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Divergent responses in growth and nutritional quality of coastal macroalgae to the combination of increased of pCO₂ and nutrients

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Abstract:

Coastal ecosystems are subjected to global and local environmental stressors, including increased atmospheric carbon dioxide (CO₂) (and subsequent ocean acidification) and nutrient loading. Here, we tested how two common macroalgal species in the Northwest Atlantic (*Ulva* spp. and *Fucus vesiculosus* Linneaus) respond to the combination of increased CO₂ and nutrient loading. We utilized two levels of pCO₂ with two levels of nutrients in a full factorial design, testing the growth rates and tissue quality of *Ulva* and *Fucus* grown for 21 days in monoculture and biculture. We found that the opportunistic, fast-growing *Ulva* exhibited increased growth rates under high pCO₂ and high nutrients, with growth rates increasing three-fold above *Ulva* grown in ambient pCO₂ and ambient nutrients. By contrast, *Fucus* growth rates were not impacted by either environmental factor. Both species exhibited a decline in carbon to nitrogen ratios (C:N) with elevated nutrients, but pCO₂ concentration did not alter tissue quality in either species. Species grown in biculture exhibited similar growth rates to those in monoculture conditions, but *Fucus* C:N increased significantly when grown with *Ulva*, indicating an effect of the presence of *Ulva* on *Fucus*. Our results suggest that the combination of ocean acidification and nutrients will enhance abundance of opportunistic algal species in coastal systems and will likely drive macroalgal community shifts, based on species-specific responses to future conditions.

Key words: ocean acidification, eutrophication, climate change, macroalgae, nutritional quality

1. Introduction

Increasing amounts of carbon dioxide (CO₂) in the earth's atmosphere are the driving force behind global climate change (Pachauri et al., 2014). Ocean acidification, a decrease in pH brought about by increased atmospheric CO₂, has garnered attention due to the overwhelmingly negative effects predicted for calcifying organisms (Comeau et al., 2014; Diaz-Pulido et al., 2011; Hoegh-Guldberg et al., 2007; Waldbusser et al., 2015). Changes in ocean chemistry associated with ocean acidification, such as lowered saturation states, are causing reductions in growth, increased shell dissolution, and declines in fitness and performance of many marine calcifying species (Ries et al., 2009; Waldbusser et al., 2015). Ocean acidification has also been shown to impact the growth, development, and sensory systems in fish (Frommel et al., 2016; Munday et al., 2009).

Conversely, less attention has been paid to non-calcifying autotrophic organisms. These species may benefit from ocean acidification and the subsequent change in ocean chemistry, as increased concentrations of both aqueous CO₂ and bicarbonate (HCO₃) may enhance photosynthesis and growth in primary producers. Enhanced growth rates under increased CO₂ conditions have been observed in fleshy macroalgae (Kübler et al., 1999; Olischläger and Wiencke, 2013; Diaz-Pulido et al., 2011; Zou, 2005, p. 200; Kroeker et al., 2010) and seagrasses (Zimmerman et al., 1997). However, the response of primary producers to increased CO₂ is highly species specific, ultimately dependent on carbon limitation and carbon acquisition ability as well as developmental stage (Gaitán-Espitia et al., 2014; Olischläger et al., 2012). As such, negative and neutral responses to CO₂ enrichment have also been observed (Gutow et al., 2014; Rautenberger et al., 2015). Divergent responses of fleshy macroalgae to CO₂ enrichment are correlated with the

presence and efficiency of the carbon concentrating mechanism (CCM) (Giordano et al., 2005; Raven and Beardall, 2003). Due to highly abundant bicarbonate ions, most macroalgae rely on CCMs to convert HCO_3^- to CO_2 for use in photosynthesis (Hepburn et al., 2011). In addition, many species also have the ability to passively diffuse CO_2 and may gain an advantage under future conditions due to reduced reliance and down-regulation of CCMs. While most marine macroalgae have CCMs, a few species within the Rhodophyta rely on passive diffusion of CO_2 for photosynthesis (Giordano et al., 2005; Raven and Beardall, 2003). These species, among others, should experience enhanced growth and photosynthesis due to the increased concentration of CO_2 associated with ocean acidification.

While ocean acidification is projected to impact all marine systems, the effects will likely vary across ecosystems (Hofmann et al. 2011). The signal of ocean acidification is easy to determine in the open ocean. Unlike in the open ocean, coastal pH is highly variable due to daily and seasonal shifts in photosynthesis and respiration, and coastal acidification may be driven more by eutrophication than increases in atmospheric CO_2 (Cai et al., 2011). In coastal and estuarine environments of the northwest Atlantic, daily pH variation ranges from 0.1 to 0.65 units, depending on the location and season (Turner, 2015). Nutrient loading (and potential eutrophication events) also impacts coastal bays and estuaries with low flow and low turnover (Lee and Olsen, 1985). Nutrients can enter these waterways via agricultural and urban runoff and sewage treatment discharge, pumping excess nitrogen and phosphorous into the water column (Nixon, 1995). While these nutrients are critical to algal growth, excess concentrations can facilitate harmful algal blooms, either composed of micro and/or macroalgae

(Anderson et al., 2002) whose decomposition or respiration can lower oxygen levels in the water column potentially leading to hypoxic events, with detrimental impacts on coinhabitants (Granger et al., 2000; Thomsen et al., 2006; Valiela et al., 1997).

Macroalgal blooms can also act as a deterrent to coastal recreation (Valiela et al., 1997; Worm and Lotze, 2006).

Our understanding of climate effects on coastal zones is critical, as these ecosystems hold high value in biodiversity as well as economic and societal importance (Harley et al., 2006). Increased CO₂, combined with increased concentrations of limiting nutrients, could act in conjunction to stimulate and enhance growth in primary producers. While acidification studies are beginning to incorporate additional environmental stressors such as light intensity and warming (Olischläger and Wiencke, 2013; Rautenberger et al., 2015; Roleda et al., 2012; Sarker et al., 2013), the combined effects of acidification and nutrients on primary producers are less understood (but see Campbell and Fourqurean, 2014; Falkenberg et al., 2013; Russell et al., 2009).

