Estuaries and Coasts (2018) 41:2340–2355
https://doi.org/10.1007/s12237-018-0437-0

The Bloom-Forming Macroalgae, *Ulva*, Outcompetes the Seagrass, *Zostera marina*, Under High CO_2 Conditions

Craig S. Young[1] · Bradley J. Peterson[1] · Christopher J. Gobler[1]

Received: 20 October 2017 / Revised: 13 June 2018 / Accepted: 2 July 2018 / Published online: 16 July 2018

Abstract
While multiple species of macroalgae and seagrass can benefit from elevated CO_2 concentrations, competition between such organisms may influence their ultimate responses. This study reports on experiments performed with a Northwest Atlantic species of the macroalgae, *Ulva*, and the seagrass, *Zostera marina*, grown under ambient and elevated levels of pCO_2, and subjected to competition with each other. When grown individually, elevated pCO_2 significantly increased growth rates and productivity of *Ulva* and *Zostera*, respectively, beyond control treatments (by threefold and 27%, respectively). For both primary producers, significant declines in tissue $\delta^{13}C$ signatures suggested that increased growth and productivity were associated with a shift from use of HCO_3^- toward CO_2 use. When grown under higher pCO_2, *Zostera* experienced significant increases in leaf and rhizome carbon content as well as significant increases in leaf carbon-to-nitrogen ratios, while sediments within which high CO_2 *Zostera* were grown had a significantly higher organic carbon content. When grown in the presence of *Ulva*; however, above- and below-ground productivity and tissue nitrogen content of *Zostera* were significantly lower, revealing an antagonistic interaction between elevated CO_2 and the presence of *Ulva*. The presence of *Zostera* had no significant effect on the growth of *Ulva*. Collectively, this study demonstrates that while *Ulva* and *Zostera* can each individually benefit from elevated pCO_2 levels, the ability of *Ulva* to grow more rapidly and inhibit seagrass productivity under elevated pCO_2, coupled with accumulation of organic C in sediments, may offset the potential benefits for *Zostera* within high CO_2 environments.

Keywords Seagrass · Macroalgae · Ocean acidification · Competition

Introduction

The shifts in carbonate chemistry due to the excessive diffusion of carbon dioxide (CO_2) from fossil fuel combustion into surface oceans is expected to initiate shifts in the community structure of marine flora and fauna. While fossil fuel combustion is expected to increase CO_2 levels 260% by 2100 (Meehl et al. 2007), coastal zones upwelling, riverine discharge, and eutrophication-enhanced microbial respiration can also significantly lower pH and increase pCO_2 levels. Eutrophication-enhanced microbial respiration can cause the seasonal accumulation of respiratory CO_2 that, in some cases, can exceed current pCO_2 projections for the open ocean (> 1000 μatm) for the end of the century (Cai et al. 2011; Melzner et al. 2013; Wallace et al. 2014). While prior studies have demonstrated the negative implications of higher pCO_2 and decreased CO_3^{2-} availability on the growth of calcifying organisms (Gazeau et al. 2007; Talmage and Gobler 2010; Kroeker et al. 2013), other studies have shown that some, but not all, photosynthetic organisms can benefit from an increase in pCO_2 (Palacios and Zimmerman 2007; Koch et al. 2013; Hattenrath-Lehmann et al. 2015; Young and Gobler 2016, 2017). Due to this, non-calcifying autotrophs may gain a competitive advantage over their calcifying counterparts under acidified conditions (Porzio et al. 2011). However, the extent to which individual, non-calcifying marine autotrophs will benefit from elevated CO_2 concentrations will depend on competition (Young and Gobler 2017) and has yet to be fully explored.

The marine photosynthetic organisms that benefit from higher CO_2 concentrations are generally non-calcifying autotrophs whose inorganic uptake is not substrate-saturated at

Communicated by Ken Dunton

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s12237-018-0437-0) contains supplementary material, which is available to authorized users.

✉ Christopher J. Gobler
Christopher.gobler@stonybrook.edu

[1] School of Marine and Atmospheric Sciences, Stony Brook University, Southampton, NY 11968, USA

current CO_2 concentrations (Koch et al. 2013). Carbon acquisition in marine photosynthetic organisms involves the active transport of CO_2 and bicarbonate (HCO_3^-) as well as the diffusive uptake of CO_2 (Badger 2003). While CO_2 is the preferred inorganic carbon source for many marine autotrophs, HCO_3^- is more abundant than CO_2 in seawater at a pH of 8. As such, marine autotrophs require carbon concentrating mechanisms (CCM) and intracellular or extracellular carbonic anhydrase (CA) to convert HCO_3^- to CO_2 to be used by RuBisCO (Badger 2003; Gao and McKinley 1994; Israel and Hophy 2002; Koch et al. 2013). Elevated CO_2 has been shown to enhance the growth of marine macroalgae, including chlorophytes (Björk et al. 1993; Olischläger et al. 2013; Young and Gobler 2016), rhodophytes (Hofmann et al. 2012; Xu et al. 2010; Young and Gobler 2016), and phaeophytes (Hepburn et al. 2011). Chlorophytes, such as *Ulva rigida*, exposed to elevated CO_2 concentrations may downregulate their CCMs, allowing more energy to be available for other biochemical processes such as vegetative growth (Koch et al. 2013; Young and Gobler 2016, 2017). Alternatively, the increased availability of CO_2 in seawater may cause a shift toward the diffusive uptake of CO_2 over use of CCM, thus relieving carbon limitation (Mercado et al. 1998; Young and Gobler 2016, 2017). Values of $\delta^{13}C$ are often used to assess the types of carbon utilized by seagrasses and macroalgae with values of − 10‰ or higher in seagrasses and macroalgae being reflective of the sole use of HCO_3^- whereas macroalgae relying wholly on diffusion of CO_2 for carbon attain a value of − 30‰ (Hepburn et al. 2011; Maberly et al. 1992; Raven et al. 2002). While $\delta^{13}C$ values of − 30‰ or lower have not been observed in seagrasses, it is suggested that increased reliance on CO_2 diffusion can significantly lower the $\delta^{13}C$ of seagrasses (Vizzini et al. 2010).

Seagrasses are another group of autotrophs that have been shown to benefit from elevated CO_2 concentrations. Most seagrass species are C_3 plants capable of utilizing CO_2 and HCO_3^- for photosynthesis, in which CCMs and external CA are used for the fixation of the carbon from HCO_3^- when CO_2 diffusion is slow (Koch et al. 2013; Touchette and Burkholder 2000). As C_3 plants, seagrasses are expected to benefit from increases in CO_2 levels, as their initial carboxylating enzyme, RuBisCO, is not substrate-saturated at current CO_2 concentrations (Koch et al. 2013). The seagrasses *Zostera marina*, *Thalassia testudinum*, and *T. hemprichii* exhibit increased photosynthetic rates, reproduction, below- and above-ground biomass, and production of non-structural carbohydrates in below- and above-ground structures when grown under high CO_2 concentrations (Beer and Koch 1996; Campbell and Fourqurean 2013; Durako 1993; Jiang et al. 2010; Palacios and Zimmerman 2007; Zimmerman et al. 1995; Zimmerman et al. 1997).

Despite the benefits of elevated CO_2 for seagrasses, the extent to which those benefits are realized in an ecosystem setting will partly depend on the outcome of competition with other estuarine autotrophs that may also benefit from such conditions (Young and Gobler 2017). Ephemeral macroalgae, such as *Ulva*, are well-known seagrass competitors (Hauxwell et al. 2001; McGlathery 2001; Valiela et al. 1997) that also benefit from elevated CO_2 and can inhibit other autotrophs such as phytoplankton (Tang and Gobler 2011; Tang et al. 2015; Young and Gobler 2017). Being rooted in sediments, seagrasses are often more light-limited than nutrient-limited (Valiela et al. 1997). In temperate estuaries, seagrasses can persist in oligotrophic estuarine regions due to their ability to acquire nutrients from both the sediments and the water column, as well as their nutrient storage capabilities (Pedersen and Borum 1992; Short and McRoy 1984; Valiela et al. 1997). As nutrient loading increases, macroalgae gain a competitive advantage over seagrass due to higher rates of maximum nutrient uptake (Pedersen and Borum 1997; Valiela et al. 1997). In persistently eutrophic estuaries, ephemeral macroalgae often overgrow and shade-out seagrasses (McGlathery 2001; Valiela et al. 1997), as well as create unfavorable biogeochemical conditions such as anoxia and potentially toxic concentrations of ammonium (NH_4^+) (Hauxwell et al. 2001).

Recent studies have demonstrated that *Ulva rigida*, a dominant macroalgae within Northwest Atlantic coastal waters (Young and Gobler 2016, 2017), and *Zostera marina*, the primary seagrass of the same region, both grow more rapidly when exposed to elevated levels of CO_2 (Palacios and Zimmerman 2007; Young and Gobler 2016, 2017; Zimmerman et al. 1995; Zimmerman et al. 1997). The objective of this study was to assess how elevated CO_2 concentrations influence competition between these autotrophs. Both primary producers were grown with and without elevated levels of pCO_2 as well as with and without the other primary producer. Growth and productivity responses, $\delta^{13}C$ signatures, and elemental composition of the primary producers were evaluated at the start and end of experiments performed throughout the summer months in a Northwest Atlantic estuary.

Methods

Eelgrass and Macroalgae Collection and Preparation

Ulva rigida used for this study was collected from a shallow-water site in Shinnecock Bay, NY, USA (40.85° N, 72.50° W), while *Zostera marina* shoots were collected from eelgrass beds located 5 km east of the macroalgae (Fig. 1; Furman and Peterson 2015). Permission to access and collect the water, *Z. marina*, and *U. rigida* was received from the Southampton Town Trustees, Southampton, NY, USA, who hold jurisdiction over Shinnecock Bay. Large, well-pigmented fronds of *U. rigida* and ~ 20-cm rooted shoots of *Z. marina*

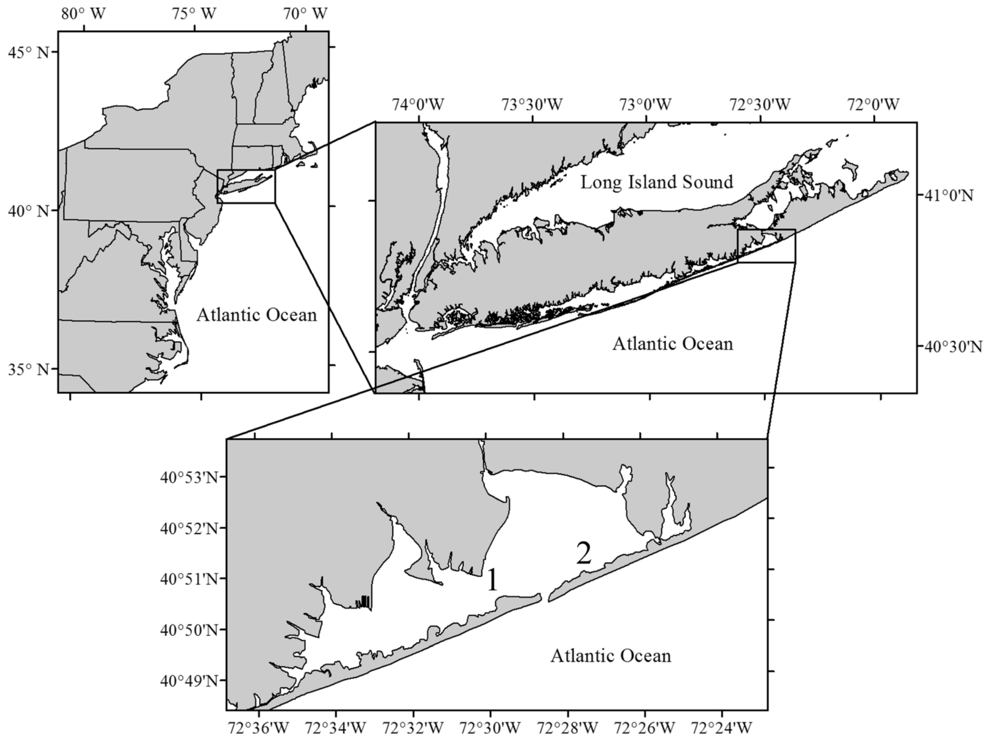

Fig. 1 Map of Shinnecock Bay, NY, USA. All maps were generated using ArcMap 10.4.1 (Esri). Each number on the map denotes a collection site for primary producers used in experiments performed May through September: (1) *Ulva rigida*, (2) *Zostera marina*

