem density (Figure 1B, C) were not altered by either $CO_{2(aq)}$ ($P = 0.088$ and $P = 0.094$, respectively) or nutrient enrichment ($P = 0.808$ and $P = 0.930$, respectively). Belowground biomass (Figure 2A) was altered by $CO_{2(aq)}$ treatment ($P = 0.024$); however, this was attributable to an increase within the open plot as compared to the ambient chambers (Holm–Sidak test, Table 1). Nutrient enrichment had no effect on belowground biomass ($P = 0.706$). Shoot/root ratios were higher within the elevated $CO_{2(aq)}$ chambers ($P = 0.029$), yet only when compared to the open plots (Holm–Sidak test, Table 1). Nutrient enrichment had no effect on shoot/root ratios ($P = 0.518$). Belowground carbohydrates were unaltered by either $CO_{2(aq)}$ or nutrient enrichment ($P = 0.476$ and $P = 0.198$, respectively).

Seagrass Productivity

Leaf growth varied during the course of the experiment (maximum in October and minimum in January) and was increased by nutrient enrichment ($P = 0.007$, Table 2; Figure 3A, B). Averaged across $CO_{2(aq)}$ treatments, growth rates (mean ± SE) were 22.4 ± 1.6 and 21.2 ± 1.5 mg g^{-1} d^{-1} within the + NP and − NP treatments, respectively. Elevated $CO_{2(aq)}$ had no effect on leaf growth or areal productivity ($P = 0.07$ and $P = 0.329$, respectively).

Seagrass Nutrient Content

Nutrient enrichment increased leaf nitrogen content and decreased leaf C/N ratios ($P = 0.013$), yet had no effect on phosphorus content (Table 2; Figure 4). Averaged across $CO_{2(aq)}$ treatments, nitrogen content (mean ± SE) was 2.28 ± 0.1% in the − NP chambers and open plots, and 2.47 ± 0.1% in the + NP chambers and open plots. C/N and N/P ratios within the + NP treatments were 17.3 ± 0.7 and 38.1 ± 2.5, respectively. C/N and N/P ratios in the − NP treatments were 18.2 ± 0.7 and 35.3 ± 2.1, respectively. Elevated $CO_{2(aq)}$ altered phosphorus content and N/P ratios ($P = 0.025$); however, significant differences were only detected between the elevated $CO_{2(aq)}$ chambers and the open plots (Holm–Sidak test, Table 2). Seagrass leaf P content (mean ± SE) was 0.14 ± 0.01% within the elevated $CO_{2(aq)}$ cham-

Table 1. Results of Two-Way ANOVA on Final Seagrass Harvest

	Source of variation		
	CO_2	Nutrients	CO_2 × nutrients
Standing crop	***F* = 3.469, *P* = 0.049**	$F = 0.177$, $P = 0.678$	$F = 0.215$, $P = 0.809$
post hoc results	(Elev. CO_2 > Amb. CO_2)		
Shoot density	$F = 2.722$, $P = 0.088$	$F = 0.061$, $P = 0.808$	$F = 0.213$, $P = 0.810$
Horizontal meristems	$F = 2.636$, $P = 0.094$	$F = 0.080$, $P = 0.930$	$F = 0.054$, $P = 0.947$
Belowground biomass	***F* = 4.346, *P* = 0.024**	$F = 0.145$, $P = 0.706$	$F = 0.290$, $P = 0.751$
post hoc results	(Open > Amb. CO_2)		
Shoot/root	***F* = 4.165, *P* = 0.029**	$F = 0.431$, $P = 0.518$	$F = 0.788$, $P = 0.467$
post hoc results	(Elev. CO_2 > Open)		
Belowground carbohydrates	$F = 0.765$, $P = 0.476$	$F = 1.749$, $P = 0.198$	$F = 0.816$, $P = 0.454$

Significant differences are indicated in bold. Post hoc results were determined by a Holm–Sidak test (α = 0.05).

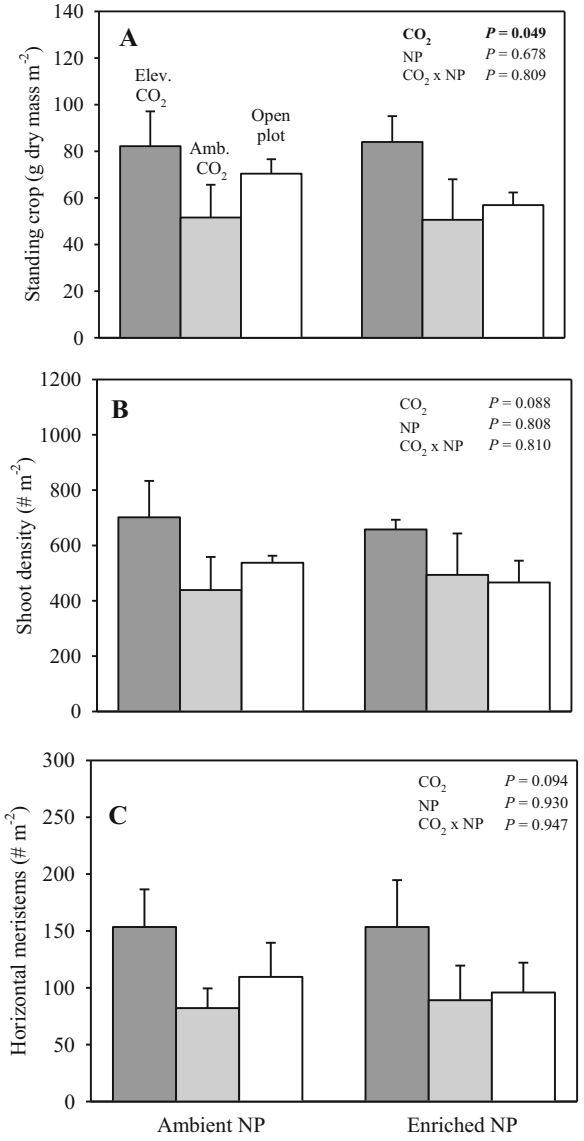

Figure 1. Seagrass standing crop (**A**), shoot density (**B**), and horizontal meristem density (**C**) (means ± 1SE) after $CO_{2(aq)}$ and nutrient enrichment. ANOVA results are displayed.