In coastal ecosystems, the green macroalga *Ulva* and the brown macroalga *Fucus* have different life history and ecological traits. *Ulva* is a fast-growing, opportunistic, ephemeral genus that thrives in a wide range of environments. *Fucus* is a long-lived, slow growing, perennial genus that creates complex, three-dimensional habitat for other organisms. These genera, among others, form the base of coastal marine food webs in the northwest Atlantic and are commonly grazed by herbivores and omnivores (Bracken et al., 2014; Lubchenco, 1983; Watson and Norton, 1985). Both genera use CCMs (Koch et al., 2013), but exhibit divergent responses, with increased growth rates for *Ulva lactuca* and decreased growth rates for *Fucus vesiculosus* under high CO₂ conditions (Gutow et

al., 2014; Olischläger et al., 2013). Similarly, *Ulva lactuca* has increased growth rates under high nutrients (Steffensen, 1976). *Fucus vesiculosus* experiences a reduction in growth and cover due to the indirect effects of added nutrients, such as increased turbidity and increased growth of epiphytic algae (Berger et al., 2004).

The objective of our research was to quantify the interaction of CO₂ and nutrients on *Ulva* spp. and *F. vesiculosus* found in Narragansett Bay, RI, by assessing growth rates, tissue quality (tissue C:N ratio), carbon and nitrogen content of algal tissues, and potential competitive effects. In the coastal ecosystems, perennial *Fucus* spp. can grow in high densities in the intertidal and shallow subtidal zones, up to 4,000 plants/m² (Creed et al., 1996). In Narragansett Bay, *Fucus* spp. can account for 5-20% of year round algal cover, whereas during the growing season, *Ulva* spp. density can range from 5-75 g/m² (Guidone et al., 2013). While *U. lactuca* was chosen for this study, recent invasions of the cryptic *U. australis* have nullified our initial identification (Guidone et al., 2013; Hofmann et al., 2010). It is likely that the tested specimens are a mix of two species: *U. lactuca* and *U. australis*. We will hereafter refer to our test organisms as *Ulva* and *Fucus*. We predicted that the growth rate and tissue quality of *Ulva* would increase with extreme pCO₂ levels and increased nutrients, and the combination of the two environmental factors would result in a synergistic effect on its growth rate (Neori et al., 1991; Russell et al., 2009). By contrast, we predicted that extreme levels of pCO₂ will decrease the growth rate of *Fucus*, which is likely to occur due to potential pH sensitivity of its CCM (Axelsson et al., 2000; Gutow et al., 2014), but increase tissue quality (as seen in Gutow et al., 2014). We hypothesized that *Fucus* growth would be unaffected by nutrient loading as this species has lower nutrient uptake rates and is typically adapted to

lower nutrient environments (Savage and Elmgren, 2004), and nutrients may indirectly reduce growth rates by promoting the growth of competitors (Hemmi et al., 2005; Pedersen and Borum, 1996; Worm and Lotze, 2006). Growth rates and tissue quality of both *Ulva* and *Fucus* were tested in a biculture experiment to inform community response, where we expect opportunistic *Ulva* to outgrow *Fucus* resulting in lower growth rates and tissue quality of *Fucus* (Connell and Russell, 2010; Falkenberg et al., 2013; Worm and Lotze, 2006). We interpret our results in the context of macroalgal response to climate change and future ecosystem structure.

2. Materials and Methods

2.1 Algal Collection and Experimental Design

We conducted experiments using the flow-through seawater facility at the US Environmental Protection Agency Atlantic Ecology Division in Narragansett, RI (*Ulva* monoculture - September 2014, *Fucus* monoculture - November 2014, and biculture - October 2014). First, thalli of *Ulva* and *Fucus* were collected from the shallow subtidal zone at the University of Rhode Island's Narragansett Bay Campus beach (41°29'26"N, -71°25'11"4W) in August 2014, September 2014, and October 2014. While much of the *Fucus* population at this site remains strictly intertidal, *Fucus* can thrive in the lower intertidal and shallow subtidal (Lubchenco, 1983). As our experiments did not replicate tidal cycles, it was critical that the collected *Fucus* came from the shallow subtidal in order to not introduce stress associated with full-time immersion. In order to capture the sites of *Fucus* growth (Moss, 1967), non-reproductive tips of *Fucus* (~3-5cm in length) were cut from adult thalli. Apical tips of *Fucus* used in experiments were taken from

unique adult thalli, so that each tip came from a different individual and that tips were not clones of one another. *Fucus* tips and *Ulva* thalli were cleaned of any epiphytes, and transferred into separate 20L glass aquaria with flow-through seawater and aeration. Algal individuals (*Ulva* thalli and *Fucus* tips) were acclimated to lab conditions for five days prior to the start of each experiment.

To set up experiments, we first spun algae 20 × in a salad spinner (Thornber et al., 2008), removed a small piece (~10% of starting mass) of the thallus which was then dried at 60°C for 24 h and then placed in a desiccator for C:N analysis (see below), and then recorded the initial algal wet mass of each remaining piece. We placed individuals into 20L aquaria with one individual per tank (for monocultures) or one individual of each species (for the biculture experiment). Starting sizes of all test individuals were about the same size (3-5 cm in length) but due to physical and structural differences in the two species the starting wet mass differed. Starting wet mass was 0.40g and 0.55g for *Ulva* and *Fucus*, respectively. Based on these starting masses and tank volumes, the density of macroalgae was 5.6 g/m² in the *Ulva* monoculture, 7.72 g/m² in the *Fucus* monoculture, and about 13.5 g/m² in the biculture experiment.

Narragansett Bay has high variation in pCO₂ and DIN on both spatial and temporal scales. Annual average pCO₂ concentration is around 400 µatm but ranges from 150 – 1000 µatm (Turner, 2015). DIN in Narragansett Bay runs along a north south gradient, where water has an annual average of 70 µM DIN (with nitrate, NO₃⁻, with annual peaks reaching concentrations greater than 40 µM) in the north and annual average of 4-10 µM DIN in the south (location of collected specimens), where certain parts of the bay can exceed 180 µM DIN (Krumholz, 2012). We experimentally tested the

response of algae to four environmental treatments, which factorially combined two levels of pCO₂ enrichment (ambient ~ 400 μatm pCO₂, and extreme projections for the year 2100 ~1100 μatm pCO₂, values aimed at, or slightly higher than, concentration pathway 8.5 (RCP8.5) projections; Moss et al., 2010) and two levels of nutrient loading (ambient ~ 4 μM nitrate, and high ~ 80 μM nitrate). Each of the four environmental treatments had a 40L headwater tank where CO₂ gas or ambient outdoor air (depending on the treatment) was bubbled in at a constant rate. We obtained high pCO₂ by bubbling in pure CO₂ gas via an Aalborg Mass Flow Controller GFC (Aalborg Instruments and Controllers, INC) into the headwater tank. Filtered, tempered seawater (18°C, kept constant) was pumped into headwater tanks to mix with CO₂ or air. Mixing of gas and water was aided via a Hydor Circulation Fan. Treated water was pumped from the headwater tank via an Eheim 1200 submersible pump to a manifold, delivering treated water to seven experimental aquaria for each treatment. Aquaria received water at a rate of 130 ± 5 mL/min, using a flow-through design we captured the natural variability of coastal pH that can fluctuate up to about 0.4 units at this location (Turner, 2015). Here, all experimental aquaria were exposed to the same amount of natural, daily variability, with pCO₂-treated aquaria starting at a reduced pH.