were collected and transported to the Stony Brook Southampton Marine Science Center of Stony Brook University in seawater-filled containers within 15 min of collection. Our sequencing efforts and microscopy during this study affirmed that *U. rigida* was the species of *Ulva* present at the macroalgal sampling site (Young and Gobler 2016). We refer to the algae as *Ulva* due to the inconsistent macroalgal taxonomic nomenclature as well as the similarity of sequences of the internal transcribed spacer (ITS) region of the ribosome among *Ulva* species (Hofmann et al. 2010; Kirkendale et al. 2013). For the sake of consistency, *Z. marina* will be referred to as *Zostera*. Individual thalli of *Ulva* approximately 10 cm in length were cut from large thalli with care taken to avoid the potentially reproductive outer region of the organism and placed in a salad spinner to remove debris and epiphytes. Thalli were then extensively rinsed with filtered (0.2 μm) seawater before being spun again to further remove any debris, epiphytes, and excess seawater (Young and Gobler 2016). Additional samples of *Ulva* were cut, cleaned, rinsed, and spun as previously described, and frozen for further analyses (see below). *Ulva* samples were weighed on an A&D EJ300 digital balance (± 0.01 g) to obtain initial wet weight. Similarly, *Zostera* shoots were extensively rinsed with filtered seawater to remove debris and epiphytes. Four individual shoots (~ 20 cm) were placed in cylindrical, sand-filled 20 × 9-cm plastic planters. The sand used to fill the planters was collected from ocean-facing areas south of Shinnecock Bay to ensure low levels of organic carbon in the sand. At the beginning of experiments, an 18-gauge hypodermic needle was used to create a hole in the leaves of the short shoot just above the sheath for quantifying *Zostera* leaf growth (Wall et al. 2008; Zieman 1974).

Assessing the Effects of Elevated pCO_2 and Competition on *Zostera* and *Ulva*

Four experiments were performed to assess the effects of competition and elevated pCO_2 on the growth of *Ulva* and *Zostera* during May, June, July, and September. Polycarbonate containers (20 L) were acid-washed (10% HCl) and liberally rinsed with deionized water before being filled with filtered (0.2 μm) seawater. The containers were placed in outdoor water baths filled with seawater heated or cooled to temperatures consistent with ambient levels (~ 20–25 °C) via the temperature control system at the Stony Brook Southampton Marine Science Center. The containers were exposed to natural light intensity (~ 1000 μmol s^{-1} m^{-2}) and duration, which was quantified via discrete and continuous measurements from a LI-COR LI-1500 light sensor logger and HOBO pendant light loggers, respectively. Light levels were measured just above the sediment surface where the *Zostera* shoots were planted, and did not differ across treatments in any experiment. For all experiments, nine containers were assigned to both ambient (~ 400 μatm) and elevated (~ 2000 μatm) concentrations of CO_2, with the level in the elevated treatment representing both concentrations present within eutrophic estuaries (Baumann et al. 2015; Cai et al. 2017; Melzner et al. 2013; Wallace et al. 2014) as well as levels projected for world oceans in the twenty-second century (Caldeira and Wickett 2003, 2005; Foster et al. 2017). Three sets of containers, in triplicate, were established for experiments: One for only *Zostera*, one for only *Ulva*, and one with both *Zostera* and *Ulva*, resulting in 18 total experimental containers. For each experiment, all containers received nutrient additions (5 μM nitrate, 0.3 μM phosphate) every day for the duration of the experiments to mimic regional nutrient loading rates (~ 4×10^6 kg N year^{-1} for Great South Bay; Kinney and Valiela 2011), mimic levels seasonally present within collection sites (Young and Gobler 2016), and to ensure levels of nitrate were not toxic to *Zostera* (< 7 μM; Burkholder et al. 1992).

All containers were aerated via a 1.5″ × 0.5″ (~ 3.8 × 1.3 cm) air diffuser (Pentair) connected to a length of Tygon tubing that was inserted to the bottom of each container and connected to an air source. A gas proportionator (Cole Parmer® Flowmeter system, multitube frame) was used to mix ambient air with 5% CO_2 gas (Talmage and Gobler 2010) to introduce the control (~ 400 μatm) and elevated (~ 2000 μatm) levels of pCO_2 into the experimental containers. The gas mixtures were delivered at a net flow rate of 2500 ± 5 mL min^{-1} through a nine-way gang valve into the tubing that was placed through a small opening in the closed lid of the container, allowing for the gases to turn over the volume of the containers > 100 times daily (Talmage and Gobler 2010). Bubbling was initiated 2 days prior to the beginning of each experiment to allow CO_2 concentrations and pH to reach a state of equilibrium. Each experiment persisted approximately 2 weeks, a duration consistent with prior studies that observed significant changes in the growth and productivity of *Ulva* and *Zostera*, respectively, in an experimental setting (Dennison and Alberte 1982; Wall et al. 2008; Young and Gobler 2016, 2017). Measurements of pH within containers were made daily using an Orion Star A321 Plus electrode (± 0.001) calibrated before each use with National Institute of Standards and Technology (NIST) traceable standards. Our prior research has found that this instrument provides pH measurements linearly consistent with measurements made spectrophotometrically and with ion-sensitive field-effect transistor-based pH meters (e.g., Durafet by Honeywell). Discrete water samples were collected at the beginning and conclusion of experiments to directly measure dissolved inorganic carbon (DIC; Wallace et al. 2014). The water samples were preserved using a saturated mercuric chloride ($HgCl_2$) solution and stored at 4 °C until analyses. The samples were analyzed by a VINDTA 3D (Versatile Instrument for the Determination of Total inorganic carbon and titration Alkalinity) delivery system coupled with a UIC coulometer (model CM5017O). Levels of pCO_2 (Table 1, Supplementary Tables S1) were calculated using measured levels of DIC, pH (NIST), temperature, and salinity, as well as the first and second dissociation constants of carbonic acid in seawater (Millero 2010) using the program CO2SYS (http://cdiac.ornl.gov/ftp/co2sys/). As a quality assurance measure, levels of DIC and pH of certified reference material (provided by Dr. Andrew Dickson of the University of California, San Diego, Scripps Institution of Oceanography; batch 158 = 2044 μmol DIC kg seawater^{-1}) were measured during analyses of every set of samples. Further analysis of samples continued only after complete recovery (99.8 ± 0.2%) of certified reference material was attained. The delivery of air and CO_2 resulted in actual pCO_2 and pH values of ~ 400 and ~ 8.1 μatm, respectively, for ambient conditions and ~ 2000 and ~ 7.3 μatm, respectively, for the elevated CO_2 conditions, mimicking the range found seasonally in estuarine environments (Baumann et al. 2015; Melzner et al. 2013; Wallace et al. 2014).

Experiments began with the introduction of *Zostera*, *Ulva*, and nutrients into the experimental containers, with discrete and continuous measurements of pH, temperature, and light made throughout the duration of experiments. At the end of experiments, final pH, temperature, and salinity measurements were made and final water samples for DIC analysis were collected and analyzed as described above. After DIC was measured, all *Ulva* samples were removed from their respective treatments, rinsed, spun, re-rinsed, re-spun, weighed as described above, and placed into small freezer bags for further analyses. *Zostera* shoots were removed from their respective treatments, with each leaf cut at the area just above the sheath. The rhizomes were measured for total length, length to the hole in the leaf created by the hypodermic needle (new growth), and width. Each leaf was cut where the hole was, separating the leaf into new and old growth, with all new and old growth from each container being dried to a constant weight at 60 °C for 24 h. For each experiment, seagrass productivity was calculated as areal productivity (cm^2 m^{-2} day^{-1}) and above-ground biomass production (g DW m^{-2} day^{-1}) (Wall et al. 2008; Zieman 1974). Additionally, the number of new leaves produced within each treatment was determined. Weight-based growth rates for *Ulva* were determined using the relative growth formula (growth day^{-1}) = $(\ln W_{final} - \ln W_{initial})/(\Delta t)$, where W_{final} and $W_{initial}$ are the final and initial weights in grams and Δt is the number of days of the experiment. Our prior research has demonstrated that such weight-based growth rates are linearly consistent with areal-based growth of *Ulva* (Young and Gobler 2016, 2017). Significant differences in growth and productivity during each experiment were assessed using three-way ANOVA within SigmaPlot 11.0, where the main treatments were pCO_2 (ambient or elevated), competition (*Zostera* or *Ulva*, alone or in the same container), and time of the experiment.

Table 1 Values of pH (NBS scale), salinity (g kg^{-1}), temperature (°C), pCO_2 (µatm), DIC (µmol $kgSW^{-1}$), HCO_3^- (µmol $kgSW^{-1}$), NO_3^- (µM), PO_4^{3-} (µM), and NH_4^+ (µM) for May through September experiments. Values represent means ± standard error. Data from individual experiments appear within supplementary tables (S1 Tables)

Treatment	pH	Salinity	Temperature	pCO_2	DIC	HCO_3^-	NO_3^-	PO_4^{3-}	NH_4^+
Zostera									
Ambient CO_2	8.17 ± 0.01	31.3 ± 0.2	19.8 ± 0.1	420 ± 40	1550 ± 50	1430 ± 50	1.03 ± 0.07	0.94 ± 0.10	7.07 ± 1.72
Elevated CO_2	7.33 ± 0.01	31.2 ± 0.2	19.8 ± 0.1	2250 ± 120	1570 ± 80	1470 ± 70	1.30 ± 0.10	0.98 ± 0.17	8.57 ± 1.40
Ulva									
Ambient CO_2	8.13 ± 0.01	31.1 ± 0.2	19.6 ± 0.1	450 ± 30	1560 ± 50	1450 ± 40	1.07 ± 0.06	0.89 ± 0.10	6.57 ± 0.35
Elevated CO_2	7.31 ± 0.01	31.1 ± 0.2	19.6 ± 0.1	2290 ± 120	1580 ± 70	1480 ± 70	1.11 ± 0.08	0.86 ± 0.11	6.68 ± 0.69
Zostera/*Ulva*									
Ambient CO_2	8.18 ± 0.01	31.4 ± 0.2	19.8 ± 0.1	440 ± 20	1550 ± 50	1440 ± 40	1.08 ± 0.07	1.22 ± 0.16	5.32 ± 0.64
Elevated CO_2	7.35 ± 0.01	31.2 ± 0.2	19.7 ± 0.1	2210 ± 110	1560 ± 80	1470 ± 80	1.07 ± 0.06	0.79 ± 0.10	7.92 ± 1.86

Above- and Below-Ground Tissue Analyses

For carbon (C), nitrogen (N), and stable carbon isotope ($\delta^{13}C$) analyses, frozen samples of *Zostera* and *Ulva* were dried at 60 °C for 48 h and then homogenized into a fine powder using a mortar and pestle. Total tissue C, N, and $\delta^{13}C$ were analyzed using an elemental analyzer interfaced to a Europa 20-20 isotope ratio mass spectrometer at the UC Davis Stable Isotope Facility (Young and Gobler 2016). Three-way ANOVA within SigmaPlot 11.0 was used to assess significant differences in above-ground tissue (leaf) content for *Zostera* and *Ulva* for each experiment where the main treatment effects were pCO_2 (ambient or elevated), the presence of *Ulva* when assessing *Zostera* or the presence of *Zostera* when assessing *Ulva*, and the time of the experiment.