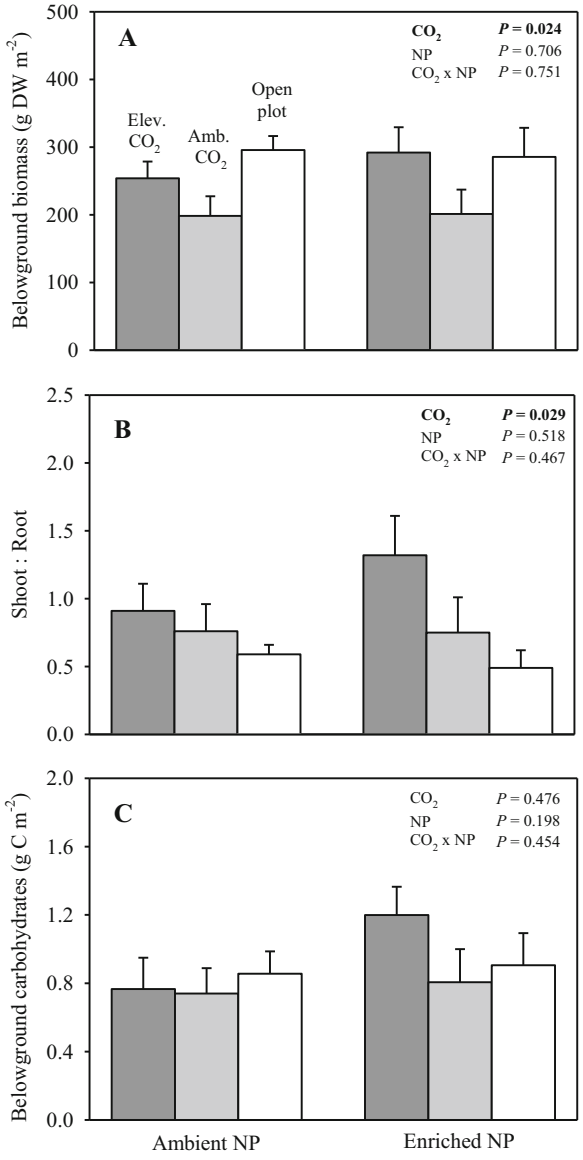

Figure 2. Seagrass belowground biomass (**A**), shoot/root ratios (**B**), and belowground carbohydrates (**C**) (mean ± 1SE) after $CO_{2(aq)}$ and nutrient enrichment. ANOVA results are displayed.

bers and was 0.15 ± 0.01% and 0.16 ± 0.01% within the ambient chambers and open plots, respectively. Seagrass N/P ratios were 38.3 ± 2.2 within the elevated $CO_{2(aq)}$ chambers, and 37.5 ± 2.3 and 34.2 ± 2.3 within the unenriched chambers and open plots, respectively. Leaf nitrogen content was not altered by $CO_{2(aq)}$ treatment. The within-subjects factor of time was significant for all nutrient response variables (Table 2), and significant time × nutrient interactions were detected for leaf N/P ratios ($P = 0.003$).

Inorganic and Organic Sediment Carbon Content

Sediment properties were not altered by $CO_{2(aq)}$ or nutrient enrichment. Across all treatments, IC (mean ± SE) ranged from a minimum of 9.3 ± 0.2% in the + NP open plots to a maximum of 10.0 ± 0.2% in the − NP ambient $CO_{2(aq)}$ chambers. OC ranged from a minimum of 3.0 ± 0.3% in the − NP ambient $CO_{2(aq)}$ chambers to a maximum of 3.7 ± 0.4% in the + NP open plots.

Table 2. Results of Two-Way Repeated-Measures ANOVA on Seagrass Growth, Shoot Mass, Leaf Area, and Nutrient Content

Source of variation	Leaf growth	Leaf C/N	Leaf C/P	Leaf N/P
Within-subjects factor				
Time	***F* = 106.9, *P* < 0.001**	***F* = 52.92, *P* < 0.001**	***F* = 20.21, *P* < 0.001**	***F* = 9.026, *P* < 0.001**
Time × CO_2	$F = 0.981$, $P = 0.453$	$F = 0.817$, $P = 0.590$	$F = 1.002$, $P = 0.435$	$F = 1.551$, $P = 0.154$
Time × nutrient	$F = 0.380$, $P = 0.804$	$F = 1.846$, $P = 0.128$	$F = 2.411$, $P = 0.072$	***F* = 4.415, *P* = 0.003**
Time × CO_2 × nutrient	$F = 0.641$, $P = 0.726$	$F = 1.134$, $P = 0.350$	$F = 2.032$, $P = 0.071$	$F = 1.353$, $P = 0.231$
Between subjects factor				
CO_2	$F = 3.029$, $P = 0.07$	$F = 1.206$, $P = 0.320$	$F = 3.428$, $P = 0.054$	***F* = 4.516, *P* = 0.025**
post hoc results				(Elev. CO_2 > Open)
Nutrient	***F* = 8.889, *P* = 0.007**	***F* = 7.437, *P* = 0.013**	$F = 0.000$, $P = 0.999$	***F* = 8.319, *P* = 0.01**
post hoc results	(+ NP > − NP)	(− NP > + NP)		(+ NP > − NP)
CO_2 × nutrient	$F = 1.862$, $P = 0.180$	$F = 0.486$, $P = 0.622$	$F = 1.646$, $P = 0.219$	$F = 2.832$, $P = 0.084$