Nutrients were added individually to each experimental aquarium, as appropriate, through slow-release agar blocks (Teichberg et al., 2010). Blocks were created to meet the desired nutrient concentration (~80 μM nitrate, ~80 μM ammonia, ~4 μM phosphate for the 20L aquaria) by adding nitrate and ammonium, in the form of KNO₃ (at 2M concentration) and NH₄CL (at 2M concentration), along with 3% agar and seawater (Tate, 1990). Phosphate was added to the blocks, in the form of KH₂PO₄ (at 1M

concentration). Blocks containing only agar and seawater were added to the ambient nutrient tanks in order to simulate the physical addition of the block. To create the blocks, nutrients, seawater, and agar were thoroughly mixed and heated, then 50 mL of mixture was poured into a petri dish and chilled for 24 hrs. Once solid, blocks were divided into fourths, and each experimental tank received the block segment. Block segments were hung in mesh bags within the tanks to ensure consistent dissolution, with full dissolution of segments taking ~4 weeks (Ober, personal observations). Water flow into experimental aquaria was measured at a rate of 150 mL/min, meaning water in each tank turned over at a rate of about once every 2.25 h, this flow rate was controlled and kept consistent for each aquaria. Prior testing of block concentrations, size, and tank flow ensured that water in each nutrient-enriched aquaria remained within 10 μM of desired concentrations. These concentrations remained consistent despite species-specific differences in uptake rates. Seawater nutrient levels were analyzed at the beginning of the experiment and midway through the experiment. 60 mL samples of seawater were filtered (GF/F) and frozen prior to analysis. Seawater samples were analyzed for nitrate and phosphate by the URI Marine Science Research Facility, an RI NSF EPSCoR Core Facility (Table 1).

Each experiment ran for a total of 21 days, and tanks were supplemented with artificial light (Sylvania Full Spectrum) at $172.4 \pm 30 \mu\text{mol photons m}^{-2}\text{sec}^{-2}$ with a light/dark rhythm of 14:10h (L:D). Light levels were slightly under saturation for both *Ulva* ($\sim 200 \mu\text{mol photons m}^{-2}\text{sec}^{-2}$) and *Fucus* ($\sim 300 \mu\text{mol photons m}^{-2}\text{sec}^{-2}$), but levels used in this study were high enough to remove undersaturation as a factor in growth (Bäck and Ruuskanen, 2000; Rautenberger et al., 2015; Rohde et al., 2008). Tanks were

scrubbed and cleaned every two days and any epiphytes growing on the algae were removed. Every seven days, algae were briefly removed, weighed, and a small piece (<10% of total wet mass) was removed for future C:N analysis. Mass of removed tissue was included in the calculation of total growth; however, this likely resulted in an underestimation of total algal growth. Algal tissue quality was determined by drying tissue samples for 24 h at 60°C. All dried samples were preserved in glass vials and placed in a desiccation chamber. Samples were ground into a powder and placed in tin capsules. All samples were analyzed for carbon and nitrogen concentrations by Dr. Brad S. Moran's laboratory at the University of Rhode Island Graduate School of Oceanography using an Exeter Analytical CE-440 elemental analyzer.

2.2 Carbonate Chemistry

Temperature, salinity, dissolved inorganic carbon (DIC), and total alkalinity (TA) were sampled during each experiment following the Best Practices Guide (Dickson et al., 2007). For each experiment, water samples were taken twice weekly from a random subset of tanks from each treatment, with sampling occurring midday (resulting in two to four total water samples per week per treatment). Samples were bottled and preserved with mercuric chloride (HgCl_2) and then analyzed for DIC using a Shimadzu DIC gas chromatograph. TA was calculated for each sample using a Metrohm 877 Titrino plus titrator. Carbonate chemistry for experimental tanks (Table 1) was calculated using CO2SYS (Pierrot et al., 2006) using TA, DIC, salinity, and temperature with constants from Mehrbach et al. (1973). Calculated values for pH are reported on the seawater scale. In addition, pH and temperature were logged for each treatment throughout the course of

the experiment using a WTW Profiline pH meter with glass electrode and HOBO temperature logging pendants, respectively, with pH recorded to help indicate daily fluctuations. All data presented in Table 1 are representative of midday values. Due to high flow rates and large aquaria, algal metabolism did not impact seawater carbonate chemistry (Roleda et al., 2015). In addition, the pH of experimental tanks were periodically compared with “blank tanks” (ones without algal cultures but receiving the same environmental treatments) to ensure that algal metabolism wasn’t altering the environmental parameters.

2.3 Statistical Analysis:

Relative growth rate (RGR, % day⁻¹) was calculated for each alga based on change in wet mass between the start and end of each experiment. Mean RGR, final carbon and nitrogen concentrations, and final C:N ratios for each species were assessed using two-way analyses of variance (ANOVA) with pCO₂ and nutrient level fixed factors. Initial C:N ratios of either species did not differ among environmental treatments (two-way ANOVA, p>0.05). As *Ulva*’s growing season does not extend into northern hemisphere winters, time was a significant restraint in our experimental design and did not allow for us to simultaneously run biculture and monoculture experiments thus making comparisons of results more difficult. However, critical environmental conditions, as well as experimental parameters, were purposely kept consistent between experiments (Table 1) in effort to assess differences between results in monoculture and biculture experiments. Initial tissue concentrations of C and N for monoculture and biculture experiments were compared using a one-way ANOVA (with culture as a fixed

factor) for both algal species. There was no significant difference in initial C:N ratios in algal tissues for both species (one-way ANOVA, *Ulva*: $F_{1,42} = 0.77$, $p = 0.38$; *Fucus*: $F_{1,43} = 0.11$, $p = 0.75$). Therefore, we compared final tissue C:N between monoculture and biculture experiments using a one-way ANOVA to determine whether culture influenced tissue-level response of macroalgae. By including culture as a factor in our analysis, we aim to understand how species might be affecting one another, but understand that our experimental design does not itself test competition. Here, using culture as a factor can help point to effects of one species on another. All statistical analyses were performed using JMP v 11 (www.jmp.com).

