NC Sea Grant and NC Coastal Reserve and National Estuarine Research Reserve Fellowship

Final Report

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Introduction

Seagrasses, or high salinity submerged aquatic vegetation (SAV), are underwater angiosperms (den Hartog 1970) that deliver essential ecosystem services to coastal areas including the provision of habitat for recreational and commercial fisheries species (Hughes et al. 2002) and improvement in local water quality conditions (Barbier et al. 2011). High salinity SAV in the Albemarle Pamlico Estuarine System (APES) declined at rate of 1.5% per year between 2007 and 2013 (Field et al. 2021). Although the cause of this decline is unknown, high salinity SAV located at other geographic transition zones are vulnerable to anthropogenic impacts (Hyndes et al. 2016), including light reductions associated with eutrophication and greater turbidity from increased coastal development. Light availability is often the primary limiting factor for SAV growth and survival in estuarine habitats (Dennison and Alberte 1985; Biber et al. 2009).

NC has two dominant high salinity SAV species, *Halodule wrightii* and *Zostera marina.* Both are at the edge of their geographic range (Short et al. 2007), with temperate *Z. marina* on the trailing or contracting edge and tropical *H. wrightii* on the leading or expanding edge (Pinksy et al. 2020). In the past 10 years climate change has lead to a significant increase in coastal ocean temperatures and the rate of sea level rise (Kunkel et al. 2020). Continued increasing water temperatures could favor the thermal preferences of *H.wrightii* over *Z. marina* given the species thermal optimum and upper limits (20-35°C) (Phillips 1960; Koch et al. 2006). *H. wrightii* could also be resilient in deeper water as it has been observed at depths of 12.9 m in locations when water clarity is not limiting (Manuel et al. 2013). However, in NC despite a decline in temperate *Z. marina* biomass and an increased patchiness in SAV meadows (Bartenfelder et al. 2022, Field et al. 2021), *H. wrghtii* biomass and extent is not increasing significantly. As a result the gaps left by *Z. marina* at the meadow deep edge remain (Kenworthy and Fonseca 1996; Manuel et al. 2013; Bartenfelder et al. 2022). Lack of SAV at the deep edge of these meadows indicates that light limitation is a potential driver of change in the system that is impacting both the temperate and tropical seagrass species (Field et al. 2021).

In NC *H. wrightii* is dominant during warmer periods (July-November) and aligns with a period where there is seasonal decline in water quality which reduces light availability (Micheli et al. 2008; Moore et al. 1997). *H. wrightii* experiences thermal cold stress when water temperatures fall below the thermal optimal range of 20 – 30°C (Phillips 1960; Bartenfelder et al. 2022). During this stressful cold period light availability is sufficient, yet plant aboveground growth is reduced (Dunton 1996). While it is not well understood for tropical species, prolonged cold temperature stress (< 20 °C) can damage to photosystems resulting in reduced photosynthetic performance and eventually can lead to death if exposures are prolonged (Zhang et al. 2021).

To avoid heat stress *Z. marina* has shifted to a seed dependent life history strategy where shoots die-back during the stressful summer months and germinate from seeds once temperature stresses have been removed (Jarvis et al. 2012). However, in NC *H. wrightii* does not produce a seed bank so it cannot escape cold stress and it is critical for clonal growth to maintain the population year-round (Ferguson et al. 1993; Sordo et al. 2011). *H. wrightii* survives thermally stressful winter periods but it is unclear how temperature plays into persistence of the species or how resilient this species would be if light conditions deteriorated during this time.

The 2021 North Carolina Coastal Habitat Protection Plan Amendment (CHPP) defines 22% surface irradiance as adequate for survival and growth of high salinity SAV populations (CHPP SAV Action 4.7) (NCDEQ 2021). Currently water clarity in the APES meets these thresholds for SAV in water depths ≤ 1.7 m (Hall 2022), indicating adequate light is only available to SAV in shallow areas. These depths are significantly shallower than historical high salinity SAV distributions in the APES system as evidenced by the continuing loss of many meadows 'deep edge' over time (Field et al. 2021). In addition, threshold values used in the CHPP were derived from empirical studies of SAV under optimal thermal conditions and do not consider differences in light penetration with season or how edge of range species light requirements may differ during thermally stressful periods (NCDEQ 2021). To ensure that current trends of high salinity SAV loss are stabilized and eventually reversed, light threshold values specific to edge of range SAV populations under the thermal conditions that plants experience in NC need to be defined. Increased light requirements may be necessary to facilitate expansion of *H. wrightii* into deeper depths to ensure the long-term survival and expansion of high salinity SAV in the system (CHPP SAV Action 4.8; Applying Science – NERRS SMP; Healthy Coastal Ecosystem - NCSG SMP). Therefore, this research will address the following question: What is most limiting to expansion of *H. wrightii* at its northern limit in the western Atlantic Ocean, light, water temperature or their interaction?