Significant results are indicated in bold. Post hoc results were determined by a Holm–Sidak test (α = 0.05).

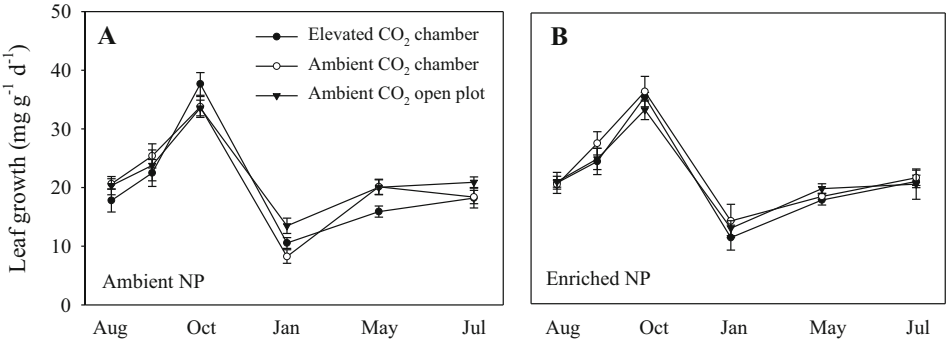

Figure 3. Time series of relative leaf growth rates (mean ± 1SE) within the nutrient-unenriched (**A**) and nutrient-enriched (**B**) $CO_{2(aq)}$ treatments.

Discussion

Our work presents modest evidence that prolonged increases in dissolved CO_2 may alter the structure of shallow seagrass habitats. It is important to note that the statistical results of these CO_2 effects ($P = 0.049$) were near our significance threshold ($\alpha = 0.05$), and thus these findings should be interpreted within this context. We assert that increased replication would have largely improved the power of our analysis, which at our given level of replication ($n = 5$) was 0.6, less than the desired 0.8 (Cohen 1988). Nutrient enrichment increased plant N content and leaf growth, yet interactions with elevated $CO_{2(aq)}$ were not detected. Thus, contrary to our original hypothesis, nutrient availability did not alter $CO_{2(aq)}$ effects at our site, suggesting that even relatively nutrient-poor plant communities may positively respond to elevated $CO_{2(aq)}$ over sufficient timescales.

$CO_{2(aq)}$ enrichment had no effect on seagrass NSC, contrary to the results of prior research (Zimmerman and others 1997; Campbell and Fourqurean 2013). These disparities suggest that the effects of $CO_{2(aq)}$ enrichment on NSC production may be variable, and the lack of a significant NSC response in the current study might be attributable to differences in harvest schedules or experimental time scales (Korner 2000). Previous work documenting increases in seagrass NSC with elevated $CO_{2(aq)}$ was recorded in short-term (weeks–months) experiments. Over the longer duration of the current study, increased rhizome NSC might have been masked by seasonal variation in carbohydrate pools or the eventual investment of rhizome NSC toward alternate biomass compartments (Chapin and others 1990), as plants can direct growth and vegetative expansion toward acquiring resources in least supply (Bloom and others 1985). Under elevated $CO_{2(aq)}$, naturally growing, intact seagrasses (with undisturbed access to sediment nutrient pools) may invest in shoot proliferation and aboveground biomass. Due to the destructive nature of harvesting rhizomes, a single