3. Results

3.1 Algal Growth

Ulva grown in monoculture under high pCO₂ and high nutrients had nearly a threefold faster relative growth rate (RGR) than monoculture *Ulva* grown under ambient conditions (7.5 ± 0.33 % day⁻¹ vs. 2.83 ± 1.06 % day⁻¹, respectively; Fig. 1A). RGR of *Ulva* grown in monoculture was significantly increased by elevated pCO₂ and elevated nutrients ($p = 0.005$, $p = 0.002$, respectively; Table 2a), with no significant interaction.

We did not observe an effect of pCO₂ ($p = 0.50$; Table 2a) or nutrient level ($p = 0.52$; Table 2a) on the RGR of *Fucus* grown in monoculture, as rates ranged from 1.91 ± 0.17 to 2.16 ± 0.20 % day⁻¹ (Fig. 1C), with a non-significant interaction ($p = 0.45$; Table 2a).

We observed similar growth rates of *Ulva* and *Fucus* between monoculture and biculture experiments. *Ulva* grown in biculture under high pCO₂ and high nutrients

experienced a similar threefold increase in RGR compared to individuals grown under ambient conditions ($7.6 \pm 0.55 \text{ \% day}^{-1}$ vs. $2.3 \pm 1.4 \text{ \% day}^{-1}$, respectively; Fig. 1B). The observed range of RGR across treatments of *Fucus* grown in biculture, 1.99 ± 0.17 and $2.18 \pm 0.11 \text{ \% day}^{-1}$ (Fig. 1D), were comparable to the range found in monoculture experiments. Elevated pCO₂ and elevated nutrients significantly increased the RGR of bicultured *Ulva* ($p = 0.012$, $p = 0.002$, respectively; Table 2b) with no significant interaction. RGR of bicultured *Fucus* was unaffected by environmental treatment, maintaining similar rates of growth regardless of nutrient or pCO₂ level (Table 2b).

3.2 Algal Tissue Content (C:N, C, N)

The C:N of both *Ulva* and *Fucus* grown in monoculture was significantly lower under high nutrient treatments ($p < 0.0001$ and $p = 0.002$, respectively; Table 3a; Fig. 2). With elevated nutrients, mean C:N in *Ulva* was measured at 14.81 compared to a mean of 19.82 under ambient nutrients. Mean C:N within *Fucus* grown under high nutrients was measured at 19.59 compared to a mean of 23.72 under ambient nutrient conditions. By contrast, pCO₂ did not significantly affect the C:N of either *Ulva* or *Fucus* ($p = 0.64$, $p = 0.11$, respectively; Table 3a), with non-significant pCO₂ × nutrient interactions (Table 3a).

Carbon concentration within tissues of *Ulva* and *Fucus* were significantly higher under high pCO₂ ($p = 0.03$, $p = 0.05$, respectively; Table 3a, Fig. 3A and 3B). Similarly, nitrogen concentration in both *Ulva* and *Fucus* tissues increased under high nutrient treatments ($p = 0.02$, $p = 0.04$, respectively; Table 3a, Fig. 3A and 3B).

Tissues of *Ulva* and *Fucus* grown in biculture were similarly affected by nutrient addition as those grown in monoculture. Here, increased nutrients lead to significant decreases in C:N for both species (*Ulva*: $p = 0.03$; *Fucus*: $p = 0.0002$; Table 3b). Tissue C:N for both species was not affected by $p\text{CO}_2$ level and no significant interaction was observed between nutrients and $p\text{CO}_2$ (Table 3b). Elevated nutrients also resulted in increased concentrations of nitrogen in the tissues of both *Ulva* and *Fucus* ($p = 0.01$, $p = 0.03$, respectively; Table 3b). In addition, elevated nutrients resulted in significantly higher concentrations of carbon in the tissues of *Ulva* growing in biculture ($p = 0.02$; Table 3b).

3.3 C:N in Monoculture vs. Biculture

As initial C:N did not differ for either *Ulva* or *Fucus* between treatments and culture experiments, we compared final C:N between monoculture and biculture treatments for both species. We found that culture significantly impacted the C:N of *Fucus* tissue ($F_{1,43} = 8.02$, $p = 0.007$), where *Fucus* grown in had higher C:N ratios than *Fucus* grown in monoculture. Monocultured *Fucus* had a mean C:N of 20.40 compared to a mean of 23.25 under biculture. Alternatively, tissue C:N of *Ulva* was not altered by culture as ratios did not differ between monoculture and biculture treatments ($F_{1,42} = 1.13$, $p = 0.30$).

4. Discussion

4.1 Algal Growth

Non-calcifying primary producers are predicted to benefit from changes in seawater chemistry due to ocean acidification (see Kroeker et al., 2013). We

hypothesized that *Ulva* would be significantly impacted by pCO₂ and experience increased growth rates. Our results support this hypothesis as we found that *Ulva* growth rates doubled under 1100 µatm pCO₂ conditions. This matches the response of *Ulva* to ocean acidification in other systems (Olischläger et al., 2013; Xu and Gao, 2012) as well as other macroalgal species (Campbell and Fourqurean, 2014; Kübler et al., 1999; Olischläger and Wiencke, 2013; Swanson and Fox, 2007). Olischläger et al. (2013) observed a doubled growth rate of *U. lactuca* when exposed to 700 µatm pCO₂, and Xu and Gao (2012) found *U. prolifera* exhibited increased growth rates of about 40% when exposed to 1000 µatm pCO₂. These studies used concentrations of pCO₂ expected in the next 50-100 years, whereas our study focused on the more extreme pCO₂ projection for the year 2100. However, the response of macroalgae is still highly species-specific, and neutral or negative impacts of ocean acidification on growth rate have been observed in non-calcifying macroalgae (Cornwall et al., 2012; Gutow et al., 2014; Israel and Hophy, 2002; Mercado and Gordillo, 2011). Divergent responses of macroalgae to acidification are likely due to the differences in CCM effectiveness, potentially giving certain species more independence from the environment, or CCMs are optimized for higher pH conditions and their activity is sensitive to pH (Axelsson et al., 2000; Moulin et al., 2011).

