Estuaries and Coasts
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# 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]

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## 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

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

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

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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*

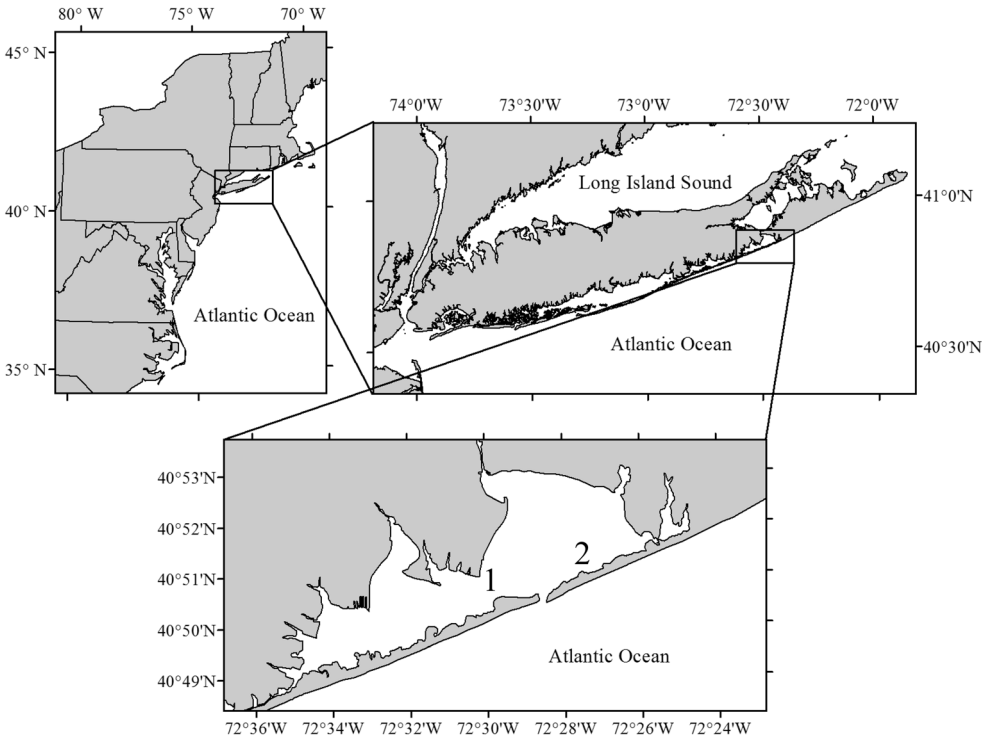


**Fig. 1** Map of Shinnecock Bay, NY, USA. All maps were generated using ArcMap 10.4.1 (Esri). Each number on the map denotes a collection site for primary producers used in experiments performed May through September: (1) *Ulva rigida*, (2) *Zostera marina*

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 (~ $4 \times 10^6$ 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 $\Delta t$ 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:

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

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 $1.11 \pm 0.03$ μM, $0.94 \pm 0.05$ μM, and $6.94 \pm 0.49$ 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

