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Cumulative effects of ocean acidification, eutrophication, and competition on the growth of two bloom-forming, estuarine macroalgae

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Abstract of the Thesis

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Abstract

While there is a growing interest in understanding how marine life will respond to future ocean acidification, many coastal ecosystems currently experience intense acidification in response to upwelling, riverine discharge, and eutrophication. Such acidification can be inhibitory to calcifying animals, but less is known regarding how non-calcifying macroalgae may respond to elevated CO_2. Additionally, while the ability of some marine autotrophs to benefit from elevated CO_2 over others may result in shifts in community structure, such shifts can also be affected by competition between primary producers. In order to examine what role ocean acidification, eutrophication, and competition plays in the growth of marine macroalgae, a series of experiments were performed during summer through fall 2014 and 2015 with North Atlantic populations of *Gracilaria tikvahiae* and *Ulva rigida* that were grown *in situ* within a mesotrophic estuary (Shinnecock Bay, NY, USA) or exposed to normal and elevated, but environmentally realistic, levels of pCO_2 and/or nutrients (nitrogen and phosphorus), as well as being subjected to

competition with each other as well as with diatom and dinoflagellate assemblages (2015). Across the 2014 and 2015 experiments, the growth rates of *Gracilaria* were significantly increased by 70% (2014) and 34% (2015) when exposed to elevated levels of pCO_2 ($p<0.05$). Under the same conditions, the growth rates of *Ulva* were increased by 30% (2014) and 41% (2015). For nearly all 2014 experiments, *Gracilaria* was unaffected by nutrient enrichment. In contrast, the growth response of *Ulva* was more complex as this alga experienced significantly ($p<0.05$) increased growth rates in response to both elevated pCO_2 and nutrients and, in two cases, pCO_2 and nutrients interacted to provide synergistically enhanced growth. For the 2015 experiments, growth rates of *Gracilaria* with or without elevated pCO_2 were unaffected by the presence of competing plankton or *Ulva*. In contrast, growth of *Ulva* was significantly reduced when grown with *Gracilaria* ($p<0.05$) and in several experiments, growth rates of *Ulva* were found to be significantly reduced when competing with plankton ($p<0.05$). Dinoflagellates grew significantly faster when exposed to elevated pCO_2 ($p<0.05$) but experienced significantly reduced growth rates grown with *Gracilaria* ($p<0.05$). Across all experiments, *Gracilaria* and *Ulva* experienced significant declines in tissue $\delta^{13}C$ signatures, suggesting that increased growth rates were associated with a shift from use of HCO_3^- to CO_2 use. This shift in carbon use coupled with significantly increased growth in response to elevated pCO_2 suggests that photosynthesis of these algae was limited by their inorganic carbon supply. For the 2015 experiments, elevated C:N ratios among macroalgae suggested that competition for N also shaped interactions among autotrophs, particularly for *Ulva*. Collectively, these study demonstrates that while several types of estuarine autotrophs can benefit from elevated pCO_2 levels, their relative benefit can change when direct competition with other primary producers is considered with *Gracilaria* outcompeting *Ulva* and dinoflagellates outcompeting diatoms under high pCO_2.

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Chapter 1

Ocean acidification accelerates the growth of two bloom-forming macroalgae

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Introduction

Ocean acidification is changing the chemistry of the ocean. Beyond reducing pH, the anthropogenic delivery of CO_2 into surface oceans this century will differentially affect various pools of inorganic carbon, with CO_2 and HCO_3^- expected to increase 260% and 20%, respectively, and CO_3^{2-} levels expected to decrease 60% (Koch et al., 2013). As the total dissolved inorganic carbon (DIC) pool shifts towards these predicted values, marine flora and fauna are expected to have a varied response with lower availability of CO_3^{2-} inhibiting the growth of calcifying organisms (Gazeau et al., 2007; Doney et al., 2009; Talmage and Gobler, 2010) and higher CO_2 levels potentially benefiting some, but not all, photosynthetic organisms (Palacios and Zimmerman, 2007; Doney et al., 2009, Hattenrath-Lehmann et al., 2015).

The extent to which uncalcified marine macroalgae benefit from anthropogenically-induced changes in carbonate chemistry is complex and not fully understood. While CO_2 is an important carbon source for photosynthesis, the likelihood of elevated CO_2 benefiting autotrophs is partly dependent on photosynthetic pathways utilized by algae. C_3 plants that utilize RuBisCO as their initial carboxylating enzyme experience loss of fixed carbon due to photorespiration and may benefit from increased CO_2 concentrations since RuBisCO is not substrate-saturated at current CO_2 levels (Reiskind et al., 1988; Koch et al., 2013). In contrast, C_4 plants that utilize phosphenolpyruvate carboxylase (PEPC) experience little photorespiratory loss due to use of carbon concentrating mechanisms (CCM) and thus may not benefit from increased CO_2 since PEPC is substrate-saturated at current CO_2 levels (Reiskind et al., 1988; Koch et al., 2013). Marine macroalgae acquire carbon through direct diffusive uptake of CO_2 as well as active transport of CO_2 and HCO_3^- (Badger, 2003). Although the majority of macroalgae are C_3 plants, they often make use of CCMs and extracellular carbonic anhydrase (CA) to convert HCO_3^- to

CO_2 for use by RuBisCO (Gao and McKinley, 1994; Israel and Hophy, 2002; Badger, 2003; Koch et al., 2013). However, there is significant variation in the photosynthetic strategies employed by different macroalgae regarding the use of extracellular CA as well as the degree to which HCO_3^- and/or CO_2 can or cannot be utilized for photosynthesis (Badger, 2003). Macroalgal growth in response to elevated pCO_2 can also be manifested through non-photosynthetic means. Webber et al. (1994) and Roger et al. (1998) found that acclimation to elevated CO_2 can result in decreased concentrations of RuBisCO, but results in an increase in soluble carbohydrate content that could enhance growth rates and alter the total carbon content of algal tissues.

Beyond the progressively increasing levels of CO_2 in the world's oceans due to the combustion of fossil fuels, there are strong sources of CO_2 in coastal zones (Waldbusser and Salisbury, 2014). One of the most prominent CO_2 sources in coastal zones appears to be eutrophication-enhanced microbial respiration (Cai et al., 2011; Melzner et al., 2013; Wallace et al., 2014). The accumulation of respiratory CO_2 from the degradation of excessive organic matter can lower seawater pH and commonly result in CO_2 levels (>1,000 µatm) not predicted to occur in open ocean regions for more than a century (Wallace et al., 2014). The combination of excessive nutrients and elevated CO_2 could have a variety of impacts on primary producers. It has been well-established that with excessive nutrient loading, dominance among benthic autotrophs can shift from seagrasses to fast-growing, ephemeral macroalgae such as *Ulva* and *Gracilaria* (Pedersen and Borum, 1997; Valiela et al., 1997). Furthermore, some species of *Ulva* including *U. rigida* and *U. lactuca* have been shown to experience increased growth under elevated CO_2 concentrations (Björk et al., 1993; Olischläger et al., 2013) while others have not (Rautenberger et al., 2015). Additionally, elevated CO_2 levels could aid in the assimilation of

nutrients by *Ulva* (Gordillo et al., 2001). *Ulva* is well-known for the formation of green tides along eutrophied coastlines such as Brittany, France, and Qingdao, China (Zhao et al., 2013). Common rhodophytes such as *Gracilaria* have been shown to bloom in response to high nutrient concentrations (Ye et al., 2013) and, like some *Ulva*, may also benefit from elevated CO_2 concentrations, although this has never been examined. In general, the dynamics of macroalgal communities in response to eutrophication and elevated CO_2 are difficult to generalize, as both the slow-growing (e.g. *Gracilaria*) and fast-growing (e.g. *Ulva*) species have been hypothesized to benefit from elevated CO_2 and nutrients and studies assessing the response of macrophytes to elevated CO_2 have been limited (Koch et al., 2013).

The objective of this study was to assess how elevated concentrations of CO_2 alone, and combined with elevated nutrient levels, affect the growth rates of two common species of temperate, bloom-forming macroalgae; the rhodophyte, *Gracilaria tikvahiae,* and the chlorophyte, *Ulva rigida*. The overabundance of these macroalgae is commonly interpreted as a symptom of eutrophication and their overgrowth within estuaries can have a series of negative impacts on marine plants and animals (Valiela et al., 1997; Nelson et al., 2003; Liu et al., 2009). Macroalgae were exposed to ambient and elevated concentrations of CO_2 with and without nutrient enrichment during experiments performed throughout their growing season and their growth responses, $\delta^{13}C$ signatures, and elemental composition were evaluated.

Methods

Macroalgae Collection and Preparation

Macroalgae used for this study were collected from shallow regions of eastern Shinnecock Bay, NY, USA (40.85º N, 72.50º W; Fig. 1) during low tide. Permission to access

the water and collect the water and macroalgae was received from the Southampton Town Trustees, Southampton, NY, USA, who hold jurisdiction over Shinnecock Bay. Collections targeted large, well-pigmented, robust fronds of *Ulva* and *Gracilaria* that were transferred to dark, temperature-controlled containers filled with seawater and returned to the Stony Brook Southampton Marine Science Center within 15 minutes of collection. Individual thalli of *Gracilaria* approximately 5 cm in length were cut from the main organism and spun in a salad spinner to remove debris and epiphytes. Samples were then extensively rinsed with filtered (0.2 μm) seawater and placed into the salad spinner a second time to further remove debris, epiphytes, and excess seawater. *Ulva* samples were prepared by use of a small brass ring to cut circular sections approximately 3 cm in diameter from a singular, large sheet of *Ulva* with care taken to avoid the outer, potentially reproductive region of the plant (Wallace and Gobler, 2015). *Ulva* circles were brought through the same cleaning procedures described for *Gracilaria*. Five additional circular samples of *Ulva* were created from the same vegetative plant and were placed between two transparency films and frozen for future analysis described below. All samples were weighed on an A&D EJ300 digital scale (± 0.01 g) to obtain initial wet weights in grams. To prevent desiccation, all samples were kept in individual, 100 mL filtered (0.2 μm) seawater-filled containers after spinning prior to use in experiments.

In situ Growth Experiments

In situ growth experiments with *Gracilaria* and *Ulva* were performed to assess the rate at which the macroalgae grew within the region of Shinnecock Bay from which they were collected. Experiments were performed monthly from June through November with two experiments performed September and October, for a total of eight experiments. Quadruplet,

0.25 m^2 incubation cages constructed from ~1 cm^2 wire mesh were attached to a four-armed (25 cm) umbrella fishing apparatus on a line with surface flotation and a bottom weight that kept cages suspended at 0.2 m below the surface (Wallace and Gobler, 2015). Discrete and continuous measurements of light and temperature present during experiments were made using a LI-COR LI-1500 light sensor logger and HOBO pendant temperature and light loggers, respectively. Quadruplet thalli of each macroalgae species were placed in each cage for ~7 days after which the samples were recovered, brought to the lab, and rinsed, spun, re-rinsed, re-spun, and weighed as described above. *Gracilaria* samples were placed into small freezer bags for further analysis, whereas *Ulva* samples were placed between two transparency films and flattened with care to minimize folds. The surface areas of the experimental *Ulva* samples, in addition to the five initial *Ulva* samples were analyzed using SigmaScan Pro 5 (Wallace and Gobler, 2015). Weight-based growth rates for both species were determined using the relative growth rate formula (growth d^{-1}) = (ln W_{final} – ln $W_{initial}$) / (Δt) where W_{final} and $W_{initial}$ are the final and initial weights in grams and Δt is the duration of the experiment in days.

Assessing the Effects of Elevated Nutrients and pCO_2

Parallel experiments were established to assess the effects of elevated nutrients and pCO_2 on the growth of *Gracilaria* and *Ulva*. Thirty-six, 2.5 L polycarbonate bottles were acid-washed (10% HCl), liberally rinsed with deionized water before use, and rinsed and filled with 0.2 µm filtered seawater from eastern Shinnecock Bay. Experimental bottles were placed in an environmental control chamber set to approximate the temperature (16 – 21°C) and light intensity (~450-500 µmol s^{-1} m^{-2} on a 14 h : 10 h light dark cycle) present during *in situ* experiments and were randomly assigned, in triplicate (n=3), to one of four treatments for each

species: a control with ambient levels of pCO_2 (~400 µatm) and no nutrients added, a treatment of enhanced nutrient levels (50µM nitrate, 3 µM phosphate), a treatment of elevated pCO_2 (~2000 µatm), and a treatment of elevated pCO_2 and nutrient levels (~2000 µatm, 50µM nitrate, 3 µM phosphate). These nutrient and pCO_2 levels were higher than levels present at the collection site, but consistent with concentrations present in eutrophic US East Coast estuaries (Wallace et al., 2014; Wallace and Gobler, 2015). Each bottle was aerated via a 1 mL, polystyrene serological pipette inserted to the bottom of each experimental bottle and via tygon tubing to an air source. Bottles were subjected to the control level of pCO_2 (~400 µatm) and elevated (~2000 µatm) via use of a gas proportionator system (Cole Parmer® Flowmeter system, multitube frame) that mixed ambient air with 5% CO_2 gas (Talmage and Gobler, 2010). The $\delta^{13}C$ of this tanked CO_2 gas was determined to be -27.7‰ by syringe injection into a split/splitless inlet of a continuous flow gas chromatograph isotope ratio mass spectrometer (cf-GCIRMS, Finnegan MAT 253) using a 0.25µm x 30m poraplot column and a secondary standard referenced to V-PDB in the laboratory of Dr. John Mak (Stony Brook University). The mixtures of air and CO_2 gas were delivered at a net flow rate of 500 ± 5 mL min^{-1} through an 18-way gang valve into the serological pipettes that fit through an opening drilled into the closed cap to the bottom of polycarbonate bottles. This delivery rate turned over the volume of experimental bottles >100 times daily, ensuring that desired pCO_2 concentrations and pH levels were generally maintained (Talmage and Gobler, 2010). Bubbling was established two days before the beginning of each experiment to ensure that pCO_2 concentrations and pH levels had reached a state of equilibrium and experiments persisted for ~ one week. Measurements of pH within bottles were made throughout each experiment using an Orion Star A321 Plus electrode (± 0.001) calibrated prior to each use using National Institute of Standards and Technology (NIST)

traceable standards. Measurements using this pH meter were highly similar to and never significantly different from scale-adjusted spectrophotometric pH measurements made using *m*-cresol purple as described by Dickson et al. (2007). DIC concentrations in bottles were measured using an EGM-4 Environmental Gas Analyzer (PP Systems) system that quantifies DIC levels after separating the gas phase from seawater via acidification using a Liqui-Cel Membrane (Membrana) (Talmage and Gobler, 2010). This instrument provided a methodological precision better than ± 1% for replicated measurements of total dissolved inorganic carbon. The levels of DIC and pH within Dr. Andrew Dickson's (University of California, San Diego, Scripps Institution of Oceanography) certified reference material (Batch 138, 141) were measured during every analytical run as a quality assurance measure; analysis of samples proceeded only after complete recovery of certified reference material was attained. pCO_2 levels (mean of t=initial and t=final, Table 1) 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 according to Roy et al. (1993) using the program CO2SYS (http://cdiac.ornl.gov/ftp/co2sys/). The targeted levels of pCO_2 resulted in actual pCO_2 and pH values of 441 ± 72 μatm and 7.9 ± 0.1, respectively, for ambient conditions and 1941 ± 141 μatm and 7.3 ± 0.1, respectively, for the elevated CO_2 conditions (Table 1).

Experiments began with the addition of nutrients and introduction of macroalgae into experimental bottles. Experiments were maintained for seven days, during which daily pH and temperature measurements of each individual bottle were made with the Orion Star A321. Continuous measurements of light and temperature present during experiments were made using a LI-COR LI-1500 light sensor logger and HOBO pendant temperature and light data loggers and continuous pH measurements were made within selected bottles using the Orion Star A321

pH meter. At the termination of experiments, final pH and temperature measurements were made and a final DIC sample from each bottle was analyzed as described above. After measuring DIC, each macroalgae sample was removed from their respective bottles and rinsed, spun, re-rinsed, re-spun, and weighed as described above. *Gracilaria* samples were placed into small freezer bags for further analysis, whereas *Ulva* samples were placed between two transparency films and flattened with care to minimize folds. The surface areas of the samples were analyzed using SigmaScan Pro 5. Weight-based growth rates for both species were determined as described above. Significant differences in growth rates during experiments were assessed using a three-way ANOVA within SigmaPlot 11.0 where the main treatment effects were pCO_2 treatment (ambient or elevated), nutrients (none or enhanced), and date of experiment.

Tissue Analyses

Identification of macroalgae was based on morphology, microscopy, known biogeography, and DNA sequencing. *Gracilaria tikvahiae* is one of the most common species of red algae along the North American east coast, is the only *Gracilaria* species native to the Northeast US (Schneider et al., 1979; Sears, 1998; Kim et al., 2014), and displays a distinct, continuous, phylogenic lineage across the Canadian-Northeast-Mid-Atlantic US region (Gurgel et al., 2004). The morphology and pigmentation of *Gracilaria* fronds used in this study were fully consistent with prior descriptions of *Gracilaria tikvahiae* in the region (Schneider et al., 1979; Sears, 1998; Gurgel et al., 2004) and this was considered to be the species of *Gracilaria* used during this study. In contrast to *Gracilaria*, identifying *Ulva* spp. across the Northeast US is more challenging due to the co-occurrence of multiple, morphologically similar species (Hofmann et al., 2010). For this study, selected frozen *Ulva* samples were dried at 55°C and

then homogenized into a fine powder using a mortar and pestle. DNA from selected samples were extracted using the CTAB method and the quality and quantity of nucleic acids were assessed by use of a Nanodrop 2000 spectrophotometer (Wallace and Gobler, 2015). Next-generation DNA sequencing of ITS1 and ITS2 regions of the ribosome of samples (Hofmann et al., 2010; Wallace and Gobler, 2015) was performed on extracted samples using an Illumina MiSeq at the Molecular Research Laboratory (Shallowater, TX, USA). Forward primer 18S1763 (5`-GGTGAACCTGCGGAGGGATCATT-3`) and reverse primer 5.8S142 (5`-TATTCCGACGCTGAGGCAG-3`) were used for amplification of ITS1 whereas for ITS2, forward primer 5.8S30 (5`-GCAACGATGAAGAACGCAGC-3`) and reverse primer ENT26S (5`-GCTTATTGATATGCTTAAGTTCAGCGGGT-3`) were used (Wallace and Gobler, 2015). The sequences in samples (Genbank Accession #KU306346) had the greatest similarity with *Ulva rigida* which has been previously identified in NY estuaries (Wallace and Gobler, 2015) and the US Northeast (Hofmann et al., 2010) and are synonymous with other *Ulva* spp. (*Ulva lactuca var. rigida*). Due to the plastic nature of macroalgal taxonomic nomenclature as well as the high similarity in ITS sequences among *Ulva* species (Hofmann et al., 2010; Kirkendale et al., 2010) for the purposes of this study, we refer to these algae simply as *Ulva* and for consistency, refer to *Gracilaria tikvahiae* as *Gracilaria*.

For carbon (C) and nitrogen (N) analyses, frozen samples were dried at 55°C, and then homogenized into a fine powder using a mortar and pestle. The total tissue nitrogen and carbon content of the homogenized samples were analyzed using a CE Instruments Flash EA 1112 elemental analyzer (Sharp, 1974). Samples were analyzed for $\delta^{13}C$ signatures using an elemental analyzer interfaced to a Europa 20-20 isotope ratio mass spectrometer at the UC Davis Stable

Isotope Facility (Wallace and Gobler, 2015). Concentrations of nitrate, ammonium, and phosphate were measured using standard wet chemical methods (Parsons, 2013).

Finally, isotope mixing models were developed to estimate the use of CO_2 and HCO_3^- during experiments. The models considered the $\delta^{13}C$ and biomass of macroalgal tissue before and after experiments, the $\delta^{13}C$ of the tanked gas used for experiments (-27.7‰), the $\delta^{13}C$ of the marine CO_2 and HCO_3^- pool (-10‰ and 0‰, respectively; Mook et al., 1974; Maberly et al., 1992; Raven et al., 2002), the fractionation of C during macroalgal uptake of CO_2 and HCO_3^- (-20‰ and -10‰, respectively; Mook et al., 1974; Maberly et al., 1992; Raven et al., 2002), the fractionation of C during conversion of tanked CO_2 bubbled into experimental vessels to HCO_3^- (+10‰, respectively; Mook et al., 1974; Maberly et al., 1992; Raven et al., 2002), and the concentration of DIC with and without the addition of tanked CO_2 with the later providing an indication of the fraction of DIC contributed by the tanked gas compared to ambient air. We assumed that during the course of the experiment, the tanked CO_2 gas came to equilibrium with the total DIC pool and thus that the HCO_3^- pool took on a lighter $\delta^{13}C$ signature in a manner proportional to the fraction of the DIC pool comprised of tanked gas compared to ambient air. Next, since we dried and homogenized the entire experimental macroalgal fronds for subsequent analyses, we assumed that the $\delta^{13}C$ signature of the algal tissue was proportionally representative of the fraction of original tissue (pre-experiment) with its original $\delta^{13}C$ signature and that the tissue grown during the experiment would take on a $\delta^{13}C$ value representative of the CO_2 or HCO_3^- pool with a value made proportionally more negative by the tanked CO_2. Finally, two sets of mixing models were run for each macroalgal species to determine what their $\delta^{13}C$ signature would be if they were using exclusively CO_2 and exclusively HCO_3^- during experiments. One-way ANOVAs were used to assess the differences between the measured $\delta^{13}C$

signature of the macroalgae and signatures calculated based on exclusive CO_2 or HCO_3^- use and Tukey tests were used to assess differences between individual groups.

Results

Gracilaria

The *in situ* growth of *Gracilaria* in Shinnecock Bay was found to be highly similar to and not significantly different from growth rates within experimental control bottles with the exception of the August experiment, when experimental growth rates exceeded those *in situ* (Two-way ANOVA; $p > 0.05$; Fig. 2; S2 Tables). *Gracilaria* growth rates differed seasonally (Three-way ANOVA; $p < 0.05$; Fig. 2; S2 Tables). The experimental growth rates of *Gracilaria* were highly sensitive to changes in CO_2 concentrations (Three-way ANOVA; $p < 0.05$; Fig. 2; S2 Tables). For six of the eight experiments, the growth of *Gracilaria* increased significantly when exposed to elevated CO_2 concentrations (Tukey test; $p < 0.05$; Fig. 2; S2 Tables) with experiments during late September and late October being the exceptions to this trend. On average, growth rates under elevated CO_2 were 70% higher than growth under ambient conditions (Fig. 2). In contrast, the addition of nutrients did not significantly alter the growth rates of *Gracilaria* or yield statistically significant interactions with elevated pCO_2 concentrations during any experiment (S2 Tables). As a final observation, there did not appear to be any obvious signs of epiphytes on the *Gracilaria* samples at the conclusion of the experiments, as per the extensive rinsing and spinning phases.

The stable carbon isotope ($\delta^{13}C$) content of *Gracilaria* was significantly reduced by exposure to elevated pCO_2, with the average $\delta^{13}C$ value of the ambient and elevated CO_2 groups being, on average, -13‰ and -21‰, respectively (Three-way ANOVA; $p<0.001$; Fig. 3; S2 and

S3 Tables). The $\delta^{13}C$ signatures of *Gracilaria* were not altered by nutrients but did differ by experiment (Three-way ANOVA; $p<0.001$; S2 and S3 Tables). Isotope mixing models demonstrated that when incubated with elevated pCO_2 concentrations, *Gracilaria* $\delta^{13}C$ signatures (-21‰) were significantly lower than values expected if their DIC was exclusively from use of HCO_3^- (-14‰) and significantly higher than expected from the use of exclusively CO_2 (-28‰; Tukey test; $p<0.001$; Fig 4; S2 and S3 Tables). Quantitatively, the model suggested *Gracilaria* was using equal amounts of HCO_3^- and CO_2 during experimental incubations with CO_2 (Fig. 4).

The nitrogen content of *Gracilaria* during experiments was found to be significantly higher in treatments that received nutrients and was found to differ seasonally (Three-way ANOVA; $p < 0.05$; S2 Tables). On average, ambient and elevated nutrient treatments were found to have tissue nitrogen concentrations of 0.029 ± 0.005 and 0.032 ± 0.004 g N per g dry tissue, respectively (Fig. 5; S4 Tables). In contrast, the carbon content of *Gracilaria* was not significantly altered by pCO_2 or nutrients, but did differ by seasonally (Three-way ANOVA; $p < 0.05$; Fig. 5; S2 and S4 Tables). The tissue C:N ratio of *Gracilaria* was found to be significantly lower under elevated nutrient treatments (10.7 ± 0.2) compared to ambient nutrient treatments (12.3 ± 0.4) and differed seasonally (Three-way ANOVA; $p < 0.05$; Fig. 5; S2 and S4 Tables). Tissue C:N ratio was not significantly changed in the CO_2 treatments (S2 Tables).

<u>*Ulva*</u>

The growth rates of *Ulva* during *in situ* experiments did not differ statistically from those found within experimental control bottles except during experiments in early October and November when experimental control growth rates were greater than those observed *in situ* (Two-way ANOVA; $p > 0.05$; Fig. 6; S2 Tables). *Ulva* growth rates differed by experiment

(Three-way ANOVA; $p < 0.05$; Fig 5; S2 Tables). *Ulva* displayed more complex responses to nutrients and CO_2 concentrations during experiments compared to *Gracilaria*. During experiments in June, July, and late October, *Ulva* growth rates significantly increased in response to elevated CO_2 concentrations (Tukey test; $p < 0.05$; Fig 6; S2 Tables; S1 Figure). In addition, *Ulva* experienced significantly higher growth rates in response to higher nutrient levels during experiments performed during July and early September (Fig. 6; Tukey test; $p < 0.05$; S2 Tables; S2 Figure). Finally, there was an interactive effect of CO_2 and nutrients during the late October and November experiments during which these two factors synergistically increased the growth rates of *Ulva* ($p < 0.05$; S2 Tables; S3 Figure). On average, for all experiments, *Ulva* growth rates when exposed to elevated CO_2 were 30% higher than ambient conditions (Fig. 5; $p<0.05$; Three-way ANOVA; S2 Tables) whereas the nutrients yielded a smaller, non-significant increase in growth rates (13%; Fig. 6). As a final observation, there did not appear to be any obvious signs of epiphytes on the *Ulva* samples at the conclusion of the experiments, as per the extensive rinsing and spinning phases.

In a manner similar to *Gracilaria*, the $\delta^{13}C$ content of *Ulva* was significantly reduced by exposure to elevated pCO_2 (to -27‰) relative to control treatments value of -7‰ (Three-way ANOVA; $p<0.001$; Fig. 3; S2 and S3 Tables). Unlike *Gracilaria*, however, the $\delta^{13}C$ of *Ulva* was also affected by nutrients that yielded significantly higher values (-5‰) relative to control treatments (-7‰) and the $\delta^{13}C$ differed by experiment (Three-way ANOVA; $p<0.05$; Fig. 3; S2 and S3 Tables). Nutrients and CO_2 did not interact to alter the $\delta^{13}C$ of *Ulva*. Isotope mixing models demonstrated that when incubated with elevated pCO_2 concentrations, *Ulva* $\delta^{13}C$ signatures (-27‰) were significantly lower than values expected from the strict use of HCO_3^- (-12‰) and significantly higher than expected from the strict use of CO_2 (-33‰; Tukey test;

p<0.001; Fig 4; S2 Tables). Quantitatively, the model suggested that for *Ulva*, during experimental incubations with elevated CO_2, 70% of their carbon came from CO_2 and 30% came from HCO_3^- (Fig. 4).

Also similar to *Gracilaria*, the nitrogen content of *Ulva* was significantly higher in elevated nutrient treatments (0.022 ± 0.004 g N per g dry tissue) compared to ambient nutrient treatments, regardless of pCO_2 concentrations (0.019 ± 0.006 g N per g dry tissue; Three-way ANOVA; $p < 0.05$; Fig. 7; S2 and S4 Tables). The carbon content of *Ulva* was not significantly altered by CO_2 but was significantly increased by nutrients and differed by experiment (Three-way ANOVA; $p < 0.05$; Fig. 7; S2 and S4 Tables). Tissue C:N was significantly lower in the elevated nutrient treatments (16.9 ± 0.6) than ambient nutrient treatments (21.5 ± 1.6) and differed by experiment (Three-way ANOVA; $p < 0.05$; Fig. 7; S2 and S4 Tables).