How *Ulva* takes advantage of increased CO₂ may be due to changes in physiological processes, such as down-regulation of CCM activity and reallocation of energy, increased nitrogen assimilation, and/or slight increases in photosynthetic activity (Olischläger et al., 2013; Xu and Gao, 2012). Algal species that lack CCMs are predicted to have increased photosynthetic activity under high pCO₂ conditions, as these species

are carbon limited (Kübler et al., 1999). *Ulva*, however, has a highly efficient CCM and does not appear carbon limited (Axelsson et al., 1999, 1995). Xu and Gao (2012) offer a few mechanisms for the success of *Ulva* under high pCO₂ environments, in which photochemical and photorespiratory pathways are mediated. However, it is important to note that although we discuss ocean acidification as an increase in pCO₂, there is also an increase in the amount of HCO₃⁻ available under these conditions. In our experiments, we observed greater concentrations of HCO₃⁻ when we increased pCO₂ (Table 1). As *Ulva* primarily uses HCO₃⁻, the enhanced growth we observe in this species under high pCO₂ conditions may very well be tied to HCO₃⁻ rather than CO₂. Fernández et al. (2015) found evidence of greater HCO₃⁻ use as an inorganic carbon source for photosynthesis in *Macrocystis pyrifera*. Photosynthetic rates of *Ulva*, as determined by oxygen production, increased by a factor of 1.2 under 700 µatm pCO₂, but this increase was not statistically significant (Olischläger et al., 2013). Conversely, Rautenberger et al. (2015) found that light levels drive photosynthetic activity and growth in *U. rigida*, not high pCO₂, and light and nutrients are typically cited as the most important factors influencing growth in *Ulva spp.* (Aldridge and Trimmer, 2009; Coutinho and Zingmark, 1993).

Our prediction that the growth rate of *Fucus* would decrease under high pCO₂ (e.g. Gutow et al. 2014) was not validated. Similar to *Ulva*, *Fucus* utilizes a CCM to help convert and supply sites of photosynthesis with usable carbon, but *Fucus* species have an internal store of usable carbon and thus are not as sensitive to changes in carbon chemistry (Kawamitsu and Boyer, 1999). It is possible that long-lived species such as *Fucus* may respond more slowly (e.g. months vs. weeks) to an altered environment.

Pedersen and Borum (1996) found that fast-growing species, like *Ulva*, exhibit increased growth rates with increased nutrient concentrations and exhibit increased uptake rates (Pederson and Borum, 1997), unlike *Fucus*, which has lower uptake rates. Pedersen and Borum (1996) also highlight a much higher nutrient uptake rate in *Ulva* (fitting a hyperbolic curve with increasing nutrient concentrations) compared to *Fucus*. Inorganic nitrogen was added as a factor in the form of both nitrate (NO_3^-) and ammonium (NH_4^+) in relatively equal concentrations. As a fast-growing, ephemeral species, *Ulva* has a higher nitrogen demand to reach maximum growth compared to *Fucus*, a slow growing species (Pederson and Borum, 1997). Nutrient uptake kinetics in *Ulva* highlight asymptotic uptake of both NO_3^- and NH_4^+ , where uptake slows between 20 and 40 μM (Pederson and Borum, 1997; Rees et al., 2007), pointing to saturation. Here, our tested values of NO_3^- and NH_4^+ fall above expected saturation levels, and perhaps *Ulva* growth under our treatment levels would be the same even if nutrients were half of what we tested. Young and Gobler (2016) elevated NO_3^- to 50 μM but found that this level of nutrient addition only significantly increases *Ulva* growth rates in seasons where nutrient levels are typically the lowest. Our experiment does not test seasonality in *Ulva*'s response, but our conditions were meant to reflect water conditions in September for Narragansett Bay, one of the months in which Young and Gobler (2016) observed a significant impact of NO_3^- on *Ulva* growth.

Increased concentrations of NH_4^+ may be a more significant factor than NO_3^- . *Ulva* exhibits an ability to uptake in NH_4^+ as an immediate surge, but ultimately, longer exposure to high levels of NH_4^+ result in reduce uptake (Pederson and Borum, 1997). Rees et al. (2007) showed significantly increased uptake rates of NH_4^+ in *Ulva*

intestinalis when NH_4^+ concentrations increased, these uptake rates exceed those for NO_3^- , and the increased uptake of NH_4^+ came with reduced uptake of NO_3^- . *Fucus* becomes saturated at NH_4^+ and NO_3^- concentrations around 10-30 μM and the maximum uptake rates in this species are less than half of those observed in *Ulva* (Pederson and Borum, 1997). *Fucus*, however, spans much of the intertidal and shallow subtidal, and Benes and Bracken (2016) showed greater uptake rates in high shore *Fucus*. We used *Fucus* that was found to be submerged at low tide. *Fucus* found in the lower zone exhibits reduced uptake rates, and in addition, *Fucus* showed that nitrogen uptake rates are plastic and individuals can adapt rates based on submersion time (Benes and Bracken, 2016). *Fucus* used in our experiments was not subjected to low tide conditions, and likely adapted to constant submersion. As such, we would expect uptake rates to be reduced even more (Benes and Bracken, 2016).

Our observed *Ulva* growth rates were also significantly enhanced by the addition of nutrients, similar to prior experimental studies (Steffensen, 1976; Teichberg et al., 2010) and field observations (Díaz et al., 2005), supporting our hypothesis. As a fast-growing opportunistic species, *Ulva* can absorb excess nutrients in the water column and form blooms that are detrimental to ecosystems (Teichberg et al., 2010; Valiela et al., 1997) and can lead to eelgrass declines (Hauxwell et al., 2001; McGlathery, 2001). By contrast, we found no effect of nutrient treatment on the growth rate of *Fucus*. *Fucus* can take up excess nutrients, but at a marginal rate (~3%) that may not enhance growth rates (Savage and Elmgren, 2004). Our analysis, however, did not allow for determining whether NO_3^- or NH_4^+ played different roles in influencing this increased growth. In addition, while nitrogen has long been thought of as the limiting resource for macroalgae,

there is evidence that has shown positive correlations between algal growth and phosphorous concentration (Villares et al., 1999). Our nutrient addition included adding phosphorous, but ultimately tissue concentrations of phosphorous were not analyzed and we make no conclusions about the role of phosphorous in macroalgal growth.

Of several studies on the combined impacts of ocean acidification and nutrients (Campbell and Fourqurean, 2014; Falkenberg et al., 2013; Russell et al., 2009), only Russell et al. (2009) found a synergism between these two factors, with turf algal percent cover multiplied when both of these factors are increased. Campbell and Fourqurean (2014) and Falkenberg et al. (2013) found that at least one of the factors increased growth rates, but with no significant interaction. Fernández et al. (2017) found that growth and photosynthetic rates of *Macrocystis pyrifera* were unaffected by pCO₂ and despite increased uptake of nitrogen, nutrient conditions were similarly ineffective. Comparing field growth and laboratory growth of *Ulva*, Young and Gobler (2016) found increased pCO₂ had a larger impact on growth in *Ulva*, compared to slight increases in nutrients, but did find evidence that increased nutrients enhances growth (Young and Gobler, 2016) and a few cases where pCO₂ and nutrients added synergistically to effect growth rates. Our results do not point to a synergism, but the significant effect of pCO₂ and nutrients on *Ulva* growth we observed supports findings in these studies as we show that growth rates in *Ulva* were the highest under high pCO₂ and high nutrients (Fig. 1A and 1C).