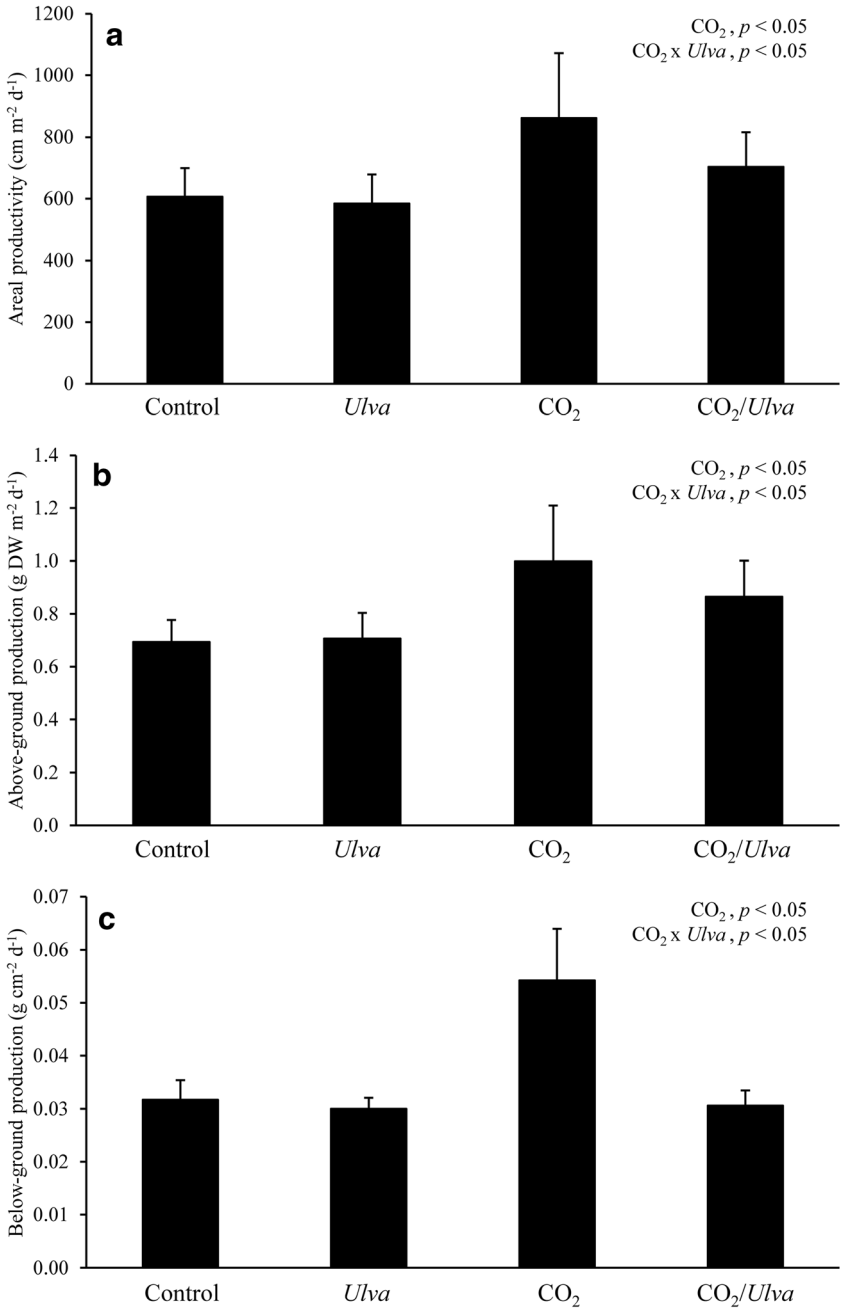


**Fig. 2** **a** Areal productivity, **b** above-ground biomass production, and **c** below-ground biomass production of *Zostera* exposed to ambient and elevated $CO_2$ concentrations, with and without competition from *Ulva* for experiments performed May through September. Growth measurements were 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