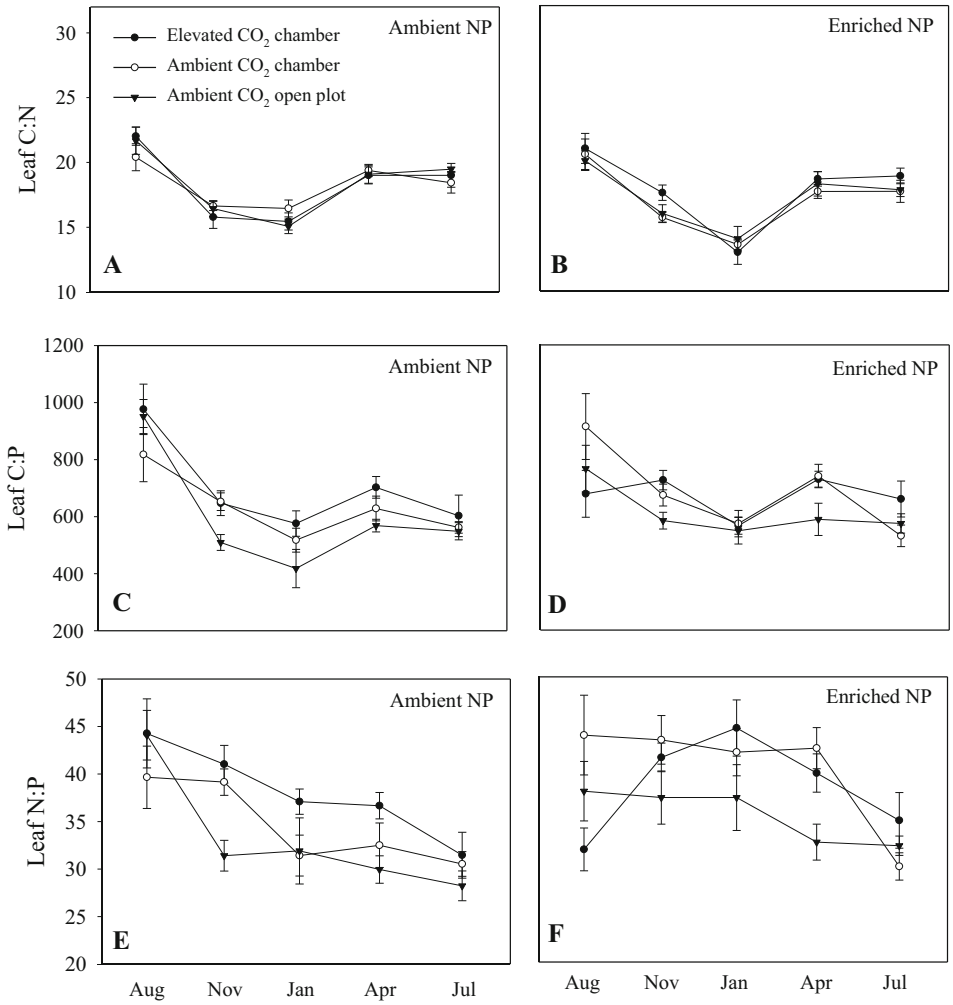

Figure 4. Time series of seagrass leaf C:N (**A**, **B**), C:P (**C**, **D**), N:P (**E**, **F**) elemental ratios (mean ± 1SE) within the nutrient-unenriched (*left column*) and nutrient-enriched (*right column*) treatments.

NSC assessment was conducted at the end of this study to maintain plot integrity. Thus, any transient gains in seagrass NSC from elevated $CO_{2(aq)}$ may have been diminished by the production of new biomass by the time of harvest. Lee and Dunton (1996) document that seagrasses with extensive rhizomes (such as *T. testudinum*) can utilize stored NSC to fuel respiration during the winter season, or provide resources necessary for leaf growth during the spring. As we present modest evidence that elevated $CO_{2(aq)}$ increased standing crop, this suggests that NSC accumulation may represent a relatively short-term response, with shifts in biomass representing the ultimate consequences of long-term carbon enrichment.

Increased aboveground biomass with elevated $CO_{2(aq)}$ follows responses from a number of terrestrial systems (Bazzaz 1990; Korner 2000; Ainsworth and Long 2005). Using ex situ mesocosms, Palacios and Zimmerman (2007) show increased shoot density and rhizome biomass of the temperate species *Zostera marina* under long-term (~ 1 year) elevated $CO_{2(aq)}$ and more recently demonstrate that $CO_{2(aq)}$ can further enhance summertime shoot survival and growth in the Chesapeake Bay (Zimmerman and others 2015; Zimmerman and others 2017). Studies of seagrasses surrounding natural CO_2 vents similarly document increases in shoot density (Hall-spencer and others 2008; Russell and others 2013), aboveground biomass (Takahashi and others 2016), and belowground root biomass (Fabricius and others 2011) with elevated $CO_{2(aq)}$. Yet, it remains important to note that (1) many vent studies document the most prominent increases in biomass at sites characterized by extremely high $CO_{2(aq)}$ and low pH (< 7.6) and (2) some studies show contradictory declines in seagrass structure and function with $CO_{2(aq)}$

enrichment, potentially attributable to the presence of harmful trace elements surrounding the vent systems (Apostolaki and others 2014; Olivé and others 2017). Together, these examples highlight the need for additional in situ manipulative studies, whereby pH offsets can be carefully maintained and CO_2 effects can be rigorously explored. Working with *Posidonia oceanica*, Cox and others (2016) represent one of the other few manipulative field studies to examine seagrass responses to elevated $CO_{2(aq)}$ (pH $\sim$ 7.88). Although they document limited effects, these conclusions are taken with a cautionary note due to poor replication (n = 1). Furthermore, as compared to the current study, Cox and others (2016) conducted in situ enrichment within a much deeper meadow (11 m) at a temperate latitude, where both light levels and average seawater temperatures (23°C) were lower. These environmental factors may have served to mute responses, as demonstrated in other experiments where $CO_{2(aq)}$ effects were minimal under light-limited conditions (Palacios and Zimmerman 2007). Similar to terrestrial systems, disparities in the effects of elevated $CO_{2(aq)}$ on plant communities may be premised around varying patterns of carbohydrate allocation, all potentially influenced by the type of study (ex situ vs in situ), duration, environmental context, and the species under consideration (see Table S2 for comparisons across seagrass $CO_{2(aq)}$ research). Specifically, careful attention must be given toward scaling the experimental duration to the turnover and response times of the species under study. For our work, demographic analysis showed that average seagrass shoot age was approximately 1.8 years (Campbell and Fourqurean, unpublished data). Thus, CO_2 effects may have been more prominently detected with extended experimentation ($>$ 1 year). Overall, we assert that long-term experiments conducted within natural systems (which limits transplant artifacts and maintains rooting volume) may be the only way to accurately and realistically assess $CO_{2(aq)}$ effects on seagrass ecosystems.