Materials and Methods

Baseline light requirements for *H. wrightii* growth and survival were quantified at the shallow and deep edge of high salinity SAV meadows under thermally stressful and non-stressful conditions using a combination of field and laboratory experiments. Field experiments compared the CHPP defined threshold light requirements for NC *H. wrightii* populations (22% SI ≤ 1.7m) *in situ* conditions under thermally optimal (≥20°C) and stressful (<15°C) conditions. A laboratory shading experiment exposing *H. wrightii* to thermally stressful conditions was also conducted to gain further insight on the species physiological response to multiple stressors.

Field Shading and Water Depth Experiment.

Shading experiments were conducted in established high salinity SAV meadows in the Rachel Carson Reserve at Middle Marsh (Sentinel Site 3; Jarvis et al. 2022) during summer optimal temperatures (16 August 2023 – 04 September 2023) and during stressful winter temperatures (20 January 2023 – 08 February 2023). All field experiments were established at 2 water depths;

shallow (≤1m MLW) and deep (≥1.7m MLW). Across both treatment depths shoots were exposed to three light treatments to look at shading stress: ambient (no shade), ambient reduced by 22% SI, and ambient reduced to 11% SI.

For all experimental treatments vertical isolation borders were inserted to ensure that seagrass outside of the experimental plot does not translocate resources to SAV within treatment plots (Short and Coles, 2001). Additionally, at the beginning of each sampling period control and light manipulated plots (0.5m) were established for each treatment depth (n = 4) (Fig 1). Two types of control plots were established using quadrats with isolation borders (Control 1) and with no isolation borders (Control 2) to quantify the effects of rhizome severing on physiological response metrics (Fig 1). For shaded treatments the plots were covered with shade cloth fixed 40 cm above the sediment surface. Shade screens were removed and cleaned weekly to reduce the effects of fouling on shade treatments. All plots were "gardened" to contain and maintain only the target species *H. wrightii*.

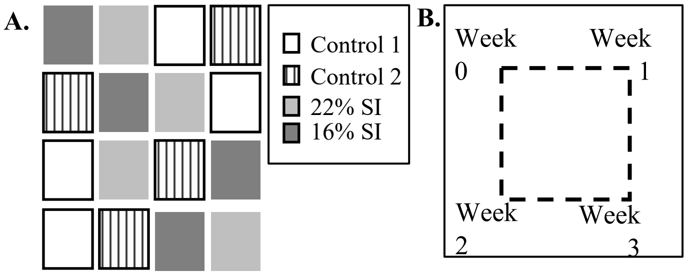

Figure 1| Sampling design for shading experiment. (A) Experimental design for all shading treatments to be replicated at Middle Marsh. (B) Sampling quadrat showing area shaded (solid line) and locations for weekly sampling (dotted line).

All experiments were conducted over 4-weeks. A GPS (meter accuracy) was used to mark the locations of plots in the meadows to ensure plots could be found during each sampling event and to avoid reusing the sample location during a different experimental period. At the sampling site an Odyssey P.A.R. wiper was placed in the shallow and deep edge of the meadow to recorded temperature (°C) and light (PAR μmol m^2 s^{-1}) values every 15 minutes. To determine if light reduction treatments are maintained throughout the tidal cycle and between weekly de-fouling an Odyssey P.A.R. wipers were also placed in one of the 22% SI and 11% SI plots in both the shallow and deep treatments. Weekly data was collected on shoot density and canopy height (cm). During each week of the experiment, initial (T_0), week 1 (T_1), week (T_2) and the final (T_3), C:N samples were collected from all treatments. On the day the experiment plots were set-up biomass cores (0.22 cm diameter, 10 cm depth) were collected in a portion of the meadow not used in the experiment to represent *H. wrightii* biomass for the experiment season (n = 4 per season). At the conclusion of the summer and winter experiment biomass cores (0.22 cm diameter, 10 cm depth) were collected from each treatment. Measurements of photosynthetic efficiency were not collected in the field given the limitations of fluorescence measurements on seagrasses under *in situ* conditions (Bhagooli et al. 2021).

Laboratory Shading Experiment.

H. wrightii was collected from Back Sound in January 2023, 2 weeks prior to the start of the experiment. Following collection, SAV was planted in pots and placed in temperature and light-controlled recirculating mesocosms at UNCW's Center for Marine Science. Shoot density was maintained at 4 shoots per pot to reflect *in situ H. wrightii* winter density (shoots m^{-2}; Jarvis Lab data). For the experiment, thermally stressful water temperatures were maintained between 10-12°C using a Teco TK chiller. Light was maintained at 180 μmol m^2 s^{-1} for ambient treatments and day-night light cycles were 10h day/14h night, aligning with the day length during February in North Carolina.

The winter thermal stress experiment was conducted over 4-weeks and shoots were exposed to three light treatments to look at shading stress: ambient, ambient reduced to 22% SI, and ambient reduced to 11% SI. For each light treatment there were 4 tanks, containing 6 pots of the target species (n = 4). This design had more pots than necessary to provide redundancy in case there was unexpected plant loss it would not lead to loss of sample point. Onset HOBO Pendant Temperature/Light Data Loggers were used to record temperature (°C) and light (PAR μmol m^2 s^{-1}) values in tanks every 15 minutes for the duration of the experiment.