Discussion

During this study, elevated levels of pCO_2 were found to significantly enhance the growth rates of two bloom-forming, estuarine macroalgae, *Gracilaria* and *Ulva*. These enhanced growth rates were accompanied by large and significant reductions in the $\delta^{13}C$ content of the macroalgae. Concurrently, nutrients were found to enhance the growth of *Ulva* but not *Gracilaria*, and the combination of elevated nutrients and pCO_2 were capable of synergistically promoting the growth of *Ulva*. Given that elevated pCO_2 and acidification of coastal ecosystems are symptoms of eutrophication and that ocean acidification is enriching pCO_2 concentrations in these systems, this study provides new insight regarding the present and future overgrowth of macroalgae in estuaries.

The effects of elevated CO_2 concentrations on the growth of algae can depend on the precise carbon acquisition pathways utilized. C_3 algae can benefit from high CO_2 as their RuBisCO is not substrate-saturated at current CO_2 levels (~400ppm) (Reiskind et al., 1988; Gao et al., 1999). Many macroalgae use HCO_3^- rather than dissolved CO_2 under current seawater pCO_2 concentrations and utilize CA to convert HCO_3^- to CO_2 for use by RuBisCO (Gao and McKinley, 1994; Israel and Hophy, 2002; Badger, 2003; Koch et al., 2013). For example, Mercado et al. (1998) found that the chlorophytes *Ulva rigida* and *U. compressa* (formerly *Enteromorpha*) do not receive enough CO_2 through diffusive uptake alone at current CO_2 levels and thus must use CCMs to acquire HCO_3^-. However, when exposed to elevated pCO_2, macroalgae may down-regulate their CCMs, reduce the use of HCO_3^-, and begin to rely on CO_2 as a primary C source (Björk et al., 1993; Gao et al., 1993; Xu et al., 2010; Cornwall et al., 2012). The energy made available from the down-regulation of the CCM may, in turn, be used for other purposes, such as increased vegetative growth (Koch et al., 2013) which we observed during this study.

Values of $\delta^{13}C$ are often used to assess the types of carbon utilized by macroalgae. The $\delta^{13}C$ of HCO_3^- is significantly higher (less negative) than that of CO_2 in seawater and values of -10‰ or higher in macroalgae are reflective of the sole use HCO_3^- and CCMs whereas macroalgae relying wholly on diffusion of CO_2 for carbon attain a value of -30‰ (Maberly et al., 1992; Raven et al., 2002; Hepburn et al., 2011). When grown in ambient seawater, *Ulva* and *Gracilaria* had $\delta^{13}C$ values of -8 and -13‰, values indicative of exclusive and near exclusive (85%) HCO_3^- use, respectively (Maberly et al., 1992; Raven et al., 2002; Hepburn et al., 2011). The use of tanked CO_2 gas with a known $\delta^{13}C$ signature (-27.7‰) permitted that CO_2 to be used as a tracer in mixing models and demonstrated that when incubated with elevated CO_2, both

macroalgal species switched their primary source of DIC. For *Ulva,* the change was the most dramatic as the three-fold decrease in $\delta^{13}C$ signature was indicative of these algae going from exclusive use of HCO_3^- to, on average, 70% of their DIC originating from CO_2 and only 30% from HCO_3^-. For *Gracilaria*, the change was less dramatic with but still notable as the alga went from ~85% HCO_3^- use under low pCO_2 conditions to 50% CO_2 use under high pCO_2 conditions. Given the switch to increasing CO_2 use by *Ulva* and *Gracilaria* and concurrent increase in growth experienced under elevated pCO_2 concentrations, these algae may have down-regulated their CCMs permitting more energy to be dedicated to vegetative growth (Koch et al., 2013). The significant increase in $\delta^{13}C$ of *Ulva* when provided with nutrients further supports these hypotheses given that they experienced enhanced growth and presumably greater photosynthetic rates due to higher nutrient levels, causing a greater use of HCO_3^- via CCMs since additional CO_2 was not available (Rautenberger et al., 2015). Finally, there are additional factors that could contribute to lowered $\delta^{13}C$ values including preferential synthesis of lipids depleted in $\delta^{13}C$ compared to proteins and carbohydrates (Hoefs, 2009) although the extent of fractionated C associated with this process is small compared to changes observed during experiments presented here. Hence, the change in $\delta^{13}C$ values during experiments suggest that when exposed to high concentrations of CO_2, these bloom-forming macroalgae obtained a significantly larger fraction of their DIC from CO_2 and often grew faster.

Elevated pCO_2 concentrations did not alter the rate at which macroalgae took up and stored carbon (C) or nitrogen (N). The lack of change in tissue C content is consistent with the findings of Gordillo et al. (2001) who reported no accumulation of soluble carbohydrates and no change in tissue C content for *Ulva rigida* fronds exposed to pCO_2-enriched conditions. Despite the unchanged tissue C content, there were expected, significant increases in tissue N content

within nutrient treatments. Both *Ulva* and *Gracilaria* have been shown to be able to rapidly assimilate and store nitrate (Ryther et al., 1981; Fan et al., 2014) and have been shown to experience enhanced tissue N content when exposed to elevated levels of nitrate (Naldi and Wheeler, 1999; Liu et al., 2009). While increases in the C:N ratio of macroalgae can reflect an increase in soluble carbohydrates during stimulation of growth rates in certain plants (Fonseca et al., 1997), during our study tissue C:N levels did not track growth rates. Given the observed changes in $\delta^{13}C$ during exposure to high pCO_2, we hypothesize that macroalgae responded to increased C availability by increasing, stoichiometrically-balanced growth rather than by storing more carbohydrates.

Eutrophication has been shown to promote coastal ocean acidification due to the accumulation of respiratory CO_2 emanating from the microbial degradation of the excessive organic matter (Wallace et al., 2014). The present study has shown that *Gracilaria* and *Ulva* are capable of enhanced growth under elevated pCO_2 levels and that *Ulva* can, on occasion, synergistically benefit from concurrently higher nutrient concentrations. Going forward, this finding may have broad implications as it demonstrates that, in some cases, the true impacts of elevated pCO_2 on macroalgae may only be realized when excessive nutrients are present. Prior studies have demonstrated that elevated CO_2 levels may have little effect on photosynthetic rates of some algae (Björk et al., 1993; Israel and Hophy, 2002; Cornwall et al., 2012) but can result in increased biomass of *Gracilaria* sp., *G. chilensis*, and *G. lemaneiformis* (Gao et al., 1993; Xu et al., 2010) and *Ulva rigida* and *U. lactuca* (Gordillo et al., 2001; Olischläger et al., 2013). While *Gracilaria* can benefit from high nutrient concentrations (Ye et al., 2013), *Ulva* is capable of undergoing more rapid growth in eutrophic settings (Wallace and Gobler, 2015) due to a high maximum rate of uptake of ammonium and nitrate (Pedersen and Borum, 1997). This was

observed during the present study as *Ulva* growth rates were significantly higher than *Gracilaria*, and *Ulva* responded to nutrients more consistently than *Gracilaria*. *Ulva* is known to outcompete slower-growing algae in eutrophic estuaries, such as Saldanha Bay, South Africa (Andersen et al., 1996), Britanny, France (Perrot et al., 2014), and Qingdao, China (Zhao et al., 2013). The current study demonstrates that within eutrophied estuaries, seasonally elevated levels of pCO_2 may be equally or more important than excessive nutrients in promoting algal growth. For example, *Gracilaria* grew faster in the presence of higher pCO_2 levels but was unaffected by nutrients. Previously, it has been noted that more pristine estuaries are characterized by numerous, slower-growing macroalgal species while eutrophic estuaries are typically dominated by fewer, fast-growing, ephemeral macroalgal species (Pedersen and Borum, 1997; Valiela et al., 1997; Smetacek and Zingone, 2013). While nutrient loading and changes in light levels have been ascribed as the factors controlling these trends, the findings presented here suggest that elevated levels of pCO_2 may be equally or more important for shaping estuarine macroalgal community composition.

The extent to which elevated levels of pCO_2 affect the growth of macroalgae in estuaries will likely be influenced, in part, by physical mixing and circulation. In poorly flushed and/or mixed estuarine regions, diffusive boundary layers around seaweeds may limit DIC uptake (Wheeler, 1980; Koch, 1993) and thus higher ambient pCO_2 may be more likely to be beneficial. In contrast, in high energy environments with fast-moving currents or wave-flow, boundary layers are less likely to develop (Wheeler, 1980; Koch, 1993) and additional pCO_2 may be less likely to affect growth. During this study, macroalgae were vigorously bubbled at a rate that turned over the dissolved gas pool more than 700-times daily, a process that was unlikely to permit the development of boundary layers. This hypothesis is supported by the highly similar

growth rates of thalli in a fairly high energy region of Shinnecock Bay during *in situ* experiments and in our control, experimental bottles for nearly all experiments. Hence, in our experiments, enhanced growth experienced during exposure to high levels of pCO_2 were more likely a consequence of an intra-cellular, photosynthetic benefit for the algae rather than changes in external conditions.

The full implications of climate change for macroalgal communities are not fully understood, as studies of the effects of processes such as ocean acidification, rising temperatures, and changes in nutrient loading rates have been performed on a limited number of species. Porzio et al. (2011) examined >100 species of macroalgae near volcanic CO_2 vents in the Gulf of Naples, Italy, and found 20 species of calcium carbonate-containing macroalgae were no longer present under the acidification, whereas the ochryophyte *Dictyota dichotoma* and the rhodophyte *Hildenbrandia rubra* were most abundant within the high CO_2 environment. Other studies have similarly found that tropical calcifying macroalgae indigenous to coral reefs are likely to be negatively impacted by ocean acidification (Harley et al., 2012; Hofmann et al., 2012; Hofmann et al., 2015). Connell and Russell (2010) found that elevated CO_2 and temperature enhanced the growth of opportunistic turf-forming algae and that expansion of this algae inhibited the growth of kelp (*Ecklonia radiata*). As climate change processes promote increased pCO_2, this and prior studies suggest that macroalgal communities may shift and favor rapid-growing and opportunistic species such as *Ulva, Gracilaria,* and turf algae, perhaps to the detriment of calcifying macroalgae and/or kelp.

The more rapid growth of some species of macroalgae will have important implications for other classes of marine autotrophs. The majority of seagrass species are C_3 plants that are not currently substrate-saturated at current CO_2 levels, with some, such as *Zostera marina*, showing

enhanced photosynthesis and growth under elevated CO_2 concentrations (Palacios and Zimmerman, 2007; Koch et al., 2013). Elevated nutrient loading, however, typically favors the dominance of macroalgae over seagrasses, as macroalgae are more competitive for high nutrient levels and can overgrow and shade seagrass (Valiela et al., 1997). Beyond CO_2, climate change-induced warming may further favor macroalgae among submerged aquatic vegetation as many temperate species of seagrass exist at or near their upper level of thermal tolerance (Short, 1993). Finally, although highly excessive nutrient loading in estuaries with extended residence times are thought to ultimately favor the growth of phytoplankton blooms over macroalgae, the ability of both *Ulva* and *Gracilaria* to allelopathically inhibit the growth of phytoplankton (Lu et al., 2011; Tang and Gobler, 2011) may allow macroalgae to remain dominant in high nutrient, high CO_2 estuaries.

Macroalgal blooms can be harmful to marine life. Specifically, the overgrowth of macroalgae can cover critical benthic habitats and promote diel hypoxia/anoxia in estuaries (Valiela et al., 1997; Valiela et al., 2002; Liu et al., 2009) and *Ulva* has been shown to cause mortality in multiple calcifying animals including bivalves, barnacles, and larval crabs (Magre, 1974; Johnson and Welsh, 1985; Nelson et al., 2003). Since these calcifying animals are also sensitive to high levels of CO_2 (Ries et al., 2009; Findlay et al., 2010; Talmage and Gobler, 2010; Long et al., 2013) the stimulation of harmful macroalgae such as *Ulva* under elevated pCO_2 levels may represent a previously unrecognized, compounding environmental threat to some ocean animals. In contrast, other animals might benefit from predicted shifts in macroalgal communities. Some herbivorous fish of the families Blenniidae, Kyphosidae, and Siganidae selectively feed on filamentous and fleshy seaweeds such as *Ulva* (Tolentino-Pablico et al., 2008) and *Ulva lactuca* can be an important nursery for juvenile blue crabs (*Callinectes sapidus*)

(Wilson et al., 1990). Furthermore, excessive nutrient loading generally enhances the nitrogen content and C:N ratio of macroalgal tissues, which could benefit herbivores feeding on such material (Hemmi and Jormalainen, 2002). Hence, while shifts in macroalgal communities caused by climate change and eutrophication may promote the prevalence of non-calcifying macroalgae over seagrasses and calcifying macroalgae and may be harmful to some marine mollusks, these shifts could benefit marine organisms that either graze on macroalgae or utilize it as a nursery.

Tables

Table 1: Values of pH (NBS scale), temperature (°C), salinity (g kg^{-1}), pCO_2 (µatm), DIC (µmol $kgSW^{-1}$), HCO_3^- (µmol $kgSW^{-1}$), alkalinity (µmol $kgSW^{-1}$), DIN (µM), and DIP (µM) for *Gracilaria* and *Ulva* for June through November experiments. Values represent means ± SE. Data from individual experiments appear within supplementary tables (S1 Tables).

Gracilaria									
Treatment	pH	Temperature	Salinity	pCO_2	DIC	HCO_3^-	Alkalinity	DIN	DIP
Control	8.23±0.02	18.4±0.1	29.5±0.8	327±58	1520±73	1380±73	1790±76	5.42±0.87	0.72±0.11
Nutrients	8.29±0.03	18.5±0.1	29.5±0.7	314±61	1400±68	1310±93	1720±71	55.42±8.86	3.72±0.58
CO_2	7.37±0.01	18.6±0.1	29.8±0.6	2530±108	1760±60	1660±48	1710±59	5.42±0.87	0.72±0.11
CO_2/Nutrients	7.38±0.01	18.6±0.1	29.7±0.7	2380±114	1710±61	1630±49	1670±58	55.42±8.86	3.72±0.53
Ulva									
Treatment	pH	Temperature	Salinity	pCO_2	DIC	HCO_3^-	Alkalinity	DIN	DIP
Control	8.27±0.02	18.5±0.1	29.3±0.8	329±55	1540±72	1380±70	1780±76	5.42±0.87	0.72±0.11
Nutrients	8.35±0.03	18.5±0.1	29.6±0.7	328±56	1440±57	1330±63	1750±89	55.42±8.86	3.72±0.58
CO_2	7.37±0.01	18.6±0.1	29.7±0.6	2510±102	1770±70	1650±56	1720±70	5.42±0.87	0.72±0.11
CO_2/Nutrients	7.40±0.01	18.6±0.1	29.8±0.6	2300±163	1740±55	1650±46	1700±58	55.42±8.86	3.72±0.53

Figures

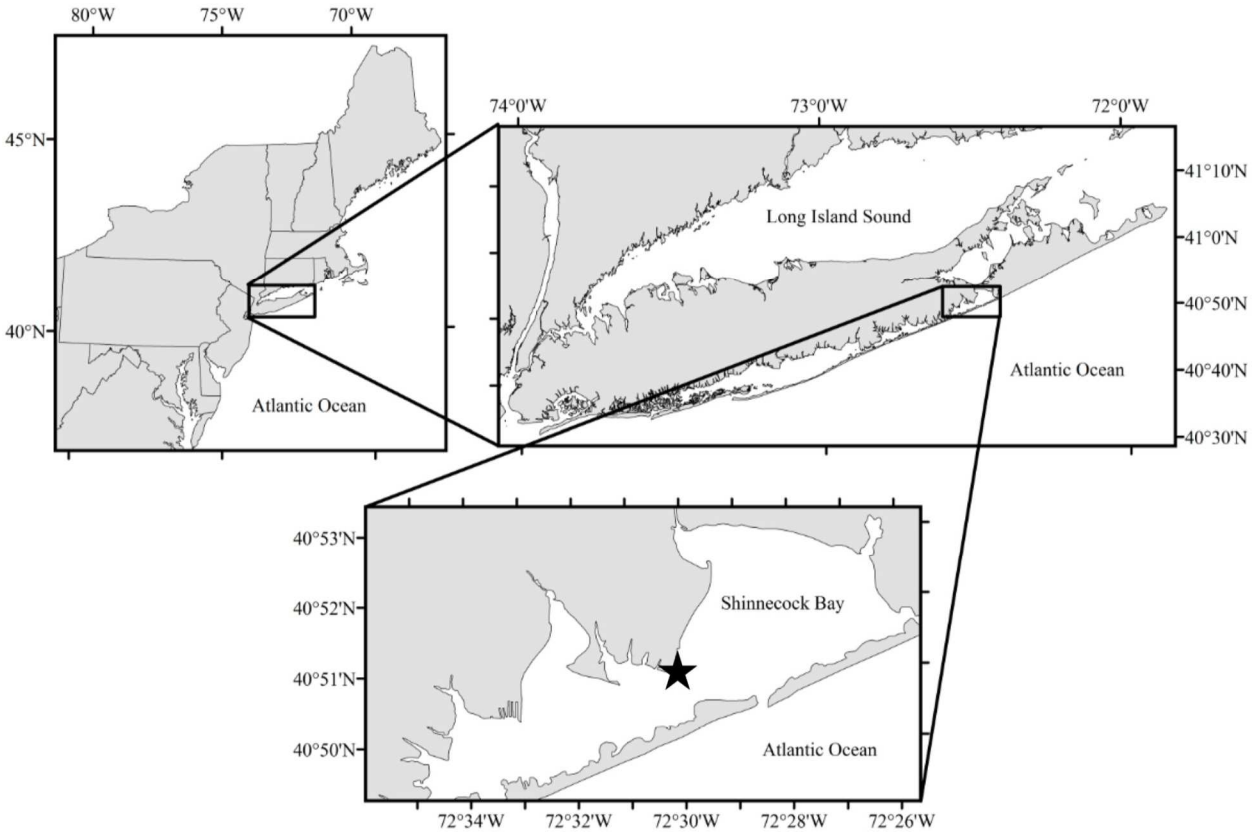

Figure 1: Map of Shinnecock Bay, NY, USA. The star represents the shallow-water region where macroalgal collections occurred and *in situ* experiments were performed.

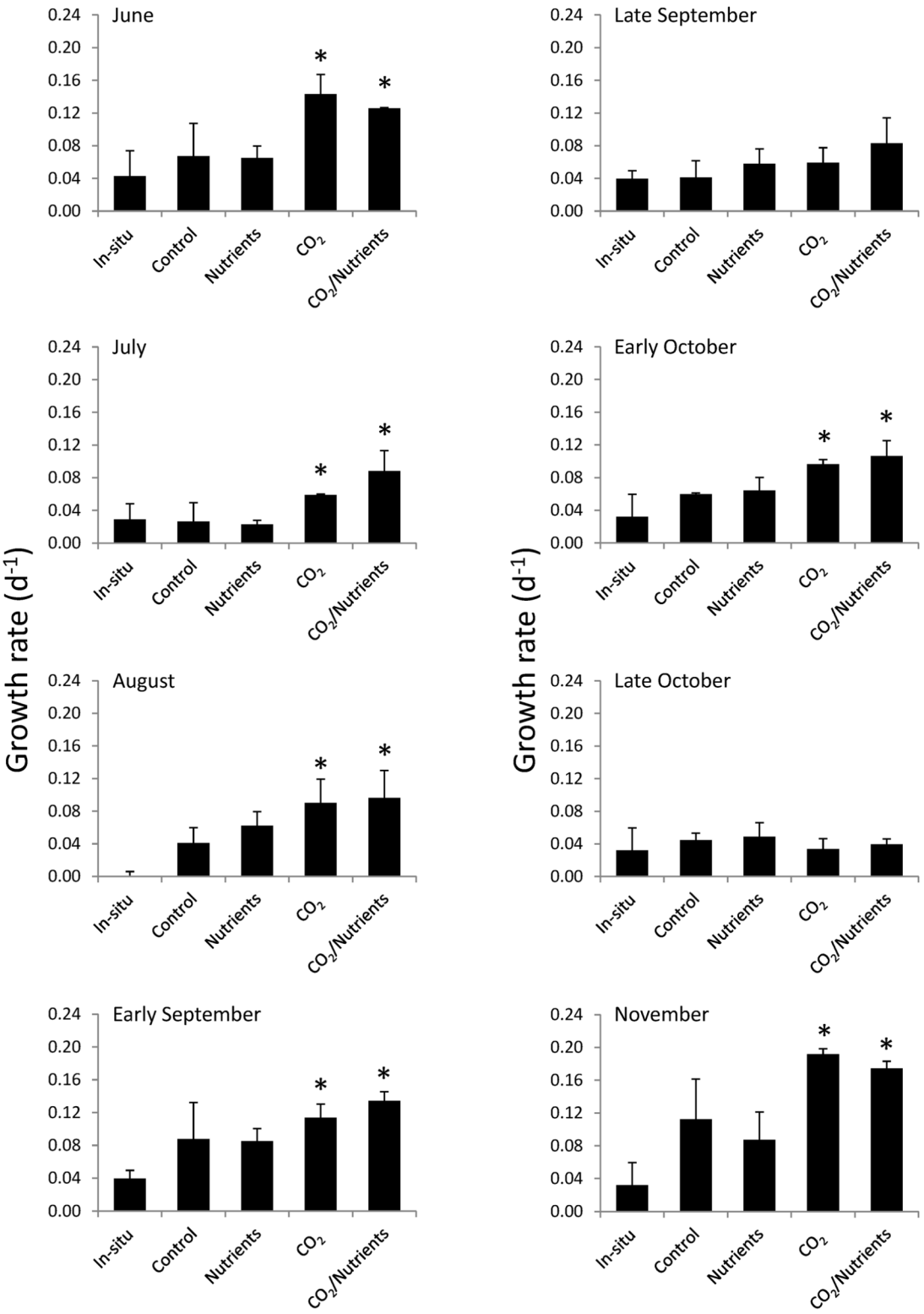

Figure 2: Growth rates of *Gracilaria* exposed ambient and elevated CO_2 conditions with and without nutrient additions for experiments performed August through November. Columns with an asterisk over them indicate significant results.

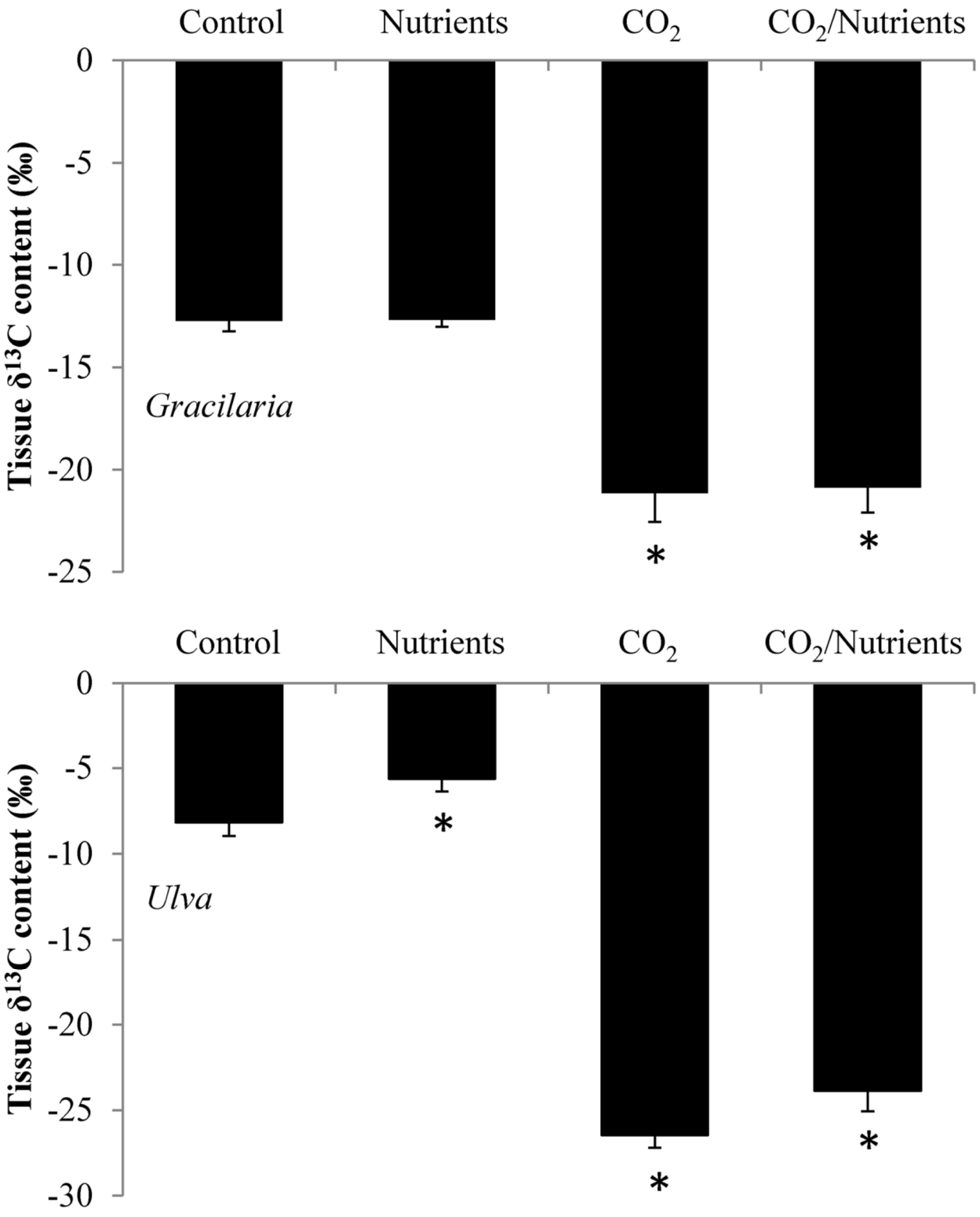

Figure 3: $\delta^{13}C$ content of *Gracilaria* and *Ulva* exposed to ambient and elevated CO_2 conditions with and without nutrient additions for experiments performed August through November.