The below-ground biomass production of *Zostera* rhizomes was determined by cutting the rhizome away from the meristem, liberally rinsing the rhizome with fresh water to remove sand and other debris, and carefully removing the roots. A fixed length (2 cm) was cut from each rhizome starting from where the rhizome was cut away from the meristem. The rhizomes and roots were then dried at 60 °C for 72 h and then weighed using a Mettler Toledo AB304-S/FACT analytical balance (± 0.0001). Below-ground production was calculated by dividing the dry weight by the total area of all rhizomes within each replicate per day (g cm^{-2} day^{-1}). After being weighed, the rhizomes were homogenized into a fine powder using a mortar and pestle. Tissue C and N content of the homogenized samples were analyzed using a CE Instruments Flash EA 1112 elemental analyzer (Sharp 1974). Three-way ANOVA within SigmaPlot 11.0 were used to assess significant differences in below-ground production and tissue content of *Zostera* rhizomes during experiments, where the main treatment effects were pCO_2 (ambient or elevated), the presence of *Ulva*, and the time of the experiment.

Isotopic mixing models were used to estimate the use of CO_2 and HCO_3^- diluted by the introduction of the isotopically lighter 5% CO_2 gas (Young and Gobler 2016). The model considered the $\delta^{13}C$ and biomass of the tissue of *Ulva* and *Zostera* before and after experiments, the $\delta^{13}C$ of the 5% CO_2 gas used for the experiments (− 80‰), the $\delta^{13}C$ of the marine CO_2 and HCO_3^- pool (− 10 and 0‰, respectively; Maberly et al. 1992; Mook et al. 1974; Raven et al. 2002), C fractionation during the uptake of CO_2 and HCO_3^- by *Ulva* and *Zostera*, which was found to be highly similar between both species and of a magnitude that did not significantly alter the results of the mixing models (− 20 and − 10‰ for both species, relative to pool, respectively; Hemminga and Mateo 1996; Maberly et al. 1992; Mook et al. 1974; Raven et al. 2002), C fractionation that occurs during the conversion of the tanked 5% CO_2 gas bubbled within the experimental containers to HCO_3^- (+ 10‰; Maberly et al. 1992; Mook et al. 1974; Raven et al. 2002), and the DIC concentration with and without exposure to the 5% CO_2 gas. The $\delta^{13}C$ of the tanked gas was determined by syringe injection into a split/splitless inlet of a continuous flow gas chromatography combustion isotope ratio mass spectrometry (GC/C/IRMS; Young and Gobler 2016). Determination of the DIC concentration with and without the addition of the 5% CO_2 gas indicated the contribution of the gas to the DIC concentration compared to ambient air. The model assumed that the 5% CO_2 gas reached equilibrium with the total DIC pool, which was highly likely given the high turnover rate of seawater within the experimental containers by the bubbled CO_2 mixture (> 100 times daily). It was further assumed that the tissue grown during the experiments took on a $\delta^{13}C$ signature that was reflective of the DIC pool (Young and Gobler

2016). Lastly, separate calculations of the same mixing model were performed for *Ulva* and *Zostera*. The following equation was used as the hypothetical mixing model to estimate the $\delta^{13}C$ signature of *Ulva* and *Zostera* in the high CO_2 treatments had they grown exclusively using HCO_3^- or CO_2, respectively:

$$\text{Final } \delta^{13}\text{C} = \left(\text{Initial } \delta^{13}\text{C}*\left(\frac{\text{Initial DW}}{\text{Final DW}}\right)\right)+$$

$$\left(\left(\left(\text{DIC } \delta^{13}\text{C}*\frac{\text{Ambient [DIC]}}{\text{Elevated [DIC]}}\right)+\left(\text{Tank } \delta^{13}\text{C}*\left(1-\frac{\text{Ambient [DIC]}}{\text{Elevated [DIC]}}\right)\right)-\text{C fractionation}\right)*\frac{\text{Final DW}-\text{Initial DW}}{\text{Final DW}}\right)$$

where initial $\delta^{13}C$ is the $\delta^{13}C$ signature of the *Ulva* or *Zostera* at the start of experiments, ambient and elevated [DIC] are the concentrations of total dissolved inorganic carbon within ambient and elevated CO_2 treatments, respectively, DIC and tank $\delta^{13}C$ are the $\delta^{13}C$ signatures found within the DIC pool for HCO_3^- (0‰) and CO_2 (− 10‰), and the tanked 5% CO_2 (− 80‰; measured via GC/C/IRMS), respectively, C fractionation is the biological fractionation by the autotrophs during the uptake of CO_2 (− 20‰) or HCO_3^- (− 10‰), and initial and final DW denotes dry tissue weights of the autotrophs at the beginning and end of the experiments, respectively. For *Zostera*, initial dry weight was the dry weights of "old" growth, while final dry weight was the sum of "old" and "new" growth (see above). For *Ulva*, initial dry weight was determined by obtaining the dry weight of the additional *Ulva* samples created at the beginning of experiments (see above), while final dry weight was the dry weights of the final samples. A one-way ANOVA was used to assess the differences between actual $\delta^{13}C$ signatures of *Ulva* and *Zostera*, and signatures calculated based on the exclusive use of HCO_3^- or CO_2, with Tukey tests used to assess the differences between the individual groups.

Sediment and Dissolved Nutrient Analyses

The organic C content of sediments from the planters that held the *Zostera* shoots from each container at the end of experiments was analyzed. A small quantity of sediment (6–8 g) was removed, dried at 60 °C for 72 h, weighed, and combusted at 450 °C for 4 h, weighed again, and compared to the original dry weights to estimate the amount of organic C in the sediments.

To determine concentrations of nitrate (NO_3^-), phosphate (PO_4^{3-}), and ammonium (NH_4^+) within experimental vessels, 20 mL of seawater was removed from each container and filtered by passing the seawater through pre-combusted (4 h at 450 °C) glass fiber filters (GF/F, 0.7 μm pore size). The filtrate was frozen in acid-washed scintillation vials for later analysis. The filtrate was analyzed colorimetrically for NO_3^-, PO_4^{3-}, and NH_3 by a QuikChem 8500 (Lachat Instruments) flow injection analysis system using methods for analysis of the nutrients highlighted by Parsons et al. (1984). Nutrients were measured at the beginning and at the end of the experiment. The average concentrations of NO_3^-, PO_4^{3-}, and NH_4^+ at the end of experiments were 1.11 ± 0.03 μM, 0.94 ± 0.05 μM, and 6.94 ± 0.49 μM, respectively. Nutrient concentrations across all experiments and treatments are reported in Table 1 and Supplementary Tables S1.

Results

Zostera

Areal productivity, above-ground production, and below-ground production of *Zostera* were all highly sensitive to changes in pCO_2 concentrations and varied seasonally (three-way ANOVA; $p < 0.05$ for time and CO_2; Fig. 2; Supplementary Tables S2). Areal productivity under elevated CO_2 was 31% higher than under ambient CO_2. There was, however, an antagonistic interaction between elevated CO_2 and the presence of *Ulva*, where despite the increased areal productivity under elevated CO_2, the presence of *Ulva* suppressed productivity by 22% (three-way ANOVA; $p < 0.05$; Fig. 2a; Supplementary Tables S2). Elevated CO_2 significantly increased the average above-ground production of *Zostera* by 33% compared to ambient CO_2 treatments (three-way ANOVA; $p < 0.05$; Fig. 2b; Supplementary Tables S2). There was an antagonistic interaction between elevated CO_2 and the presence of *Ulva*, in which above-ground production, under elevated CO_2, was significantly reduced by competition with *Ulva* ($p < 0.05$; Fig. 2b). Lastly, the below-ground production of *Zostera* was significantly higher (35%) when exposed to elevated CO_2 concentrations (Three-way ANOVA; $p < 0.05$; Fig. 2c; Supplementary Tables S2). In a manner similar to areal productivity and above-ground production, the below-ground production of *Zostera*, despite being

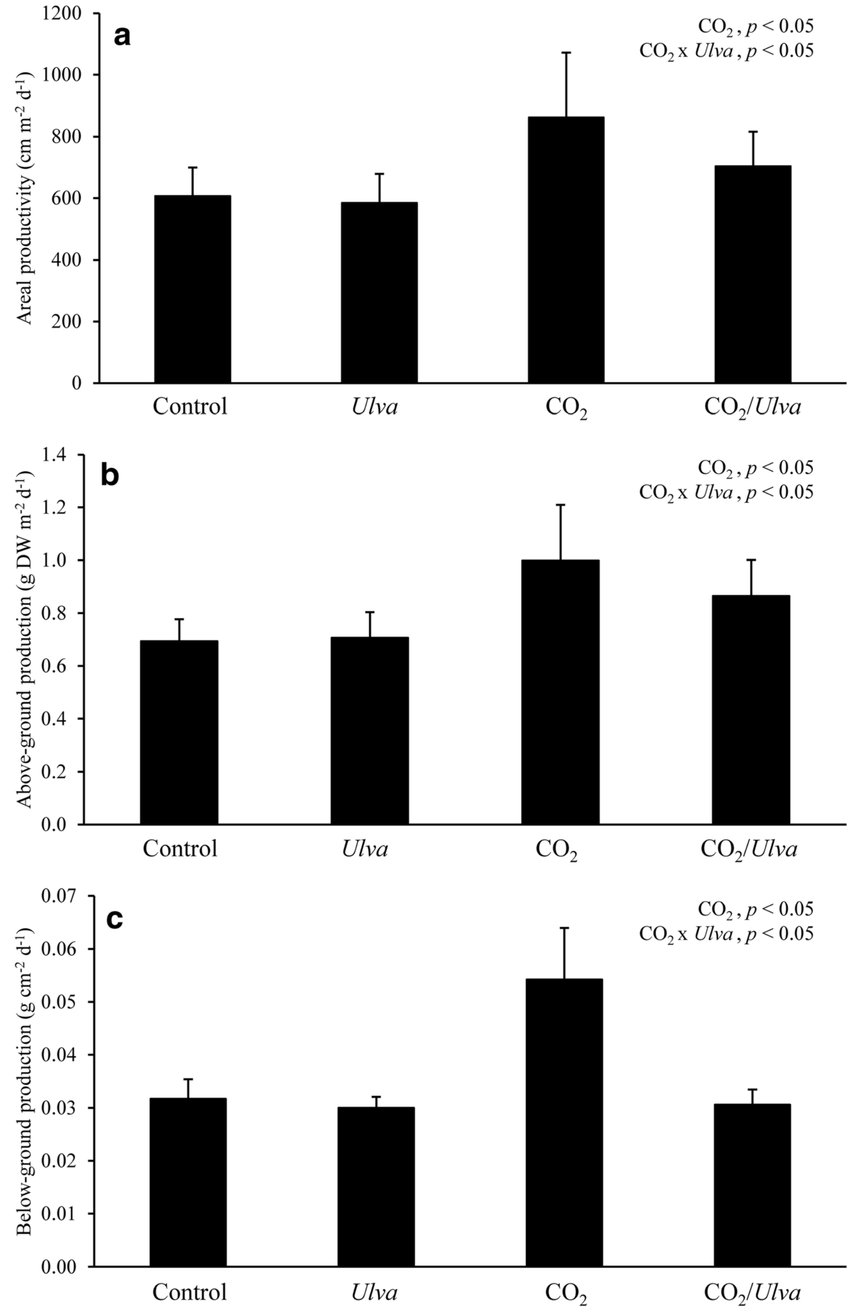

Fig. 2 **a** Areal productivity, **b** above-ground biomass production, and **c** below-ground biomass production of *Zostera* exposed to ambient and elevated CO_2 concentrations, with and without competition from *Ulva* for experiments performed May through September. Growth measurements were taken at the end of experiments. Columns represent means ± standard error. Significant main treatment effects (CO_2 and *Ulva*) appear on the top right of each figure

significantly higher when exposed to elevated CO_2, was reduced by 20% when competing with *Ulva*, demonstrating an antagonistic interaction between elevated CO_2 and competition with *Ulva* ($p < 0.05$; Fig. 2c; Supplementary Tables S2).