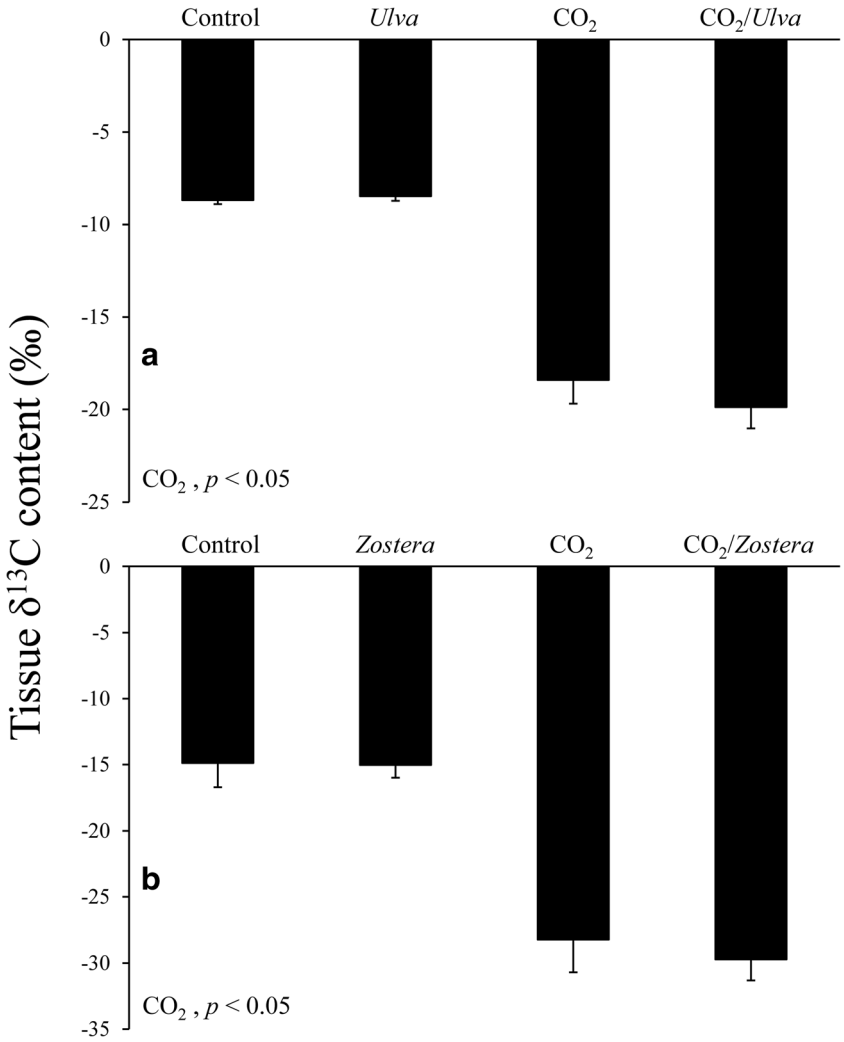


**Fig. 3** $\delta^{13}C$ content of **a** *Zostera* and **b** *Ulva* exposed to ambient and elevated $CO_2$ concentrations, with and without competition from the other primary producer for experiments performed May through September. Measurements were taken at the end of experiments. Columns represent 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;

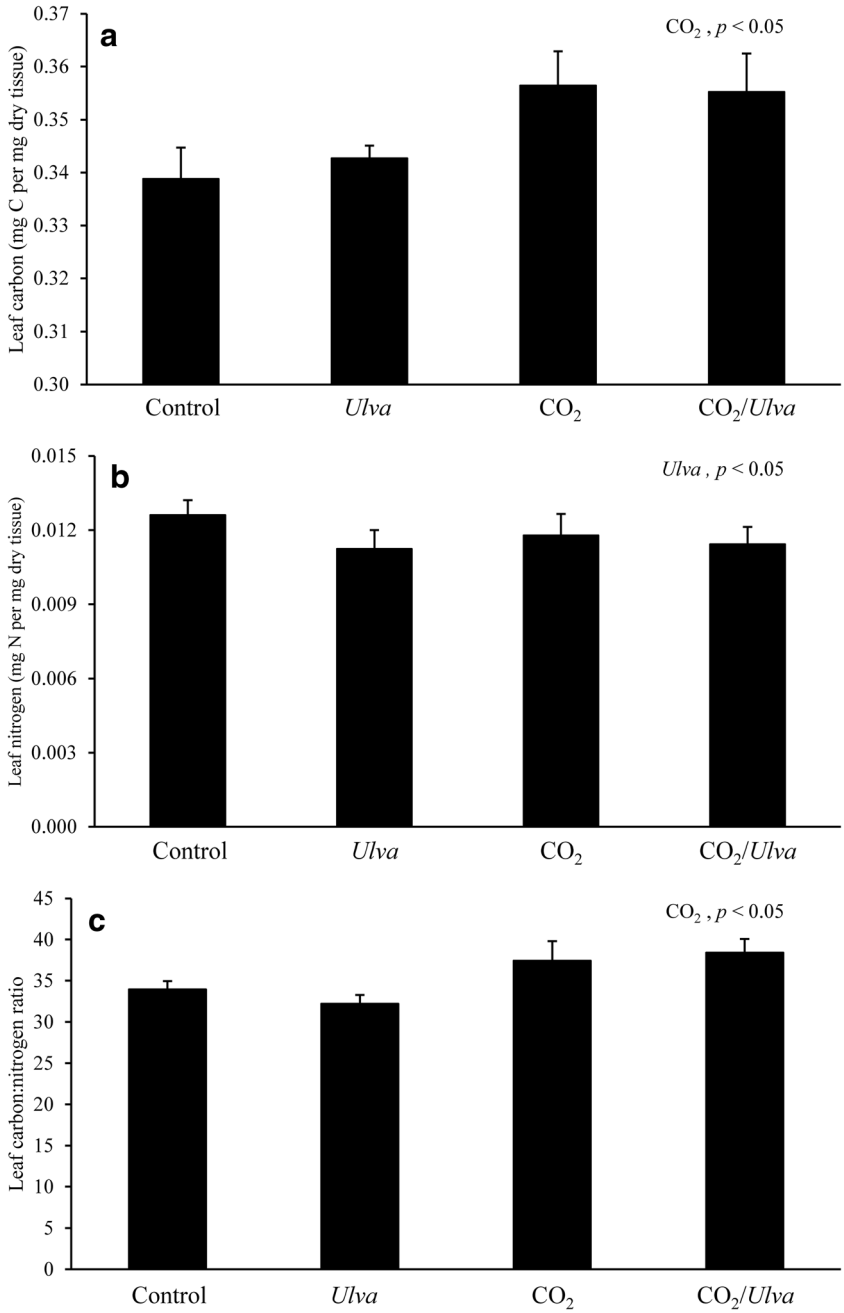


**Fig. 4** **a** Leaf C, **b** leaf N, and **c** leaf C:N content of *Zostera* exposed to ambient and elevated $CO_2$ concentrations, with and without competition from *Ulva* for experiments performed May through September. Measurements were made at the end of experiments. Columns represent means ± standard 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