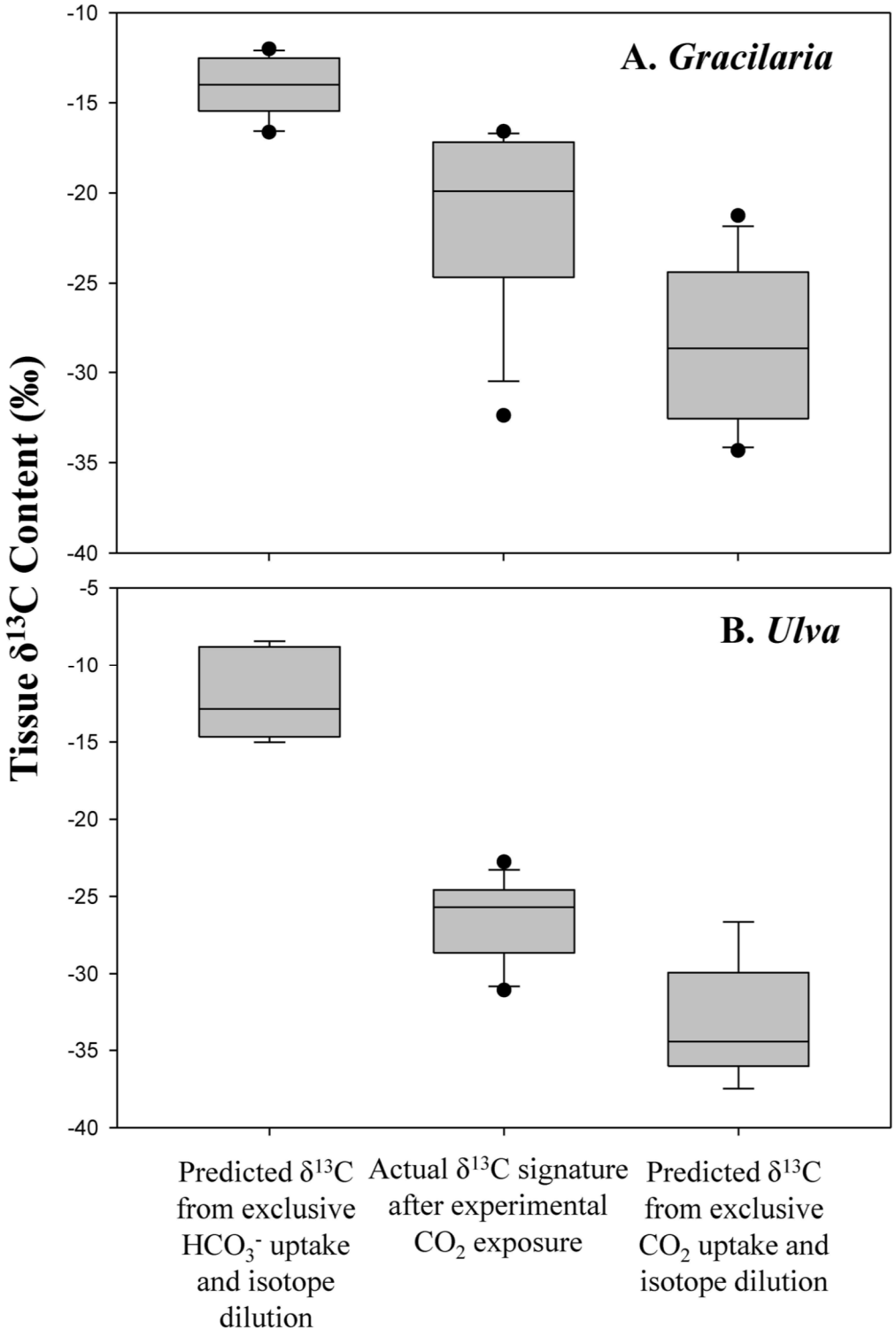

Figure 4: $\delta^{13}C$ content of A) *Gracilaria* and B) *Ulva* exposed to elevated CO_2 conditions compared with the $\delta^{13}C$ signature expected from the exclusive use of CO_2 or the exclusive use of HCO_3^-. Box plots depict the mean median (line within the boxes), 25th and 75th percentiles (lower and upper edges of the boxes), and 10th and 90th percentiles of the data (lower and upper error bars).

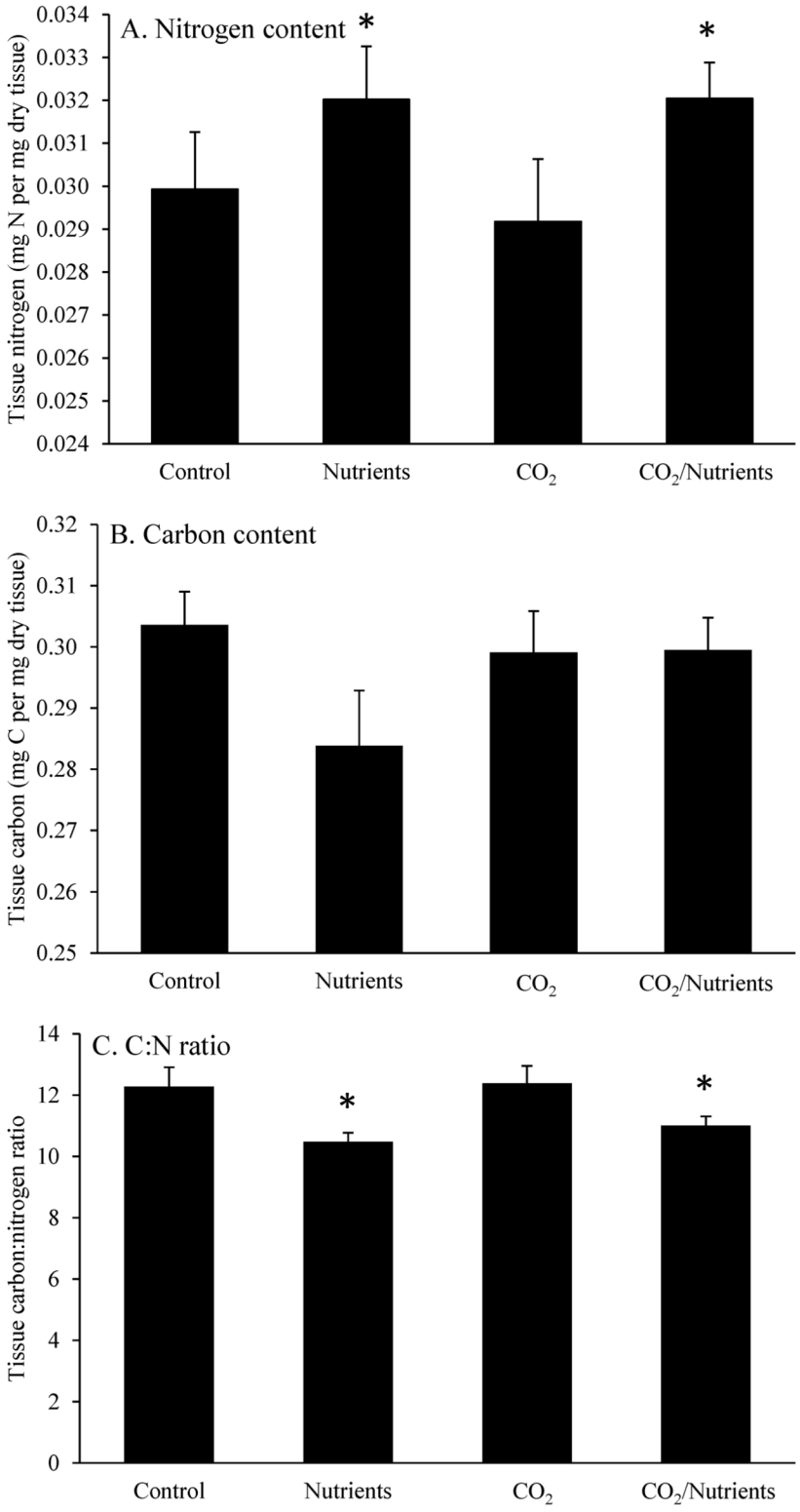

Figure 5: Tissue nitrogen, carbon, and C:N content of *Gracilaria* exposed to ambient and elevated CO_2 conditions with and without nutrient additions for experiments performed August through November.

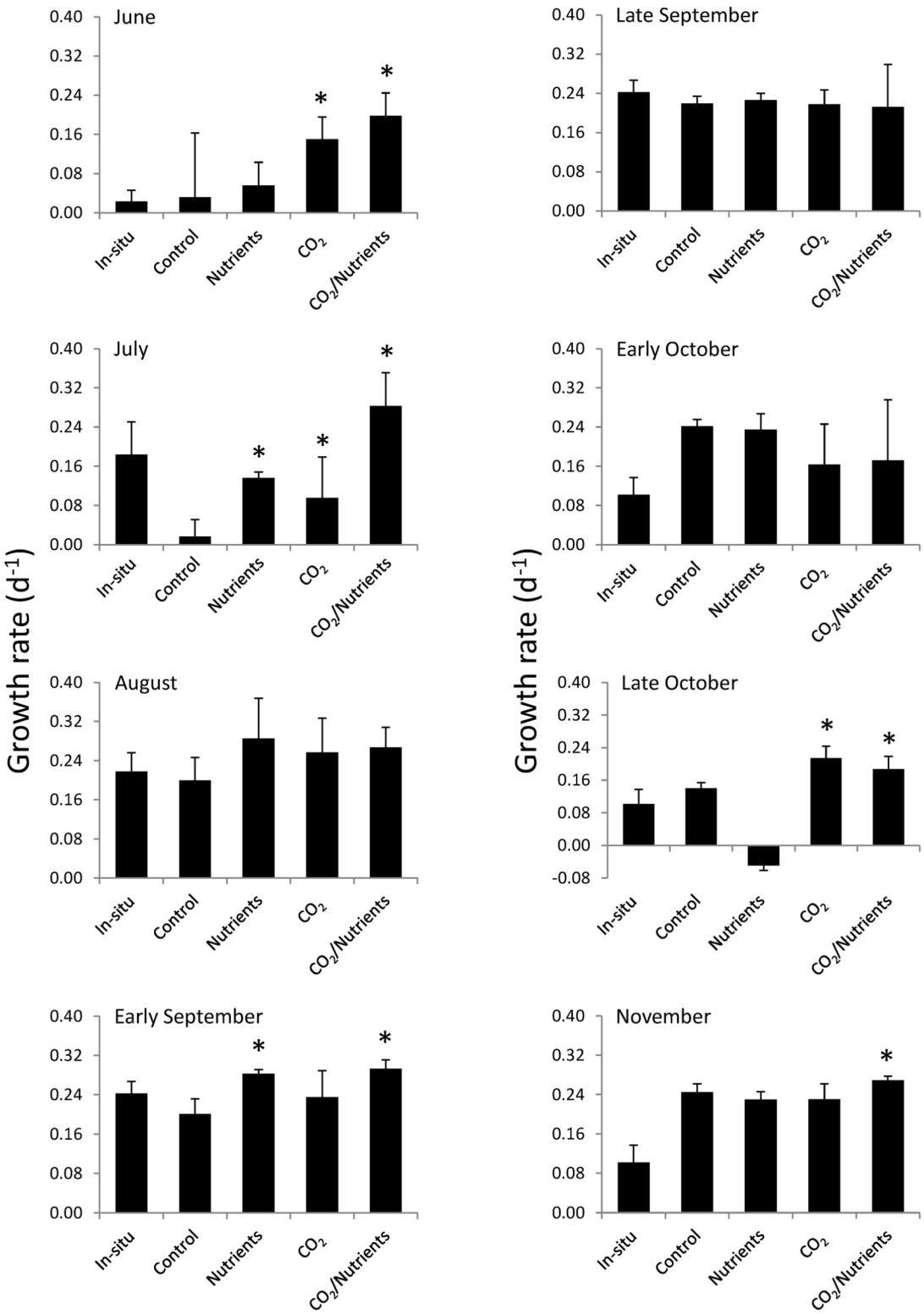

Figure 6: Growth rates of *Ulva* exposed to ambient and elevated CO_2 conditions with and without nutrient additions for experiments performed August through November. Columns with an asterisk over them indicate significant results.

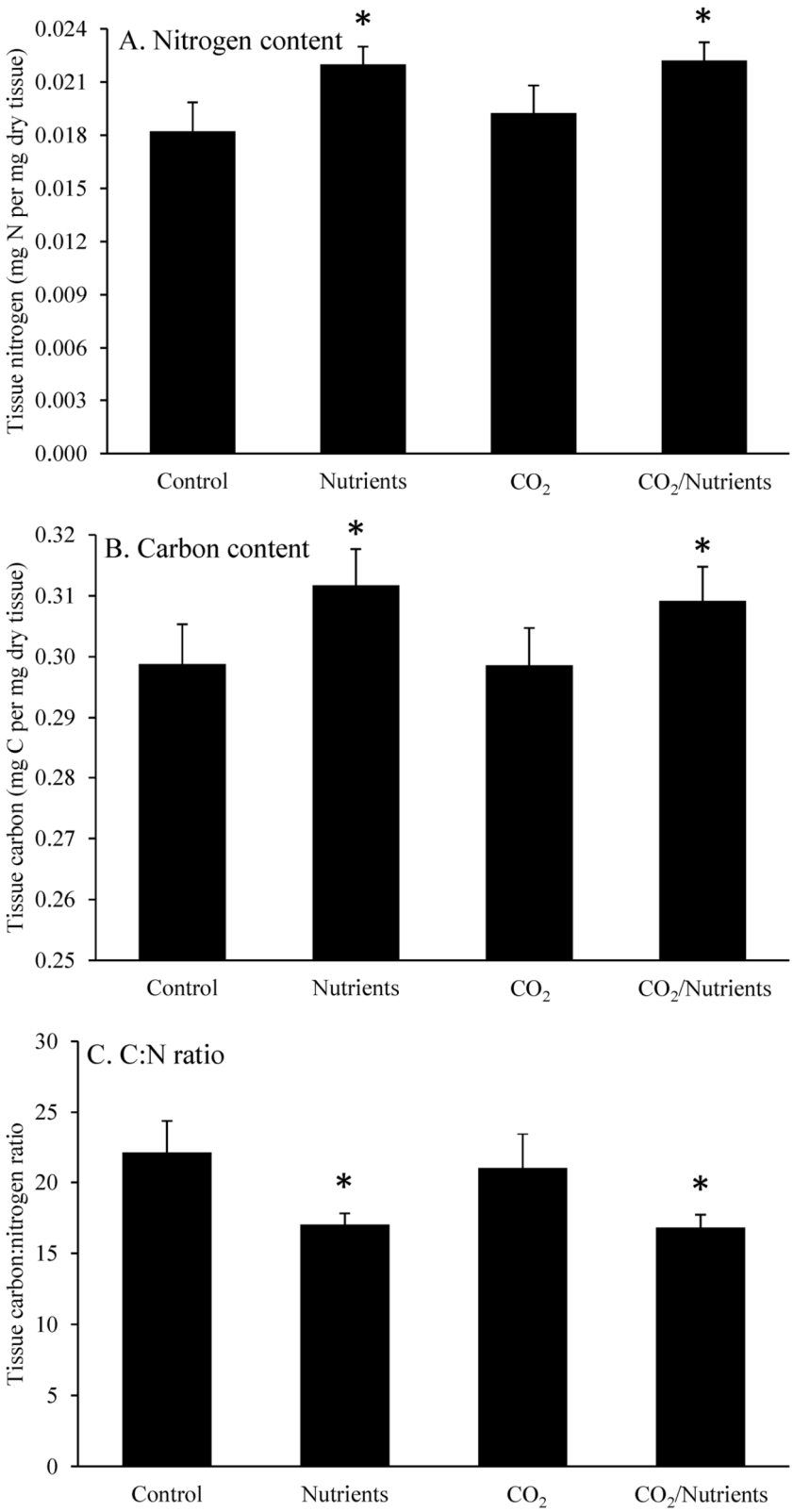

Figure 7: Tissue nitrogen, carbon, and C:N content of *Ulva* exposed to ambient and elevated CO_2 conditions with and without nutrient additions for experiments performed August through November.

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Chapter 2

The organizing effects of elevated CO_2 on competition among estuarine primary producers

Introduction

By the end of the century, the diffusion of CO_2 from fossil fuel combustion into surface oceans is expected to cause CO_2 and HCO_3^- levels to increase 260% and 20%, respectively (Meehl et al., 2007). Beyond the combustion of fossil fuels, upwelling, and riverine discharge, another prominent CO_2 source in coastal ecosystems is eutrophication-enhanced microbial respiration (Cai et al., 2011; Melzner et al., 2013; Wallace et al., 2014). The degradation of excessive organic matter can lead to the seasonal accumulation of respiratory CO_2 which lowers seawater pH and increases pCO_2 to levels not expected in the open ocean until next century (>1,000 µatm; Wallace et al., 2014). Shifts in the concentrations of various inorganic carbon sources within the total dissolved inorganic carbon (DIC) pool are likely to elicit a variety of responses from marine flora and fauna. Decreased availability of CO_3^{2-} can inhibit the growth of calcifying organisms (Talmage and Gobler, 2010; Gazeau et al., 2013; Kroeker et al., 2013) while the increased availability of CO_2 may benefit some, but not all, photosynthetic organisms (Palacios and Zimmerman, 2007; Koch et al., 2013; Hattenrath-Lehmann et al., 2015; Young and Gobler, 2016). The photosynthetic organisms most likely to benefit from an increase in CO_2 levels are non-calcifying autotrophs whose inorganic carbon uptake is not substrate-saturated at present CO_2 concentrations (Koch et al., 2013).

Numerous non-calcified marine autotrophs have been shown to benefit from anthropogenically-induced changes in carbonate chemistry. Marine photosynthetic organisms acquire carbon through the active transport of CO_2 and HCO_3^- as well as the diffusive uptake of CO_2 (Badger, 2003). Since HCO_3^- is more abundant than CO_2 in seawater, many marine autotrophs rely on carbon concentrating mechanisms (CCM) and intracellular or extracellular carbonic anhydrase (CA) to convert HCO_3^- to CO_2 for use by RuBisCO (Gao and McKinley,

1994; Israel and Hophy, 2002; Badger, 2003; Koch et al., 2013). For marine macroalgae, a variety of chlorophytes, phaeophytes, and rhodophytes are able to utilize HCO_3^- and CO_2 for photosynthesis (Gao and McKinley, 1994). When exposed to elevated CO_2, some chlorophytes such as *Ulva rigida* and *U. lactuca* experience increased growth (Björk et al., 1993; Olischläger et al., 2013; Young and Gobler, 2016), while others do not (Rautenberger et al., 2015). Non-calcifying rhodophytes such as *Gracilaria lemaneiformis, G. tikvahiae*, *Chondrus crispus* (Xu et al., 2010; Hofmann et al., 2012; Young and Gobler, 2016), and phaeophytes such as the giant kelp (*Macrocystis pyrifera*) (Hepburn et al., 2011) have been shown to benefit from elevated CO_2 concentrations. Elevated CO_2 can also accelerate the growth of individual species of phytoplankton within multiple classes, including dinoflagellates (*Karlodinium veneficum, Alexandrium fundyense, Alexandrium ostenfeldii*) (Fu et al., 2010; Kremp et al., 2012; Hattenrath-Lehmann et al., 2015), diatoms (*Skeletonema costatum*, *Pseudo-nitzschia fraudulenta*, *Pseudo-nitzschia multiseries*) (Kim et al., 2006; Sun et al., 2011; Tatters et al., 2012), and raphidophytes (*Heterosigma akashiwo*) (Fu et al., 2008). However, not all species within these groups benefit, as is the case of several dinoflagellates (Fu et al., 2008; Kremp et al., 2012; Hattenrath-Lehmann et al., 2015). Additionally, some studies have found that natural phytoplankton community growth and composition will be unaffected by increases in pCO_2 to levels predicted by 2100 (Berge et al., 2010; Nielsen et al., 2012).

The community structure of marine autotrophs is strongly shaped by competition, which can be affected by relative abundance of resources such as nutrients, light, and inorganic carbon. For example, as nutrient loading increases, macroalgae gain a competitive advantage over seagrass (Valiela et al., 1997). A similar trend can be found within macroalgal communities, as increased nitrogen loading can favor fast-growing species, such as *Ulva* spp. over slower-

growing ones (Valiela et al., 1997) due to the former possessing higher rates of maximum nutrient uptake (Pedersen and Borum, 1997). Continued nitrogen loading, however, can shift the competitive advantage in favor of phytoplankton, which often have a higher V_{max}, a lower K_m, and a higher α than macroalgae (Hein et al., 1995), thus allowing for faster nutrient acquisition and dominance under conditions of extreme nutrient loading rates and extended residence times (Valiela et al., 1997). Shifts in the concentration and speciation of inorganic carbon in estuaries may also drive competition among autotrophs. In the presence of high CO_2, some species of macroalgae may down-regulate their CCMs, thus permitting more energy to be available for other processes such as vegetative growth (Koch et al., 2013; Young and Gobler, 2016) or may shift towards diffusive uptake of CO_2 over use of a CCM to relieve carbon limitation (Mercado et al., 1998; Young and Gobler, 2016). Some algal species rely strictly on the diffusive uptake of CO_2 or the active transport of HCO_3^-, with most species being capable of using both forms of carbon (Gao and McKinley, 1994). Thus, the physiological responses of individual algae to increased CO_2 may alter community structure.

Recently, I have demonstrated that populations of *Ulva rigida* and *Gracilaria tikvahiae* from Northwest Atlantic coastal waters experience accelerated growth and likely CO_2 uptake when exposed to elevated pCO_2 (Young and Gobler, 2016). The objective of this study was to assess how elevated concentrations of CO_2 influences competition among estuarine autotrophs including *Ulva rigida*, *Gracilaria tikvahiae*, diatoms, and dinoflagellates. Each macroalgal population was grown with and without elevated levels of pCO_2 as well as with and without the other alga and with and without ambient plankton populations. The growth responses, $\delta^{13}C$ signatures, and elemental composition of algae were evaluated at the start and end of experiments performed through the growing season of these macroalgal populations.

Methods

Macroalgae Collection and Preparation

Macroalgae used for this study were collected from Shinnecock Bay, NY, USA (40.85° N, 72.50°) during low tide. Permission to access the water and collect the water and macroalgae was received from the Southampton Town Trustees, Southampton, NY, USA, who hold jurisdiction over Shinnecock Bay. Large, well-pigmented, robust fronds of *Ulva* and *Gracilaria* were collected and transported to the Stony Brook Southampton Marine Science Center in seawater-filled containers within 15 minutes of collection. My prior research has used DNA sequencing and microscopy to determine that *Ulva rigida* and *Gracilaria tikvahiae* are the species *Ulva* and *Gracilaria* present at my sampling site during summer and fall (Young and Gobler, 2016). The visual and microscopic analyses during this study affirmed that identification. Due to the plastic nature of macroalgal taxonomic nomenclature as well as the high similarity of ITS sequences among *Ulva* species (Hofmann et al., 2010; Kirkendale et al., 2013), for the purposes of this study and consistency with prior studies (Young and Gobler, 2016), we refer to these algae simply as *Ulva* and *Gracilaria*. Individual thalli of *Gracilaria* approximately 5 cm in length were cut from the main plant and placed in a salad spinner to remove debris and epiphytes. Samples were extensively rinsed with filtered (0.2 μm) seawater and placed back into the salad spinner to further remove debris, epiphytes, and excess seawater. Circular sections of similar length of *Ulva* were cut from large thalli with care taken to avoid the outer, potentially reproductive region of the plant (Wallace and Gobler, 2015). Samples of *Ulva* were prepared using the same cleaning procedures as *Gracilaria*. All samples were weighed on an A&D EJ300 digital balance (± 0.01 g) to obtain initial wet weight in grams. To prevent

desiccation, all samples were kept in 100 mL filtered (0.2 μm) seawater-filled containers after spinning and weighing but prior to use in experiments.

In situ Growth Experiments

To assess growth rates of *Gracilaria* and *Ulva* within the region of Shinnecock Bay from which they were collected, *in situ* growth experiments were performed monthly from June through October. Quadruplet, 0.25 m^2 incubation cages constructed from 1 cm^2 wire mesh were attached to a four-armed (25 cm) umbrella fishing apparatus on a line with surface flotation and a bottom weight to keep the cages suspended at 0.2 m (Wallace and Gobler, 2015; Young and Gobler, 2016). Continuous measurements of light and temperature were made using HOBO pendant temperature and light loggers. Thalli of each species of macroalgae were placed in each quadruplet cage for approximately one week in parallel with laboratory experiments (*described below*) after which thalli were recovered, brought to the lab, and rinsed, spun, re-rinsed, re-spun, and weighed as described above. Samples of *Gracilaria* and *Ulva* were frozen for further tissue analysis. Weight-based growth rates for both species were determined using the relative growth rate formula (growth d^{-1}) = (ln W_{final} – ln $W_{initial}$) / (Δt), where W_{final} and $W_{initial}$ are the final and initial weights in grams and Δt is the number of days of the experiment.

Assessing the Effects of Elevated pCO_2 and Competition

Five laboratory experiments were performed to assess the effects of competition and elevated pCO_2 on the growth of *Gracilaria, Ulva*, and natural plankton communities during early July, late July, August, September and October. Polycarbonate bottles (2.5 L) were acid washed (10% HCl) and liberally rinsed with deionized water before use. Experimental bottles were

placed in an environmental control chamber set to the approximate temperature (~16-21°C) and light intensity (~400 µmol s^{-1} m^{-2}) and duration (14 h: 10 h light : dark cycle) present during *in situ* experiments. Bottles were filled with filtered (0.2µm polysulfone filter capsule, Pall) with the plankton community removed or unfiltered seawater with the full plankton community. For the early and late July, and August experiments, bottles were randomly assigned, in triplicate to one of four treatments: a control with ambient levels of pCO_2 (~400 µatm) in filtered seawater (no plankton), a treatment with ambient pCO_2 in unfiltered seawater (with plankton), a treatment with elevated pCO_2 (~2,500 µatm) in filtered seawater (no plankton), and a treatment with elevated pCO_2 in unfiltered seawater (with plankton). Three sets of these bottles were established: One for *Ulva*, one for *Gracilaria*, and one with both *Ulva* and *Gracilaria* resulting in a total of 36 experimental bottles. For the September and October experiments, bottles were randomly assigned to the aforementioned treatments, but in quadruplicate. Additionally, eight bottles were filled with seawater only with four bottles being subjected to ambient pCO_2, and the other four being subjected to elevated pCO_2. All bottles for each experiment received nutrient additions (50µM nitrate, 3 µM phosphate) at the beginning of the experiment to ensure nutrient replete growth. The nutrient and pCO_2 concentrations used during experiments were higher than what is present at the collection site, but are within the range of concentrations present in eutrophic US East Coast estuaries (Wallace et al. 2014; Wallace and Gobler 2015) and used during prior experiments with *Ulva* and *Gracilaria* from Shinnecock Bay, NY, USA (Young and Gobler, 2016).

Each bottle was aerated via a 1.5" x 0.5" (~3.8 x 1.3 cm) air diffuser (Pentair) connected to a 1 mL, polystyrene serological pipette inserted to the bottom of each bottle and connected via tygon tubing to an air source. Bottles were subjected to the control (~400 µatm) and elevated

(~2500 µatm) levels of pCO_2 via a gas proportionator system (Cole Parmer® Flowmeter system, multitube frame) that mixed ambient air with 5% CO_2 gas (Talmage and Gobler, 2010). The gas mixtures were delivered at a net flow rate of 2500 ± 5 mL min^{-1} through an 18- or 14-way gang valve into the serological pipettes that fit through an opening in the closed cap of the bottle. The delivery rate of gases turned over the volume of the experimental bottles >1,00 times daily (Talmage and Gobler, 2010). Bubbling began two days prior to beginning each experiment allowing pCO_2 concentrations and pH levels to reach a state of equilibrium. Experiments persisted for one week. Measurements of pH within bottles were made daily through use of an Orion Star A321 Plus electrode (± 0.001) calibrated prior to use with National Institute of Standards and Technology (NIST) traceable standards. DIC concentrations in bottles were measured using an EGM-4 Environmental Gas Analyzer (PP Systems) system that quantifies DIC levels after separating the gas phase from seawater by acidification using a Liqui-Cel Membrane (Membrana) (Talmage and Gobler 2010). As a quality assurance measure, the levels of DIC and pH with Dr. Andrew Dickson's (University of California, San Diego, Scripps Institution of Oceanography) certified reference material (batches 142, 147, 151) were measured during analyses of every set of samples. The analysis of samples continued only after complete recovery of the certified reference material was attained. Levels of pCO_2 (mean of t=initial and t=final, Table 1) 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 according to Millero et al. (2010) using the program CO2SYS (http://cdiac.ornl.gov/ftp/co2sys/). The targeted levels of pCO_2 resulted in actual pCO_2 and pH values of ~400 µatm and ~8.0, respectively, for ambient conditions and ~2600 µatm and ~7.2, respectively, for the elevated CO_2

conditions, mimicking the range found seasonally in estuarine environments (Melzner et al., 2013; Wallace et al., 2014; Baumann et al., 2015).

Experiments began with the introduction of macroalgae and nutrients into experimental bottles. HOBO pendant temperature and light data loggers were used to continuously monitor light levels. At the end of experiments, final pH, temperature, and salinity measurements were made and a final DIC was collected and analyzed as described above. After measuring DIC, all macroalgae samples were removed from their respective bottles and rinsed, spun, re-rinsed, re-spun, and weighed as described above. *Gracilaria* and *Ulva* samples were placed into small freezer bags for further analyses. Weight-based growth rates for both species were determined as described above. Significant differences in growth rates were assessed using three-way ANOVA with SigmaPlot 11.0, where the main treatments were pCO_2 treatment (ambient or elevated), the presence of plankton (filtered or unfiltered seawater), and competition (each macroalgal species alone or in the same bottle). Additionally, one-way ANOVA were used to compare the growth rates of the control group and the *in situ* experiments.