The $\delta^{13}C$ content of *Zostera* varied throughout the summer and was significantly reduced by elevated CO_2, with the average $\delta^{13}C$ of the ambient and elevated CO_2 treatments being about − 8 and − 19‰, respectively (three-way ANOVA; $p <$ 0.05 for time and CO_2; Fig. 3; Supplementary Tables S2–S3). The presence of *Ulva* had no significant effect on the $\delta^{13}C$ content of *Zostera* (three-way ANOVA; $p > 0.05$; Fig. 3a; Supplementary Tables S2-S3). Isotopic mixing models demonstrated that *Zostera*, when exposed to elevated CO_2 concentrations, had $\delta^{13}C$ signatures (− 18.3‰) that were significantly lower than values expected if C was obtained exclusively from the use of HCO_3^- (− 13.8‰; Tukey test; $p <$ 0.001; Supplementary Fig. S1 and Tables S2), but significantly higher than expected from the exclusive use of CO_2 (− 21.9‰; Tukey test; $p < 0.001$; Supplementary Fig. S1 and Tables S2).

Above-ground tissue (leaf) C of *Zostera* varied seasonally and was significantly higher when exposed to elevated CO_2 concentrations (three-way ANOVA; $p < 0.05$ for time and CO_2; Fig. 4a; Supplementary Tables S2 and S4) but was

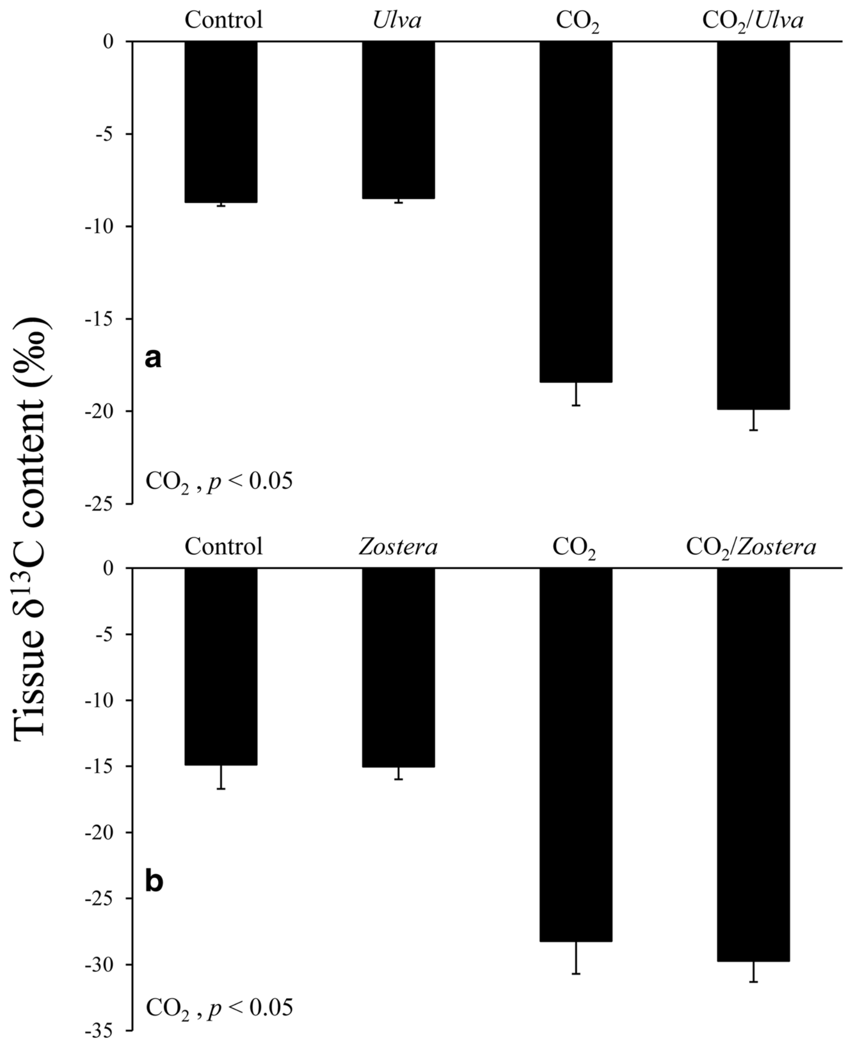

Fig. 3 $\delta^{13}C$ content of **a** *Zostera* and **b** *Ulva* exposed to ambient and elevated CO_2 concentrations, with and without competition from the other primary producer for experiments performed May through September. Measurements were taken at the end of experiments. Columns represent means ± standard error. Significant main treatment effects (CO_2 and *Ulva*) appear on the bottom left of each figure

unaffected by the presence of *Ulva* ($p > 0.05$). Leaf N was significantly lower in the presence of *Ulva* and varied with time (three-way ANOVA; $p < 0.05$ for time and *Ulva*; Fig. 4b; Supplementary Tables S2 and S4) but was unaffected by exposure to elevated CO_2 concentrations ($p > 0.05$). The leaf C:N ratio of *Zostera* was significantly higher when exposed to elevated CO_2 concentrations and changed seasonally (three-way ANOVA; $p < 0.05$ for time and CO_2; Fig. 4c; Supplementary Tables S2 and S4), but unaffected by the presence of *Ulva* ($p > 0.05$).

Below-ground tissue (rhizome) C was significantly higher when exposed to elevated CO_2 concentrations compared to ambient concentrations and changed over the summer (three-way ANOVA; $p < 0.05$ for CO_2 and time; Fig. 5a; Supplementary Tables S2 and S4) while the presence of *Ulva* had no effect ($p > 0.05$). The rhizome N content of *Zostera* was significantly lower in the presence of *Ulva* and changed through the summer (three-way ANOVA; $p < 0.05$ for *Ulva* and time; Fig. 5b; Supplementary Tables S2 and S4) but was unaffected by exposure to elevated CO_2 concentrations ($p > 0.05$). Overall, the rhizome C:N was not significantly affected when exposed to elevated CO_2 concentrations or in the presence of *Ulva* (three-way ANOVA; $p > 0.05$ for all; Fig. 5c; Supplementary Tables S2 and S4). The organic C content of sediments with *Zostera* shoots was significantly higher under elevated CO_2 levels relative to ambient levels (three-way ANOVA; $p < 0.05$; Fig. 5d; Supplementary Tables S2 and S4). Organic C content of the sediments was not significantly affected by the presence of *Ulva* or time (three-way ANOVA; $p > 0.05$).

Ulva

The growth of *Ulva* was highly sensitive to changes in CO_2 concentrations and differed by season (three-way ANOVA; $p < 0.05$ for CO_2 and time; Fig. 6; Supplementary Tables S2). Under elevated CO_2 concentrations, growth was three-to-four times higher relative to growth under ambient concentrations (Fig. 6). Overall, *Ulva* growth was unaffected by the presence of *Zostera* (three-way ANOVA; $p > 0.05$; Fig. 6;

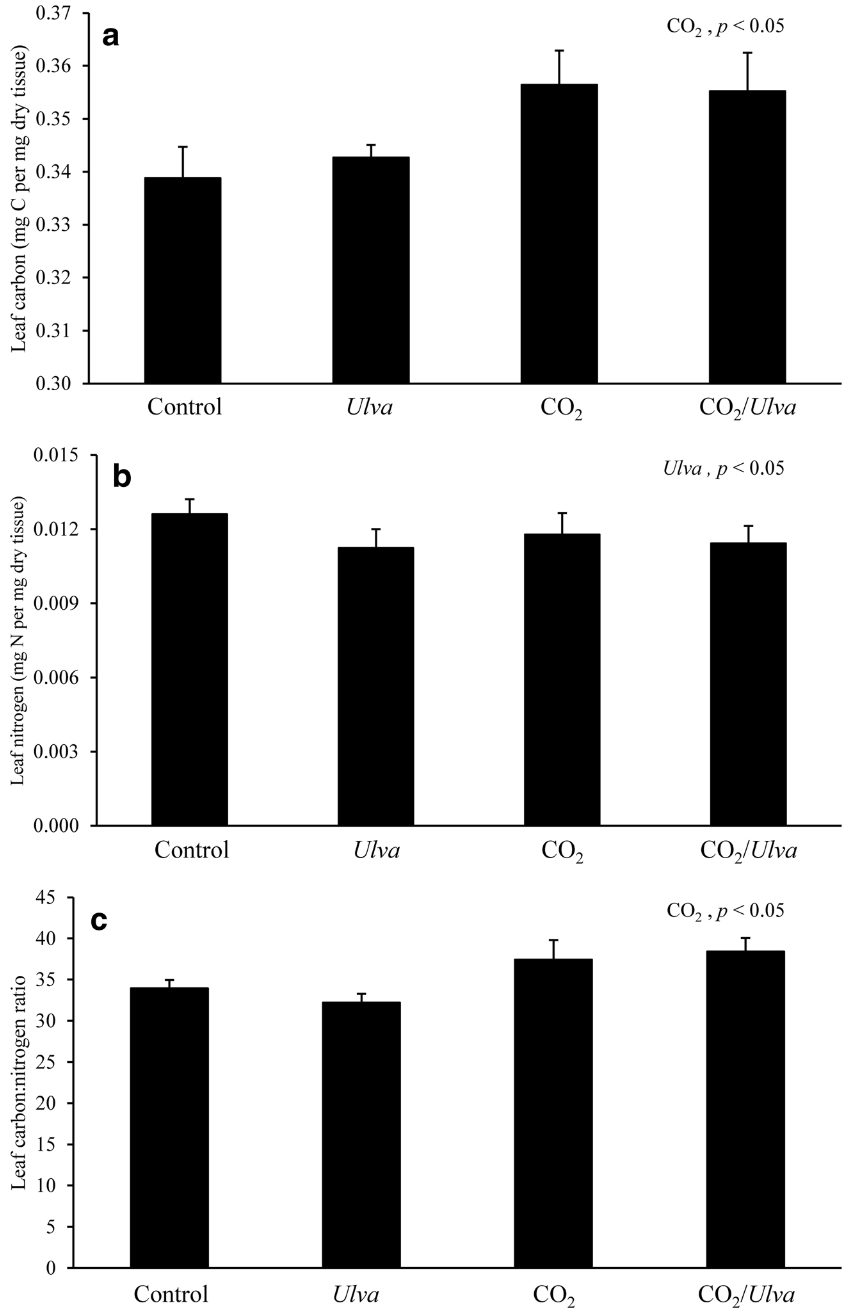

Fig. 4 **a** Leaf C, **b** leaf N, and **c** leaf C:N content of *Zostera* exposed to ambient and elevated CO_2 concentrations, with and without competition from *Ulva* for experiments performed May through September. Measurements were made at the end of experiments. Columns represent means ± standard error. Significant main treatment effects (CO_2 and *Ulva*) appear on the top right of each figure

Supplementary Tables S2). The $\delta^{13}C$ content of *Ulva* was significantly lower when exposed to elevated CO_2 concentrations, with the average $\delta^{13}C$ of ambient and elevated CO_2 treatments being approximately − 15 and − 28‰, respectively (three-way ANOVA; $p < 0.001$; Fig. 3b; Supplementary Tables S2-S3), while the presence of *Zostera* had no effect on the $\delta^{13}C$ content of *Ulva* ($p > 0.05$). Isotopic mixing models demonstrated that when exposed to elevated CO_2 concentrations *Ulva* yielded $\delta^{13}C$ signatures (− 28‰) that were significantly lower than values expected if C was obtained exclusively from the use of HCO_3^- (− 17.9‰; Tukey test; $p < 0.001$; Supplementary Fig. S1 and Tables S2), but not significantly different than expected from the exclusive use of CO_2 (− 28.9‰; Tukey test; $p > 0.05$; Supplementary Fig. S1 and Tables S2). Tissue C, N, and C:N of *Ulva* were unaffected by CO_2 concentration or the presence of *Zostera* (three-way ANOVA; $p > 0.05$; Fig. 7; Supplementary Tables S2 and S4).