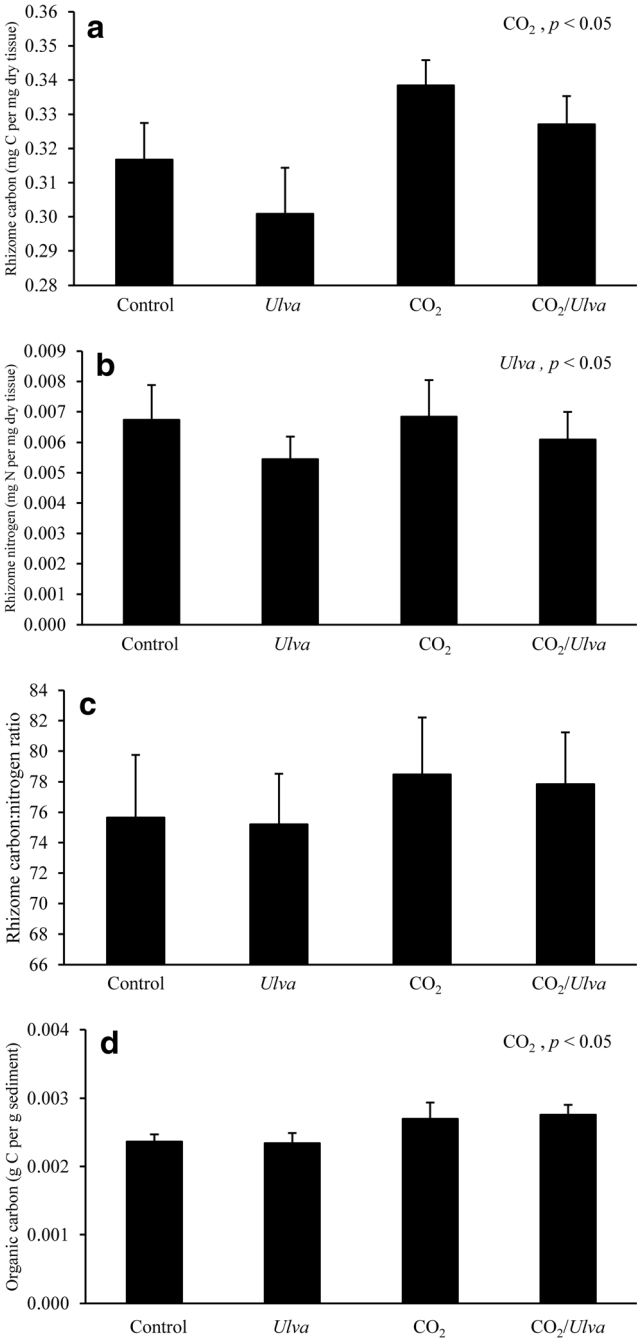


**Fig. 5** **a** Rhizome C, **b** rhizome N, **c** rhizome C:N content of *Zostera*, and **d** *Zostera*-containing sediment organic carbon exposed to ambient and elevated $CO_2$ concentrations, with and without competition from *Ulva* for experiments performed May through September. Measurements were 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

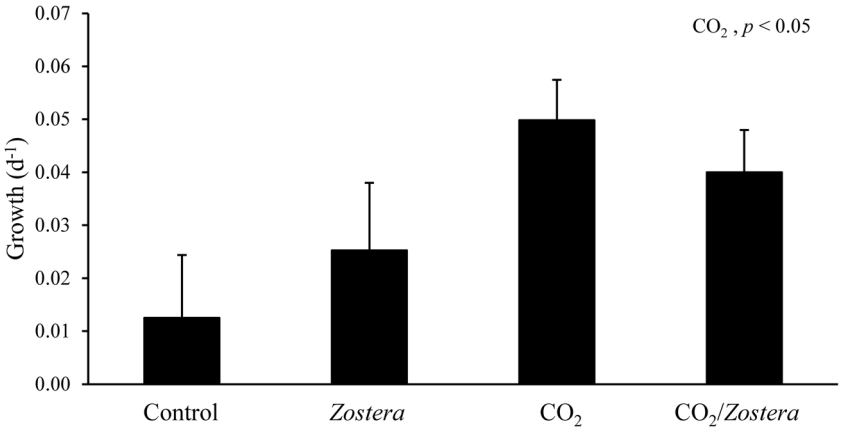


**Fig. 6** Growth rates of *Ulva* exposed to ambient and elevated $CO_2$ concentrations, with and without competition from *Zostera* for experiments performed May through September. Growth measurements were made at the end of experiments. Columns represent means ± standard error. Significant main treatment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