The growth and composition of the plankton community was assessed during the September and October experiments by removing 50 mL aliquots of seawater from experimental bottles in unfiltered seawater treatments at the beginning and at the conclusion of each experiment and preserving samples with Lugol's iodine. Aliquots were placed in Sedgewick-Rafter chambers and enumerated using a light microscope, an approach that permitted the quantification of plankton > 10 μm (Wallace and Gobler, 2015). More than 200 cells were quantified per sample. For the purposes of this study, the most abundant plankton groups were quantified, specifically diatoms and dinoflagellates. Significant differences in abundance were

assessed using three-way ANOVA with SigmaPlot 11 where the main treatments were pCO_2 (ambient or elevated), *Ulva* (with or without *Ulva*), and *Gracilaria* (with or without *Gracilaria*).

Tissue Analyses

For carbon (C), nitrogen (N), and stable carbon isotope ($\delta^{13}C$) analyses, frozen samples of *Gracilaria* and *Ulva* were dried at 55°C for 48 h and then homogenized into a fine powder using a mortar and pestle. The 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. Significant differences in tissue content for each species of algae and class of plankton during experiments were assessed using three-way ANOVA within SigmaPlot 11.0 where the main treatment effects were pCO_2 treatment (ambient or elevated), the presence of plankton (filtered or unfiltered seawater), and competition (each macroalgal species alone or in the same bottle).

Results

Gracilaria

The *in situ* growth of *Gracilaria* in Shinnecock Bay was found to be similar to and not significantly different from growth rates within the control groups of experiments with the exception of the early July and August experiment, when experimental growth rates were slightly lower and higher, respectively, than those *in situ* (One-way ANOVA; $p < 0.05$; Fig. 2; S6 Tables). The growth rates of *Gracilaria* within the experimental groups were found to be sensitive to changes in CO_2 concentrations (Fig. 2). During experiments in late July, August, and

October, the growth of *Gracilaria* increased significantly when exposed to elevated CO_2 concentrations (Three-way ANOVA; $p < 0.05$; Fig. 2; S6 Tables). On average, growth rates under elevated CO_2 were 37% higher and 30% higher than growth under ambient conditions in experimental bottles filled with filtered and unfiltered seawater, respectively (Fig. 2). Growth rates of *Gracilaria* were not affected by the presence of *Ulva* and were mostly unaffected by the presence of plankton with the exception of the early July experiment when plankton significantly slowed the growth of *Gracilaria* (Three-way ANOVA; $p < 0.05$; Fig. 2; S6 Tables). During the August experiment, there was an interaction between CO_2, competition with *Ulva*, and competition with plankton, whereby elevated CO_2 significantly enhanced growth rates within filtered treatments (Three-way ANOVA; $p < 0.05$; Fig. 2; S6 Tables) but not within unfiltered treatments (Three-way ANOVA; $p > 0.05$; Fig. 2; S6 Tables). Additionally, in this same experiment, growth was significantly higher under elevated CO_2 in treatments without *Ulva* (Three-way ANOVA; $p < 0.05$; Fig. 2; S6 Tables), but not in treatments with competition from *Ulva*, demonstrating that *Ulva* altered the response of *Gracilaria* to CO_2 in this experiment.

The $\delta^{13}C$ content of *Gracilaria* was significantly reduced by elevated CO_2 delivery, with the average of the ambient and elevated CO_2 treatments being, on average, -13‰ and -24‰, respectively (Three-way ANOVA; $p<0.001$; Fig. 3; S6-S7 Tables). Overall, there was no significant difference in $\delta^{13}C$ between filtered and unfiltered seawater treatments, regardless of CO_2 concentration (Three-way ANOVA; $p>0.05$; S6-S7 Tables). Additionally, there was no significant difference in $\delta^{13}C$ caused by exposure to *Ulva*. On average, the tissue C content of *Gracilaria* was largely unaffected by CO_2 concentration, competition with *Ulva*, and competition with plankton (Three-way ANOVA; $p>0.05$; Fig. 4; S6 and S8 Tables). However, elevated CO_2 was found to have significantly increased the tissue C content relative to the ambient

concentration for the late July experiment (Three-way ANOVA; $p<0.05$; S6 Tables). Competition with *Ulva* significantly reduced tissue N of *Gracilaria* for the August, September, and October experiments, while competition with plankton significantly decreased tissue N for all experiments with the exception of the August experiment (Three-way ANOVA; $p < 0.05$; S6 and S8 Tables). Elevated CO_2 treatments resulted in decreased tissue N for only the September experiment (S6 and S8 Tables). The tissue C:N ratio of *Gracilaria* was unaffected by elevated CO_2 concentrations (Three-way ANOVA; $p > 0.05$; S6 and S8 Tables), but was found to be significantly higher during competition with *Ulva* during the August experiment and during competition plankton assemblages during the early and late July experiments (Three-way ANOVA; $p<0.05$; Fig. 4; S6 and S8 Tables).

Ulva

The growth rates of *Ulva* during *in situ* experiments did not differ statistically from those found within the control treatment of experiments (One-way ANOVA; $p > 0.05$; Fig. 5; S6 Tables). The response of *Ulva* to the different variables within the experimental bottles was more complex compared to *Gracilaria*. Overall, growth by *Ulva* was found to be significantly higher under elevated pCO_2 concentrations and significantly higher in treatments without *Gracilaria* and competing plankton (Three-way ANOVA; $p < 0.05$; Fig. 5; S6 Tables). During four of the five experiments (early and late July, August, and September), the growth of *Ulva* increased significantly when exposed to elevated pCO_2 concentration, increasing, on average, 38% and 44% relative to ambient treatments in filtered and unfiltered treatments, respectively (Three-way ANOVA; $p < 0.05$; Fig. 2; S6 Tables). On average, *Ulva* growth rates were ~ 20% lower when grown in the presence of plankton, and 12% lower when grown in the presence of *Gracilaria*

(Fig. 5). During the early July experiment, the presence of plankton depressed the growth of *Ulva* as did the presence of *Gracilaria* (Three-way ANOVA; $p < 0.05$; Fig. 5; S6 Tables). *Ulva* growth in the presence of plankton was also significantly reduced during the late July experiment (Three-way ANOVA; $p < 0.05$; Fig. 5; S6 Tables). As independent variables, plankton and *Gracilaria* did not significantly alter *Ulva* growth rates during the September experiment, but there was a synergistic interaction between elevated pCO_2 and the absence of plankton in slowing *Ulva* growth (Three-way ANOVA; $p < 0.05$; Fig. 5; S6 Tables). During the October experiment, the growth of *Ulva* was not affected by any treatment.

The $\delta^{13}C$ content of *Ulva* was significantly reduced by exposure to elevated CO_2 concentrations, with the average $\delta^{13}C$ of the ambient and elevated CO_2 treatments being -12‰ and -33‰, respectively (Three-way ANOVA; $p<0.001$; Fig. 3; S6-S7 Tables). For the entire study, the $\delta^{13}C$ of *Ulva* was not significantly altered by the presence of *Gracilaria* or plankton (Three-way ANOVA; $p>0.05$; S6-S7 Tables). The $\delta^{13}C$ was, however, found to be significantly lower in treatments with plankton present for the August and September experiments (Three-way ANOVA; $p<0.05$; S6-S7 Tables). Tissue C content of *Ulva* was not significantly affected by elevated CO_2 concentrations, competition with *Gracilaria*, or competition with plankton (Three-way ANOVA; $p<0.05$; Fig. 6; S6 and S8 Tables). In contrast, during each experiment tissue N content was significantly lower when *Ulva* was grown in the presence of plankton, with the exception of the October experiment (Three-way ANOVA; $p<0.05$; Fig. 6; S6 and S8 Tables). The tissue C:N ratio of *Ulva* was significantly higher in the presence of plankton during each experiment except October (Three-way ANOVA; $p<0.05$; Fig. 6; S6 and S8 Tables).

Plankton

Regarding plankton communities, at the onset of the September and October experiments, the dominant plankton >10 μm were diatoms, whereas at the end of experiments, the abundance of diatoms diminished and dinoflagellates became more prominent. The growth rates of diatoms and dinoflagellates were found to significantly decrease and increase, respectively, during exposure to elevated CO_2 during the September and October experiments (Three-way ANOVA; $p < 0.05$; S6 Tables). Diatoms and dinoflagellate growth rates were also affected by the species of macroalgae present. Diatom growth rates were significantly higher in treatments containing *Ulva* compared to treatments without (Three-way ANOVA; $p < 0.05$; Fig. 7; S6 Tables). Dinoflagellates growth was significantly decreased in the presence of *Gracilaria* (Three-way ANOVA; $p < 0.05$; Fig. 7; S6 Tables).

Discussion

During this study, elevated CO_2 concentrations significantly enhanced the growth rates of *Gracilaria*, *Ulva,* and dinoflagellates, but not diatoms. For *Gracilaria*, growth rates were largely unaffected by the presence of *Ulva* and plankton whereas the growth rates of *Ulva* were significantly depressed when grown with *Gracilaria* or the full plankton community. Among the phytoplankton, diatom growth benefited from the presence of *Ulva*, while the growth rates of dinoflagellates were slowed by *Gracilaria.* For both macroalgae, the $\delta^{13}C$ was significantly lowered by elevated pCO_2 while their N content was reduced by competition with the other macroalgae species and/or plankton. Together these findings provide novel insight regarding the outcomes of competition among primary producers under high CO_2 conditions.

Most macroalgae are capable of active transport of HCO_3^- or CO_2 into their CCM or the diffusive uptake of CO_2 (Badger, 2003). High CO_2 concentrations may cause macroalgae to down-regulate CCMs that convert HCO_3^- to CO_2 (Björk et al., 1993; Gao et al., 1993; Xu et al., 2010; Cornwall et al., 2012) resulting in more energy available for other processes such as vegetative growth (Koch et al., 2013; Young and Gobler, 2016). The amount of energy saved by this process is not fully clear, as the process depends on several external factors, such as PAR, and internal factors, such as type of CCM used by the macroalgae, or the potential leakage of carbon dioxide from the CCM (Raven et al., 2014). The $\delta^{13}C$ signatures of macroalgae during this study suggested these species switched from HCO_3^- to CO_2 use and potentially downregulated their CCMs as values prior to the start of the experiments (-12-13‰) were reflective of HCO_3^- and CCM use whereas the more negative values of macroalgae at the end of the experiment (-23.6 ± 5‰ and -33.5 ± 5‰ for *Gracilaria* and *Ulva*, respectively) were within the range expected of macroalgae relying more diffusion of CO_2 (Maberly et al., 1992; Raven et al., 2011; Hepburn et al., 2011) using isotope mixing models to account for the lighter CO_2 gas used in experiments (Young and Gobler, 2016). It is also possible that higher pCO_2 alleviated inorganic C limitation and enhanced growth rates. Mercado et al. (1998) reported that *U. rigida* and *U. compressa* (formerly *Enteromorpha*) do not receive enough CO_2 through diffusive uptake at current CO_2 levels, a finding consistent with the enhanced growth of *Ulva* during this study and supported by the shift in $\delta^{13}C$ during this study for both *Ulva* and *Gracilaria*. Regardless, the enhanced growth rates for these macroalgae under higher CO_2 indicate that inorganic C limitation was alleviated.

Consistent with prior studies of macroalgae, changes in CO_2 levels did not alter tissue C and N content (Gordillo et al. 2001, Young and Gobler 2016) and competition with other

autotrophs did not alter their C content. In contrast, competition with other autotrophs resulted in significantly decreased N content and decreased tissue C:N ratios for *Gracilaria* and *Ulva*. Both macroalgal species are able to rapidly assimilate and store nitrate (Ryther et al., 1981; Fan et al., 2014) and have been shown to experience enhanced tissue N content when exposed to excessive nitrate concentrations (Naldi and Wheeler, 1999; Liu et al., 2009). Compared to *Gracilaria*, *Ulva* is capable of undergoing more rapid growth in eutrophic settings (Valiela et al., 1997; Wallace and Gobler, 2015) due to a high maximum rate of uptake of nutrients such as nitrate (Pedersen and Borum, 1997). Phytoplankton are superior competitors for N compared to macroalgae (Hein et al., 1995; Valiela et al., 1997). The significant declines in N content of macroalgae when grown with phytoplankton and elevated C:N ratios of macroalgae at the end of experiments (15 – 40), despite the high levels of N present at the start of experiments (50μM), affirms the role of N as a limiting element in this (Mulholland et al., 2002) and other estuaries (Nixon, 1995) and suggests this N was likely depleted over the course of the experiment. This is almost certainly the case in experiments with the full plankton community intact as uptake rates of plankton communities can exceed 25 μM per day in Shinnecock Bay (Mulholland et al., 2002). The precise outcomes of competition among estuarine autotrophs exposed to high CO_2, therefore, will be partly dependent upon ambient nutrient supplies.

Beyond tissue content of macroalgae, the importance of both N and pCO_2 in shaping algal community composition was also evident in the competitive growth responses of macroalgae. Nutrient loading favors fast-growing macroalgae with rapid uptake rates of nutrients over slower-growing counterparts (Pedersen and Borum, 1997; Valiela et al., 1997). The growth rates of *Ulva* were, on average, three-times faster than *Gracilaria* during experiments and thus, despite a 55-60% lower tissue N content, had a significantly larger N

demand making it more prone to N limitation, especially when placed in competition with other autotrophs. This hypothesis is supported by the C:N ratios of *Ulva* which were significantly higher than those of *Gracilaria* throughout this study ($p<0.001$; T-test), suggesting *Ulva* was more N-limited. Similarly, the presence of phytoplankton, which are able to outcompete macroalgae for nutrients, may have further depleted nutrient concentrations during experiments, thus causing the decreased growth of *Ulva* in unfiltered treatments for some of the experiments (Valiela et al., 1997). Again, this hypothesis is supported by the significant increase in the C:N ratio that *Ulva* experienced when grown in the presence of phytoplankton communities. Collectively these findings suggest that while pCO_2 enhances the growth of *Ulva* and *Gracilaria*, the slower-growing *Gracilaria* is better adapted for persisting at more dynamic nutrient concentrations than *Ulva* (Pedersen and Borum, 1996 & 1997). In a field experiment by Fujita (1985), when N was introduced in pulses every five days, *Gracilaria tikvahiae* was able to outcompete *Ulva lactuca* in mixed macroalgal beds, despite the latter possessing a more rapid N uptake rate. Furthermore, the *Gracilaria vermiculophylla*, normally found in the West Pacific, has invaded northern European estuaries as early as 2002, and has become among the most abundant macroalgae in the region, despite competition with *Ulva* and other ephemeral algae (Thomsen et al., 2007). However, were nutrients continuously added during experiments, it is plausible that the growth of *Ulva* would have been unaffected by other autotrophs. Hence, the outcome of competition among estuarine autotrophs exposed to high CO_2 depend, at least in part, on ambient N levels.

Dinoflagellates experienced more rapid growth when exposed to high CO_2 while diatoms did not. Results from prior studies suggest that the response of phytoplankton communities to elevated CO_2 concentrations are likely to depend on the species present but that dinoflagellates

are more prone to C-limitation than diatoms as dinoflagellates possess form II RubisCO, which has a low affinity for CO_2 (Rost et al., 2006; Reinfelder, 2011). The dinoflagellates *Protoceratium reticulatum* (Ratti et al. 2007), *Karlodinium veneficum* (Fu et al., 2010) and *Karenia brevis* (Errera et al., 2014) all grow more rapidly under high CO_2 as do *Alexandrium* species from Europe (*Alexandrium minutum*, Flores-Moya et al. 2012; *Alexandrium ostenfeldii*, Kremp et al. 2012) and the North America (*Alexandrium catenella*; Fu et al. 2012; Tatters et al. 2013a; *Alexandrium fundyense* Hattenrath-Lehmann et al., 2015). While the general response of diatoms to elevated CO_2 also appears to be species-specific, they seem to be generally less sensitive to changes in pCO_2. Dozens of diatom species realize maximal growth rates under a wide range of pH/ pCO_2 levels (Chen and Durbin 1994; Taraldsvik and Myklestad 2000; Hinga 2002; Berge et al., 2010), although elevated CO_2 enhances the growth rates of some species including *Pseudo-nitzschia fraudulenta* (Tatters et al., 2012), *Pseudo-nitzschia multiseries* (Sun et al., 2011), and *Chaetoceros debilis* (Trimborn et al., 2013). Hence, my findings of CO_2-stimulated growth of dinoflagellates but not diatoms are generally consistent with prior studies, but specific responses will depend on, among other factors, nutrient levels, the species of phytoplankton present within a community, as well as competition with other autotrophs. I did not identify phytoplankton to the species level during this study. Regardless, given dinoflagellates are responsible for most harmful algal blooms (HABs; Smayda, 1997) and that HABs are common within eutrophic settings (Heisler et al., 2008), the findings here suggest that high CO_2, eutrophic estuaries may be more likely to host HABs with negative ecosystem consequences (Hattenrath-Lehmann et al., 2015).

Diatom and dinoflagellate growth rates were also affected by macroalgae with dinoflagellates growth being inhibited by *Gracilaria* but *Ulva* promoting the growth of diatoms.

Prior studies have found that dinoflagellates in temperate estuaries are vulnerable to allelopathic inhibition by macroalgae (Tang and Gobler, 2011; Tang et al., 2014) and *Gracilaria* spp. have been shown to allelopathically depress dinoflagellate growth rates (Wang et al., 2007; Lu et al., 2011). While *Ulva* has been found to allelopathically inhibit the growth of individual dinoflagellate species in culture (Tang and Gobler, 2011), during this study *Ulva* was found to have no effect on dinoflagellates but promoted the growth of diatoms. This finding indicates that *Ulva* may generally promote a succession within phytoplankton communities from dinoflagellates to diatoms, potentially via the remineralization of nutrients (Wang et al., 2012) that promotes the growth of diatoms. The growth promotion of diatoms may be associated with the ability of *Ulva* to release and regenerate nutrients such as ammonium and phosphate (Lyngby et al., 1999; Wang et al., 2012). Another possibility is that vitamin B_{12}-producing epiphytic bacteria on *Ulva* may have promoted the growth of diatoms. Diatoms are unable to synthesize vitamin B_{12} and as such, require bacteria for the production of the vitamin (Haines and Guillard, 1974; Croft et al., 2006). Udell et al. (1969) found samples of *Ulva lactuca* in the same contiguous water body as my study site to be rich in vitamin B_{12} likely due to epiphytic bacteria. It is possible that the synthesis of vitamin B_{12} by epiphytic bacteria could have promoted the growth of diatoms in treatments containing *Ulva.*

There are numerous ecosystem implications of the overgrowth of macroalgae, such as *Ulva* and *Gracilaria*, due to the ability to outcompete autotrophs due to increased nutrient loading and CO_2 concentrations. The overgrowth of bloom-forming macroalgae has been shown to have negative effects on seagrass meadows (Valiela et al., 1997; Hauxwell et al., 2001), kelp forests (Connell et al., 2013), coral reefs (Anthony et al., 2011; Connell et al., 2013) and even phytoplankton communities (Tang and Gobler, 2011; Lu et al., 2011; Tang et al., 2014).

Although seagrass can experience enhanced growth in the presence of elevated CO_2 concentrations (Palacios and Zimmerman, 2007), increased nutrient loading favors macroalgal growth that can lead to the demise of seagrass (Valiela et al., 1997; McGlathery, 2001). Aside from the direct deleterious effects of ocean acidification on coral reefs and calcifying invertebrates (Doney et al., 2009), continued eutrophication and ocean acidification may allow fast-growing macroalgae to overgrow substrate used by coral (Hughes et al., 2003). The overgrowth of macroalgae also can cause mortality in some invertebrates (Magre, 1974; Johnson and Welsh, 1985; Nelson et al., 2003). While the giant kelp (*Macrocystis pyrifera*), may benefit from elevated CO_2 concentrations (Hepburn et al., 2011), overgrowth of bloom-forming macroalgae by the same CO_2 concentrations may allow the algae to overtake substrate, thus inhibiting kelp recruitment (Kennelly, 1987; Connell et al., 2010 & 2013). While excessive nutrient loading in estuaries with relatively long residence times favor phytoplankton over macroalgae, the ability of both *Ulva* and *Gracilaria* to benefit from high CO_2 and to inhibit the growth of some phytoplankton via allelopathy may allow macroalgae to remain dominant in some high nutrient, high CO_2 estuaries (Tang and Gobler, 2011; Lu et al., 2011; Tang et al., 2014). Finally, macroalgae blooms may overgrow seagrass beds (Valielia et al., 1999; Hauxwell et al. 2001; Connell et al., 2013) to the detriment of invertebrate and fish species that use seagrass for food, cover, and as nurseries (Heck et al., 1995; Perkins-Visser et al., 1996; Francour, 1997; Blanc and Daguzan, 1998; McGlathery, 2001).

Tables

Table 1. Values of pH (NBS scale), temperature (°C), salinity (g kg^{-1}), pCO_2 (µatm), DIC (µmol $kgSW^{-1}$), HCO_3^- (µmol $kgSW^{-1}$) for *Gracilaria* and *Ulva* for June through October experiments. Values represent means ± standard error. Data from individual experiments appear within supplementary tables (S5 Tables).

Ulva						
Treatment	pH	Salinity	Temperature	pCO_2	DIC	HCO_3^-
Ambient/Filtered	8.14±0.04	30.9±0.5	16.6±0.5	270±30	1230±30	1140±30
Ambient/Unfiltered	8.23±0.04	30.7±0.6	16.5±0.6	270±30	1490±60	1360±50
CO_2/Filtered	7.17±0.04	30.3±0.3	15.7±0.5	2600±200	1490±60	1370±50
CO_2/Unfiltered	7.26±0.04	30.8±0.5	15.9±0.7	2660±240	1630±50	1520±40
Gracilaria						
Treatment	pH	Salinity	Temperature	pCO_2	DIC	HCO_3^-
Ambient/Filtered	8.10±0.04	30.9±0.5	16.0±0.7	300±30	1280±30	1190±30
Ambient/Unfiltered	8.19±0.05	30.7±0.6	16.5±0.6	310±40	1630±100	1490±90
CO_2/Filtered	7.17±0.04	30.4±0.4	15.0±0.6	2670±260	1450±60	1330±50
CO_2/Unfiltered	7.26±0.4	30.7±0.6	15.5±0.5	2550±250	1670±60	1550±50
Gracilaria and *Ulva*						
Treatment	pH	Salinity	Temperature	pCO_2	DIC	HCO_3^-
Ambient/Filtered	8.15±0.04	30.9±0.5	16.4±0.7	270±20	1240±30	1150±30
Ambient/Unfiltered	8.22±0.06	30.6±0.5	16.3±0.5	280±40	1540±40	1410±30
CO_2/Filtered	7.16±0.04	30.5±0.4	15.6±0.5	2520±180	1450±50	1320±50
CO_2/Unfiltered	7.27±0.04	30.6±0.5	15.8±0.5	2700±230	1660±50	1550±50

Figures

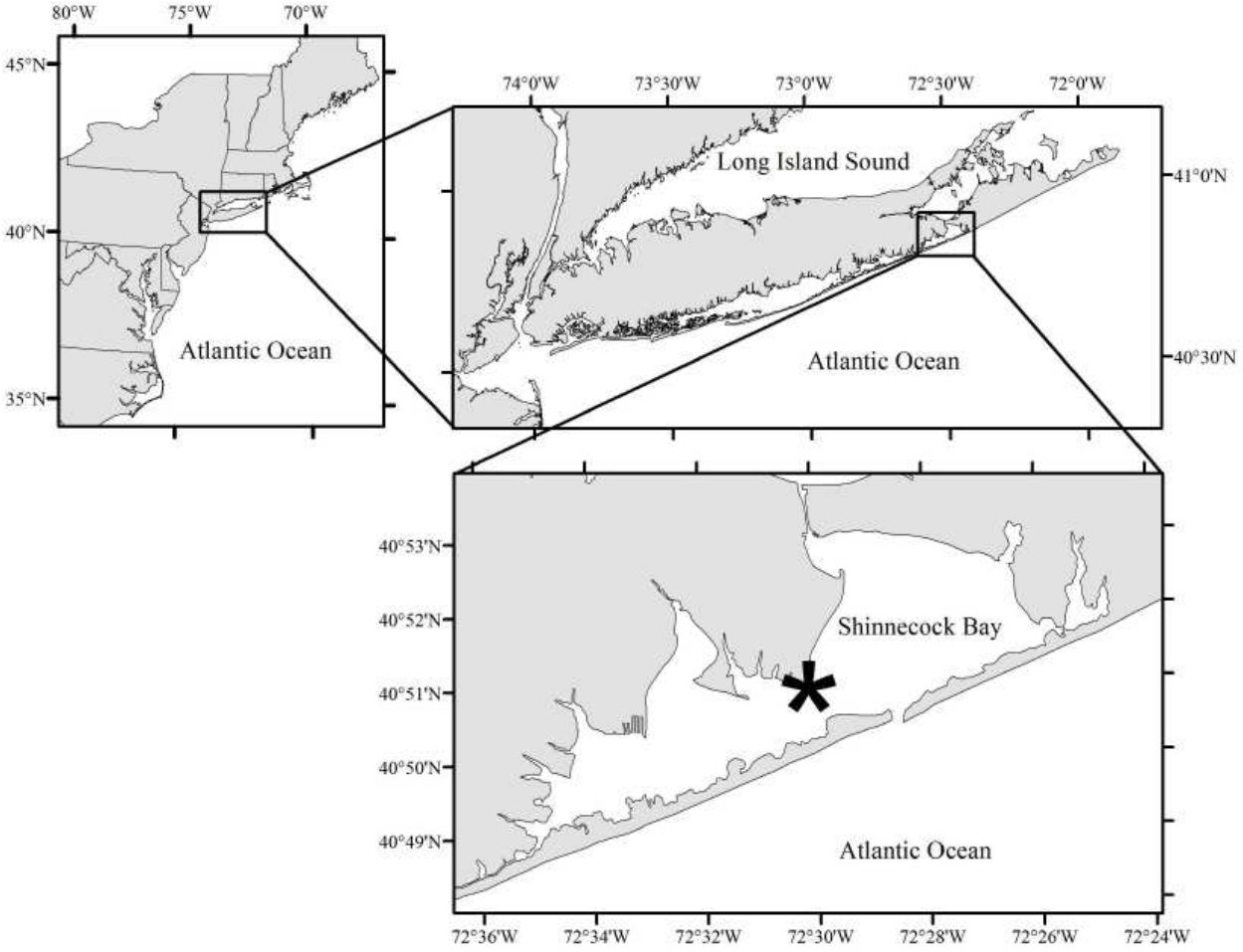

Figure 1: Map of Shinnecock Bay, NY, USA. The asterisk represents the shallow-water region where macroalgal collections occurred and *in situ* experiments were performed.