Nutrient enrichment produced relatively minor effects on leaf nutrient content, leaf growth rates, or final biomass metrics. This indicated that factors other than nutrient availability likely controlled seagrass productivity at this site. Although N and P enrichment increased leaf growth rates, the effect was minor (mean increase of 5.5% over the controls) and, in isolation, did not result in increased biomass. Although the seagrass landscape in south Florida is generally one of marked nutrient limitation (Fourqurean and Zieman 2002), seagrass beds toward the western boundary of Florida Bay are not strongly responsive to nutrient enrichment, displaying minor changes in either leaf tissue chemistry, growth, or biomass because of a balanced environmental NP supply relative to plant demand (Armitage and others 2005). Seagrasses at our site displayed N/P ratios near 30:1, suggesting our study site was not strongly nutrient limited. Prior studies have suggested that seagrasses with N content above 1.8% are generally not limited by N supply, and seagrasses with P content above 0.2% are generally not limited by P supply (Duarte 1990). Mean seagrass nutrient content at our site was 2.37% N (above the N threshold), and P content was 0.15% DW (suggestive of slight P limitation). However, these thresholds may not hold true for all locations, as other studies have documented minor seagrass responses to P enrichment despite relatively low tissue concentrations ($\approx$ 0.10%P) (Ferdie and Fourqurean 2004). Moreover, we find no long-term effect of elevated $CO_{2(aq)}$ on seagrass nutrient content, contrasting with prior intermediate-term experiments which document $CO_{2(aq)}$-mediated declines in leaf N and P content (Campbell and Fourqurean 2013). It is likely that the transient declines reported by Campbell and Fourqurean (2013) were primarily driven by NSC dilution, as opposed to broader mismatches between plant nutrient demand and environmental supply.

$CO_{2(aq)}$ $\times$ nutrient interactions were not detected across the timescale of the current study, indicating that N and P amendments did not alter seagrass responses to $CO_{2(aq)}$, and background nutrient supply was sufficient to meet plant demand, similar to the results of prior ex situ work documenting limited $CO_{2(aq)}$ $\times$ nutrient interactions for two tropical seagrasses, *Halodule uninervis* and *Thalassia hemprichii* (Ow and others 2016), and the temperate seagrass *Zostera noltii* (Martinez-Crego and others 2014). In terrestrial studies, progressive nitrogen limitation (PNL) has been proposed as a mechanism constraining plant responses to elevated $CO_{2(aq)}$, as additional elemental resources may be required to sustain increased growth (Luo and others 2004; Norby and others 2010). Resulting tests of this hypothesis with FACE experimentation have provided conflicting results, with PNL being demonstrated in some studies and not in others (Norby and Zak 2011). These trends may be explained by the ability to compensate for reduced N availability via increased soil exploration by fine roots (Finzi and others 2007). In seagrass meadows, particularly those dominated by large-bodied species such as *T. testudinum*, investments in belowground biomass (root-rhizome complexes)

may allow for sufficient exploration of sediment nutrient pools to support increased demand under elevated $CO_{2(aq)}$, as *T. testudinum* has the ability to liberate sediment bound P via the exudation of organic acids (Long and others 2008). Thus, we suggest that seagrasses may respond to elevated $CO_{2(aq)}$ across a range of nutrient regimes. We further note that belowground biomass was higher within our open plots as compared to our ambient $CO_{2(aq)}$ chambers, yet intriguingly, there was no difference in belowground biomass between these open plots and the elevated $CO_{2(aq)}$ chambers (Figure 2A). It is unclear what might be driving such trends; however, any negative chamber effect on belowground biomass may have been partially mitigated by $CO_{2(aq)}$ addition, as prior work has demonstrated positive $CO_{2(aq)}$ effects on belowground compartments (Palacios and Zimmerman 2007).

$CO_{2(aq)}$ or nutrient enrichment did not alter soil organic matter (OM) or organic carbon (OC) content. Similar to terrestrial systems, seagrass meadows are now being recognized as substantial hotspots for long-term carbon storage (Fourqurean and others 2012), and thus any $CO_{2(aq)}$ mediated increase in seagrass productivity might serve to promote C storage within these systems via either increased belowground biomass or aboveground canopy structure, which facilitates particle trapping (Hendriks and others 2008). Although some terrestrial work has demonstrated increases in belowground C stocks with elevated $CO_{2(aq)}$ (Jastrow and others 2005; Luo and others 2006), these findings tend to be evident over extended time scales (multiple years). Thus, the duration of the current experiment may not have been sufficient to detect changes in soil properties via the before mentioned mechanisms. Further research will be required to sufficiently determine how seagrass ecosystem C dynamics may be ultimately influenced by $CO_{2(aq)}$, as effects on long-term C storage will critically depend upon plant partitioning and the distribution of C across biomass pools of varying residence times (Norby and Zak 2011).