4.2 Algal Tissue Content

Overall, we found that increased pCO₂ did not affect the C:N ratio of either *Ulva* spp. or *Fucus vesiculosus*. Although adding carbon is counterintuitive to decreasing the

C:N ratio, Gordillo et al. (2001) found that increased pCO₂ resulted in increased uptake of nitrate in *U. rigida*, thus lowering the tissue C:N ratio. While our results don't show decreased C:N with high pCO₂, analysis of *Ulva* tissues grown in biculture and under high pCO₂ show an uptick in nitrogen concentration. Our results, however, indicate that nutrient level was the primary driver of tissue C:N ratio in both *Ulva* and *Fucus*, and this may ultimately obscure changes in nitrogen uptake due to increased pCO₂. High nutrient treatments resulted in decreased C:N ratios, which increases tissue quality. Falkenberg et al. (2013) found that increasing each factor resulted in decreased C:N ratios for turf algae, but only nutrients effectively lowered the C:N in the kelp, *Ecklonia radiata*. We did not find a significant interaction between acidification and nutrients loading on tissue C:N, similar to Falkenberg et al. (2013). Our results contradict those from Gutow et al. (2014), where high pCO₂ resulted in a decreased C:N ratio for *F. vesiculosus*.

Our findings indicate that both acidification and nutrients have the ability to alter tissue composition. The addition of pCO₂ significantly increased the percent carbon found in *Ulva* and *Fucus* tissues, and increased nutrient concentrations raised tissue nitrogen in both. However, pCO₂ did not affect the C:N rate. Therefore, in our system, nutrients are the key driver of C:N ratios in *Ulva* and *Fucus*. While we observed higher concentrations of N within *Ulva* tissues grown under high pCO₂ treated individuals, the difference was not significant and our results do not match Gordillo et al. (2001) and Xu and Gao (2012), who found that high pCO₂ facilitates nitrate uptake in *U. rigida* and *U. prolifera*, respectively.

The uptake of nutrients and carbon is limited by how much of the resource is available per unit biomass. We did not alter the concentrations of nutrients or pCO₂ to

reflect changes in algal biomass between experiments, where biculture experiments had significantly greater algal biomass. However, concentrations of these resources were greater than saturation levels for both algal species, therefore, we do not anticipate that changes in biomass resulted in changes in nutrient uptake and ultimately any changes in tissue C or N. In addition, there is documented evidence that *Ulva* allelochemicals harm the growth of *Fucus* germlings (Nelson et al., 2003), but with high flow rates and balanced algal concentrations, we do not expect these compounds to have played a role in determining the health of *Fucus* grown in biculture.

4.3 Monoculture vs. Biculture:

Our experimental design and execution was impacted by the seasonality of *Ulva* in Narragansett Bay. As such, experiments were designed and planned in effort to utilize current growing seasons of both macroalgal species. This limited our ability to simultaneously run monocultures and bicultures. However, as this study was done under laboratory conditions, we had the ability to control and maintain environmental parameters throughout. Comparisons can be made between monoculture and biculture experiments, but arguments about the impact of one algal species on another could be bolstered by a more randomized experimental design. While we observed almost identical growth rates of both *Ulva* and *Fucus* between monoculture and biculture experiments, we did find an effect of *Ulva* on *Fucus* as evidence of altered C:N ratios of *Fucus* in biculture experiments, where C:N of *Fucus* was significantly higher in tissues when grown with *Ulva*. This is potentially due to the inability of *Fucus* to acquire nutrients in the presence of fast-growing, nutrient limited species like *Ulva* (Duarte,

1995). While growth rates of either species seemed unaffected by culture, finding changes in tissue composition and ultimately access to nutrients as a result of biculture may point to longer-term impacts of one species on another. Experimental duration and within tank algal densities likely played a role in masking any tangible effects of one species on another in terms of growth rates. Alestra and Schiel, 2015 tracked fucoid growth under different environmental and culture conditions (also using *Ulva*) over a 6 week period, and while they observed decreased size in fucoid germlings grown alongside *Ulva*, the percent cover of *Ulva* didn't start significantly expanding until 4-6 weeks into the experiment. At 21 days, our experiment might not have allowed for enough time to observe size discrepancies between *Fucus* grown in monoculture and in biculture.

Interspecific competition and interactions for space and resources drive algal community composition and function (Olson and Lubchenco, 1990; Stachowicz, 2001), and algal communities may undergo assemblage shifts under climate change (Connell and Russell, 2010). However, to more completely understand the interactions between algal species (and ultimately any competitive effects) we would need to expose algal species to the same environmental treatments using a replacement-additive design. As such, while we found evidence of *Ulva* affecting *Fucus*, we cannot say with confidence that this is evidence of resource competition.

4.4 Impacts to Coastal Ecosystems

In coastal systems where anthropogenic nutrient loading is prevalent, algal blooms are likely occur. The role of nutrients in facilitating macroalgal blooms is well

established (Lapointe, 1997; Lapointe and Bedford, 2010). While both nutrients and CO₂ are both resources for primary producers, few studies have assessed the role of pCO₂ in contributing to blooms. As our study shows, increased nutrients and pCO₂ result in faster Ulva growth, and their combination results in the largest RGR. Quantifying this effect may allow for better prediction of opportunistic and bloom forming macroalgal response to common coastal environmental stressors. As such, ocean acidification may ultimately end up contributing to the timing, frequency, and duration of macroalgal blooms. Ocean acidification has been shown to not only enhance growth rates in some algal species but also may enhance the uptake of nutrients (Gordillo et al., 2001), increasing growth rates indirectly. In addition, acidification can alleviate the cold-driven temperature stress in a species of red algae, resulting in higher growth rates in colder waters (Olischläger and Wiencke, 2013).

Seaweed takeover of critical ecosystems such as coral reefs (Diaz-Pulido et al., 2011) and the turf algal dominance in kelp forest ecosystems (Connell and Russell, 2010) highlight the prediction that many macroalgal species are expected to flourish under future climate scenarios. Field studies of algal diversity at naturally low pH vent sites indicate shifting assemblages as water become more acidic (Porzio et al., 2011). Our results highlight the ability of fast-growing, ephemeral species to take advantage of changing conditions. In other systems, these fast-growing species are predicted to outcompete larger, slow-growing species (Connell and Russell, 2010; Falkenberg et al., 2013). While long-lived species like *Fucus* may not be directly impacted by environmental change as our results show, they may, however be indirectly affected by the overgrowth of epiphytes (Berger et al., 2004) or competing species. *Fucus* dominates

space in the intertidal and shallow subtidal and helps create complex, 3-D structure that is critically important as a source of refuge and habitat for other organisms in the community. Our results do not describe a direct, negative effect of acidification or nutrients on *Fucus*, but these indirect effects may ultimately result in *Fucus* decline, altering algal community assemblages and ecosystem services. As *Ulva* is a seasonal macroalga, a takeover of *Fucus*, or other perennial species is not likely, but as Olischläger and Wiencke (2013) showed an ability for increased pCO₂ to alleviate cold temperature stress, the growing season of *Ulva* may be extended, which could have implications for perennial species.