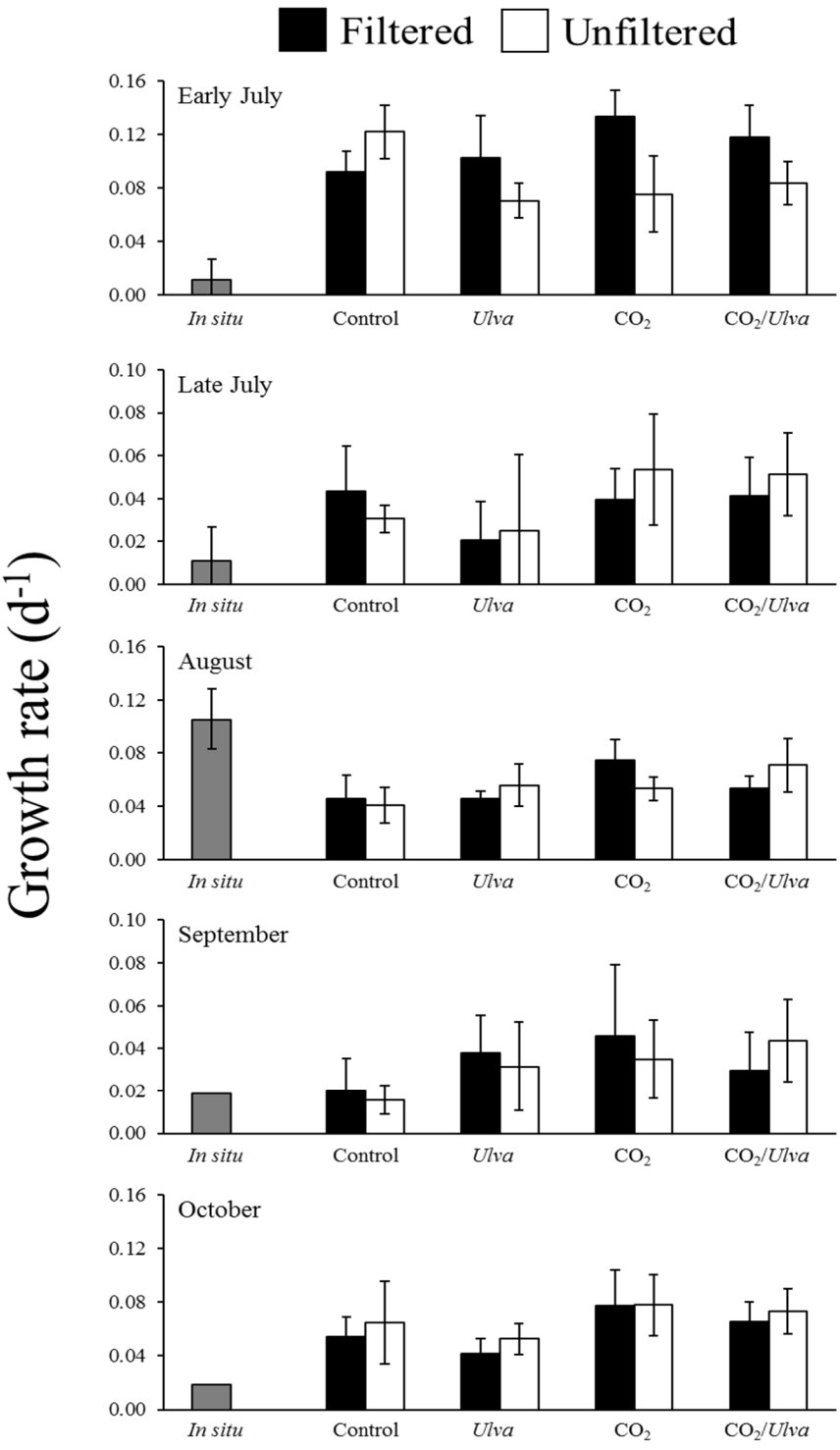

Figure 2: Growth rates of *Gracilaria* exposed to ambient and elevated CO_2 conditions, with and without competition from *Ulva*, and with and without competition from phytoplankton for experiments performed July through October.

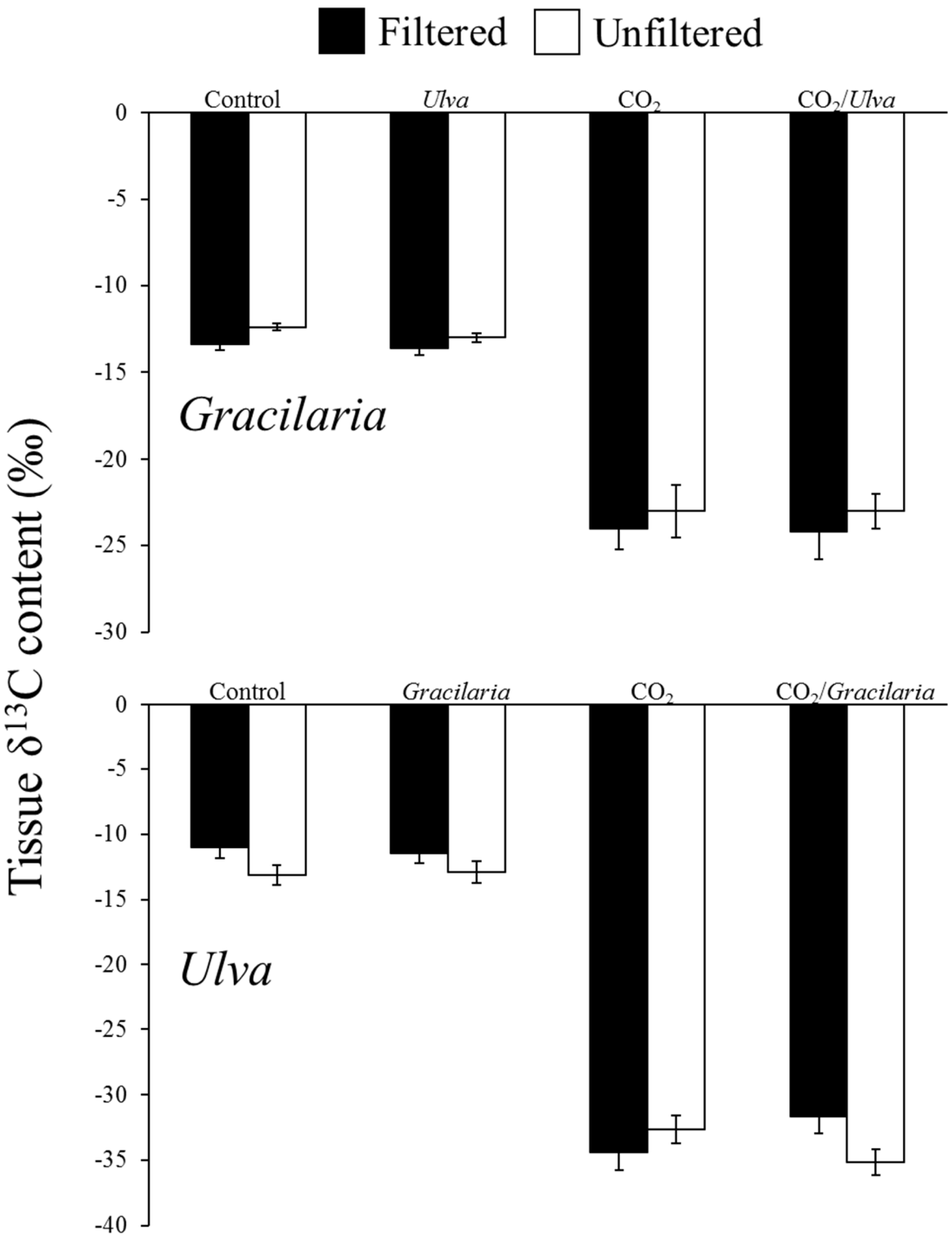

Figure 3: $\delta^{13}C$ content of *Gracilaria* and *Ulva* exposed to ambient and elevated CO_2 conditions, with and without competition from *Ulva*, and with and without competition from phytoplankton for experiments performed July through October.

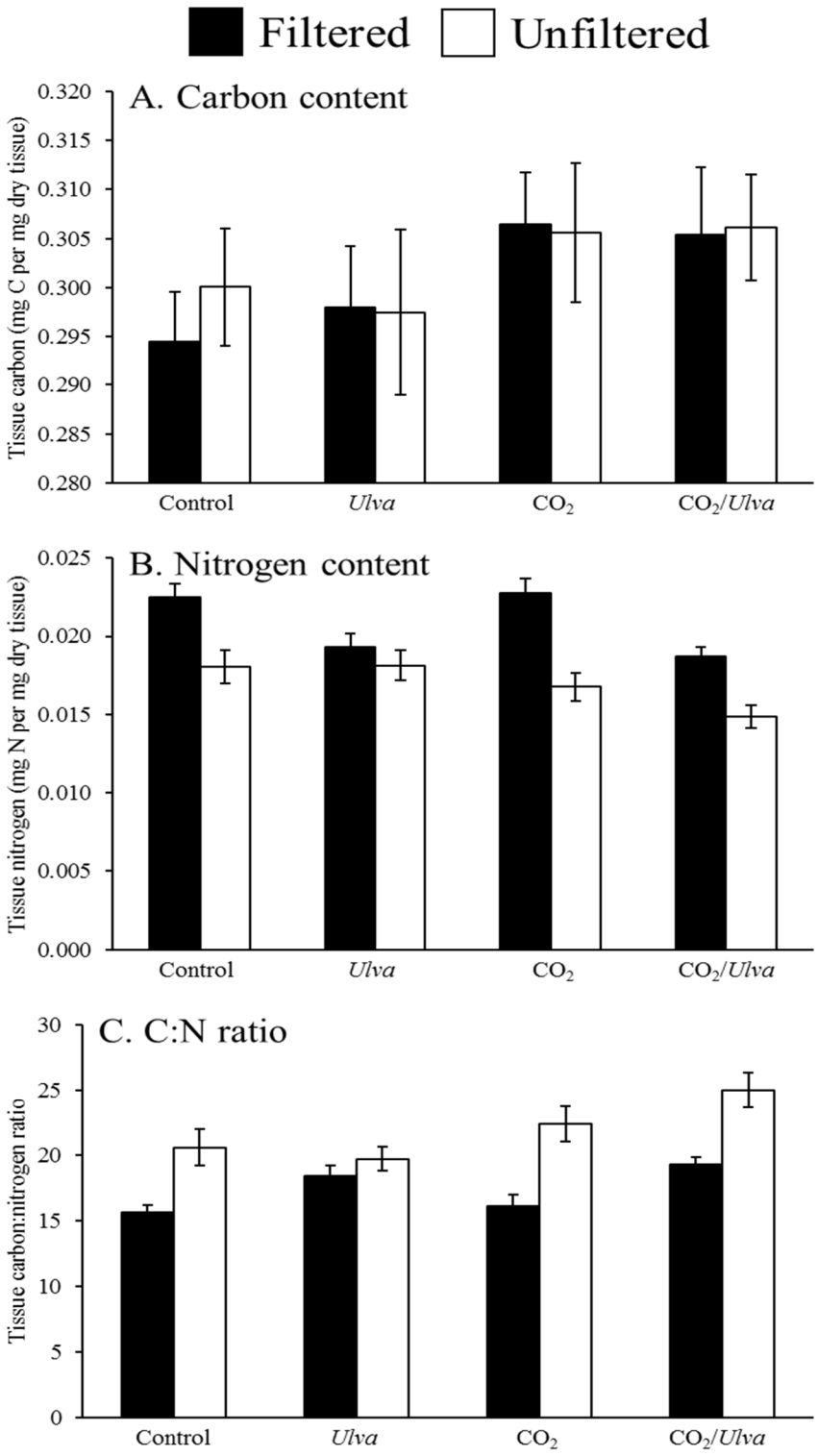

Figure 4: Tissue nitrogen, carbon, and C:N content of *Gracilaria* exposed to ambient and elevated CO_2 conditions, with and without competition from *Ulva*, and with and without competition from phytoplankton for experiments performed July through October.

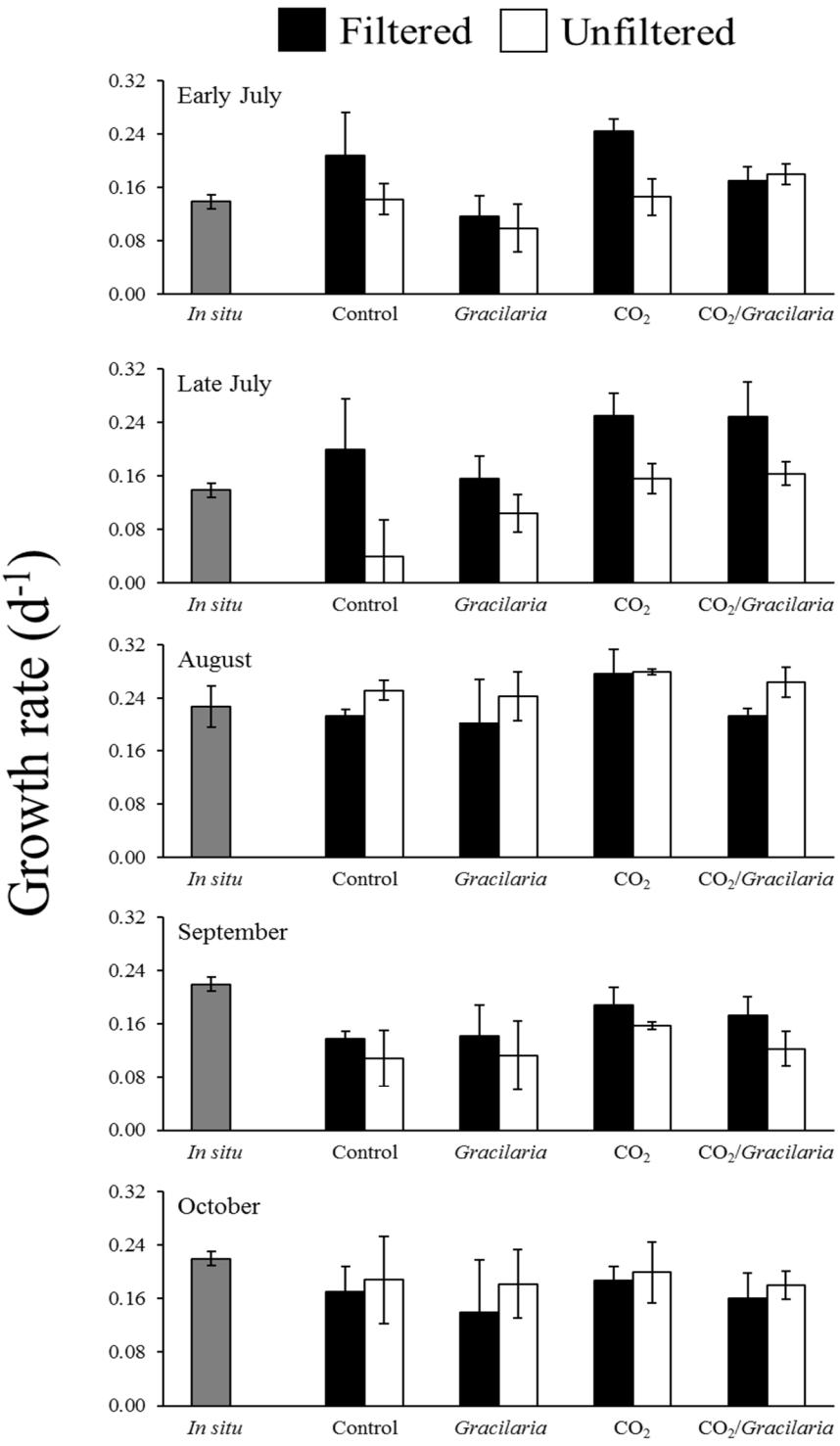

Figure 5: Growth rates of *Ulva* exposed to ambient and elevated CO_2 conditions, with and without competition from *Gracilaria*, and with and without competition from phytoplankton for experiments performed July through October.

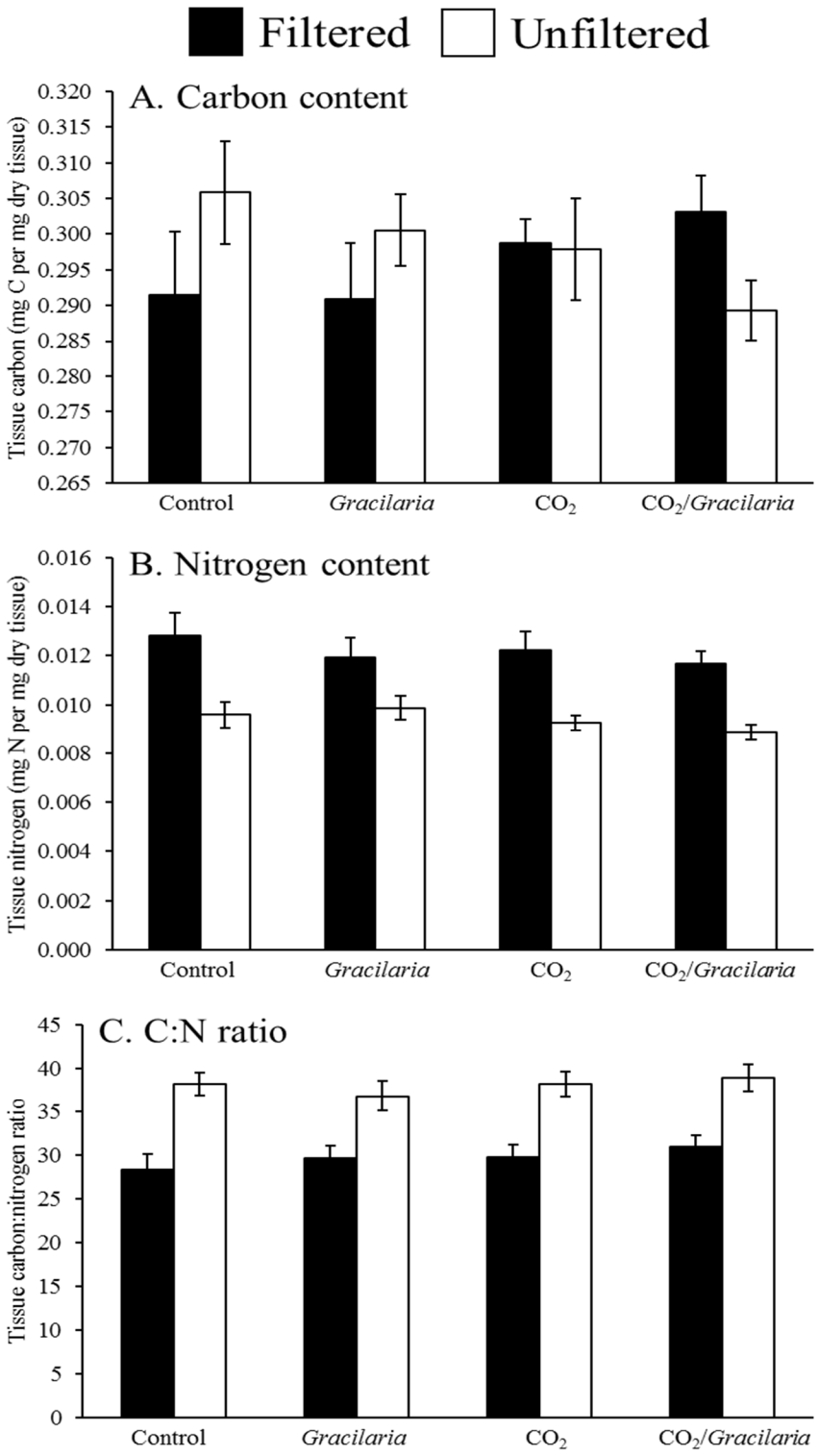

Figure 6: Tissue nitrogen, carbon, and C:N content of *Ulva* exposed to ambient and elevated CO_2 conditions, with and without competition from *Gracilaria*, and with and without competition from phytoplankton for experiments performed July through October.

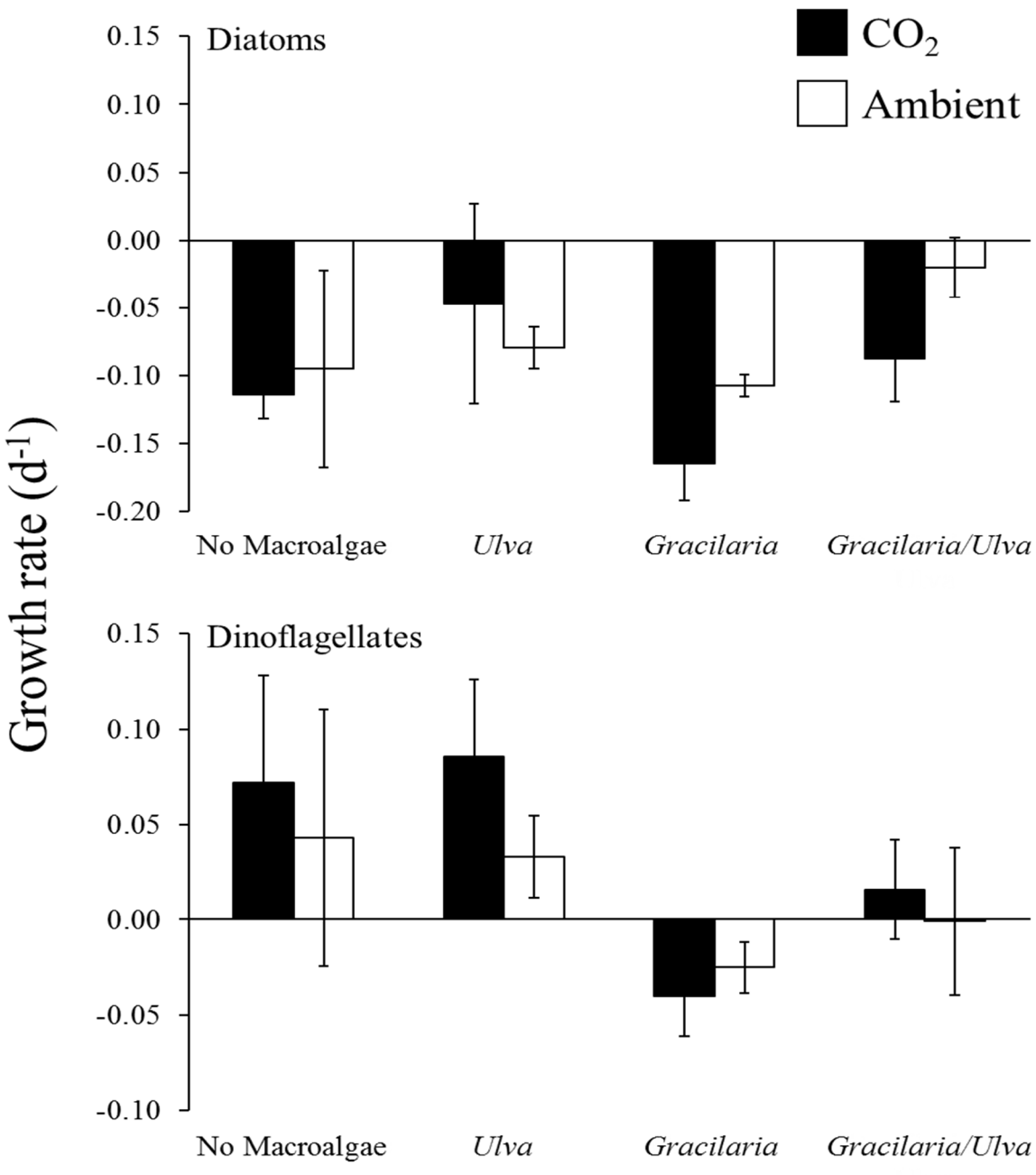

Figure 7: Growth rates of diatoms and dinoflagellates exposed to ambient and elevated CO_2 conditions, with and without competition from *Gracilaria* and/or *Ulva*.

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Appendix

Supplementary Tables

S1 Tables: Values of pH (NBS scale), temperature (°C), salinity (g kg^{-1}), and pCO_2 (µatm) for *Gracilaria* and *Ulva* for June through November experiments. Values represent means ± SE.

Gracilaria

June

Treatment	pH	Temperature	Salinity
Control	8.31±0.09	18.7±0.1	31.1±0.1
Nutrients	8.38±0.09	18.7±0.1	31.1±0.1
CO_2	7.44±0.01	18.7±0.1	31.1±0.1
CO_2/Nutrients	7.44±0.01	18.7±0.1	31.1±0.1

July

Treatment	pH	Temperature	Salinity	pCO_2
Control	8.28±0.04	18.8±0.1	30.8±0.8	377±149
Nutrients	8.48±0.09	18.7±0.1	30.6±0.6	287±240
CO_2	7.40±0.02	18.7±0.1	30.8±0.8	3120±97
CO_2/Nutrients	7.42±0.03	18.7±0.1	30.8±0.8	3210±0.6

August

Treatment	pH	Temperature	Salinity	pCO_2
Control	8.20±0.04	18.4±0.1	31.0±0.1	296±212
Nutrients	8.31±0.07	18.5±0.1	31.0±0.1	343±163
CO_2	7.39±0.03	18.6±0.1	31.0±0.1	2340±92
CO_2/Nutrients	7.40±0.02	18.7±0.1	31.0±0.1	2030±457

Early September

Treatment	pH	Temperature	Salinity	pCO_2
Control	8.20±0.05	18.6±0.1	31.4±0.1	310±201
Nutrients	8.24±0.06	18.6±0.1	31.5±0.1	306±212
CO_2	7.37±0.01	18.7±0.1	31.4±0.1	2570±26
CO_2/Nutrients	7.41±0.02	18.6±0.1	31.3±0.1	1640±220

Late September

Treatment	pH	Temperature	Salinity	pCO_2
Control	8.30±0.08	18.6±0.1	32.1±0.1	317±275
Nutrients	8.26±0.07	18.6±0.1	32.1±0.1	316±276
CO_2	7.33±0.01	18.8±0.1	32.1±0.1	2220±70
CO_2/Nutrients	7.34±0.02	18.7±0.1	32.1±0.1	2210±64

Early October

Treatment	pH	Temperature	Salinity	pCO_2
Control	8.20±0.06	18.3±0.6	25.4±3.4	324±224
Nutrients	8.21±0.06	19.0±0.1	25.7±3.7	303±223
CO_2	7.33±0.01	19.1±0.1	27.2±2.4	2770±391
CO_2/Nutrients	7.34±0.01	19.1±0.1	26.5±3.5	2610±674

Late October

Treatment	pH	Temperature	Salinity	pCO_2
Control	8.11±0.05	17.8±0.2	28.7±0.2	350±215
Nutrients	8.12±0.05	17.8±0.2	28.9±0.8	478±219
CO_2	7.31±0.01	17.9±0.2	28.5±0.1	2330±78
CO_2/Nutrients	7.32±0.01	17.9±0.2	29.1±0.5	2380±116

November

Treatment	pH	Temperature	Salinity	pCO_2
Control	8.22±0.06	17.8±0.1	26.1±0.3	334±147
Nutrients	8.28±0.07	17.7±0.1	26.6±0.1	307±151
CO_2	7.37±0.02	18.0±0.1	27.4±0.1	2460±205
CO_2/Nutrients	7.35±0.01	18.0±0.1	27.3±0.2	2340±107

Ulva

June

Treatment	pH	Temperature	Salinity
Control	8.37±0.08	18.7±0.1	31.1±0.1
Nutrients	8.42±0.09	18.7±0.1	31.1±0.1
CO_2	7.48±0.02	18.7±0.1	31.1±0.1
CO_2/Nutrients	7.46±0.01	18.7±0.1	31.1±0.1

July

Treatment	pH	Temperature	Salinity	pCO_2
Control	8.23±0.05	18.7±0.1	30.8±0.8	385±142
Nutrients	8.59±0.12	18.8±0.1	30.8±0.8	271±256
CO_2	7.40±0.02	18.7±0.1	30.7±0.7	3080±132
CO_2/Nutrients	7.45±0.03	18.7±0.1	30.8±0.8	2910±300

August

Treatment	pH	Temperature	Salinity	pCO_2
Control	8.27±0.03	18.5±0.1	31.0±0.1	283±141
Nutrients	8.35±0.06	18.4±0.1	31.0±0.1	321±119
CO_2	7.39±0.02	18.6±0.1	31.0±0.1	2110±257
CO_2/Nutrients	7.41±0.02	18.8±0.1	31.0±0.1	2130±446

Early September

Treatment	pH	Temperature	Salinity	pCO_2
Control	8.29±0.05	18.6±0.1	31.4±0.2	310±187
Nutrients	8.44±0.12	18.6±0.1	31.5±0.1	305±227
CO_2	7.38±0.02	18.7±0.1	31.4±0.1	2570±291
CO_2/Nutrients	7.50±0.05	18.6±0.1	31.3±0.3	1635±981

Late September

Treatment	pH	Temperature	Salinity	pCO_2
Control	8.37±0.08	18.6±0.1	32.1±0.1	317±275
Nutrients	8.40±0.09	18.6±0.1	32.1±0.1	316±276
CO_2	7.33±0.02	18.7±0.1	32.1±0.1	2220±69
CO_2/Nutrients	7.38±0.03	18.7±0.1	32.1±0.1	2210±64

Early October

Treatment	pH	Temperature	Salinity	pCO_2
Control	8.25±0.06	19.0±0.1	25.4±3.4	324±227
Nutrients	8.22±0.05	18.9±0.1	25.7±3.7	303±181
CO_2	7.32±0.01	19.1±0.1	27.2±1.8	2770±306
CO_2/Nutrients	7.32±0.01	19.0±0.1	26.5±2.9	2610±401

Late October

Treatment	pH	Temperature	Salinity	pCO_2
Control	8.17±0.04	17.8±0.2	28.7±0.7	350±207
Nutrients	8.09±0.03	17.9±0.2	28.9±0.2	478±59
CO_2	7.30±0.01	17.9±0.2	28.5±0.5	2330±70
CO_2/Nutrients	7.29±0.01	17.9±0.2	29.1±0.7	2380±29

November

Treatment	pH	Temperature	Salinity	pCO_2
Control	8.21±0.04	17.7±0.1	26.1±0.3	334±151
Nutrients	8.27±0.06	17.8±0.1	26.6±0.2	307±178
CO_2	7.34±0.02	17.8±0.1	27.4±0.1	2460±2
CO_2/Nutrients	7.38±0.02	18.0±0.1	27.3±0.1	2340±122

S2 Tables: Statistical analyses of variance for laboratory and *in situ* experiments (June through November 2014) for *Gracilaria* and *Ulva*.