Elevated $CO_{2(aq)}$ modestly increased seagrass standing crop, providing evidence that carbon enrichment will likely prove beneficial to submerged plants beyond enhancements in photosynthetic output. These responses were not influenced by NP addition, suggesting that elevated $CO_{2(aq)}$ may alter plant functioning, even in relatively nutrient-poor systems. Such findings may be attributable to substantial seagrass investment in belowground structures, capable of efficiently exploiting sediment NP pools, thereby meeting any $CO_{2(aq)}$-driven increases in nutrient demand. However, as seagrass N and P content widely varies across south Florida (Fourqurean and Zieman 2002), we assert that valuable information would be gained by conducting additional seagrass $CO_{2(aq)}$ research across a broader range of nutrient availability, particularly in locations experiencing extreme limitation. Increases in ecosystem C storage under elevated $CO_{2(aq)}$ are currently unknown, as the ultimate effects will largely depend upon the capability of these systems to increase organic C inputs toward refractory soil compartments, which are stable over extended periods (Fourqurean and others 2012). Although our current work provides some evidence for increases in seagrass standing crop, other experiments have highlighted the negative effects of CO_2 on calcifying epiphytes (across both in situ and ex situ studies), suggesting that future rates of calcification and biogenic sediment production may be altered with climate change (Hall-Spencer and others 2008; Campbell and Fourqurean 2014; Cox and others 2015). Furthermore, other environmental parameters, such as light and/or temperature, may additionally influence $CO_{2(aq)}$ effects, likely in complex ways that will be difficult to assess without carefully designed multi-factorial experiments. While some studies document that light enhances seagrass responses to $CO_{2(aq)}$ via increases in photosynthetic carbon demand (Palacios and Zimmerman 2007), others show that increased light can promote negative $CO_{2(aq)}$ effects by fueling the growth of epiphytic algae (Burnell and others 2014). Interactions with temperature have also recently received attention, whereby $CO_{2(aq)}$ can serve to mitigate the effects of thermal stress by promoting whole plant carbon balance (Zimmerman and others 2015). Overall, similar to terrestrial systems, we argue that these foundational habitats will change with future increases in carbon supply and should be targeted as a priority for further assessments of the effects of climate change on coastal ecosystems.

ACKNOWLEDGEMENTS

We thank Dr. Patrick Rice and the Florida Keys Community College for logistical support. Jennifer Sweatman, Bryan Dewsbury, Nathan Lemoine and Thomas Frankovich provided assistance in the field. This work was conducted in the Florida Keys National Marine Sanctuary under permits FKNMS-2010-015 and FKNMS-2010-015A and was supported by the National Science Foundation through the Florida Coastal Everglades Long-Term Ecologi-

cal Research Program under Grant No. DBI-0620409, and a Graduate School Doctoral Evidence Acquisition Fellowship awarded to J.E.C by Florida International University. This is contribution #74 from the Marine Education and Research Center in the Institute for Water and Environment at Florida International University.

REFERENCES

Ainsworth EA, Long SP. 2005. What have we learned from 15 years of free-air CO_2 enrichment (FACE)? A meta-analytic review of the responses of photosynthesis, canopy properties and plant production. New Phytol 165:351–71.

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 2:2625–35.

Apostolaki ET, Vizzini S, Hendriks IE, Olsen YS. 2014. Seagrass ecosystem response to long-term high CO_2 in a Mediterranean volcanic vent. Mar Environ Res 99:9–15.

Armitage AR, Frankovich TA, Heck KL, Fourqurean JW. 2005. Experimental nutrient enrichment causes complex changes in seagrass, microalgae, and macroalgae community structure in Florida Bay. Estuaries 28:422–34.

Armitage AR, Frankovich TA, Fourqurean JW. 2006. Variable responses within epiphytic and benthic microalgal communities to nutrient enrichment. Hydrobiologia 569:423–35.

Armitage AR, Frankovich TA, Fourqurean JW. 2011. Long-term effects of adding nutrients to an oligotrophic coastal environment. Ecosystems 14:430–44.

Bazzaz FA. 1990. The response of natural ecosystems to the rising global CO_2 levels. Annu Rev Ecol Syst 21:167–96.

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

Bloom AJ, Chapin FS, Mooney HA. 1985. Resource limitation in plants—an economic analogy. Annu Rev Ecol Syst 16:363–92.

Burnell OW, Russell BD, Irving AD, Connell SD. 2014. Seagrass response to CO_2 contingent on epiphytic algae: indirect effects can overwhelm direct effects. Oecologia 176:871–82.

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

Campbell JE, Fourqurean JW. 2011. Novel methodology for in situ carbon dioxide enrichment of benthic ecosystems. Limnol Oceanogr Methods 9:97–109.

Campbell JE, Fourqurean JW. 2013. Effects of in situ CO_2 enrichment on the structural and chemical characteristics of the seagrass *Thalassia testudinum*. Mar Biol 160:1465–75.

Campbell JE, Fourqurean JW. 2014. Ocean acidification outweighs nutrient effects in structuring seagrass epiphyte communities. J Ecol 102:730–7.

Chapin FS, Schulze ED, Mooney HA. 1990. The ecology and economics of storage in plants. Annu Rev Ecol Syst 21:423–47.

Cohen J. 1988. Statistical power analysis for the behavioral sciences. New York: Routledge Academic.

Cox TE, Schenone S, Delille J, Díaz-Castañeda V, Alliouane S, Gattuso J-P, Gazeau F. 2015. Effects of ocean acidification on *Posidonia oceanica* epiphytic community and shoot productivity. J Ecol 103:1594–609.

Cox TE, Gazeau F, Alliouane S, Hendriks IE, Mahacek P, Le Fur A, Gattuso JP. 2016. Effects of in situ CO_2 enrichment on structural characteristics, photosynthesis, and growth of the Mediterranean seagrass *Posidonia oceanica*. Biogeosciences 13:2179–94.

Dickson AG, Millero FJ. 1987. A comparison of the equilibrium constants for the dissociation of carbonic acid in seawater media. Deep Sea Res 34:1733–43.