Discussion

During this study, elevated CO_2 concentrations significantly enhanced the growth of *Ulva* and the areal productivity and

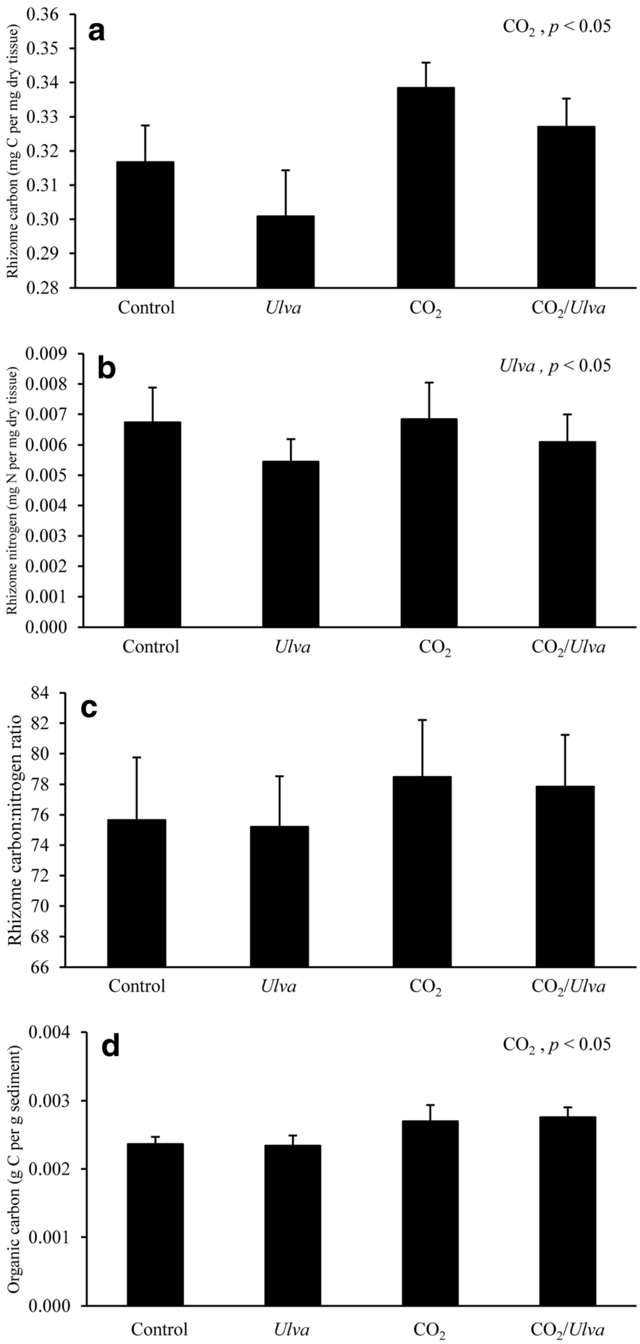

Fig. 5 **a** Rhizome C, **b** rhizome N, **c** rhizome C:N content of *Zostera*, and **d** *Zostera*-containing sediment organic carbon exposed to ambient and elevated CO_2 concentrations, with and without competition from *Ulva* for experiments performed May through September. Measurements were made at the end of experiments. Columns represent means ± standard error. Significant main treatment effects (CO_2 and *Ulva*) appear on the top right of each figure

above- and below-ground biomass production of *Zostera*. For *Zostera*, areal productivity and above- and below-ground production were significantly repressed by the presence of *Ulva*. In contrast, *Ulva* was largely unaffected by *Zostera*. For both primary producers, tissue $\delta^{13}C$ was significantly lowered by elevated CO_2 concentrations. While the tissue C, N, and C:N of *Ulva* was unaffected by the presence of *Zostera* or elevated CO_2 levels, *Zostera* experienced more complex responses. *Zostera* experienced increased leaf C and C:N ratios in response to elevated CO_2, and reduced N content in the presence of *Ulva*. Elevated CO_2 concentrations significantly increased *Zostera* rhizome C, while competition with *Ulva* significantly reduced the rhizome N of *Zostera*. Sediment organic C levels were significantly higher in treatments exposed to elevated CO_2 but were not affected by *Ulva*. Together, these findings provide insight regarding how competition between seagrass and macroalgae may be altered by current and future high CO_2 concentrations.

Time/season was a significant treatment effect for many of the parameters measured for *Zostera* and *Ulva* during this study, an outcome consistent with the time span during which experiments were performed (May through September). Over the course of experiments, temperature and photoperiods differed, two factors that likely drove seasonal changes in growth rates of *Zostera* (Bulthuis 1987; Hauxwell et al. 2006; Zimmerman et al. 1989) and *Ulva* (Henley 1992, 1993; Sand-Jensen 1988). Despite these seasonal changes, responses of *Zostera* and *Ulva* to elevated pCO_2 and competition with each other were markedly consistent.

The physiological response of macroalgae and seagrass to elevated CO_2 concentrations depends on their mode of carbon acquisition as well as if the inorganic carbon uptake of the organism is substrate-saturated at present CO_2 concentrations (Badger 2003; Koch et al. 2013). Prior studies have shown that elevated CO_2 concentrations may cause macroalgae to downregulate their CCMs that convert HCO_3^- to CO_2 which may, in turn, allow more energy to be available for other processes such as vegetative growth (Björk et al. 1993; Cornwall et al. 2012; Koch et al. 2013). Numerous species of seagrass, such as *Z. marina*, have been shown to possess C_3 photosynthetic pathways along with CCMs and external CA to utilize HCO_3^- for photosynthesis (Beer and Rehnberg 1997; Beer and Wetzel 1982; Invers et al. 1999) that may downregulate under high CO_2. The $\delta^{13}C$ signatures of *Ulva* and *Zostera* during this study decreased significantly when exposed to higher CO_2 and isotopic mixing models suggest that these autotrophs switched from primarily HCO_3^- use to primarily CO_2 use and potentially downregulated their CCMs, although further study is needed to definitively affirm this. The precise change in tissue $\delta^{13}C$ at the beginning of experiments (− 9.8 and − 15.7‰ for *Zostera* and *Ulva*, respectively) to the conclusion of experiments (− 18.3 and − 28‰ for *Zostera* and *Ulva*, respectively), indicate a greater reliance on the diffusive uptake of CO_2 (Hepburn et al. 2011; Maberly et al. 1992; Raven et al. 2011). Another possibility is that elevated CO_2 levels may alleviate inorganic C limitation that may occur during photosynthesis, allowing for enhanced growth and productivity. *Ulva rigida* and *U. compressa* (formerly *Enteromorpha*) utilize CCMs, as they

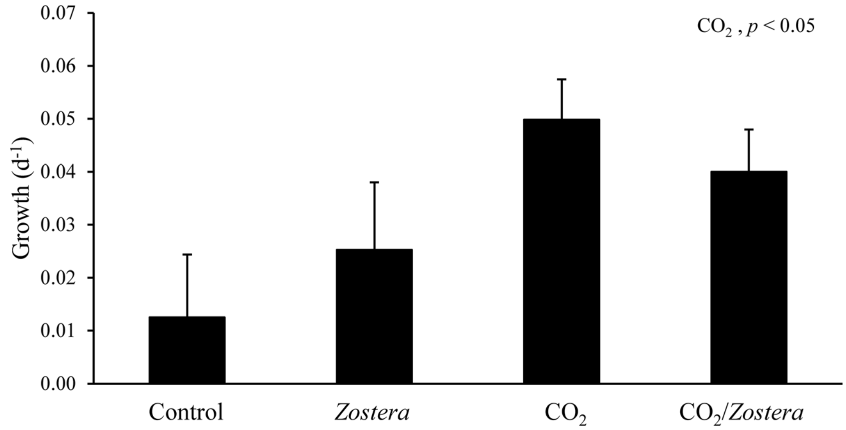

Fig. 6 Growth rates of *Ulva* exposed to ambient and elevated CO_2 concentrations, with and without competition from *Zostera* for experiments performed May through September. Growth measurements were made at the end of experiments. Columns represent means ± standard error. Significant main treatment effects (CO_2 and *Zostera*) appear on the top right of each figure

do not receive enough CO_2 through diffusive uptake alone at present CO_2 concentrations (Mercado et al. 1998). Similarly, for many seagrasses, the diffusive supply of CO_2 to leaves is slow and inefficient, causing them to rely on the active transport of HCO_3^-, along with CCMs and external CA (Beer 1989; Beer and Koch 1996; Koch et al. 2013). Thus, it is plausible that enhanced growth and productivity of *Ulva* and *Zostera* was the result of elevated CO_2 levels alleviating inorganic C limitation.

The benefits of elevated CO_2 levels for the growth of *Ulva* and productivity of *Zostera* in the present study are consistent with prior studies of this algae (Björk et al. 1993; Olischläger et al. 2013; Young and Gobler 2016) and seagrass (Beer and Koch 1996; Palacios and Zimmerman 2007; Zimmerman et al. 1997). Direct competition between these primary producers under high CO_2 has not been previously explored and may, however, offset some of the benefits of elevated CO_2 for *Zostera*. For example, there existed an antagonistic interaction between elevated CO_2 and the presence of *Ulva* for the areal productivity and above- and below-ground production of *Zostera*, whereby the benefit of high CO_2 was partly or largely negated by *Ulva*. The overgrowth of macroalgae can decrease water clarity, shade seagrass, and reduce productivity (Valiela et al. 1997). In this study, *Ulva* outgrew *Zostera* by 21 and 5% under elevated and ambient CO_2 concentrations, respectively, but light levels reaching *Zostera* shoots did not differ across treatments, making light limitation unlikely to have altered *Zostera* productivity in these experiments.

Macroalgae may also directly compete with *Zostera* for nutrients which can limit seagrass growth (Duarte 1995). The decline in leaf and rhizome N and increase in leaf C:N for *Zostera* in the presence of *Ulva* is consistent with the findings of Davis and Fourqurean (2001), suggesting that the rapid use of N by *Ulva* deprived *Zostera* of an adequate N supply. This hypothesis is further supported by significantly lower concentrations of nitrate and ammonium in the presence of *Ulva* (three-way ANOVA; $p < 0.05$; Supplementary Tables S1-S2). In a natural setting, excessive nutrient loading favors the growth of fast-growing, ephemeral macroalgae such as *Ulva* due to the ability to rapidly assimilate and store nitrogenous nutrients (Fan et al. 2014; Liu et al. 2009; Naldi and Wheeler 1999; Pedersen and Borum 1997). In contrast, seagrasses generally dominate more oligotrophic estuaries due to their ability to acquire nutrients through their roots and to store N in their leaves, stems, and rhizomes for use (Pedersen and Borum 1992; Short and McRoy 1984; Valiela et al. 1997).

There was likely increased nutrient competition in the combined *Ulva-Zostera* treatment under high CO_2 given the higher levels of total autotrophic biomass in this treatment and the faster growth rates for both species within this treatment. There were some signs of N-stress in *Zostera* in the combined *Ulva-Zostera* treatment under high CO_2, as productivity and leaf and root N content were significantly reduced. Prior research has indicated that maximum growth of *Zostera* occurs at ammonium and nitrate concentrations of ~2 and ~3–4 µM, respectively (Zimmerman et al. 1987). During experiments, 5 µM of nitrate was added to each container daily and the final concentrations of ammonium in all vessels were consistently above 5 µM, data suggesting that the growth of *Zostera* was not fully N-limited. While it is plausible that the nitrate concentrations were rapidly depleted to < 2 µM due to uptake by *Ulva* each day and that the ammonium concentrations only rose to above 5 µM at the end of experiments, we do not have measurements to support such hypotheses. We emphasize that, in an ecosystem setting, when *Ulva* and *Zostera* compete under high CO_2, nutrient-loading rates will likely not change and thus any nutrient competition that may have emerged within the *Ulva-Zostera* treatment under high CO_2 would be likely to occur in estuaries as well. Nutrient levels added to each experimental vessel here were carefully chosen to be environmentally realistic and consistent with regional N loading rates (Kinney and Valiela 2011) but also to ensure N levels were not toxic (< 7 µM nitrate per day; Burkholder et al. 1992). Future studies with *Ulva* and *Zostera* that examine both changing N levels and changing CO_2 levels will be able to provide a clearer sense of the extent to which *Zostera* inhibition by *Ulva* under high CO_2 is caused by nutrient competition, among other factors.