Three-way analysis of variance for *Gracilaria* growth for June through November experiments

Source of Variation	DF	SS	MS	F	P
CO_2	1	0.0385	0.0385	77.274	<0.001
Nutrients	1	0.000486	0.000486	0.974	0.328
Time	7	0.0921	0.0132	26.367	<0.001
CO_2 x Nutrients	1	0.000204	0.000204	0.409	0.525
CO_2 x Time	7	0.0168	0.0024	4.807	<0.001
Nutrients x Time	7	0.00388	0.000554	1.111	0.368
CO_2 x Nutrients x Time	7	0.00142	0.000202	0.406	0.895
Residual	60	0.0299	0.000499		
Total	91	0.184	0.00203		

Three-way analysis of variance for *Ulva* growth for June through November experiments

Source of Variation	DF	SS	MS	F	P
CO_2	1	0.0469	0.0469	17.413	<0.001
Nutrients	1	0.00887	0.00887	3.295	0.075
Time	7	0.239	0.0342	12.704	<0.001
CO_2 x Nutrients	1	0.00386	0.00386	1.432	0.236
CO_2 x Time	7	0.109	0.0156	5.805	<0.001
Nutrients x Time	7	0.077	0.011	4.085	0.001
CO_2 x Nutrients x Time	7	0.0228	0.00326	1.21	0.312
Residual	57	0.153	0.00269		
Total	88	0.659	0.00748		

Two-way analysis of variance for *Gracilaria* growth under control and *in situ* conditions

Source of Variation	DF	SS	MS	F	P
Location	1	0.00158	0.00158	2.844	0.104
Time	7	0.00507	0.00072	1.303	0.288
Location x Time	7	0.0208	0.00297	5.347	<0.001
Residual	26	0.0144	0.00056		
Total	41	0.0427	0.00104		

Two-way analysis of variance for *Ulva* growth under control and *in situ* conditions

Source of Variation	DF	SS	MS	F	P
Location	1	0.00068	0.00068	0.35	0.559
Time	7	0.203	0.029	14.847	<0.001
Location x Time	7	0.252	0.0359	18.398	<0.001
Residual	31	0.0606	0.00195		
Total	46	0.519	0.0113		

Three-way analysis of variance of the tissue nitrogen for *Gracilaria* for June through November experiments

Source of Variation	DF	SS	MS	F	P
CO_2	1	2.4E-06	2.4E-06	0.199	0.658
Nutrients	1	0.00011	0.00011	9.328	0.004
Time	5	0.00113	0.00023	18.989	<0.001
CO_2 x Nutrients	1	2.7E-06	2.7E-06	0.229	0.634
CO_2 x Time	5	7.5E-05	1.5E-05	1.26	0.297
Nutrients x Time	5	5.8E-05	1.2E-05	0.97	0.446
CO_2 x Nutrients x Time	5	4.1E-05	8.2E-06	0.694	0.63
Residual	48	0.00057	1.2E-05		
Total	71	0.00198	2.8E-05		

Three-way analysis of variance of the tissue carbon for *Gracilaria* for June through November experiments

Source of Variation	DF	SS	MS	F	P
CO_2	1	0.00056	0.00056	1.103	0.299
Nutrients	1	0.00168	0.00168	3.334	0.074
Time	5	0.0182	0.00363	7.183	<0.001
CO_2 x Nutrients	1	0.00183	0.00183	3.621	0.063
CO_2 x Time	5	0.00296	0.00059	1.171	0.337
Nutrients x Time	5	0.00247	0.0005	0.978	0.441
CO_2 x Nutrients x Time	5	0.00836	0.00167	3.308	0.012
Residual	48	0.0243	0.00051		
Total	71	0.0603	0.00085		

Three-way analysis of variance of the tissue C:N for *Gracilaria* for June through November experiments

Source of Variation	DF	SS	MS	F	P
CO_2	1	1.841	1.841	1.618	0.209
Nutrients	1	45.616	45.616	40.098	<0.001
Time	5	177.656	35.531	31.233	<0.001
CO_2 x Nutrients	1	0.796	0.796	0.7	0.407
CO_2 x Time	5	3.425	0.685	0.602	0.698
Nutrients x Time	5	32.098	6.42	5.643	<0.001
CO_2 x Nutrients x Time	5	2.763	0.553	0.486	0.785
Residual	48	54.606	1.138		
Total	71	318.799	4.49		

Three-way analysis of variance of the tissue nitrogen for *Ulva* for June through November experiments

Source of Variation	DF	SS	MS	F	P
CO_2	1	1.9E-06	1.9E-06	0.22	0.642
Nutrients	1	0.00018	0.00018	20.333	<0.001
Time	5	0.00097	0.0002	22.413	<0.001
CO_2 x Nutrients	1	4.7E-07	4.7E-07	0.054	0.817
CO_2 x Time	5	1.8E-05	3.6E-06	0.414	0.837
Nutrients x Time	5	0.00053	0.00011	12.147	<0.001
CO_2 x Nutrients x Time	5	1.4E-05	2.8E-06	0.317	0.9
Residual	44	0.00038	8.7E-06		
Total	67	0.00214	3.2E-05		

Three-way analysis of variance of the tissue carbon for *Ulva* for June through November experiments

Source of Variation	DF	SS	MS	F	P
CO_2	1	7.7E-05	7.7E-05	0.173	0.68
Nutrients	1	0.00285	0.00285	6.394	0.015
Time	5	0.0161	0.00321	7.201	<0.001
CO_2 x Nutrients	1	3.4E-07	3.4E-07	0.00077	0.978
CO_2 x Time	5	0.00102	0.0002	0.455	0.807
Nutrients x Time	5	0.0026	0.00052	1.164	0.342
CO_2 x Nutrients x Time	5	0.00042	8.4E-05	0.188	0.966
Residual	44	0.0196	0.00045		
Total	67	0.0425	0.00063		

S3 Tables: Tissue $\delta^{13}C$ content (‰) of dry tissue samples of *Gracilaria* and *Ulva* for August through November experiments. Values represent means ± SE.

Gracilaria

Treatment	August	Early September	Early October	November
Control	-13.32±1.29	-10.63±0.16	-13.22±0.47	-13.82±0.61
Nutrients	-12.32±0.49	-11.81±0.51	-13.19±0.44	-13.42±0.97
CO_2	-18.21±0.79	-21.09±2.30	-18.08±1.05	-27.35±2.61
CO_2/Nutrients	-15.26±2.24	-18.58±2.27	-18.71±1.40	-25.47±1.64

Ulva

Treatment	August	Early September	November
Control	-6.57±1.09	-6.70±1.16	-11.10±0.34
Nutrients	-4.64±0.83	-3.87±0.21	-8.36±0.39
CO_2	-24.15±0.73	-27.67±1.75	-26.02±0.82
CO_2/Nutrients	-19.02±1.24	-23.29±1.34	-27.46±1.32

Three-way analysis of variance of the tissue C:N for *Ulva* for June through November experiments

Source of Variation	DF	SS	MS	F	P
CO_2	1	4.052	4.052	0.303	0.585
Nutrients	1	312.069	312.069	23.321	<0.001
Time	5	1803.45	360.689	26.954	<0.001
CO_2 x Nutrients	1	1.895	1.895	0.142	0.709
CO_2 x Time	5	11.709	2.342	0.175	0.971
Nutrients x Time	5	931.409	186.282	13.921	<0.001
CO_2 x Nutrients x Time	5	25.172	5.034	0.376	0.862
Residual	44	588.79	13.382		
Total	67	3754.06	56.031		

Three-way analysis of variance of the tissue $\delta^{13}C$ for *Gracilaria* for August through November experiments

Source of Variation	DF	SS	MS	F	P
CO_2	1	755.986	755.986	166.194	<0.001
Nutrients	1	1.128	1.128	0.248	0.622
Time	3	164.397	54.799	12.047	<0.001
CO_2 x Nutrients	1	0.731	0.731	0.161	0.691
CO_2 x Time	3	123.895	41.298	9.079	<0.001
Nutrients x Time	3	5.598	1.866	0.41	0.747
CO_2 x Nutrients x Time	3	2.675	0.892	0.196	0.898
Residual	30	136.465	4.549		
Total	45	1229.61	27.325		

Three-way analysis of variance of the tissue $\delta^{13}C$ for *Ulva* for the August through November experiments

Source of Variation	DF	SS	MS	F	P
CO_2	1	2828.64	2828.64	878.483	<0.001
Nutrients	1	60.71	60.71	18.855	<0.001
Time	2	131.475	65.737	20.416	<0.001
CO_2 x Nutrients	1	0.0851	0.0851	0.0264	0.872
CO_2 x Time	2	28.94	14.47	4.494	0.022
Nutrients x Time	2	16.984	8.492	2.637	0.092
CO_2 x Nutrients x Time	2	22.594	11.297	3.508	0.046
Residual	24	77.278	3.22		
Total	35	3166.71	90.477		

One-way ANOVA of the $\delta^{13}C$ content of *Gracilaria* exposed elevated CO_2 conditions compared with the $\delta^{13}C$ signature expected from the exclusive use of CO_2 or the exclusive use of HCO_3^- (Fig 4). Tukey tests indicated each group was significantly different from each other.

Source of Variation	DF	SS	MS	F	P
Between Groups	2	2124.84	1062.42	119.986	<0.001
Residual	27	239.072	8.855		
Total	29	2363.91			

One-way ANOVA of the $\delta^{13}C$ content of *Ulva* exposed elevated CO_2 conditions compared with the $\delta^{13}C$ signature expected from the exclusive use of CO_2 or the exclusive use of HCO_3^- (Fig 4). Tukey tests indicated each group was significantly different from each other.

Source of Variation	DF	SS	MS	F	P
Between Groups	2	1210.59	605.296	47.185	<0.001
Residual	33	423.331	12.828		
Total	35	1633.92			

S4 Tables: Tissue nitrogen content (g N per g dry tissue), tissue carbon content (g N per g dry tissue), and tissue C:N of dry tissue samples of *Gracilaria* and *Ulva* for August through November experiments. Values represent means ± SE.

Gracilaria

Tissue nitrogen content

Treatment	August	Early September	Late September	Early October	Late October	November
Control	0.021±0.002	0.027±0.002	0.031±0.004	0.033±0.001	0.035±0.002	0.032±0.001
Nutrients	0.021±0.001	0.033±0.001	0.033±0.002	0.034±0.001	0.036±0.001	0.035±0.001
CO_2	0.021±0.004	0.028±0.001	0.031±0.004	0.034±0.001	0.035±0.002	0.027±0.002
CO_2/Nutrients	0.027±0.002	0.031±0.001	0.034±0.001	0.033±0.001	0.034±0.001	0.032±0.002

Tissue carbon content

Treatment	August	Early September	Late September	Early October	Late October	November
Control	0.308±0.022	0.315±0.009	0.293±0.019	0.291±0.001	0.311±0.017	0.303±0.006
Nutrients	0.225±0.015	0.321±0.015	0.263±0.008	0.298±0.009	0.317±0.015	0.278±0.008
CO_2	0.280±0.022	0.330±0.001	0.284±0.013	0.322±0.008	0.303±0.003	0.275±0.014
CO_2/Nutrients	0.298±0.014	0.316±0.017	0.292±0.009	0.302±0.011	0.310±0.005	0.278±0.016

Tissue C:N

Treatment	August	Early September	Late September	Early October	Late October	November
Control	17.2±0.3	13.6±0.6	11.1±0.8	10.2±0.1	10.5±1.0	11.1±0.2
Nutrients	12.3±0.2	11.2±0.5	9.3±0.3	10.3±0.5	10.4±0.5	9.4±0.3
CO_2	15.9±1.6	14.0±0.5	11.1±0.9	11.1±0.6	10.2±0.5	12.1±0.6
CO_2/Nutrients	12.8±0.6	12.1±0.5	10.0±0.4	10.5±0.1	10.5±0.1	10.1±0.6

Ulva

Tissue nitrogen content

Treatment	August	Early September	Late September	Early October	Late October	November
Control	0.012±0.001	0.011±0.001	0.015±0.001	0.024±0.003	0.027±0.001	0.023±0.001
Nutrients	0.026±0.001	0.018±0.003	0.018±0.001	0.024±0.003	0.025±0.001	0.023±0.001
CO_2	0.012±0.001	0.012±0.003	0.019±0.001	0.024±0.001	0.025±0.001	0.024±0.002
CO_2/Nutrients	0.027±0.002	0.018±0.001	0.019±0.003	0.025±0.001	0.024±0.001	0.022±0.001

Tissue carbon content

Treatment	August	Early September	Late September	Early October	Late October	November
Control	0.302±0.003	0.335±0.009	0.267±0.003	0.295±0.019	0.301±0.019	0.293±0.011
Nutrients	0.335±0.013	0.322±0.007	0.282±0.018	0.309±0.013	0.320±0.003	0.306±0.010
CO_2	0.295±0.005	0.327±0.009	0.268±0.034	0.306±0.011	0.286±0.011	0.300±0.010
CO_2/Nutrients	0.313±0.017	0.321±0.016	0.282±0.011	0.317±0.013	0.322±0.004	0.304±0.007

Tissue C:N

Treatment	August	Early September	Late September	Early October	Late October	November
Control	29.3±2.8	36.7±2.0	21.5±1.9	14.5±0.8	12.9±0.3	14.7±0.3
Nutrients	15.3±0.6	21.9±2.9	18.0±0.7	15.3±1.2	15.2±0.4	15.8±0.5
CO_2	29.8±3.2	35.1±6.3	16.5±1.1	14.7±0.5	13.5±0.8	14.8±1.4
CO_2/Nutrients	13.8±0.5	21.2±1.7	19.0±3.7	15.1±0.5	15.6±0.1	15.8±0.4

S5 Tables**:** Values of pH (NBS scale), salinity (g kg^{-1}), temperature (°C), and pCO_2 (µatm) for *Gracilaria* and *Ulva* for July through October experiments. Values represent means ± standard deviation.

Ulva

Early July

Treatment	pH	Salinity	Temperature	pCO_2
Ambient/Filtered	8.23±0.11	31.0±1.4	16.9±0.9	210±20
Ambient/Unfiltered	8.38±0.10	30.0±0.0	17.1±0.9	190±10
CO_2/Filtered	7.19±0.10	31.0±1.4	16.3±0.7	2790±30
CO_2/Unfiltered	7.32±0.03	30.5±0.7	16.7±0.9	2580±60

Late July

Treatment	pH	Salinity	Temperature	pCO_2
Ambient/Filtered	8.26±0.11	32.0±0.1	15.7±0.6	220±60
Ambient/Unfiltered	8.23±0.02	32.0±0.0	16.4±0.6	290±30
CO_2/Filtered	7.14±0.02	30.0±0.1	15.8±0.4	2760±20
CO_2/Unfiltered	7.21±0.02	32.0±0.0	16.5±0.2	3140±350

August

Treatment	pH	Salinity	Temperature	pCO_2
Ambient/Filtered	8.10±0.04	29.4±0.1	17.9±2.6	290±50
Ambient/Unfiltered	8.22±0.02	29.4±0.3	17.5±3.3	260±20
CO_2/Filtered	7.10±0.01	29.3±0.1	17.1±1.1	2490±260
CO_2/Unfiltered	7.21±0.01	29.4±0.1	17.5±1.3	2990±100

September

Treatment	pH	Salinity	Temperature	pCO_2
Ambient/Filtered	8.11±0.10	32.0±0.1	17.4±3.5	280±40
Ambient/Unfiltered	8.20±0.11	32.0±0.1	17.3±2.4	240±60
CO_2/Filtered	7.11±0.10	31.0±0.1	15.0±0.4	3090±230
CO_2/Unfiltered	7.20±0.02	32.0±0.2	14.8±0.1	2840±10

October

Treatment	pH	Salinity	Temperature	pCO_2
Ambient/Filtered	8.02±0.02	30.0±0.1	15.0±0.7	350±30
Ambient/Unfiltered	8.10±0.02	29.9±0.0	14.0±0.7	350±1
CO_2/Filtered	7.32±0.01	30.1±0.2	14.2±1.7	1890±210
CO_2/Unfiltered	7.38±0.03	30.0±0.0	14.0±1.0	1760±180

Gracilaria

Early July

Treatment	pH	Salinity	Temperature	pCO_2
Ambient/Filtered	8.19±0.15	31.5±2.1	16.1±0.6	230±10
Ambient/Unfiltered	8.35±0.10	30.0±0.1	17.8±1.3	210±10
CO_2/Filtered	7.18±0.13	31.0±1.4	15.2±0.6	2850±160
CO_2/Unfiltered	7.32±0.12	30.0±0.1	15.4±0.6	2370±170

Late July

Treatment	pH	Salinity	Temperature	pCO_2
Ambient/Filtered	8.17±0.01	32.0±0.1	15.7±0.6	270±20
Ambient/Unfiltered	8.19±0.04	32.0±0.1	15.8±0.1	300±40
CO_2/Filtered	7.17±0.01	30.0±0.2	14.8±0.1	3380±220
CO_2/Unfiltered	7.20±0.02	32.0±0.1	15.1±0.1	3150±10

August

Treatment	pH	Salinity	Temperature	pCO_2
Ambient/Filtered	8.12±0.07	29.4±0.1	17.8±2.7	280±60
Ambient/Unfiltered	8.19±0.02	29.4±0.5	17.8±2.8	260±10
CO_2/Filtered	7.08±0.02	29.3±0.2	17.0±1.3	2470±240
CO_2/Unfiltered	7.20±0.01	29.3±0.1	17.3±1.6	3000±20

September

Treatment	pH	Salinity	Temperature	pCO_2
Ambient/Filtered	8.04±0.04	31.5±0.2	16.6±3.8	370±20
Ambient/Unfiltered	8.16±0.02	32.0±0.2	16.7±4.1	410±100
CO_2/Filtered	7.11±0.11	31.5±0.1	13.3±0.4	2850±10
CO_2/Unfiltered	7.18±0.03	32.0±0.0	15.3±0.2	2500±490

October

Treatment	pH	Salinity	Temperature	pCO_2
Ambient/Filtered	8.00±0.01	30.2±0.0	13.6±0.8	370±20
Ambient/Unfiltered	8.07±0.02	30.0±0.1	14.5±0.8	390±10
CO_2/Filtered	7.31±0.02	30.1±0.1	14.4±0.4	1800±220
CO_2/Unfiltered	7.39±0.01	30.0±0.0	14.2±2.5	1750±50

Gracilaria* and *Ulva

Early July

Treatment	pH	Salinity	Temperature	pCO_2
Ambient/Filtered	8.25±0.10	31.0±1.4	16.6±0.6	210±10
Ambient/Unfiltered	8.45±0.11	30.0±0.1	17.0±0.6	160±10
CO_2/Filtered	7.18±0.12	31.5±2.1	16.3±0.8	2800±60
CO_2/Unfiltered	7.33±0.10	30.0±0.0	16.4±0.6	2740±110

Late July

Treatment	pH	Salinity	Temperature	pCO_2
Ambient/Filtered	8.23±0.09	32.0±0.2	15.7±1.1	230±60
Ambient/Unfiltered	8.22±0.04	32.0±0.1	15.6±0.6	290±40
CO_2/Filtered	7.13±0.02	30.0±0.0	15.2±1.3	2610±10
CO_2/Unfiltered	7.22±0.01	32.0±0.1	15.6±0.8	2950±80

August

Treatment	pH	Salinity	Temperature	pCO_2
Ambient/Filtered	8.07±0.01	29.4±0.1	17.9±2.6	310±20
Ambient/Unfiltered	8.22±0.02	29.4±0.1	17.7±3.0	270±10
CO_2/Filtered	7.09±0.01	29.3±0.1	17.0±1.3	2435±190
CO_2/Unfiltered	7.20±0.01	29.4±0.1	17.3±1.6	3000±70

September

Treatment	pH	Salinity	Temperature	pCO_2
Ambient/Filtered	8.14±0.01	32.0±0.1	17.8±3.0	250±40
Ambient/Unfiltered	8.13±0.02	31.5±0.0	16.4±3.8	320±50
CO_2/Filtered	7.11±0.10	31.5±0.5	15.0±0.8	2890±160
CO_2/Unfiltered	7.19±0.03	31.5±0.1	15.5±0.1	3000±190

October

Treatment	pH	Salinity	Temperature	pCO_2
Ambient/Filtered	8.05±0.05	30.0±0.1	14.2±0.7	330±20
Ambient/Unfiltered	8.09±0.01	29.9±0.2	15.0±0.9	370±10
CO_2/Filtered	7.31±0.02	30.1±0.1	14.4±1.6	1850±290
CO_2/Unfiltered	7.39±0.05	29.9±0.2	14.3±2.2	1820±110

S6 Tables: Statistical analyses of variance for laboratory and *in situ* experiments (July through October 2015) for *Gracilaria* and *Ulva*.

Three-way analysis of variance for *Gracilaria* growth for July through October experiments

Source of Variation	DF	SS	MS	F	P
CO_2	1	0.00731	0.00731	7.001	0.009
Filtered	1	0.000136	0.000136	0.13	0.719
Competition	1	0.000249	0.000249	0.239	0.626
CO_2 x Filtered	1	0.000117	0.000117	0.112	0.739
CO_2 x Competition	1	0.000126	0.000126	0.12	0.729
Filtered x Competition	1	0.000368	0.000368	0.352	0.554
CO_2 x Filtered x Competition	1	0.000181	0.000181	0.173	0.678
Residual	128	0.134	0.00104		
Total	135	0.142	0.00105		

Three-way analysis of variance for *Ulva* growth for July through October experiments

Source of Variation	DF	SS	MS	F	P
CO_2	1	0.0832	0.0832	17.696	<0.001
Filtered	1	0.00881	0.00881	1.876	0.173
Competition	1	0.0413	0.0413	8.793	0.004
CO_2 x Filtered	1	0.00218	0.00218	0.464	0.497
CO_2 x Competition	1	0.00619	0.00619	1.316	0.253
Filtered x Competition	1	0.0112	0.0112	2.393	0.124
CO_2 x Filtered x Competition	1	0.000885	0.000885	0.188	0.665
Residual	124	0.583	0.0047		
Total	131	0.739	0.00564		

One-way analysis of variance for *Gracilaria* growth under control and *in situ* conditions

Source of Variation	DF	SS	MS	F	P
Between Groups	4	0.000459	0.000115	0.0526	0.995
Residual	79	0.172	0.00218		
Total	83	0.173			

One-way analysis of variance for *Ulva* growth under control and *in situ* conditions

Source of Variation	DF	SS	MS	F	P
Between Groups	4	0.0362	0.00905	1.979	0.106
Residual	78	0.357	0.00457		
Total	82	0.393			

Three-way analysis of variance for *Ulva* growth for the early July experiment

Source of Variation	DF	SS	MS	F	P
CO_2	1	0.0106	0.0106	9.157	0.009
Filtered	1	0.0105	0.0105	9.069	0.009
Competition	1	0.0109	0.0109	9.43	0.008
CO_2 x Filtered	1	0.0000119	0.0000119	0.0103	0.92
CO_2 x Competition	1	0.00304	0.00304	2.622	0.126
Filtered x Competition	1	0.00868	0.00868	7.494	0.015
CO_2 x Filtered x Competition	1	0.00134	0.00134	1.16	0.298
Residual	15	0.0174	0.00116		
Total	22	0.065	0.00296		

Three-way analysis of variance for *Ulva* growth for the late July experiment

Source of Variation	DF	SS	MS	F	P
CO_2	1	0.0933	0.0933	11.504	0.004
Filtered	1	0.122	0.122	15.017	0.001
Competition	1	0.00184	0.00184	0.227	0.64
CO_2 x Filtered	1	0.0172	0.0172	2.12	0.165
CO_2 x Competition	1	0.00137	0.00137	0.169	0.687
Filtered x Competition	1	0.00985	0.00985	1.215	0.287
CO_2 x Filtered x Competition	1	0.00781	0.00781	0.963	0.341
Residual	16	0.13	0.00811		
Total	23	0.383	0.0166		

Three-way analysis of variance for *Ulva* growth for the August experiment

Source of Variation	DF	SS	MS	F	P
CO_2	1	0.0115	0.0115	5.908	0.027
Filtered	1	0.00238	0.00238	1.226	0.285
Competition	1	0.0089	0.0089	4.576	0.048
CO_2 x Filtered	1	0.000283	0.000283	0.145	0.708
CO_2 x Competition	1	0.0000132	0.0000132	0.00679	0.935
Filtered x Competition	1	1.21E-06	1.21E-06	0.000625	0.98
CO_2 x Filtered x Competition	1	0.00357	0.00357	1.834	0.194
Residual	16	0.0311	0.00195		
Total	23	0.0578	0.00251		

Three-way analysis of variance for *Ulva* growth for the September experiment

Source of Variation	DF	SS	MS	F	P
CO_2	1	0.0582	0.0582	12.559	0.002
Filtered	1	0.00245	0.00245	0.528	0.475
Competition	1	0.0114	0.0114	2.456	0.131
CO_2 x Filtered	1	0.0256	0.0256	5.518	0.028
CO_2 x Competition	1	0.00139	0.00139	0.3	0.589
Filtered x Competition	1	0.00411	0.00411	0.886	0.356
CO_2 x Filtered x Competition	1	0.00834	0.00834	1.8	0.193
Residual	23	0.107	0.00464		
Total	30	0.223	0.00742		

Three-way analysis of variance for *Ulva* growth for the October experiment

Source of Variation	DF	SS	MS	F	P
CO_2	1	0.00109	0.00109	0.461	0.503
Filtered	1	0.00423	0.00423	1.786	0.194
Competition	1	0.00348	0.00348	1.469	0.237
CO_2 x Filtered	1	0.000394	0.000394	0.167	0.687
CO_2 x Competition	1	0.0000488	0.0000488	0.0206	0.887
Filtered x Competition	1	0.000529	0.000529	0.224	0.641
CO_2 x Filtered x Competition	1	0.000128	0.000128	0.0539	0.818
Residual	24	0.0568	0.00237		
Total	31	0.0667	0.00215		

Three-way analysis of variance for *Gracilaria* growth for the early July experiment

Source of Variation	DF	SS	MS	F	P
CO_2	1	0.000941	0.000941	1.304	0.27
Filtered	1	0.00555	0.00555	7.697	0.014
Competition	1	0.000154	0.000154	0.213	0.651
CO_2 x Filtered	1	0.00149	0.00149	2.065	0.17
CO_2 x Competition	1	0.0000196	0.0000196	0.0272	0.871
Filtered x Competition	1	0.0000531	0.0000531	0.0736	0.79
CO_2 x Filtered x Competition	1	0.00128	0.00128	1.77	0.202
Residual	16	0.0115	0.000722		
Total	23	0.021	0.000915		

Three-way analysis of variance for *Gracilaria* growth for the late July experiment

Source of Variation	DF	SS	MS	F	P
CO_2	1	0.00206	0.00206	5.088	0.039
Filtered	1	9.42E-06	9.42E-06	0.0233	0.881
Competition	1	0.000556	0.000556	1.373	0.26
CO_2 x Filtered	1	0.000666	0.000666	1.643	0.219
CO_2 x Competition	1	0.000525	0.000525	1.295	0.273
Filtered x Competition	1	0.0000027	0.0000027	0.00667	0.936
CO_2 x Filtered x Competition	1	0.0000374	0.0000374	0.0924	0.765
Residual	15	0.00608	0.000405		
Total	22	0.00989	0.000449		