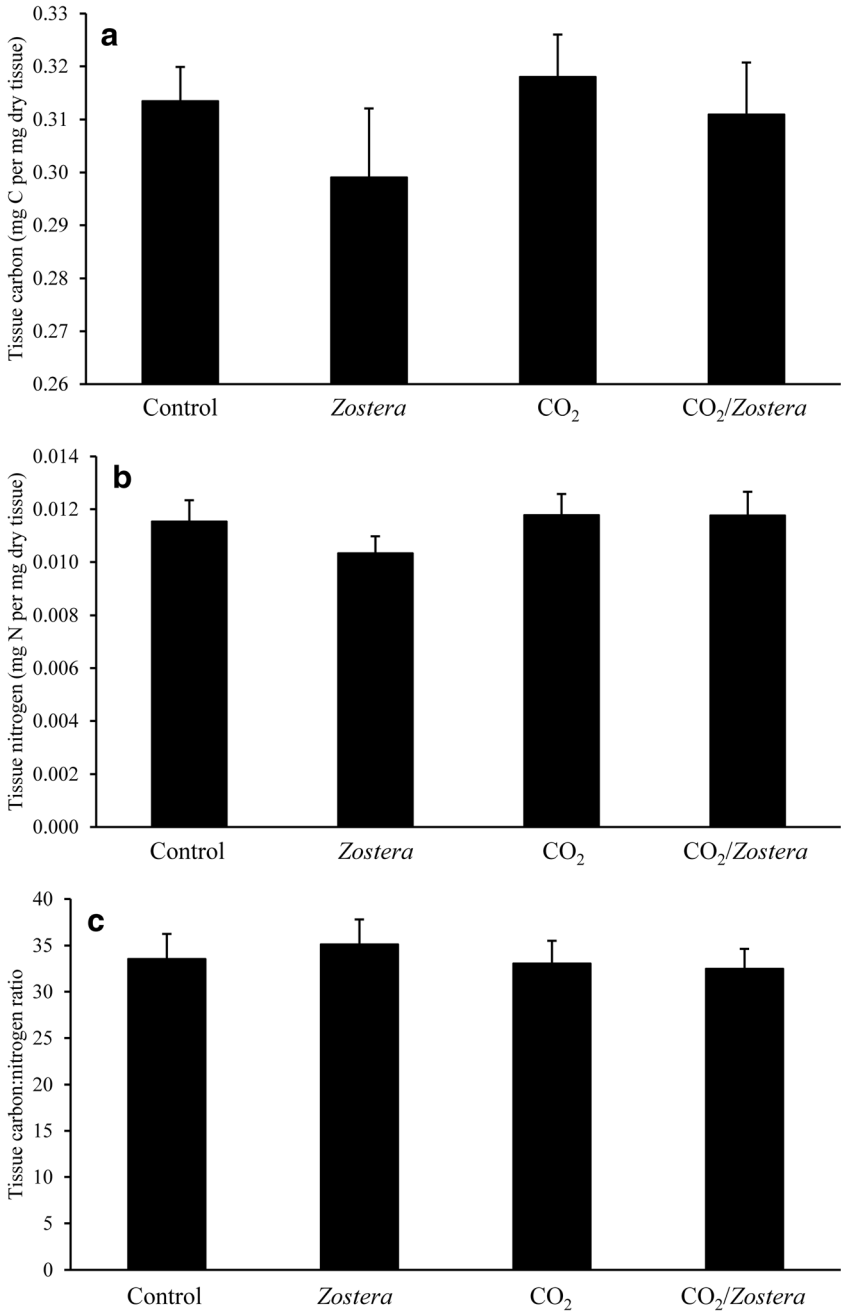


**Fig. 7** **a** Tissue C, **b** tissue N, and **c** tissue C:N content of *Ulva* exposed to ambient and elevated $CO_2$ concentrations, with and without competition from *Zostera* for experiments performed May through September. Measurements were made at the end of experiments. Columns represent means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

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