Duarte CM. 1990. Seagrass nutrient content. Mar Ecol Prog Ser 67:201–7.

Duarte CM. 1991. Seagrass depth limits. Aquat Bot 40:363–77.

Duarte CM, Chiscano CL. 1999. Seagrass biomass and production: a reassessment. Aquat Bot 65:159–74.

Ellsworth DS, Anderson IC, Crous KY, Cooke J, Drake JE, Gherlenda AN, Gimeno TE, Macdonald CA, Medlyn BE, Powell JR, Tjoelker MG, Reich PB. 2017. Elevated CO_2 does not increase eucalypt forest productivity on a low-phosphorus soil. Nat Clim Change 7:279–82.

Fabricius KE, Langdon C, Uthicke S, Humphrey C, Noonan S, De'ath G, Okazaki R, Muehllehner N, Glas MS, Lough JM. 2011. Losers and winners in coral reefs acclimatized to elevated carbon dioxide concentrations. Nat Clim Change 1:165–9.

Ferdie M, Fourqurean JW. 2004. Responses of seagrass communities to fertilization along a gradient of relative availability of nitrogen and phosphorus in a carbonate environment. Limnol Oceanogr 49:2082–94.

Finzi AC, Norby RJ, Calfapietra C, Gallet-Budynek A, Gielen B, Holmes WE, Hoosbeek MR, Iversen CM, Jackson RB, Kubiske ME, Ledford J, Liberloo M, Oren R, Polle A, Pritchard S, Zak DR, Schlesinger WH, Ceulemans R. 2007. Increases in nitrogen uptake rather than nitrogen-use efficiency support higher rates of temperate forest productivity under elevated CO_2. Proc Natl Acad Sci 104:14014–19.

Fourqurean JW, Zieman JC. 2002. Nutrient content of the seagrass *Thalassia testudinum* reveals regional patterns of relative availability of nitrogen and phosphorus in the Florida Keys USA. Biogeochemistry 61:229–45.

Fourqurean JW, Zieman JC, Powell GVN. 1992. Phosphorus limitation of primary production in Florida Bay—evidence from C–N–P ratios of the dominant seagrass *Thalassia testudinum*. Limnol Oceanogr 37:162–71.

Fourqurean JW, Willsie A, Rose CD, Rutten LM. 2001. Spatial and temporal pattern in seagrass community composition and productivity in south Florida. Mar Biol 138:341–54.

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 5:505–9.

Gattuso JP, Kirkwood W, Barry JP, Cox E, Gazeau F, Hansson L, Hendriks I, Kline DI, Mahacek P, Martin S, McElhany P, Peltzer ET, Reeve J, Roberts D, Saderne V, Tait K, Widdicombe S, Brewer PG. 2014. Free-ocean CO_2 enrichment (FOCE) systems: present status and future developments. Biogeosciences 11:4057–75.

Gil M, Armitage AR, Fourqurean JW. 2006. Nutrient impacts on epifaunal density and species composition in a subtropical seagrass bed. Hydrobiologia 569:437–47.

Hall-Spencer JM, Rodolfo-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 454:96–9.

Hendriks IE, Sintes T, Bouma TJ, Duarte CM. 2008. Experimental assessment and modeling evaluation of the effects of

the seagrass *Posidonia oceanica* on flow and particle trapping. Mar Ecol Prog Ser 356:163–73.

Hungate BA, Van Groenigen K-J, Six J, Jastrow JD, Luo Y, De Graaff M-A, Van Kessel C, Osenberg CW. 2009. Assessing the effect of elevated carbon dioxide on soil carbon: a comparison of four meta-analyses. Glob Change Biol 15:2020–34.

Jastrow JD, Miller RM, Matamala R, Norby RJ, Boutton TW, Rice CW, Owensby CE. 2005. Elevated atmospheric carbon dioxide increases soil carbon. Glob Change Biol 11:2057–64.

Jiang ZJ, Huang XP, Zhang JP. 2010. Effects of CO_2 enrichment on photosynthesis, growth, and biochemical composition of seagrass *Thalassia hemprichii* (Ehrenb.) Aschers. J Integr Plant Biol 52:904–13.

Johnson KM, Burney CM, Sieburth JM. 1981. Doubling the production and precision of the MBTH spectrophotometric assay for dissolved carbohydrates in seawater. Mar Chem 10:467–73.

Kline DI, Teneva L, Schneider K, Miard T, Chai A, Marker M, Headley K, Opdyke B, Nash M, Valetich M, Caves JK, Russell BD, Connell SD, Kirkwood BJ, Brewer P, Peltzer E, Silverman J, Caldeira K, Dunbar RB, Koseff JR, Monismith SG, Mitchell BG, Dove S, Hoegh-Guldberg O. 2012. A short-term in situ CO_2 enrichment experiment on Heron Island (GBR). Sci Rep 2:413.

Koch M, Bowes G, Ross C, Zhang X-H. 2013. Climate change and ocean acidification effects on seagrasses and marine macroalgae. Glob Change Biol 19:103–32.

Korner C. 2000. Biosphere responses to CO_2 enrichment. Ecol Appl 10:1590–619.

Kroeker KJ, Kordas RL, Crim RN, Singh GG. 2010. REVIEW AND SYNTHESIS: meta-analysis reveals negative yet variable effects of ocean acidification on marine organisms. Ecol Lett 13:1419–34.