Three-way analysis of variance for *Gracilaria* growth for the August experiment

Source of Variation	DF	SS	MS	F	P
CO_2	1	0.000999	0.000999	5.763	0.031
Filtered	1	0.0000351	0.0000351	0.203	0.659
Competition	1	0.000141	0.000141	0.811	0.383
CO_2 x Filtered	1	0.000114	0.000114	0.658	0.431
CO_2 x Competition	1	0.000234	0.000234	1.351	0.265
Filtered x Competition	1	0.00131	0.00131	7.563	0.016
CO_2 x Filtered x Competition	1	0.0000623	0.0000623	0.359	0.558
Residual	14	0.00243	0.000173		
Total	21	0.00524	0.00025		

Three-way analysis of variance for *Gracilaria* growth for the September experiment

Source of Variation	DF	SS	MS	F	P
CO_2	1	0.00132	0.00132	2.466	0.129
Filtered	1	0.000429	0.000429	0.798	0.381
Competition	1	0.000297	0.000297	0.553	0.464
CO_2 x Filtered	1	0.0000624	0.0000624	0.116	0.736
CO_2 x Competition	1	8.93E-06	8.93E-06	0.0166	0.899
Filtered x Competition	1	0.000307	0.000307	0.571	0.457
CO_2 x Filtered x Competition	1	0.0000483	0.0000483	0.0898	0.767
Residual	24	0.0129	0.000537		
Total	31	0.0154	0.000496		

Three-way analysis of variance for *Gracilaria* growth for the October experiment

Source of Variation	DF	SS	MS	F	P
CO_2	1	0.00257	0.00257	6.01	0.022
Filtered	1	0.000228	0.000228	0.532	0.473
Competition	1	0.00129	0.00129	3.007	0.096
CO_2 x Filtered	1	0.000257	0.000257	0.599	0.447
CO_2 x Competition	1	4.51E-07	4.51E-07	0.00105	0.974
Filtered x Competition	1	1.01E-07	1.01E-07	0.000236	0.988
CO_2 x Filtered x Competition	1	2.31E-06	2.31E-06	0.0054	0.942
Residual	24	0.0103	0.000428		
Total	31	0.0146	0.000472		

Three-way analysis of variance for diatom growth for the September experiment

Source of Variation	DF	SS	MS	F	P
CO_2	1	0.00127	0.00127	0.139	0.712
Ulva	1	0.047	0.047	5.17	0.032
Gracilaria	1	0.00542	0.00542	0.596	0.448
CO_2 x *Ulva*	1	0.00513	0.00513	0.565	0.46
CO_2 x *Gracilaria*	1	0.0196	0.0196	2.159	0.155
Ulva x *Gracilaria*	1	0.000237	0.000237	0.0261	0.873
CO_2 x *Ulva* x *Gracilaria*	1	0.0075	0.0075	0.825	0.373
Residual	24	0.218	0.00909		
Total	31	0.304	0.00982		

Three-way analysis of variance for dinoflagellate growth for the September experiment

Source of Variation	DF	SS	MS	F	P
CO_2	1	0.00347	0.00347	2.203	0.151
Ulva	1	0.00354	0.00354	2.246	0.147
Gracilaria	1	0.0403	0.0403	25.611	<0.001
CO_2 x *Ulva*	1	0.00154	0.00154	0.975	0.333
CO_2 x *Gracilaria*	1	0.00316	0.00316	2.005	0.17
Ulva x *Gracilaria*	1	0.00291	0.00291	1.849	0.187
CO_2 x *Ulva* x *Gracilaria*	1	0.0000306	0.0000306	0.0194	0.89
Residual	24	0.0378	0.00158		
Total	31	0.0928	0.00299		

One-way analysis of variance for changes in diatom abundance in elevated CO_2 treatments during the September experiment

Source of Variation	DF	SS	MS	F	P
Between Groups	1	20930450	20930450	44.91	<0.001
Residual	6	2796350	466058.33		
Total	7	23726800			

One-way analysis of variance for changes in dinoflagellate abundance in elevated CO_2 treatments during the September experiment

Source of Variation	DF	SS	MS	F	P
Between Groups	1	2420000	2420000	10.204	0.019
Residual	6	1423000	237166.67		
Total	7	3843000			

One-way analysis of variance for changes in diatom abundance in ambient treatments during the September experiment

Source of Variation	DF	SS	MS	F	P
Between Groups	1	7761800	7761800	12.669	0.012
Residual	6	3675950	612658.33		
Total	7	11437750			

One-way analysis of variance for changes in dinoflagellate abundance in ambient treatments during the September experiment

Source of Variation	DF	SS	MS	F	P
Between Groups	1	11685344	11685344	17.003	0.009
Residual	5	3436341.7	687268.33		
Total	6	15121686			

One-way analysis of variance for changes in diatom abundance in elevated CO_2 treatments during the October experiment

Source of Variation	DF	SS	MS	F	P
Between Groups	1	30186450	30186450	20.589	0.004
Residual	6	8796700	1466116.7		
Total	7	38983150			

One-way analysis of variance for changes in dinoflagellate abundance in elevated CO_2 treatments during the October experiment

Source of Variation	DF	SS	MS	F	P
Between Groups	1	13912813	13912813	73.452	<0.001
Residual	6	1136475	189412.5		
Total	7	15049288			

One-way analysis of variance for changes in diatom abundance in ambient treatments during the October experiment

Source of Variation	DF	SS	MS	F	P
Between Groups	1	35490313	35490313	61.082	<0.001
Residual	6	3486175	581029.17		
Total	7	38976488			

One-way analysis of variance for changes in dinoflagellate abundance in ambient treatments during the October experiment

Source of Variation	DF	SS	MS	F	P
Between Groups	1	48856613	48856613	38.12	<0.001
Residual	6	7689875	1281645.8		
Total	7	56546488			

Three-way analysis of variance of the tissue $\delta^{13}C$ for *Ulva* for the early July experiment

Source of Variation	DF	SS	MS	F	P
CO_2	1	3287.232	3287.232	217.325	<0.001
Gracilaria	1	70.658	70.658	4.671	0.046
Filtered	1	0.06	0.06	0.00397	0.951
CO_2 x *Gracilaria*	1	2.968	2.968	0.196	0.664
CO_2 x Filtered	1	0.443	0.443	0.0293	0.866
Gracilaria x Filtered	1	121.5	121.5	8.033	0.012
CO_2 x *Gracilaria* x Filtered	1	87.937	87.937	5.814	0.028
Residual	16	242.014	15.126		
Total	23	3812.812	165.774		

Three-way analysis of variance of the tissue $\delta^{13}C$ for *Ulva* for the late July experiment

Source of Variation	DF	SS	MS	F	P
CO_2	1	4319.63	4319.63	359.192	<0.001
Gracilaria	1	1.197	1.197	0.0995	0.756
Filtered	1	11.399	11.399	0.948	0.345
CO_2 x *Gracilaria*	1	1.197	1.197	0.0995	0.756
CO_2 x Filtered	1	52.747	52.747	4.386	0.053
Gracilaria x Filtered	1	1.52	1.52	0.126	0.727
CO_2 x *Gracilaria* x Filtered	1	13.321	13.321	1.108	0.308
Residual	16	192.415	12.026		
Total	23	4593.426	199.714		

Three-way analysis of variance of the tissue $\delta^{13}C$ for *Ulva* for the August experiment

Source of Variation	DF	SS	MS	F	P
CO_2	1	2026.395	2026.395	273.867	<0.001
Gracilaria	1	2.142	2.142	0.289	0.598
Filtered	1	75.935	75.935	10.263	0.006
CO_2 x *Gracilaria*	1	1.832	1.832	0.248	0.626
CO_2 x Filtered	1	1.744	1.744	0.236	0.634
Gracilaria x Filtered	1	4.208	4.208	0.569	0.462
CO_2 x *Gracilaria* x Filtered	1	5.273	5.273	0.713	0.411
Residual	16	118.387	7.399		
Total	23	2235.917	97.214		

Three-way analysis of variance of the tissue $\delta^{13}C$ for *Ulva* for the September experiment

Source of Variation	DF	SS	MS	F	P
CO_2	1	2195.378	2195.378	667.221	<0.001
Gracilaria	1	9.573	9.573	2.909	0.102
Filtered	1	27.114	27.114	8.241	0.009
CO_2 x *Gracilaria*	1	0.667	0.667	0.203	0.657
CO_2 x Filtered	1	10.879	10.879	3.306	0.082
Gracilaria x Filtered	1	0.781	0.781	0.237	0.631
CO_2 x *Gracilaria* x Filtered	1	2.813	2.813	0.855	0.365
Residual	23	75.678	3.29		
Total	30	2378.782	79.293		

Three-way analysis of variance of the tissue $\delta^{13}C$ for *Ulva* for the October experiment

Source of Variation	DF	SS	MS	F	P
CO_2	1	3886.313	3886.313	265.278	<0.001
Gracilaria	1	5.977	5.977	0.408	0.529
Filtered	1	1.562	1.562	0.107	0.747
CO_2 x *Gracilaria*	1	11.198	11.198	0.764	0.391
CO_2 x Filtered	1	10.615	10.615	0.725	0.403
Gracilaria x Filtered	1	23.822	23.822	1.626	0.214
CO_2 x *Gracilaria* x Filtered	1	30.988	30.988	2.115	0.159
Residual	24	351.599	14.65		
Total	31	4322.075	139.422		

Three-way analysis of variance of the tissue $\delta^{13}C$ for *Gracilaria* for the early July experiment

Source of Variation	DF	SS	MS	F	P
CO_2	1	2005.865	2005.865	456.536	<0.001
Ulva	1	1.799	1.799	0.409	0.531
Filtered	1	13.039	13.039	2.968	0.104
CO_2 x *Ulva*	1	11.551	11.551	2.629	0.124
CO_2 x Filtered	1	0.746	0.746	0.17	0.686
Ulva x Filtered	1	25.523	25.523	5.809	0.028
CO_2 x *Ulva* x Filtered	1	20.963	20.963	4.771	0.044
Residual	16	70.299	4.394		
Total	23	2149.783	93.469		

Three-way analysis of variance of the tissue $\delta^{13}C$ for *Gracilaria* for the late July experiment

Source of Variation	DF	SS	MS	F	P
CO_2	1	807.244	807.244	62.43	<0.001
Ulva	1	16.187	16.187	1.252	0.28
Filtered	1	33.868	33.868	2.619	0.125
CO_2 x *Ulva*	1	14.837	14.837	1.147	0.3
CO_2 x Filtered	1	1.515	1.515	0.117	0.737
Ulva x Filtered	1	5.772	5.772	0.446	0.514
CO_2 x *Ulva* x Filtered	1	4.412	4.412	0.341	0.567
Residual	16	206.885	12.93		
Total	23	1090.719	47.423		

Three-way analysis of variance of the tissue $\delta^{13}C$ for *Gracilaria* for the August experiment

Source of Variation	DF	SS	MS	F	P
CO_2	1	307.459	307.459	266.635	<0.001
Ulva	1	2.262	2.262	1.962	0.182
Filtered	1	2.256	2.256	1.957	0.182
CO_2 x *Ulva*	1	0.00217	0.00217	0.00188	0.966
CO_2 x Filtered	1	7.398	7.398	6.416	0.023
Ulva x Filtered	1	0.487	0.487	0.423	0.525
CO_2 x *Ulva* x Filtered	1	9.983	9.983	8.657	0.01
Residual	15	17.297	1.153		
Total	22	360.766	16.398		

Three-way analysis of variance of the tissue $\delta^{13}C$ for *Gracilaria* for the September experiment

Source of Variation	DF	SS	MS	F	P
CO_2	1	260.833	260.833	266.558	<0.001
Ulva	1	0.0242	0.0242	0.0247	0.876
Filtered	1	0.324	0.324	0.331	0.57
CO_2 x *Ulva*	1	0.461	0.461	0.471	0.499
CO_2 x Filtered	1	0.019	0.019	0.0194	0.89
Ulva x Filtered	1	0.183	0.183	0.187	0.669
CO_2 x *Ulva* x Filtered	1	0.316	0.316	0.323	0.575
Residual	24	23.485	0.979		
Total	31	285.644	9.214		

Three-way analysis of variance of the tissue $\delta^{13}C$ for *Gracilaria* for the October experiment

Source of Variation	DF	SS	MS	F	P
CO_2	1	885.354	885.354	399.976	<0.001
Ulva	1	0.329	0.329	0.148	0.704
Filtered	1	2.032	2.032	0.918	0.348
CO_2 x *Ulva*	1	2.864	2.864	1.294	0.267
CO_2 x Filtered	1	0.833	0.833	0.376	0.546
Ulva x Filtered	1	9.388	9.388	4.241	0.051
CO_2 x *Ulva* x Filtered	1	6.562	6.562	2.965	0.099
Residual	23	50.911	2.214		
Total	30	973.57	32.452		

Three-way analysis of variance of the tissue carbon for *Gracilaria* for the early July experiment

Source of Variation	DF	SS	MS	F	P
CO_2	1	4.78E-05	4.78E-05	0.102	0.753
Ulva	1	9.93E-05	9.93E-05	0.213	0.651
Filtered	1	9.98E-05	9.98E-05	0.214	0.65
CO_2 x *Ulva*	1	8.59E-05	8.59E-05	0.184	0.674
CO_2 x Filtered	1	0.000302	0.000302	0.647	0.433
Ulva x Filtered	1	0.00264	0.00264	5.648	0.03
CO_2 x *Ulva* x Filtered	1	0.000032	0.000032	0.0685	0.797
Residual	16	0.00747	0.000467		
Total	23	0.0108	0.000468		

Three-way analysis of variance of the tissue carbon for *Gracilaria* for the late July experiment

Source of Variation	DF	SS	MS	F	P
CO_2	1	0.00144	0.00144	5.247	0.036
Ulva	1	1.33E-05	1.33E-05	0.0486	0.828
Filtered	1	0.0026	0.0026	9.506	0.007
CO_2 x *Ulva*	1	0.00129	0.00129	4.722	0.045
CO_2 x Filtered	1	0.000171	0.000171	0.625	0.441
Ulva x Filtered	1	0.0019	0.0019	6.943	0.018
CO_2 x *Ulva* x Filtered	1	1.53E-05	1.53E-05	0.0559	0.816
Residual	16	0.00438	0.000274		
Total	23	0.0118	0.000513		

Three-way analysis of variance of the tissue carbon for *Gracilaria* for the August experiment

Source of Variation	DF	SS	MS	F	P
CO_2	1	0.00139	0.00139	2.438	0.138
Ulva	1	0.000205	0.000205	0.36	0.557
Filtered	1	0.000139	0.000139	0.244	0.628
CO_2 x *Ulva*	1	9.57E-06	9.57E-06	0.0167	0.899
CO_2 x Filtered	1	0.000235	0.000235	0.41	0.531
Ulva x Filtered	1	0.00102	0.00102	1.788	0.2
CO_2 x *Ulva* x Filtered	1	3.93E-08	3.93E-08	6.89E-05	0.993
Residual	16	0.00914	0.000571		
Total	23	0.0121	0.000528		

Three-way analysis of variance of the tissue carbon for *Gracilaria* for the September experiment

Source of Variation	DF	SS	MS	F	P
CO_2	1	0.00279	0.00279	3.026	0.095
Ulva	1	1.33E-05	1.33E-05	0.0144	0.905
Filtered	1	8.49E-06	8.49E-06	0.00921	0.924
CO_2 x *Ulva*	1	0.000177	0.000177	0.193	0.665
CO_2 x Filtered	1	9.27E-05	9.27E-05	0.101	0.754
Ulva x Filtered	1	1.39E-09	1.39E-09	1.51E-06	0.999
CO_2 x *Ulva* x Filtered	1	0.000142	0.000142	0.154	0.698
Residual	24	0.0221	0.000921		
Total	31	0.0253	0.000817		

Three-way analysis of variance of the tissue carbon for *Gracilaria* for the October experiment

Source of Variation	DF	SS	MS	F	P
CO_2	1	0.000717	0.000717	1.115	0.302
Ulva	1	3.35E-05	3.35E-05	0.0521	0.821
Filtered	1	0.00121	0.00121	1.878	0.184
CO_2 x *Ulva*	1	3.29E-06	3.29E-06	0.00511	0.944
CO_2 x Filtered	1	0.000663	0.000663	1.031	0.32
Ulva x Filtered	1	5.55E-05	5.55E-05	0.0863	0.772
CO_2 x *Ulva* x Filtered	1	0.00232	0.00232	3.603	0.07
Residual	23	0.0148	0.000643		
Total	30	0.0198	0.000661		

Three-way analysis of variance of the tissue nitrogen for *Gracilaria* for the early July experiment

Source of Variation	DF	SS	MS	F	P
CO_2	1	2.22E-05	2.22E-05	1.469	0.243
Ulva	1	6.4E-07	6.4E-07	0.0424	0.839
Filtered	1	0.000215	0.000215	14.231	0.002
CO_2 x *Ulva*	1	2.07E-05	2.07E-05	1.372	0.259
CO_2 x Filtered	1	4.88E-05	4.88E-05	3.236	0.091
Ulva x Filtered	1	0.000136	0.000136	9.014	0.008
CO_2 x *Ulva* x Filtered	1	2.57E-05	2.57E-05	1.705	0.21
Residual	16	0.000241	1.51E-05		
Total	23	0.00071	3.09E-05		

Three-way analysis of variance of the tissue nitrogen for *Gracilaria* for the late July experiment

Source of Variation	DF	SS	MS	F	P
CO_2	1	1.82E-05	1.82E-05	3.86	0.067
Ulva	1	1.51E-05	1.51E-05	3.197	0.093
Filtered	1	0.000191	0.000191	40.666	<0.001
CO_2 x *Ulva*	1	6.45E-05	6.45E-05	13.706	0.002
CO_2 x Filtered	1	1.53E-06	1.53E-06	0.324	0.577
Ulva x Filtered	1	2.17E-09	2.17E-09	0.000462	0.983
CO_2 x *Ulva* x Filtered	1	9.2E-06	9.2E-06	1.953	0.181
Residual	16	7.53E-05	4.71E-06		
Total	23	0.000375	1.63E-05		

Three-way analysis of variance of the tissue nitrogen for *Gracilaria* for the August experiment

Source of Variation	DF	SS	MS	F	P
CO_2	1	2.43E-08	2.43E-08	0.00305	0.957
Ulva	1	4.39E-05	0.0000439	5.512	0.032
Filtered	1	2.11E-05	0.0000211	2.648	0.123
CO_2 x *Ulva*	1	3.68E-06	3.68E-06	0.461	0.507
CO_2 x Filtered	1	4.6E-06	0.0000046	0.577	0.459
Ulva x Filtered	1	4.29E-09	4.29E-09	0.000538	0.982
CO_2 x *Ulva* x Filtered	1	7.11E-06	7.11E-06	0.892	0.359
Residual	16	0.000128	7.97E-06		
Total	23	0.000208	9.05E-06		

Three-way analysis of variance of the tissue nitrogen for *Gracilaria* for the September experiment

Source of Variation	DF	SS	MS	F	P
CO_2	1	0.000022	0.000022	6.983	0.014
Ulva	1	3.59E-05	3.59E-05	11.416	0.002
Filtered	1	0.000101	0.000101	31.959	<0.001
CO_2 x *Ulva*	1	8.3E-06	8.3E-06	2.635	0.118
CO_2 x Filtered	1	0.000018	0.000018	5.714	0.025
Ulva x Filtered	1	1.08E-07	1.08E-07	0.0344	0.854
CO_2 x *Ulva* x Filtered	1	7.43E-08	7.43E-08	0.0236	0.879
Residual	24	7.56E-05	3.15E-06		
Total	31	0.000261	8.41E-06		

Three-way analysis of variance of the tissue nitrogen for *Gracilaria* for the October experiment

Source of Variation	DF	SS	MS	F	P
CO_2	1	1.17E-05	0.0000117	1.283	0.269
Ulva	1	0.000131	0.000131	14.353	<0.001
Filtered	1	0.000091	0.000091	9.985	0.004
CO_2 x *Ulva*	1	1.87E-05	0.0000187	2.046	0.166
CO_2 x Filtered	1	2.13E-06	2.13E-06	0.233	0.634
Ulva x Filtered	1	2.17E-05	0.0000217	2.38	0.137
CO_2 x *Ulva* x Filtered	1	7.12E-06	7.12E-06	0.781	0.386
Residual	23	0.00021	9.11E-06		
Total	30	0.000479	0.000016		

Three-way analysis of variance of the tissue C:N for *Gracilaria* for the early July experiment

Source of Variation	DF	SS	MS	F	P
CO_2	1	57.836	57.836	3.625	0.075
Ulva	1	17.293	17.293	1.084	0.313
Filtered	1	533.096	533.096	33.41	<0.001
CO_2 x *Ulva*	1	17.141	17.141	1.074	0.315
CO_2 x Filtered	1	68.366	68.366	4.285	0.055
Ulva x Filtered	1	97.61	97.61	6.117	0.025
CO_2 x *Ulva* x Filtered	1	47.512	47.512	2.978	0.104
Residual	16	255.296	15.956		
Total	23	1094.15	47.572		

Three-way analysis of variance of the tissue C:N for *Gracilaria* for the late July experiment

Source of Variation	DF	SS	MS	F	P
CO_2	1	22.778	22.778	2.254	0.153
Ulva	1	32.128	32.128	3.179	0.094
Filtered	1	441.833	441.833	43.718	<0.001
CO_2 x *Ulva*	1	68.615	68.615	6.789	0.019
CO_2 x Filtered	1	18.677	18.677	1.848	0.193
Ulva x Filtered	1	0.0995	0.0995	0.00985	0.922
CO_2 x *Ulva* x Filtered	1	0.019	0.019	0.00188	0.966
Residual	16	161.705	10.107		
Total	23	745.855	32.428		

Three-way analysis of variance of the tissue C:N for *Gracilaria* for the August experiment

Source of Variation	DF	SS	MS	F	P
CO_2	1	3.495	3.495	0.356	0.559
Ulva	1	52.291	52.291	5.334	0.035
Filtered	1	6.656	6.656	0.679	0.422
CO_2 x *Ulva*	1	6.612	6.612	0.674	0.424
CO_2 x Filtered	1	3.627	3.627	0.37	0.552
Ulva x Filtered	1	1.194	1.194	0.122	0.732
CO_2 x *Ulva* x Filtered	1	13.916	13.916	1.42	0.251
Residual	16	156.855	9.803		
Total	23	244.644	10.637		

Three-way analysis of variance of the tissue C:N for *Gracilaria* for the September experiment

Source of Variation	DF	SS	MS	F	P
CO_2	1	66.139	66.139	12.563	0.002
Ulva	1	28.729	28.729	5.457	0.029
Filtered	1	74.106	74.106	14.077	0.001
CO_2 x *Ulva*	1	0.578	0.578	0.11	0.743
CO_2 x Filtered	1	11.299	11.299	2.146	0.156
Ulva x Filtered	1	0.126	0.126	0.0239	0.878
CO_2 x *Ulva* x Filtered	1	3.086	3.086	0.586	0.452
Residual	23	121.084	5.265		
Total	30	312.539	10.418		

Three-way analysis of variance of the tissue C:N for *Gracilaria* for the October experiment

Source of Variation	DF	SS	MS	F	P
CO_2	1	31.069	31.069	5.109	0.034
Ulva	1	104.004	104.004	17.104	<0.001
Filtered	1	28.785	28.785	4.734	0.04
CO_2 x *Ulva*	1	24.465	24.465	4.023	0.057
CO_2 x Filtered	1	0.0994	0.0994	0.0163	0.899
Ulva x Filtered	1	7.524	7.524	1.237	0.277
CO_2 x *Ulva* x Filtered	1	0.677	0.677	0.111	0.742
Residual	23	139.857	6.081		
Total	30	334.766	11.159		

Three-way analysis of variance of the tissue carbon for *Ulva* for the early July experiment

Source of Variation	DF	SS	MS	F	P
CO_2	1	0.000155	0.000155	0.903	0.356
Gracilaria	1	2.99E-05	2.99E-05	0.174	0.682
Filtered	1	0.000084	0.000084	0.49	0.494
CO_2 x *Gracilaria*	1	0.000683	0.000683	3.984	0.063
CO_2 x Filtered	1	0.000011	0.000011	0.0639	0.804
Gracilaria x Filtered	1	0.00131	0.00131	7.617	0.014
CO_2 x *Gracilaria* x Filtered	1	0.00053	0.00053	3.088	0.098
Residual	16	0.00274	0.000172		
Total	23	0.00554	0.000241		

Three-way analysis of variance of the tissue carbon for *Ulva* for the late July experiment

Source of Variation	DF	SS	MS	F	P
CO_2	1	0.000687	0.000687	0.584	0.456
Gracilaria	1	8.2E-08	8.2E-08	6.97E-05	0.993
Filtered	1	0.00111	0.00111	0.942	0.346
CO_2 x *Gracilaria*	1	0.000543	0.000543	0.462	0.506
CO_2 x Filtered	1	0.00162	0.00162	1.377	0.258
Gracilaria x Filtered	1	0.0023	0.0023	1.955	0.181
CO_2 x *Gracilaria* x Filtered	1	0.000757	0.000757	0.644	0.434
Residual	16	0.0188	0.00118		
Total	23	0.0258	0.00112		

Three-way analysis of variance of the tissue carbon for *Ulva* for the August experiment

Source of Variation	DF	SS	MS	F	P
CO_2	1	0.000125	0.000125	0.41	0.531
Gracilaria	1	0.000295	0.000295	0.965	0.341
Filtered	1	0.000138	0.000138	0.451	0.512
CO_2 x *Gracilaria*	1	9.52E-05	9.52E-05	0.312	0.584
CO_2 x Filtered	1	0.000059	0.000059	0.193	0.666
Gracilaria x Filtered	1	5.53E-05	5.53E-05	0.181	0.676
CO_2 x *Gracilaria* x Filtered	1	6.23E-08	6.23E-08	0.000204	0.989
Residual	16	0.00488	0.000305		
Total	23	0.00565	0.000246		

Three-way analysis of variance of the tissue carbon for *Ulva* for the September experiment

Source of Variation	DF	SS	MS	F	P
CO_2	1	0.000678	0.000678	1.555	0.225
Gracilaria	1	0.00049	0.00049	1.124	0.3
Filtered	1	8.16E-05	8.16E-05	0.187	0.669
CO_2 x *Gracilaria*	1	0.00114	0.00114	2.614	0.12
CO_2 x Filtered	1	0.00454	0.00454	10.412	0.004
Gracilaria x Filtered	1	0.000552	0.000552	1.267	0.272
CO_2 x *Gracilaria* x Filtered	1	4E-08	4E-08	9.18E-05	0.992
Residual	23	0.01	0.000436		
Total	30	0.0178	0.000594		

Three-way analysis of variance of the tissue carbon for *Ulva* for the October experiment

Source of Variation	DF	SS	MS	F	P
CO_2	1	0.00176	0.00176	2.109	0.159
Gracilaria	1	1.65E-05	1.65E-05	0.0198	0.889
Filtered	1	4.8E-07	4.8E-07	0.000575	0.981
CO_2 x *Gracilaria*	1	0.000157	0.000157	0.188	0.668
CO_2 x Filtered	1	0.000204	0.000204	0.244	0.626
Gracilaria x Filtered	1	0.000031	0.000031	0.0372	0.849
CO_2 x *Gracilaria* x Filtered	1	0.000433	0.000433	0.518	0.479
Residual	24	0.02	0.000835		
Total	31	0.0226	0.00073		

Three-way analysis of variance of the tissue nitrogen for *Ulva* for the early July experiment