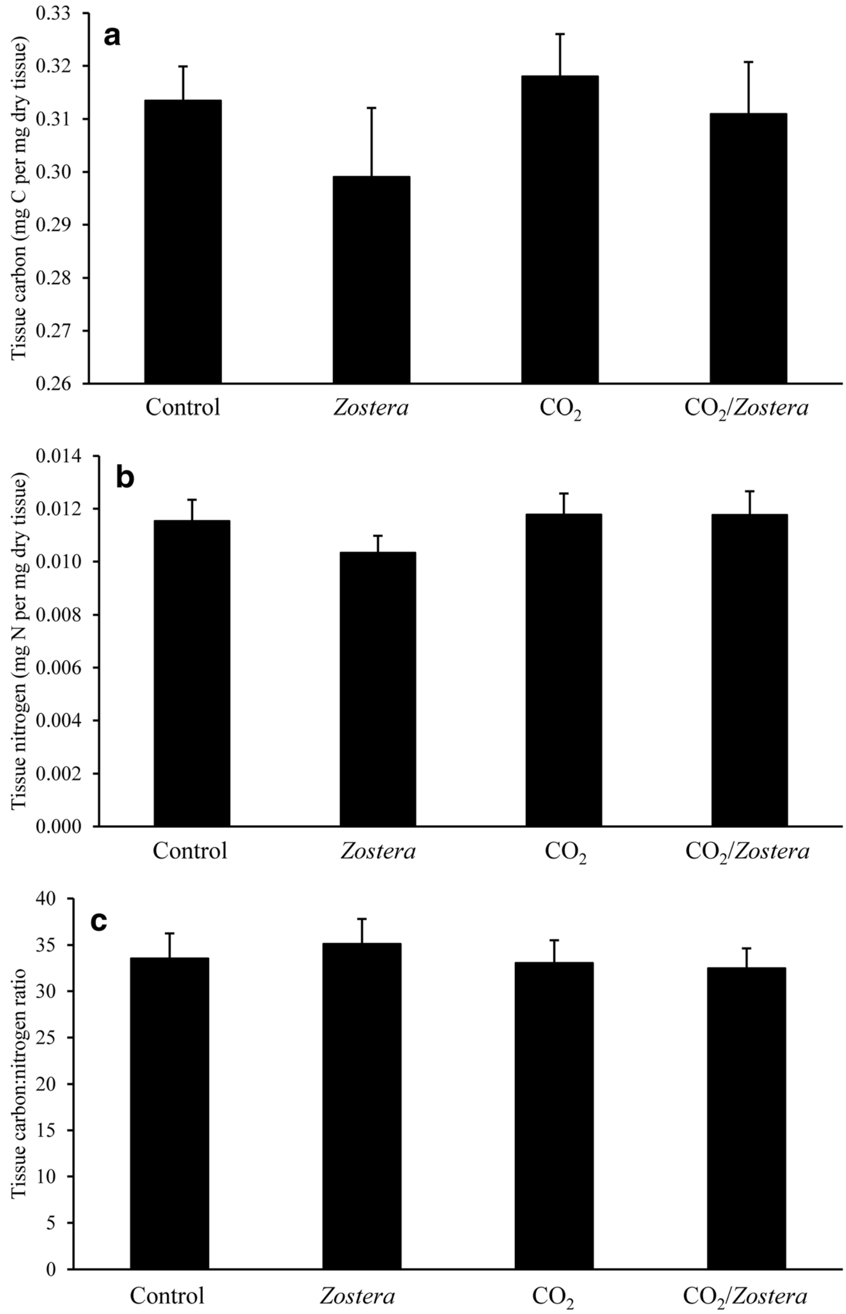

Fig. 7 **a** Tissue C, **b** tissue N, and **c** tissue C:N content of *Ulva* exposed to ambient and elevated CO_2 concentrations, with and without competition from *Zostera* for experiments performed May through September. Measurements were made at the end of experiments. Columns represent means ± standard error

Macroalgae can indirectly inhibit the productivity of seagrass through changes in the biogeochemical environment (Hauxwell et al. 2001). While not measured in the present study, the accumulation of sulfides in sediments as the result of anoxia caused by the decomposition of macroalgal mats can decrease seagrass productivity and cause mortality (Koch et al. 2007). Additionally, as nutrient concentrations increase, the reduced dissolved O_2 concentrations as a result of increased respiratory demands of rapidly growing macroalgae (i.e., *Ulva*) may increase energy costs for seagrass translocating oxygen between the above-ground structures and the roots (Cabello-Pasini et al. 2011; Hauxwell et al. 2001; Pregnall et al. 1984). While excessive NH_4^+ concentrations in the water column can also lower seagrass productivity, NH_4^+ concentrations rarely exceeded 10 μM during the present study, making it unlikely that macroalgae-driven NH_4^+ toxicity played a role in decreased *Zostera* productivity in the presence of *Ulva* (McGlathery et al. 1997; Valiela et al. 1997). Finally, given that *Ulva* spp. have been shown to produce allelochemicals that inhibit the growth of microalgae (Nan et al. 2004; Tang and Gobler 2011), it is plausible that the inhibition of *Zostera* by *Ulva* was mechanistically facilitated via allelopathy, which has been reported in a recent study by Alexandre et al. (2017). Further, given the stronger inhibitory effects of *Ulva* on *Zostera* under elevated pCO_2, it is possible that allelochemical production was strengthened under high pCO_2 perhaps because the active allelochemicals are C-rich compounds (Fajer et al. 1992; Hattenrath-Lehmann et al. 2015).

Consistent with prior studies of macroalgae, the tissue C and N content of *Ulva* was mostly unaffected by changes in CO_2 concentration (Gordillo et al. 2001; Young and Gobler 2016, 2017) nor was it affected by competition with *Zostera*. However, the leaf and rhizome C of *Zostera* was responsive to changes in CO_2 concentration. On average, leaf and rhizome C was significantly increased when exposed to elevated CO_2 concentrations. This increase in leaf and rhizome C content may be due to an increase in non-structural carbohydrates, which is consistent with other studies on the response of seagrasses to elevated CO_2 levels (Jiang et al. 2010; Zimmerman et al. 1995; Zimmerman et al. 1997). Seagrasses, like many terrestrial C_3 plants, can store carbohydrates when supply exceeds demand (Campbell and Fourqurean 2013), and use the carbohydrates for various functions, such as growth, lost tissue replacement, and for defensive compounds (Campbell and Fourqurean 2013; Chapin III et al. 1990; Dawes and Lawrence 1979). In the context of the present study, increased leaf and rhizome C may have been a consequence of increased areal productivity and above- and below-ground production from elevated CO_2 concentrations. These trends also account for the significantly higher leaf C:N was under elevated CO_2 levels. These complex changes in the macroelemental content of *Zostera* when exposed to elevated CO_2 concentrations could have important implications for their palatability to herbivores (Arnold et al. 2012; Stiling and Cornelissen 2007) as many anti-feeding compounds are C-rich compounds (Fajer et al. 1992).

The ~ 20% increase in the organic C of the *Zostera*-bearing sediments when exposed to elevated CO_2 concentrations has not been previously reported but has a series of important implications. The present study, along with prior studies (Jiang et al. 2010; Palacios and Zimmerman 2007), have shown that elevated CO_2 increases the below-ground biomass production of seagrasses as well as rhizome C content when exposed to elevated CO_2 concentrations. Organic C released by seagrass production, as well as the leakage of photosynthates by the rhizomes can influence sediment sulfate reduction, N fixation, and bacterial activity (Hansen et al. 2000; Pollard and Moriarty 1991; Welsh 2000). The accelerated consumption of oxygen by organic carbon-fueled microbial respiration can create an anaerobic environment within sediments which, when coupled with the high concentration of sulfate in seawater, provides a more suitable environment for sulfate-reducing bacteria, which account for more than 50% of organic C fixation in marine sediments (Moriarty et al. 1985; Pollard and Moriarty 1991; Welsh 2000). While sulfate-reducing bacteria play an important role in maintaining a suitable biogeochemical environment in seagrass-inhabited sediments (Pollard and Moriarty 1991), continued accumulation of sulfides in the sediments can harm seagrasses. Holmer et al. (2005) found that sulfides intruding into the below-ground structures of *Z. marina* can be re-oxidized into elemental sulfur which, after continued accumulation, can degrade seagrass meristems and cause mortality. Additionally, Goodman et al. (1995) found that increased sulfides in sediments containing *Z. marina* significantly reduced P_{max}, increased the light requirement for photosynthesis to equal respiration, and decreased the initial slope of the PI curve. Hence, elevated CO_2 concentrations that increase below-ground production significantly increase sediment organic C, which could ultimately stunt the growth and photosynthetic abilities of seagrass meadows. The relative effect of excess organic matter production within sediments on seagrass exposed to elevated CO_2 will likely be influenced by the initial sediment composition as well as the amount of oxygen transported to seagrass rhizomes that could offset some of the negative impacts. Regardless, it is possible that prior studies that have assessed the effects of elevated CO_2 concentrations on *Zostera*, but not sediments, may have overestimated the long-term benefits by not considering changes to sediment biogeochemistry.

The overgrowth of seagrass beds by macroalgae in high CO_2 settings can have a variety of ecosystem-wide consequences. Temperate seagrass meadows have high species richness, host a high abundance of invertebrate species, are used as nurseries by numerous species of juvenile shellfish (Heck Jr. et al. 1995) and crustaceans (Heck and Thoman 1984; Perkins-Visser et al. 1996), and often serve as a habitat for demersal fish to brood or produce eggs (Blanc and Daguzan 1998; Francour 1997). The overgrowth of macroalgae associated with rising levels of CO_2 can decrease seagrass shoot density, recruitment, and growth (Hauxwell et al. 2001) as well as result in shifts in trophic interactions, including the loss of invertebrates and fish that rely on seagrass for food, cover, and as nurseries (McGlathery 2001). Secondary metabolites released by *Ulva* can also directly cause mortality in some invertebrates (Johnson and Welsh 1985; Magre 1974; Nelson et al. 2003) and larval fish (Johnson 1980). The extent to which the synthesis of such compounds may be altered via exposure to excessive CO_2 has yet to be determined.

In conclusion, while both *Ulva* and *Zostera* may experience growth benefits when exposed to high, but realistic, levels of pCO_2, such benefits for *Zostera* may be offset by *Ulva* both directly (shading: Valiela et al. 1997; competition for N: Duarte 1995; allelopathy: Alexandre et al. 2017) and indirectly (changing the biogeochemical environment: Hauxwell et al. 2001). Elevated CO_2 increased below-ground production and rhizome C content of *Zostera*, but such increased below-ground production may cause long-term harm for *Zostera* as increases in sediment organic C may promote sulfide toxicity. Finally, despite the benefits that *Zostera* gains from elevated CO_2, rapidly growing macroalgae such as *Ulva* have a growth advantage in eutrophic estuaries and, as the results of the present study suggest, may lower the productivity of *Zostera*. To date, shifts in the dominance of macroalgae over seagrasses in estuaries have been primarily attributed to nutrient overloading and light limitation. This study demonstrates that in estuaries where *Ulva* and *Zostera* co-exist and compete, climate change and eutrophication-driven increases in pCO_2 are likely to be important in promoting the dominance of *Ulva* over *Zostera*.

Acknowledgements We gratefully acknowledge the assistance of Jennifer Goleski during the analysis of dissolved nutrients during the study, and of members of Bradley Peterson's laboratory in the retrieval of the *Zostera* shoots. We thank David Hirschberg of the School of Marine and Atmospheric Sciences (Stony Brook) for the elemental composition analysis of rhizomes. We are appreciative of the logistical support provided by the Stony Brook Southampton Marine Science Center staff throughout this study.