While results from our study highlight the divergent responses of two macroalgal species to climate and environmental change, we only tested these species at one density, an important caveat when putting context to the results of our biculture study. Although our densities were within the range of ecological relevance, seasonal changes in recruitment, growth, and density will likely play a role in shaping community response to climate change. Seaweed density has long been known as an important factor in determining the settlement and success of other species within a community (Schiel 1985, Ansell et al., 1998). We showed that increases in pCO₂ and nutrients can significantly enhance *Ulva* growth rates, and that *Ulva* presence and success appears to have a quantifiable impact on other macroalgae potentially impacting the timing, duration, and size of macroalgal blooms. The design of this study, however, did not allow for determining how different densities of certain species play a role in community dynamics. Blooms of green alga, like *Ulva*, can have a profound impact on the survival of other macroalgae and in some instances have resulted in the decline and replacement of

coinhabitants (Valiela et al., 1997). To better understand the dynamics of change in macroalgal communities in context of climate change, studies investigating different densities are needed. In addition, to fully quantify how macroalgal communities will respond to change more work needs to be done investigating how larger, more diverse communities respond. It is necessary to quantify algal community response over long periods of time, as the seasonality of algal species in temperate coastal ecosystems will likely play a role in determining community dynamics.

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Table 1. Carbonate chemistry and nutrient parameters averaged across all experiments. Water samples were collected at four times over a two day period in each experiment, resulting in four water samples per treatment per week. Dissolved inorganic carbon (DIC), total alkalinity (TA), temperature, and salinity were measured directly. CO2SYS (Pierrot et al. 2006) was used to calculate pH, pCO₂, CO₂, HCO₃⁻, and CO₃²⁻. Nitrate (NO₃⁻), Ammonium (NH₄⁺) and phosphate (PO₄³⁻) were measured from water samples that were taken weekly over the course of experimentation. All values represent means ± standard error.

<i>PARAMETER</i>	Ambient pCO₂		High pCO₂	
	Ambient Nutrients	High Nutrients	Ambient Nutrients	High Nutrients
pH	7.96 ± 0.02	7.97 ± 0.06	7.62 ± 0.02	7.62 ± 0.04
pCO ₂ (µatm)	469.27 ± 19	459.58 ± 15	1119.07 ± 60	1134.82 ± 99
CO ₂ (µmol/kgSW)	16.36 ± 0.7	16.03 ± 0.5	39.25 ± 2.0	39.73 ± 4.0
HCO ₃ ⁻ (µmol/kgSW)	1808.3 ± 11	1802.7 ± 7.0	1964.2 ± 14	1959.9 ± 10
CO ₃ ²⁻ (µmol/kgSW)	128.03 ± 4.5	129.80 ± 8.0	62.78 ± 3.3	62.13 ± 5.7
DIC (µmol/kgSW)	1952.7 ± 10	1948.7 ± 6.9	2066.0 ± 15	2062.0 ± 11
TA (µmol/kgSW)	2126.6 ± 12	2125.1 ± 10	2120.3 ± 16	2114.4 ± 19
Temp. (°C)	18.1 ± 0.07	18.07 ± 0.04	17.96 ± 0.05	17.98 ± 0.05
Salinity (ppt)	30.8 ± 0.02	30.8 ± 0.02	30.7 ± 0.02	30.7 ± 0.03
NO ₃ ⁻ (µM)	3.40 ± 1.5	84.78 ± 2.3	3.32 ± 1.5	88.15 ± 5.2
NH ₄ ⁺ (µM)	5.65 ± 0.56	88.34 ± 2.1	5.22 ± 0.46	92.08 ± 4.7
PO ₄ ³⁻ (µM)	1.68 ± 0.2	3.68 ± 0.9	1.49 ± 0.3	3.97 ± 1.7

Table 2. Summary of two-way ANOVAS showing the effects of ocean acidification (pCO₂) and nutrient addition (Nutrients) and their interactions on the growth rates (RGR %g day⁻¹) of *Ulva* and *Fucus* grown in monoculture (a) and biculture (b). P-values that are significant (P ≤ 0.05) are in bold.

a.

	<i>Ulva</i>				<i>Fucus</i>			
Model Source	df	SS	F	P	df	SS	F	P
RGR (%g day ⁻¹)								
<i>p</i> CO ₂	1	24.61	10.06	0.005	1	0.10	0.47	0.50
<i>Nutrients</i>	1	33.92	13.87	0.002	1	0.09	0.43	0.52
<i>p</i> CO ₂ X <i>Nutrients</i>	1	0.11	0.04	0.84	1	0.12	0.60	0.45
<i>Error</i>	18	44.01			24	4.92		

b.

	<i>Ulva</i>				<i>Fucus</i>			
Model Source	df	SS	F	P	df	SS	F	P
RGR (%g day ⁻¹)								
<i>p</i> CO ₂	1	25.60	7.71	0.012	1	0.001	0.01	0.92
<i>Nutrients</i>	1	53.85	16.23	0.001	1	0.08	0.62	0.44
<i>p</i> CO ₂ X <i>Nutrients</i>	1	1.41	0.42	0.52	1	0.04	0.31	0.58
<i>Error</i>	18	59.73			24	3.22		

($\mu\text{mol}/\text{mg}$)								
<i>pCO₂</i>	1	6.82	0.37	0.55	1	4.72	0.23	0.64
<i>Nutrients</i>	1	116.8	6.30	0.02	1	1.70	0.08	0.78
<i>pCO₂ X Nutrients</i>	1	37.67	2.03	0.17	1	0.18	0.01	0.93
<i>Error</i>	18	333.7			17	353.5		
N concentration ($\mu\text{mol}/\text{mg}$)								
<i>pCO₂</i>	1	0.17	1.01	0.33	1	0.003	0.03	0.86
<i>Nutrients</i>	1	1.31	7.98	0.01	1	0.40	5.32	0.03
<i>pCO₂ X Nutrients</i>	1	0.003	0.02	0.89	1	0.0001	0.001	0.97
<i>Error</i>	18	2.95			17	1.29		

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Fig.1 (A-D): Mean algal growth rates (% g day⁻¹ +/- SE). *Ulva lactuca* monoculture (A), *F. vesiculosus* monoculture (B), *U. lactuca* biculture (C), and *F. vesiculosus* biculture (D).