Lee KS, Dunton KH. 1996. Production and carbon reserve dynamics of the seagrass *Thalassia testudinum* in Corpus Christi bay, Texas, USA. Mar Ecol Prog Ser 143:201–10.

Lee KS, Dunton KH. 1997. Effects of in situ light reduction on the maintenance, growth and partitioning of carbon resources in *Thalassia testudinum* Banks ex Konig. J Exp Mar Biol Ecol 210:53–73.

Lewis E, Wallace DWR. 1998. Program developed for CO_2 system calculations. ORNL/CDIAC-105. Oak Ridge: Carbon Dioxide Information Analysis Center, Oak Ridge National Laboratory, U.S. Department of Energy.

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

Long MH, McGlathery KJ, Zieman JC, Berg P. 2008. The role of organic acid exudates in liberating phosphorus from seagrass-vegetated carbonate sediments. Limnol Oceanogr 53:2616–26.

Luo Y, Su B, Currie WS, Dukes JS, Finzi A, Hartwig U, Hungate B, McMurtrie RE, Oren R, Parton WJ, Pataki DE, Shaw MR, Zak DR, Field CB. 2004. Progressive nitrogen limitation of ecosystem responses to rising atmospheric carbon dioxide. Bioscience 54:731–9.

Luo YQ, Hui DF, Zhang DQ. 2006. Elevated CO_2 stimulates net accumulations of carbon and nitrogen in land ecosystems: A meta-analysis. Ecology 87:53–63.

Martinez-Crego B, Olive I, Santos R. 2014. CO_2 and nutrient-driven changes across multiple levels of organization in *Zostera noltii* ecosystems. Biogeosciences 11:7237–49.

Mehrbach C, Culberson CH, Hawley JE, Pytkowicz RM. 1973. Measurement of the apparent dissociation constants of carbonic acid in seawater at atmospheric pressure. Limnol Oceanogr 18:897–907.

Mutchler T, Sullivan MJ, Fry B. 2004. Potential of N-14 isotope enrichment to resolve ambiguities in coastal trophic relationships. Mar Ecol Prog Ser 266:27–33.

Norby RJ, Zak DR. 2011. Ecological lessons from free-air CO_2 enrichment (FACE) experiments. Annu Rev Ecol Evol Syst 42:181–203.

Norby RJ, Warren JM, Iversen CM, Medlyn BE, McMurtrie RE. 2010. CO_2 enhancement of forest productivity constrained by limited nitrogen availability. Proc Natl Acad Sci USA 107:19368–73.

Olivé I, Silva J, Lauritano C, Costa MM, Ruocco M, Procaccini G, Santos R. 2017. Linking gene expression to productivity to unravel long- and short-term responses of seagrasses exposed to CO_2 in volcanic vents. Sci Rep 7:42278.

Oren R, Ellsworth DS, Johnsen KH, Phillips N, Ewers BE, Maier C, Schafer KVR, McCarthy H, Hendrey G, McNulty SG, Katul GG. 2001. Soil fertility limits carbon sequestration by forest ecosystems in a CO_2-enriched atmosphere. Nature 411:469–72.

Ow YX, Collier CJ, Uthicke S. 2015. Responses of three tropical seagrass species to CO_2 enrichment. Mar Biol 162:1005–17.

Ow YX, Vogel N, Collier CJ, Holtum JAM, Flores F, Uthicke S. 2016. Nitrate fertilisation does not enhance CO_2 responses in two tropical seagrass species. Sci Rep 6:23093.

Pakulski JD, Benner R. 1992. An improved method for the hydrolysis and MBTH analysis of dissolved and particulate carbohydrates in seawater. Mar Chem 40:143–60.

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.

Pritchard SG, Rogers HH, Prior SA, Peterson CM. 1999. Elevated CO_2 and plant structure: a review. Glob Change Biol 5:807–37.

Rastetter EB, Agren GI, Shaver GR. 1997. Responses of N-limited ecosystems to increased CO_2: a balanced-nutrition, coupled-element-cycles model. Ecol Appl 7:444–60.

Reich PB, Hobbie SE, Lee T, Ellsworth DS, West JB, Tilman D, Knops JMH, Naeem S, Trost J. 2006. Nitrogen limitation constrains sustainability of ecosystem response to CO_2. Nature 440:922–5.

Reich PB, Hobbie SE, Lee TD. 2014. Plant growth enhancement by elevated CO_2 eliminated by joint water and nitrogen limitation. Nat Geosci 7:920–4.

Russell BD, Connell SD, Uthicke S, Muehllehner N, Fabricius KE, Hall-Spencer JM. 2013. Future seagrass beds: can increased productivity lead to increased carbon storage? Mar Pollut Bull 73:463–9.

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 SHC, Fabricius KE, Collier CJ. 2016. The effects of long-term in situ CO_2 enrichment on tropical seagrass communities at volcanic vents. ICES J Mar Sci 73:876–86.

Zieman JC. 1974. Methods for the study of the growth and production of turtle grass, *Thalassia testudinum* Konig. Aquaculture 4:139–43.

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

Zimmerman RC, Hill VJ, Gallegos CL. 2015. Predicting effects of ocean warming, acidification, and water quality on Chesapeake region eelgrass. Limnol Oceanogr 60:1781–804.

Zimmerman RC, Hill VJ, Jinuntuya M, Celebi B, Ruble D, Smith M, Cedeno T, Swingle WM. 2017. Experimental impacts of climate warming and ocean carbonation on eelgrass *Zostera marina*. Mar Ecol Prog Ser 566:1–15.