Funding information Funding for these experiments was obtained from the New York Sea Grant (award #R-FMB-38), the Laurie Landeau Foundation, the Simmons Foundation, and the Chicago Community Trust.

References

Alexandre, A., A. Baeta, A.H. Engelen, and R. Santos. 2017. Interactions between seagrasses and seaweeds during surge nitrogen acquisition determine interspecific competition. *Scientific Reports* 7 (1): 13651.

Arnold, T., C. Mealy, H. Leahey, A.W. Miller, J.M. Hall-Spencer, M. Milazzo, and K. Maers. 2012. Ocean acidification and the loss of phenolic substances in marine plants. *PLoS One* 7 (4): e35107.

Badger, M. 2003. The role of carbonic anhydrases in photosynthetic CO_2 concentrating mechanisms. *Photosynthesis Research* 77: 83–94.

Baumann, H., R.B. Wallace, T. Tagliaferri, and C.J. Gobler. 2015. Large natural pH, CO_2 and O_2 fluctuations in a temperate tidal salt marsh on diel, seasonal, and interannual time scales. *Estuaries and Coasts* 38 (1): 220–231.

Beer, S. 1989. Photosynthesis and photorespiration of marine angiosperms. *Aquatic Botany* 34: 153–166.

Beer, S., and E. Koch. 1996. Photosynthesis of marine macroalgae and seagrasses in globally changing CO_2 environments. *Marine Ecology Progress Series* 141: 199–204.

Beer, S., and J. Rehnberg. 1997. The acquisition of inorganic carbon by the seagrass *Zostera marina*. *Aquatic Botany* 56: 277–283.

Beer, S., and R.G. Wetzel. 1982. Photosynthetic carbon fixation pathways in *Zostera marina* and three Florida seagrasses. *Aquatic Botany* 13: 141–146.

Björk, M., K. Haglund, Z. Ramazanov, and M. Pedersén. 1993. Inducible mechanisms for HCO_3^- utilization and repression of photorespiration in protoplasts and thalli of three species of *Ulva* (Chlorophyta). *Journal of Phycology* 29: 166–173.

Blanc, A., and J. Daguzan. 1998. Artificial surfaces for cuttlefish eggs (*Sepia officinalis* L.) in Morbihan Bay, France. *Fisheries Research* 38 (3): 225–231.

Bulthuis, D.A. 1987. Effects of temperature on photosynthesis and growth of seagrasses. *Aquatic Botany* 27 (1): 27–40.

Burkholder, J.M., K.M. Mason, and H.B. Glasgow Jr. 1992. Water-column nitrate enrichment promotes decline of eelgrass *Zostera marina*: Evidence from seasonal mesocosm experiments. *Marine Ecology Progress Series* 81: 163–178.

Cabello-Pasini, A., V. Macías-Carranza, R. Abdala, N. Korbee, and F.L. Figueroa. 2011. Effect of nitrate concentration and UVR on photosynthesis, respiration, nitrate reductase activity, and phenolic compounds in *Ulva rigida* (Chlorophyta). *Journal of Applied Phycology* 23: 363–369.

Cai, W.-J., X. Hu, W.-J. Huang, M.C. Murrell, J.C. Lehrter, S.E. Lohrenz, W.-C. Chou, et al. 2011. Acidification of subsurface coastal waters enhanced by eutrophication. *Nature Geoscience* 4: 766–770.

Cai, W.-J., W.-J. Huang, G.W. Luther, D. Pierrot, M. Li, J. Testa, M. Xue, et al. 2017. Redox reactions and weak buffering capacity lead to acidification in the Chesapeake Bay. *Nature Communications* 8 (1): 369.

Caldeira, K., and M.E. Wickett. 2003. Oceanography: Anthropogenic carbon and ocean pH. *Nature* 425: 365.

Caldeira, K., and M. E. Wickett. 2005. Ocean model predictions of chemistry changes from carbon dioxide emissions to the atmosphere and ocean. *Journal of Geophysical Research: Oceans* 110:C09S04.

Campbell, J.E., and J.W. Fourqurean. 2013. Effects of in situ CO_2 enrichment on the structural and chemical characteristics of the seagrass *Thalassia testudinum*. *Marine Biology* 160: 1465–1475.

Chapin, F.S., III, E.-D. Schulze, and H.A. Mooney. 1990. The ecology and economics of storage in plants. *Annual Review of Ecology and Systematics* 21: 423–447.

Cornwall, C.E., C.D. Hepburn, D. Pritchard, K.I. Currie, C.M. McGraw, K.A. Hunter, and C.L. Hurd. 2012. Carbon-use strategies in macroalgae: Differential responses to lowered pH and implications for ocean acidification. *Journal of Phycology* 48 (1): 137–144.

Davis, B.C., and J.W. Fourqurean. 2001. Competition between the tropical alga, *Halimeda incrassat*, and the seagrass, *Thalassia testudinum*. *Aquatic Botany* 71: 217–232.

Dawes, C.J., and J.M. Lawrence. 1979. Effects of blade removal on the proximate composition of the rhizome of the seagrass *Thalassia testudinum* banks ex könig. *Aquatic Botany* 7: 255–266.

Dennison, W.C., and R.S. Alberte. 1982. Photosynthetic responses of *Zostera marina* L. (eelgrass) to in situ manipulations of light intensity. *Oecologia* 55: 137–144.

Duarte, C.M. 1995. Submerged aquatic vegetation in relation to different nutrient regimes. *Ophelia* 41 (1): 87–112.

Durako, M.J. 1993. Photosynthetic utilization of CO_2 (aq) and HCO_3 in *Thalassia testudinium* (Hydrocharitacae). *Marine Biology* 115 (3): 373–380.

Fajer, E.D., M.D. Bowers, and F.A. Bazzaz. 1992. The effect of nutrients and enriched CO_2 environments on production of carbon-based allelochemicals in Plantago: A test of the carbon/nutrient balance hypothesis. *The American Naturalist* 140 (4): 707–723.

Fan, X., D. Xu, Y. Wang, X. Zhang, S. Cao, S. Mou, and N. Ye. 2014. The effect of nutrient concentrations, nutrient ratios and temperature on photosynthesis and nutrient uptake by *Ulva prolifera*: Implications for the explosion in green tides. *Journal of Applied Phycology* 26: 537–544.

Foster, G.L., D.L. Royer, and D.J. Lunt. 2017. Future climate forcing potentially without precedent in the last 420 million years. *Nature Communications* 8: 14845.

Francour, P. 1997. Fish assemblages of *Posidonia oceanica* beds at port-Cros (France, NW Mediterranean): Assessment of composition and long-term fluctuations by visual census. *Marine Ecology* 18 (2): 157–173.

Furman, B.T., and B.J. Peterson. 2015. Sexual recruitment in *Zostera marina*: Progress toward a predictive model. *PLoS One* 10 (9): e0138206.

Gao, K., and K.R. McKinley. 1994. Use of macroalgae for marine biomass production and CO_2 remediation: A review. *Journal of Applied Phycology* 6: 45–60.

Gazeau, F., C. Quiblier, J.M. Jansen, J.-P. Gattuso, J.J. Middelburg, and C.H.R. Heip. 2007. Impact of elevated CO_2 on shellfish calcification. *Geophysical Research Letters* 34: L07603.

Goodman, J.L., K.A. Moore, and W.C. Dennison. 1995. Photosynthetic responses of eelgrass (*Zostera marina*) to light and sediment sulfide in a shallow barrier island lagoon. *Aquatic Botany* 50: 37–47.

Gordillo, F.J.L., F.X. Niella, and F.L. Figueroa. 2001. Non-photosynthetic enhancement of growth by high CO_2 level in the nitrophilic seaweed *Ulva rigida* C. Agardh (Chlorophyta). *Planta* 213: 64–70.

Hansen, J.W., J.W. Udy, C.J. Perry, W.C. Dennison, and B.A. Lomstein. 2000. Effect of the seagrass *Zostera capricorni* on sediment microbial processes. *Marine Ecology Progress Series* 199: 83–96.

Hattenrath-Lehmann, T.K., J.L. Smith, R.B. Wallace, L.R. Merlo, F. Koch, H. Mittelsdorf, J.A. Goleski, D.M. Anderson, and C.J. Gobler. 2015. The effects of elevated CO_2 on the growth and toxicity of field populations and cultures of the saxitoxin-producing dinoflagellate, *Alexandrium fundyense*. *Limnology and Oceanography* 60: 198–214.

Hauxwell, J., J. Cebrian, C. Furlong, and I. Valiela. 2001. Macroalgal canopies contribute to eelgrass (*Zostera marina*) decline in temperate estuarine ecosystems. *Ecology* 82 (4): 1007–1022.

Hauxwell, J., J. Cebrian, and I. Valiela. 2006. Light dependence of *Zostera marina* annual growth dynamics in estuaries subject to different degrees of eutrophication. *Aquatic Botany* 84 (1): 17–25.

Heck, K.L., Jr., K.W. Able, C.T. Roman, and M.P. Fahay. 1995. Composition, abundance, biomass, and production of macrofauna in a New England estuary: Comparisons among eelgrass meadows and other nursery habitats. *Estuaries* 18 (2): 379–389.

Heck, K.L., and T.A. Thoman. 1984. The nursery role of seagrass meadows in the upper and lower reaches of the Chesapeake Bay. *Coastal and Estuarine Research Federation* 7 (1): 70–92.

Hemminga, M. A., and M. A. Mateo. 1996. 3. Marine Ecology Progress Series 140:285–298.

Henley, W.J. 1992. Growth and photosynthesis of *Ulva rotundata* (Chlorophyta) as a function of temperature and square wave irradiance in indoor culture. *Journal of Phycology* 28 (5): 625–634.

Henley, W.J. 1993. Measurement and interpretation of photosynthetic light-response curves in algae in the context of photoinhibition and diel changes. *Journal of Phycology* 29 (6): 729–739.

Hepburn, C.D., D.W. Pritchard, C.E. Cornwall, R.J. McLeod, J. Beardall, and J.A. Raven. 2011. Diversity of carbon use strategies in a kelp forest community: Implications for a high CO_2 ocean. *Global Change Biology* 17 (7): 2488–2497.

Hofmann, L.C., J.C. Nettleton, C.D. Neefus, and A.C. Mathieson. 2010. Cryptic diversity of *Ulva* (Ulvales, Chlorophyta) in the Great Bay estuarine system (Atlantic USA): Introduced and indigenous distromatic species. *European Journal of Phycology* 45 (3): 230–239.

Hofmann, L.C., S. Straub, and K. Bischof. 2012. Competition between calcifying and noncalcifying temperate marine macroalgae under elevated CO_2 levels. *Marine Ecology Progress Series* 464: 89–105.

Holmer, M., M.S. Frederiksen, and H. Møllegaard. 2005. Sulfur accumulation in eelgrass (*Zostera marina*) and effect of sulfur on eelgrass growth. *Aquatic Botany* 81: 367–379.

Invers, O., M. Pérez, and J. Romero. 1999. Bicarbonate utilization in seagrass photosynthesis: Role of carbonic anhydrase in *Posidonia oceanica* (L.) Delile and *Cymodocea nodosa* (Ucria) Ascherson. *Journal of Experimental Marine Biology and Ecology* 235 (1): 125–133.

Israel, A., and M. Hophy. 2002. Growth, photosynthetic properties and Rubisco activities and amounts of marine macroalgae grown under current and elevated seawater CO_2 concentrations. *Global Change Biology* 8: 831–840.

Jiang, Z.J., X.-P. Huang, and J.-P. Zhang. 2010. Effects of CO_2 enrichment on photosynthesis, growth, and biochemical composition of seagrass *Thalassia hemprichii* (Ehrenb.) aschers. *Journal of Integrative Plant Biology* 52 (10): 904–913.

Johnson, D. A. 1980. Effects of phytoplankton and macroalgae on larval and juvenile winter flounder culture. University of Rhode Island, Kingston, Rhode Island, U.S.A.