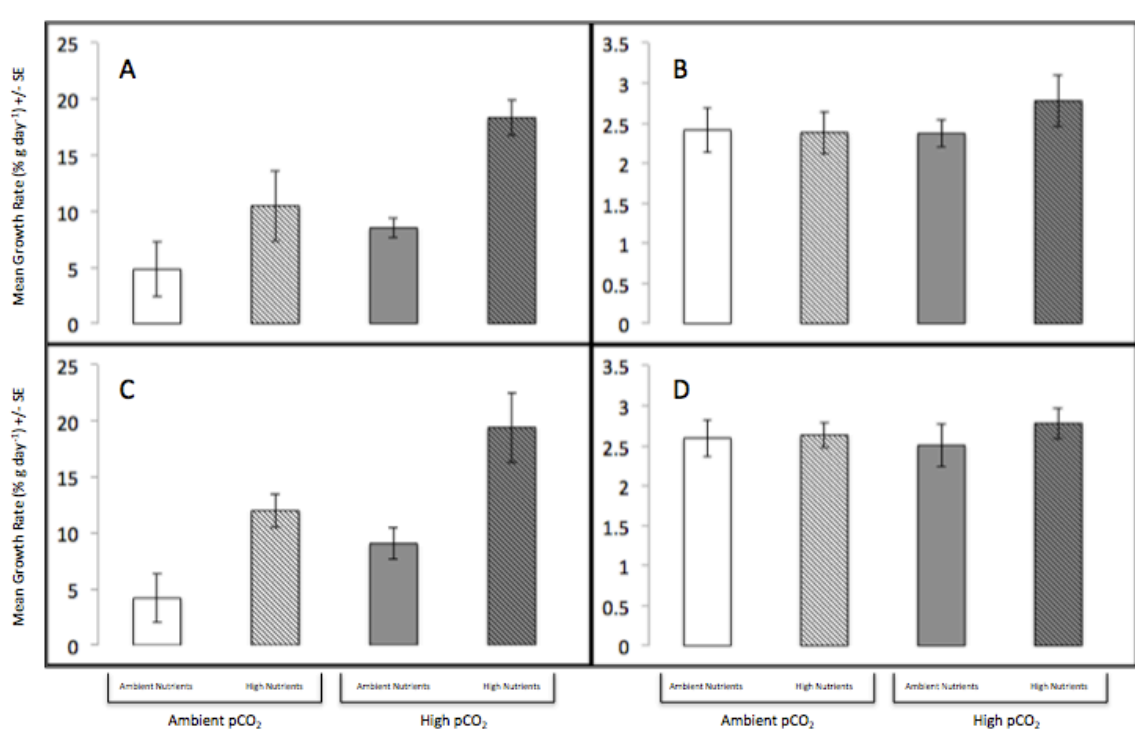


Fig.2 (A-D): Mean algal C:N Ratios (+/- SE). *Ulva lactuca* monoculture (A), *F. vesiculosus* monoculture (B), *U. lactuca* biculture (C), and *F. vesiculosus* biculture (D). We observed a significant, negative effect of nutrient addition on the C:N of both *Ulva* and *Fucus* (** represents $p < 0.0001$). In addition, we observed a significant difference between the C:N of *Fucus* tissue by culture treatment where C:N was lower in monoculture ($C < D$, $p = 0.002$).

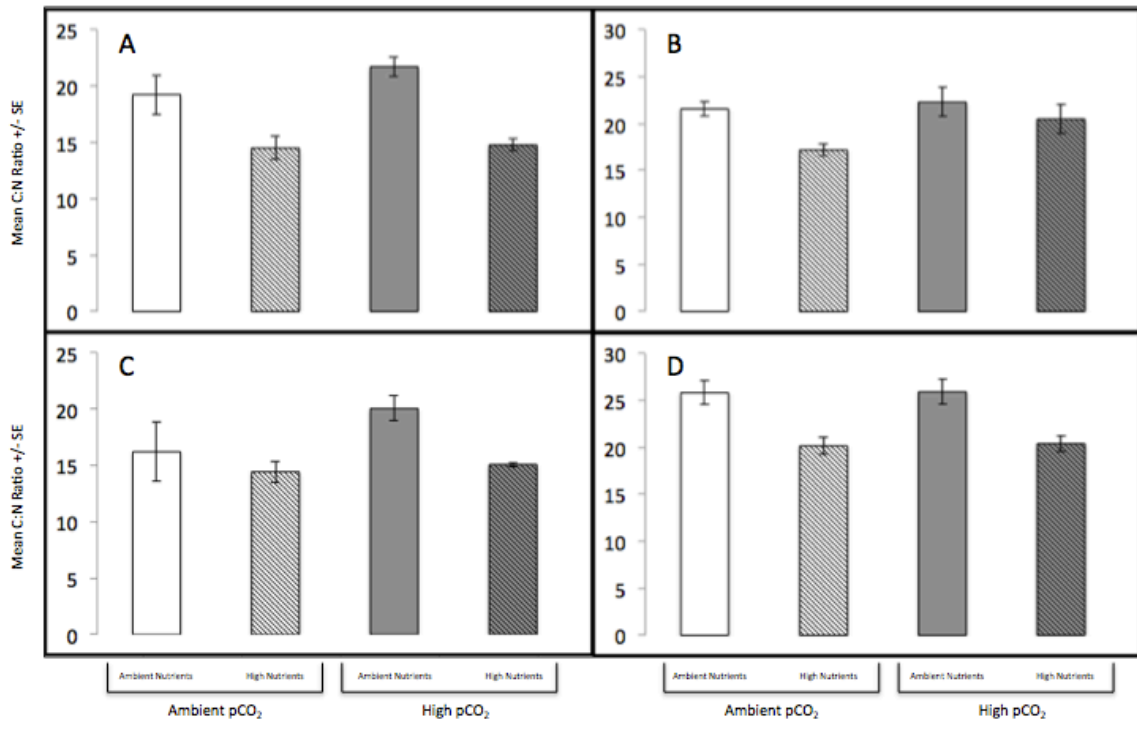


Figure 3A + B: Carbon and Nitrogen Concentrations in *U. lactuca* (A) and *F. vesiculosus* (B) by treatment. Ambient pCO₂ and ambient nutrients (open circles), Ambient pCO₂ and high nutrients (closed circles), high pCO₂ and ambient nutrients (open triangles), and high pCO₂ and high nutrients (closed triangles).