Source of Variation	DF	SS	MS	F	P
CO_2	1	3.17E-06	3.17E-06	1.414	0.252
Gracilaria	1	5.26E-06	5.26E-06	2.343	0.145
Filtered	1	0.000191	0.000191	85.053	<0.001
CO_2 x *Gracilaria*	1	1.69E-06	1.69E-06	0.751	0.399
CO_2 x Filtered	1	6.17E-08	6.17E-08	0.0275	0.87
Gracilaria x Filtered	1	1.18E-05	1.18E-05	5.254	0.036
CO_2 x *Gracilaria* x Filtered	1	7.32E-07	7.32E-07	0.326	0.576
Residual	16	3.59E-05	2.25E-06		
Total	23	0.00025	1.09E-05		

Three-way analysis of variance of the tissue nitrogen for *Ulva* for the late July experiment

Source of Variation	DF	SS	MS	F	P
CO_2	1	2.38E-06	2.38E-06	0.216	0.648
Gracilaria	1	8.07E-07	8.07E-07	0.0733	0.79
Filtered	1	6.64E-05	0.0000664	6.021	0.026
CO_2 x *Gracilaria*	1	1.64E-06	1.64E-06	0.148	0.705
CO_2 x Filtered	1	3.5E-07	3.5E-07	0.0318	0.861
Gracilaria x Filtered	1	4.31E-06	4.31E-06	0.391	0.54
CO_2 x *Gracilaria* x Filtered	1	2.33E-06	2.33E-06	0.211	0.652
Residual	16	0.000176	0.000011		
Total	23	0.000254	0.0000111		

Three-way analysis of variance of the tissue nitrogen for *Ulva* for the August experiment

Source of Variation	DF	SS	MS	F	P
CO_2	1	5.27E-06	5.27E-06	0.658	0.429
Gracilaria	1	1.67E-05	0.0000167	2.091	0.167
Filtered	1	8.51E-05	0.0000851	10.634	0.005
CO_2 x *Gracilaria*	1	2.8E-06	0.0000028	0.349	0.563
CO_2 x Filtered	1	2.79E-06	2.79E-06	0.349	0.563
Gracilaria x Filtered	1	5.27E-05	0.0000527	6.585	0.021
CO_2 x *Gracilaria* x Filtered	1	3.42E-06	3.42E-06	0.427	0.523
Residual	16	0.000128	8.01E-06		
Total	23	0.000297	0.0000129		

Three-way analysis of variance of the tissue nitrogen for *Ulva* for the September experiment

Source of Variation	DF	SS	MS	F	P
CO_2	1	2.9E-06	0.0000029	1.488	0.235
Gracilaria	1	1.88E-06	1.88E-06	0.963	0.337
Filtered	1	2.13E-05	0.0000213	10.912	0.003
CO_2 x *Gracilaria*	1	1.93E-06	1.93E-06	0.991	0.33
CO_2 x Filtered	1	5.78E-09	5.78E-09	0.00296	0.957
Gracilaria x Filtered	1	1.18E-05	0.0000118	6.049	0.022
CO_2 x *Gracilaria* x Filtered	1	2.61E-06	2.61E-06	1.341	0.259
Residual	23	4.48E-05	1.95E-06		
Total	30	0.000089	2.97E-06		

Three-way analysis of variance of the tissue nitrogen for *Ulva* for the October experiment

Source of Variation	DF	SS	MS	F	P
CO_2	1	3.18E-09	3.18E-09	0.001	0.975
Gracilaria	1	5.93E-06	5.93E-06	1.867	0.184
Filtered	1	3.06E-06	3.06E-06	0.964	0.336
CO_2 x *Gracilaria*	1	7.32E-07	7.32E-07	0.231	0.635
CO_2 x Filtered	1	8.15E-08	8.15E-08	0.0257	0.874
Gracilaria x Filtered	1	0.0000015	0.0000015	0.474	0.498
CO_2 x *Gracilaria* x Filtered	1	2.06E-07	2.06E-07	0.065	0.801
Residual	24	0.0000762	3.17E-06		
Total	31	0.0000877	2.83E-06		

Three-way analysis of variance of the tissue C:N for *Ulva* for the early July experiment

Source of Variation	DF	SS	MS	F	P
CO_2	1	60.304	60.304	3.115	0.097
Gracilaria	1	5.327	5.327	0.275	0.607
Filtered	1	2024.355	2024.355	104.559	<0.001
CO_2 x *Gracilaria*	1	3.167	3.167	0.164	0.691
CO_2 x Filtered	1	22.906	22.906	1.183	0.293
Gracilaria x Filtered	1	28.628	28.628	1.479	0.242
CO_2 x *Gracilaria* x Filtered	1	6.405	6.405	0.331	0.573
Residual	16	309.776	19.361		
Total	23	2460.867	106.994		

Three-way analysis of variance of the tissue C:N for *Ulva* for the late July experiment

Source of Variation	DF	SS	MS	F	P
CO_2	1	6.605	6.605	0.192	0.667
Gracilaria	1	14.493	14.493	0.422	0.525
Filtered	1	661.367	661.367	19.261	<0.001
CO_2 x *Gracilaria*	1	27.963	27.963	0.814	0.38
CO_2 x Filtered	1	11.549	11.549	0.336	0.57
Gracilaria x Filtered	1	0.00146	0.00146	4.24E-05	0.995
CO_2 x *Gracilaria* x Filtered	1	6.484	6.484	0.189	0.67
Residual	16	549.389	34.337		
Total	23	1277.85	55.559		

Three-way analysis of variance of the tissue C:N for *Ulva* for the August experiment

Source of Variation	DF	SS	MS	F	P
CO_2	1	21.361	21.361	1.211	0.287
Gracilaria	1	8.219	8.219	0.466	0.505
Filtered	1	305.054	305.054	17.289	<0.001
CO_2 x *Gracilaria*	1	18.795	18.795	1.065	0.317
CO_2 x Filtered	1	15.42	15.42	0.874	0.364
Gracilaria x Filtered	1	146.02	146.02	8.276	0.011
CO_2 x *Gracilaria* x Filtered	1	23.864	23.864	1.352	0.262
Residual	16	282.314	17.645		
Total	23	821.046	35.698		

Three-way analysis of variance of the tissue C:N for *Ulva* for the September experiment

Source of Variation	DF	SS	MS	F	P
CO_2	1	55.191	55.191	3.585	0.071
Gracilaria	1	0.675	0.675	0.0439	0.836
Filtered	1	272.751	272.751	17.717	<0.001
CO_2 x *Gracilaria*	1	1.711	1.711	0.111	0.742
CO_2 x Filtered	1	27.663	27.663	1.797	0.193
Gracilaria x Filtered	1	61.005	61.005	3.963	0.059
CO_2 x *Gracilaria* x Filtered	1	17.035	17.035	1.107	0.304
Residual	23	354.08	15.395		
Total	30	808.275	26.943		

Three-way analysis of variance of the tissue C:N for *Ulva* for the October experiment

Source of Variation	DF	SS	MS	F	P
CO_2	1	26.543	26.543	1.646	0.212
Gracilaria	1	73.064	73.064	4.531	0.044
Filtered	1	34.41	34.41	2.134	0.157
CO_2 x *Gracilaria*	1	22.149	22.149	1.373	0.253
CO_2 x Filtered	1	0.101	0.101	0.00626	0.938
Gracilaria x Filtered	1	13.702	13.702	0.85	0.366
CO_2 x *Gracilaria* x Filtered	1	0.45	0.45	0.0279	0.869
Residual	24	387.028	16.126		
Total	31	557.447	17.982		

S7 Tables: Tissue $\delta^{13}C$ content (‰) of dry tissue samples of *Gracilaria* and *Ulva* for July through October experiments. Values represent means ± standard deviation.

Gracilaria

	Treatment	Early July	Late July	August	September	October
Filtered	Control	-12.76±1.67	-12.46±0.77	-12.51±0.90	-12.90±0.48	-12.05±0.30
	Ulva	-13.41±0.22	-12.65±0.81	-15.19±2.14	-13.15±1.47	-12.63±1.21
	CO_2	-34.66±2.99	-21.12±1.70	-19.34±1.28	-18.60±0.24	-22.80±0.92
	CO_2/Ulva	-28.79±0.85	-26.18±2.02	-18.33±1.15	-18.77±1.29	-24.01±0.92
Unfiltered	Control	-14.40±1.41	-14.45±1.13	-12.28±0.42	-13.00±1.38	-13.07±1.78
	Ulva	-15.43±0.63	-14.40±2.24	-11.90±0.62	-13.35±0.95	-13.30±0.56
	CO_2	-31.85±3.77	-25.84±2.99	-19.49±1.31	-19.20±0.79	-25.02±1.77
	CO_2/Ulva	-33.84±2.47	-27.22±8.94	-21.73±1.63	-18.67±0.55	-22.17±2.72

Ulva

	Treatment	Early July	Late July	August	September	October
Filtered	Control	-11.88±2.35	-8.58±2.46	-8.10±0.64	-12.61±3.30	-12.90±4.50
	Gracilaria	-8.48±1.12	-9.57±2.43	-9.92±4.35	-12.51±0.90	-15.19±2.14
	CO_2	-40.09±0.73	-39.42±2.82	-26.32±1.73	-29.81±0.95	-36.94±1.44
	CO_2/Gracilaria	-27.63±5.59	-38.32±4.60	-27.37±3.95	-31.51±0.86	-32.92±5.28
Unfiltered	Control	-11.38±1.31	-13.91±2.73	-12.89±1.77	-14.76±2.70	-12.43±5.41
	Gracilaria	-9.33±2.71	-12.92±1.25	-11.16±2.45	-16.50±0.91	-14.23±3.87
	CO_2	-31.39±7.33	-35.84±4.75	-30.32±2.49	-30.79±0.88	-34.84±4.08
	CO_2/Gracilaria	-35.59±4.44	-38.73±4.85	-31.57±2.43	-31.91±1.81	-38.21±1.26

S8 Tables: Tissue nitrogen content (g N per g dry tissue), tissue carbon content (g N per g dry tissue), and tissue C:N of dry tissue samples of *Gracilaria* and *Ulva* for July through October experiments. Values represent means ± standard deviation.

Gracilaria

Tissue C content

	Treatment	Early July	Late July	August	September	October
Filtered	Control	0.292±0.030	0.294±0.026	0.278±0.016	0.295±0.026	0.309±0.015
	Ulva	0.274±0.017	0.329±0.030	0.298±0.012	0.287±0.032	0.330±0.008
	CO_2	0.309±0.018	0.289±0.012	0.300±0.026	0.308±0.028	0.326±0.012
	CO_2/Ulva	0.277±0.021	0.292±0.011	0.318±0.041	0.318±0.036	0.314±0.008
Unfiltered	Control	0.277±0.015	0.328±0.010	0.292±0.032	0.295±0.029	0.307±0.004
	Ulva	0.295±0.022	0.325±0.010	0.286±0.018	0.296±0.025	0.288±0.065
	CO_2	0.274±0.024	0.331±0.011	0.302±0.016	0.310±0.042	0.308±0.008
	CO_2/Ulva	0.289±0.021	0.302±0.001	0.294±0.013	0.312±0.021	0.325±0.013

Tissue N content

	Treatment	Early July	Late July	August	September	October
Filtered	Control	0.021±0.004	0.019±0.001	0.022±0.004	0.024±0.001	0.025±0.004
	Ulva	0.016±0.004	0.022±0.002	0.017±0.004	0.021±0.001	0.022±0.002
	CO_2	0.022±0.005	0.023±0.002	0.021±0.005	0.023±0.002	0.026±0.005
	CO_2/Ulva	0.018±0.002	0.017±0.002	0.020±0.001	0.022±0.001	0.017±0.002
Unfiltered	Control	0.011±0.001	0.015±0.002	0.019±0.003	0.022±0.002	0.020±0.002
	Ulva	0.020±0.008	0.016±0.001	0.017±0.001	0.019±0.003	0.018±0.004
	CO_2	0.010±0.001	0.015±0.004	0.019±0.001	0.018±0.002	0.020±0.001
	CO_2/Ulva	0.011±0.002	0.012±0.001	0.016±0.002	0.017±0.001	0.017±0.003

Tissue C:N

		Early July	Late July	August	September	October
Filtered	Control	16.4±2.1	18.1±3.1	15.3±2.4	14.3±1.3	14.7±2.2
	Ulva	19.9±2.9	17.2±0.4	21.3±5.7	16.0±1.4	17.9±1.6
	CO_2	17.3±4.6	15.0±1.4	17.9±5.8	15.6±1.1	15.1±2.5
	CO_2/Ulva	18.5±0.5	20.7±2.0	18.7±1.5	17.1±2.0	21.3±2.5
Unfiltered	Control	29.3±3.3	25.1±3.2	17.6±0.9	15.6±1.6	17.8±1.9
	Ulva	19.1±7.7	23.9±0.8	19.6±0.1	18.4±1.7	18.4±3.2
	CO_2	31.3±1.8	25.4±0.4	18.6±1.4	20.7±4.9	17.8±0.9
	CO_2/Ulva	30.1±4.6	31.0±7.4	21.6±1.3	21.6±2.4	22.6±3.8

Ulva

Tissue C content

	Treatment	Early July	Late July	August	September	October
Filtered	Control	0.302±0.004	0.259±0.073	0.296±0.008	0.280±0.015	0.315±0.036
	Gracilaria	0.315±0.008	0.300±0.044	0.288±0.022	0.251±0.023	0.307±0.019
	CO_2	0.307±0.008	0.307±0.003	0.285±0.014	0.301±0.014	0.294±0.018
	CO_2/Gracilaria	0.318±0.019	0.306±0.014	0.285±0.028	0.297±0.018	0.309±0.023
Unfiltered	Control	0.302±0.015	0.320±0.033	0.292±0.005	0.299±0.033	0.315±0.046
	Gracilaria	0.305±0.009	0.299±0.008	0.278±0.016	0.287±0.017	0.318±0.022
	CO_2	0.328±0.006	0.313±0.027	0.286±0.014	0.272±0.019	0.298±0.037
	CO_2/Gracilaria	0.291±0.024	0.295±0.013	0.280±0.022	0.285±0.020	0.295±0.014

Tissue N content

	Treatment	Early July	Late July	August	September	October
Filtered	Control	0.013±0.001	0.013±0.006	0.017±0.004	0.012±0.003	0.011±0.003
	Gracilaria	0.015±0.003	0.014±0.005	0.012±0.001	0.009±0.001	0.010±0.002
	CO_2	0.012±0.001	0.012±0.001	0.017±0.006	0.010±0.001	0.011±0.001
	CO_2/Gracilaria	0.014±0.001	0.013±0.001	0.012±0.001	0.010±0.001	0.010±0.001
Unfiltered	Control	0.008±0.001	0.011±0.004	0.010±0.001	0.009±0.001	0.010±0.003
	Gracilaria	0.009±0.002	0.009±0.001	0.013±0.003	0.009±0.001	0.009±0.001
	CO_2	0.008±0.001	0.009±0.001	0.010±0.001	0.008±0.001	0.011±0.002
	CO_2/Gracilaria	0.007±0.001	0.010±0.001	0.010±0.001	0.009±0.001	0.009±0.001

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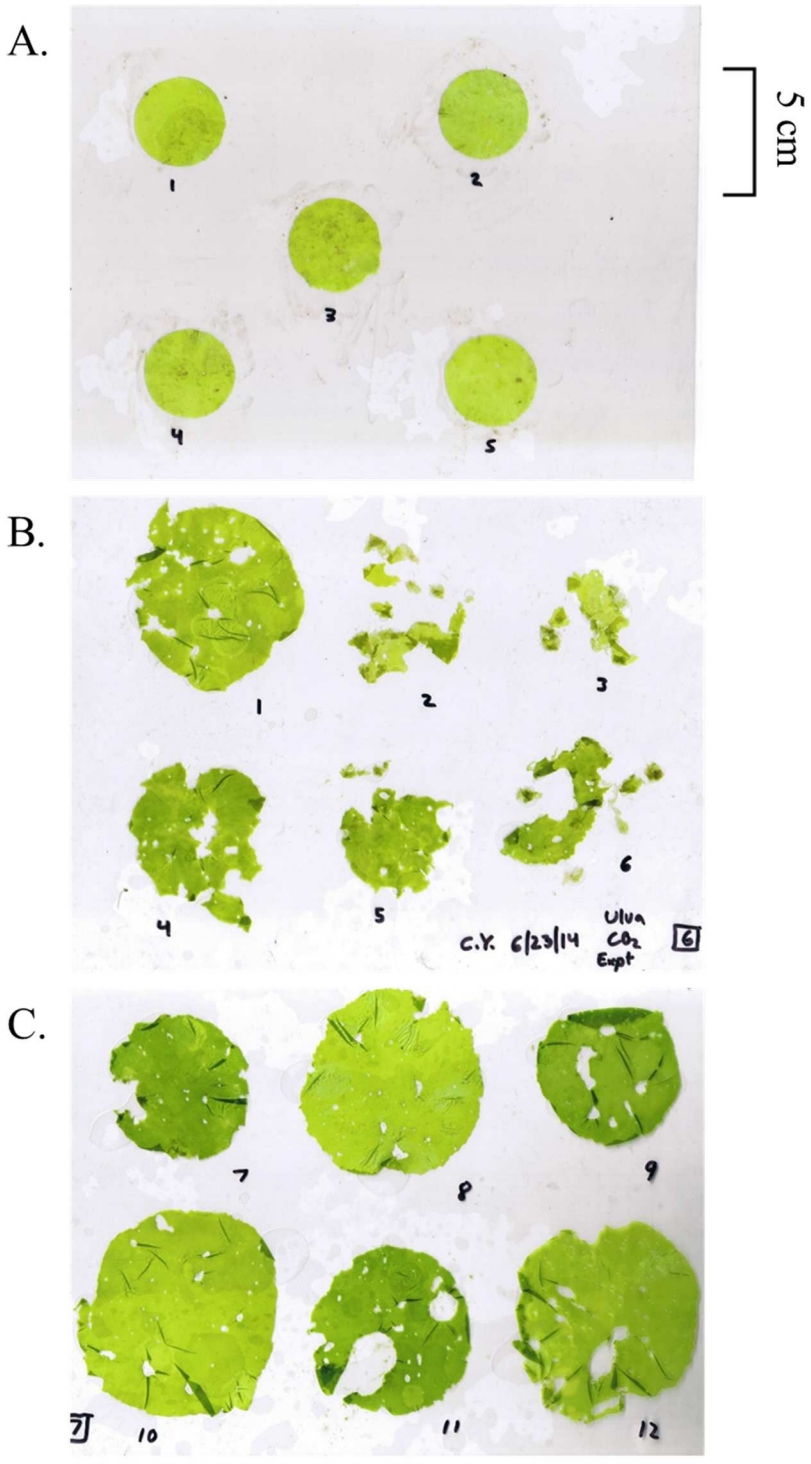
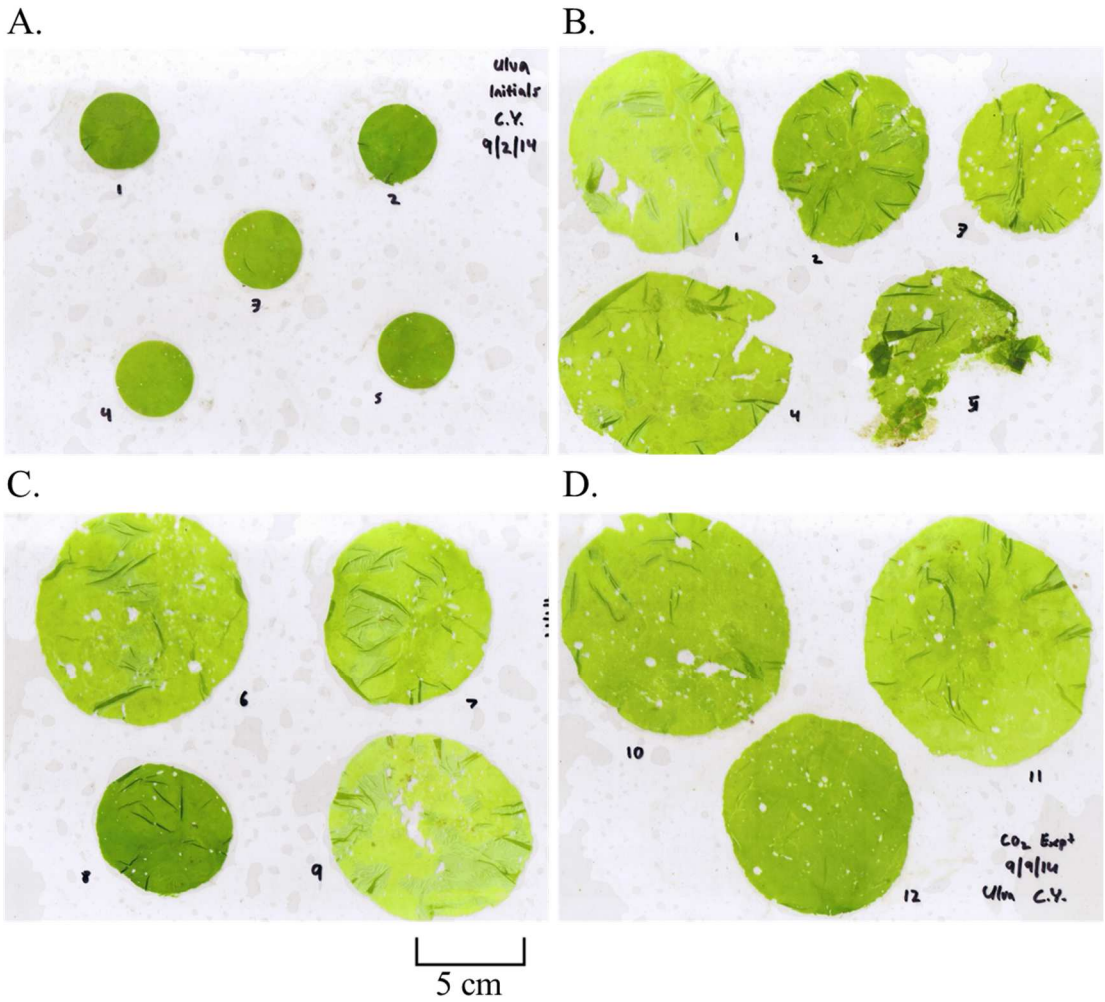
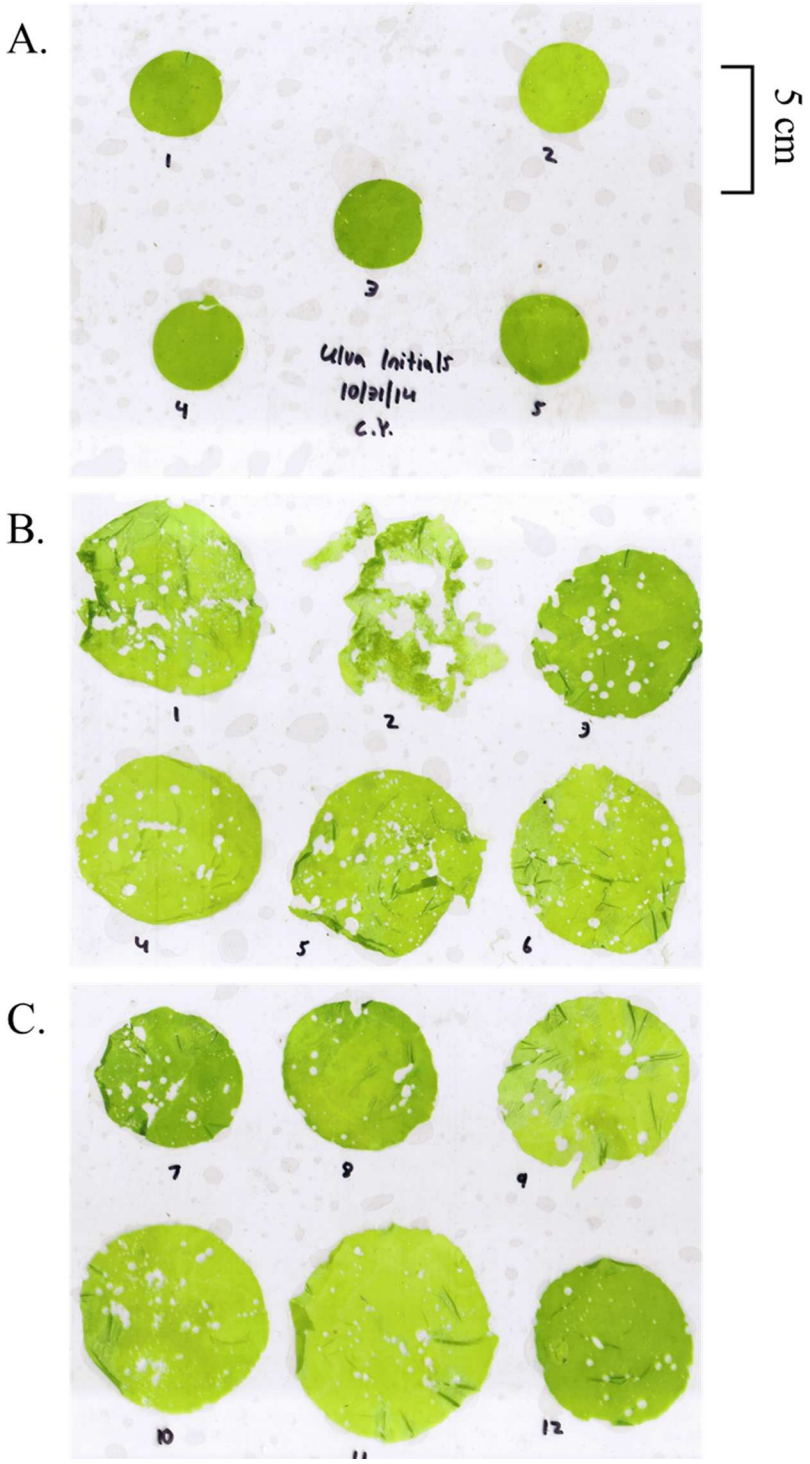
		Early July	Late July	August	September	October
Filtered	Control	27.7±0.6	26.7±8.8	21.0±4.8	28.3±6.8	35.6±5.1
	Gracilaria	24.9±4.9	26.3±5.2	27.3±1.3	31.8±4.9	35.5±4.7
	CO_2	29.2±1.7	30.3±2.9	21.5±7.2	33.8±2.8	32.0±4.1
	CO_2/Gracilaria	25.8±1.3	27.6±3.2	27.4±0.9	35.4±1.2	35.6±4.7
Unfiltered	Control	42.9±3.5	37.6±9.3	33.5±3.2	40.4±2.2	36.3±3.7
	Gracilaria	42.4±9.5	39.2±4.0	25.9±6.9	35.3±2.2	39.2±2.1
	CO_2	46.3±1.0	40.4±5.1	33.2±0.7	39.2±3.7	32.9±4.5
	CO_2/Gracilaria	49.3±4.7	35.7±4.6	33.2±2.4	38.1±4.0	38.7±1.9



S1 Figure: Samples of *Ulva rigida* from the June experiment of the CO_2/Nutrient experiments, which represents enhanced growth by *Ulva* due to elevated CO_2 concentrations. A) Initial samples; B) Samples grown under ambient CO_2 concentrations, with samples 1-3 and 4-6 representing treatments without and with nutrient additions, respectively; C) Samples grown under elevated CO_2 concentrations, with samples 7-9 and 10-12 representing treatments without and with nutrient additions, respectively.



S2 Figure: Samples of *Ulva rigida* from the early September experiment of the CO_2/Nutrient experiments, which represents enhanced growth by *Ulva* under elevated nutrient concentrations. A) Initial samples; B) Samples grown under ambient CO_2 concentrations, with samples 1-3 and 4-5 representing treatments without and with nutrient additions, respectively; C) Sample 6 was grown under ambient CO_2 and with nutrient enrichment, while samples 7-9 were grown under elevated CO_2 concentrations, without nutrient additions; D) Samples grown under elevated CO_2 and nutrient additions.



S3 Figure: Samples of *Ulva rigida* from the November experiment of the CO_2/Nutrient experiments, which represents a synergistic relationship between elevated CO_2 and nutrient concentrations. A) Initial samples; B) Samples grown under ambient CO_2 concentrations, with samples 1-3 and 4-6 representing treatments without and with nutrient additions, respectively; C) Samples grown under elevated CO_2 concentrations, with samples 7-9 and 10-12 representing treatments without and with nutrient additions, respectively.