Johnson, D.A., and B.L. Welsh. 1985. Detrimental effects of *Ulva lactuca* (L.) exudates and low oxygen on estuarine crab larvae. *Journal of Experimental Marine Biology and Ecology* 86 (1): 73–83.

Kinney, E.L., and I. Valiela. 2011. Nitrogen loading to great South Bay: Land use, sources, retention, and transport from land to bay. *Journal of Coastal Research* 27 (4): 672–686.

Kirkendale, L., G.W. Saunders, and P. Winberg. 2013. A molecular survey of *Ulva* (Chlorophyta) in temperate Australia reveals enhanced levels of cosmopolitanism. *Journal of Phycology* 49 (1): 69–81.

Koch, M., G. Bowes, C. Ross, and X.-H. Zhang. 2013. Climate change and ocean acidification effects on seagrasses and marine macroalgae. *Global Change Biology* 19: 103–132.

Koch, M.S., S. Schopmeyer, C. Kyhn-Hansen, and C.J. Madden. 2007. Synergistic effects of high temperature and sulfide on tropical seagrasses. *Journal of Experimental Marine Biology and Ecology* 341: 91–101.

Kroeker, K.J., F. Micheli, and M.C. Gambi. 2013. Ocean acidification causes ecosystem shifts via altered competitive interactions. *Nature Climate Change* 3: 156–159.

Liu, D., J.K. Keesing, Q. Xing, and P. Shi. 2009. World's largest macroalgal bloom caused by expansion of seaweed aquaculture in China. *Marine Pollution Bulletin* 58 (6): 888–895.

Maberly, S.C., J.A. Raven, and A.M. Johnston. 1992. Discrimination between ^{12}C and ^{13}C by marine plants. *Oecologia* 91 (4): 481–492.

Magre, E.J. 1974. *Ulva lactuca* L. negatively affects *Balanus balanoides* (L.) (Cirripedia Thoracica) in tidepools. *Crustaceana* 27 (3): 231–234.

McGlathery, K.J. 2001. Macroalgal blooms contribute to the decline of seagrass in nutrient-enriched coastal waters. *Journal of Phycology* 37 (4): 453–456.

McGlathery, K.J., D. Krause-Jensen, S. Rysgaard, and P.B. Christensen. 1997. Patterns of ammonium uptake within dense mats of the filamentous macroalga *Chaetomorpha linum*. *Aquatic Botany* 59: 99–115.

Meehl, G.A., T.F. Stocker, W.D. Collins, P. Friedlingstein, A.T. Gaye, J.M. Gregory, A. Kitoh, et al. 2007. Global climate projections. In *Climate change 2007: The physical science basis. Contribution of working group I to the fourth assessment report of the intergovernmental panel on climate change*, ed. S.D. Solomon, Manning M. Qin, Z. Chen, M. Marquis, K.B. Averyt, M. Tignor, and H.L. Miller. Cambridge: Cambridge University Press.

Melzner, F., T. Jörn, W. Koeve, A. Oschlies, M.A. Gutowska, H.W. Bange, H.P. Hansen, and A. Körtzinger. 2013. Future ocean acidification will be amplified by hypoxia in coastal habitats. *Marine Biology* 160: 1875–1888.

Mercado, J.M., F.J.L. Gordillo, F.X. Niella, and F.L. Figueroa. 1998. External carbonic anhydrase and affinity for inorganic carbon in intertidal macroalgae. *Journal of Experimental Marine Biology and Ecology* 221: 209–220.

Millero, F.J. 2010. History of the equation of state of seawater. *Oceanography* 23 (3): 18–33.

Mook, W.G., J.C. Bommerson, and W.H. Staverman. 1974. Carbon isotope fractionation between dissolved bicarbonate and gaseous carbon dioxide. *Earth and Planetary Science Letters* 22: 169–176.

Moriarty, D.J.W., P.I. Boon, J.A. hansen, and D.C. White. 1985. Microbial biomass and productivity in seagrass beds. *Geomicrobiology* 4 (1): 21–51.

Naldi, M., and P.A. Wheeler. 1999. Changes in nitrogen pools in *Ulva fenestrata* (Chlorophyta) and *Gracilaria pacifica* (Rhodophyta) under nitrate and ammonium enrichment. *Journal of Phycology* 35 (1): 70–77.

Nan, C., H. Zhang, and G. Zhao. 2004. Allelopathic interactions between the macroalga *Ulva pertusa* and eight microalgal species. *Journal of Sea Research* 52 (4): 259–268.

Nelson, T.A., D.J. Lee, and B.C. Smith. 2003. Are "green tides" harmful algal blooms? Toxic properties of water-soluble extracts from two bloom-forming macroalgae, *Ulva fenestrate* and *Ulvaria obscura* (Ulvophyceae). *Journal of Phycology* 39: 874–879.

Olischläger, M., I. Bartsch, L. Gutow, and C. Wiencke. 2013. Effects of ocean acidification on growth and physiology of *Ulva lactuca* (Chlorophyta) in a rockpool-scenario. *Phycological Research* 61 (3): 180–190.

Palacios, S.L., and R.C. Zimmerman. 2007. Response of eelgrass *Zostera marina* to CO_2 enrichment: Possible impacts of climate change and potential for remediation of coastal habitats. *Marine Ecology Progress Series* 344: 1–13.

Parsons, T.R., Y. Maita, and C.M. Lalli. 1984. *A manual of chemical and biological methods for seawater analysis*. Oxford: Pergamon Press.

Pedersen, M.F., and J. Borum. 1992. Nitrogen dynamics of eelgrass *Zostera marina* during a late summer period of high growth and low nutrient availability. *Marine Ecology Progress Series* 80: 65–73.

Pedersen, M.F., and J. Borum. 1997. Nutrient control of estuarine macroalgae: Growth strategy and the balance between nitrogen requirements and uptake. *Marine Ecology Progress Series* 161: 155–163.

Perkins-Visser, E., T.G. Wolcott, and D.L. Wolcott. 1996. Nursery role of seagrass beds: Enhanced growth of juvenile blue crabs (*Callinectes sapidus* Rathbun). *Journal of Experimental Marine Biology and Ecology* 198 (2): 155–173.

Pollard, P.C., and D.J.W. Moriarty. 1991. Organic carbon decomposition, primary and bacterial productivity, and sulphate reduction, in tropical seagrass beds of the Gulf of Carpentaria, Australia. *Marine Ecology Progress Series* 69: 149–159.

Porzio, L., M.C. Buia, and J.M. Hall-Spencer. 2011. Effects of ocean acidification on macroalgal communities. *Journal of Experimental Marine Biology and Ecology* 400 (1): 278–287.

Pregnall, A.M., R.D. Smith, T.A. Kursar, and R.S. Alberte. 1984. Metabolic adaptation of *Zostera marina* (eelgrass) to diurnal periods of root anoxia. *Marine Biology* 83 (2): 141–147.

Raven, J.A., M. Giordano, J. Beardall, and S.C. Maberly. 2011. Algal and aquatic plant carbon concentrating mechanisms in relation to environmental change. *Photosynthesis Research* 109 (1): 281–296.

Raven, J.A., A.M. Johnston, J.E. Kübler, R. Korb, S.G. McInroy, L.L. Handley, C.M. Scrimgeour, et al. 2002. Mechanistic interpretation of carbon isotope discrimination by marine macroalgae and seagrasses. *Functional Plant Biology* 29 (3): 355–378.

Sand-Jensen, K. 1988. Minimum light requirements for growth in *Ulva lactuca*. *Marine Ecology Progress Series* 50: 187–193.

Sharp, J.H. 1974. Improved analysis for particulate organic carbon and nitrogen from seawater. *Limnology and Oceanography* 19: 984–989.

Short, F.T., and P.C. McRoy. 1984. Nitrogen uptake by leaves and roots of the seagrass *Zostera marina* L. *Botanica Marina* 27 (12): 547–556.

Stiling, P., and T. Cornelissen. 2007. How does elevated carbon dioxide (CO_2) affect plant-herbivore interactions? A field experiment and meta-analysis of CO_2-mediated changes on plant chemistry and herbivore performance. *Global Change Biology* 13 (9): 1823–1842.

Talmage, S.C., and C.J. Gobler. 2010. Effects of past, present, and future ocean carbon dioxide concentrations on the growth and survival of larval shellfish. *Proceedings of the National Academy of Sciences of the United States of America* 107 (40): 17246–17251.

Tang, Y.Z., and C.J. Gobler. 2011. The green macroalga, *Ulva lactuca*, inhibits the growth of seven common harmful algal bloom species via allelopathy. *Harmful Algae* 10 (5): 480–488.

Tang, Y.Z., Y. Kang, D. Berry, and C.J. Gobler. 2015. The ability of the red macroalga, *Porphyra purpurea* (Rhodophyceae) to inhibit the proliferation of seven common harmful microalgae. *Journal of Applied Phycology* 27 (1): 531–544.

Touchette, B.W., and J.M. Burkholder. 2000. Overview of the physiological ecology of carbon metabolism in seagrasses. *Journal of Experimental Marine Biology and Ecology* 250: 169–205.

Valiela, I., J. McClelland, J. Hauxwell, P.J. Behr, D. Hersh, and K. Foreman. 1997. Macroalgal blooms in shallow estuaries: Controls and ecophysiological and ecosystem consequences. *Limnology and Oceanography* 42: 1105–1118.

Vizzini, S., A. Tomasello, G.D. Maida, M. Pirrotta, A. Mazzola, and S. Calvo. 2010. Effect of explosive shallow hydrothermal vents on $\delta^{13}C$ and growth performance in the seagrass *Posidonia oceanica*. *Journal of Ecology* 98: 1284–1291.

Wall, C.C., B.J. Peterson, and C.J. Gobler. 2008. Facilitation of seagrass *Zostera marina* productivity by suspension-feeding bivalves. *Marine Ecology Progress Series* 357: 165–174.

Wallace, R.B., H. Baumann, J.S. Grear, R.C. Aller, and C.J. Gobler. 2014. Coastal Ocean acidification: The other eutrophication problem. *Estuarine, Coastal and Shelf Science* 148: 1–13.

Welsh, D.T. 2000. Nitrogen fixation in seagrass meadows: Regulation, plant-bacteria interactions and significance to primary productivity. *Ecology Letters* 3: 58–71.

Xu, Z., D. Zou, and K. Gao. 2010. Effects of elevated CO_2 and phosphorus supply on growth, photosynthesis and nutrient uptake in the marine macroalga *Gracilaria lemaneiformis* (Rhodophyta). *Botanica Marina* 53 (2): 123–129.

Young, C.S., and C.J. Gobler. 2016. Ocean acidification accelerates the growth of two bloom-forming, estuarine macroalgae. *PLoS One* 11 (5): e0155152.

Young, C.S., and C.J. Gobler. 2017. The organizing effects of elevated CO_2 on competition among estuarine primary producers. *Scientific Reports* 7: 7667.

Zieman, J.C. 1974. Methods for the study of the growth and production of turtle grass, *Thalassia testudinum* König. *Aquaculture* 4: 139–143.

Zimmerman, R.C., D.G. Kohrs, D.L. Steller, and R. Alberte. 1995. Sucrose partitioning in *Zostera marina* L. in relation to photosynthesis and the daily light-dark cycle. *Plant Physiology* 108: 1665–1671.

Zimmerman, R.C., D.G. Kohrs, D.L. Steller, and R.S. Alberte. 1997. Impacts of CO_2 enrichment on productivity and light requirements of eelgrass. *Plant Physiology* 115: 599–607.

Zimmerman, R.C., R.D. Smith, and R.S. Alberte. 1987. Is growth of eelgrass nitrogen limited? A numerical simulation of the effects of light and nitrogen on the growth dynamics of *Zostera marina*. *Marine Ecology Progress Series* 41: 167–176.

Zimmerman, R.C., R.D. Smith, and R.S. Alberte. 1989. Thermal acclimation and whole-plant carbon balance in *Zostera marina* L. (eelgrass). *Journal of Experimental Marine Biology and Ecology* 130 (2): 93–109.