A suite of physiological and structural variables was measured as indicators of SAV response. A rapid indicator of physiological response, effective quantum yield (F_V/F_M) using pulse amplitude modulated fluorometry (PAM), was collected bi-weekly. Structural measurements, shoot density and canopy height (cm), collected weekly as an integrated physiological response throughout the duration of the experiment. Replicate samples from all treatments were destructively sampled for C:N weekly. For C:N analysis *H. wrightii* from each replicate was separated into aboveground (leaf) and belowground (rhizome and roots) material, dried and ground. A 3.000 – 8.000mg of ground samples was weighed using a microbalance then placed into tin capsules. Capsules were processed using a CE Elantech NC 2100 CHN elemental analyzer. For each capsule the elemental analyzer determined %N and %C.

At the start (T_0) and conclusion of the experiment (T_3) *H. wrightii* biomass was collected across all replicates. For each replicate *H. wrightii* was sectioned into aboveground and belowground material. Material was placed in pre-weighed aluminum envelopes and dried at 60°C for five consecutive days or until a constant dry weight was reached. Dry weight of aboveground belowground material was reported as above- and belowground biomass (g DW m^{-2}).

Statistical analysis

Seagrass metrics

All field experiment data was analyzed by season (summer, winter) using the statistical program *R* (R Core Team 2022). Data was examined for outliers, collinearity, relationships between variables, interactions, zero inflation, covariates, and normal distribution (Zuur et al. 2010). Residuals were also inspected visually. The best fit model was considered to be the simplest model with the lowest Akaike information criterion (AIC) score (Zuur et al., 2007). For the field experiment all seagrass data was analyzed using generalized linear mixed effects model (GLMM) with replicate set as a random factor. For the lab experiment seagrass data was analyzed using generalized linear mixed effects model (GLMM) with tank number set as a random factor. GLMM were run using the 'lme4' and 'glmmTMB' packages. *H. wrightii* total biomass (above and belowground biomass combined), aboveground biomass, belowground biomass, shoot density, leaf length, and F_v/F_m were analyzed using a gamma distribution. F_v/F_m percent change throughout the experiment was calculated as the difference in F_v/F_m from the start of the experiment (T_0) compared to each experiment week (T_1, T_2, and T_3). F_v/F_m percent change and aboveground and belowground C:N ratios across all weeks (T_0 – T_3) were analyzed using a gaussian distribution. Overall effects of categorical variables were quantified via analysis of deviance for all model parameters using a type II ANOVA. During post hoc analysis all pairwise comparisons were computed from the contrasts between factors with a 'tukey' adjustment using 'lsmeans' package (Lenth 2016).

Environmental data

For the field experiment mean daylight PAR was calculated by removing all data points that were 1 hour before sunrise and 1 hour after sunset (NOAA GML). Across both seasons PAR is reported as percent change between treatment types: shallow ambient - deep ambient, deep ambient – deep 22% SI, deep ambient – deep 11% SI, and shallow ambient - shallow 22% SI (winter only). PAR data for summer and winter shallow 11% SI and summer shallow 22% SI treatments were lost due to sensors flooding with water. Water temperature data was corrected to remove temperatures 1h before and 1h after low tide to remove data when sensors were exposed during spring or king tides (NOAA CO-OPS). For the lab experiment light was collected using HOBO sensors measuring lux (lumens m^2). Lux were converted to PAR ($\mu mol\ m^{-2}\ s^{-1}$) using a calibration between a HOBO and PAR sensors (Long et al. 2012).

<u>Results</u>

Field Experiment

SAV Metrics:

Biomass

In the winter *H. wrightii* total biomass (combined aboveground and belowground) was impacted by depth (Chi-sq = 26.693, DF = 1, p = 0.045) with greater total biomass at deep depths (t.ratio = 5.191, DF = 34, p < 0.001) (Fig 2a). Belowground biomass was impacted by depth (Chi-sq = 27.999, DF = 1, p < 0.0001) with greater belowground biomass observed at deep water depths compared to shallow (t.ratio = 5.319, DF = 34, p < 0.001) (Fig 2b). In the summer experiment total biomass was impacted by depth (Chi-sq = 12.201, DF = 1, p < 0.001) with greater total biomass at deep depths (t.ratio = 3.538, df = 37, p < 0.001) (Fig 2c). Belowground biomass was impacted by depth in the summer (Chi-sq = 30.939, DF = 1, p < 0.001) wither greater belowground biomass observed at deep water depths compared to shallow (summer t.ratio = 5.642, DF = 37, p < 0.001) (Fig 2d). In the summer aboveground biomass was impacted by depth (Chi-sq = 6.9036, DF = 1, p = 0.009) where there was greater aboveground biomass at the deep treatments (t.ratio = 2.642, DF = 37, p = 0.012) (Figure 2d).

Shoot Density

In the winter experiment *H. wrightii* shoot density had an effect with treatment water depth (Chi-sq = 5.494, DF = 1, p = 0.019) with greater shoot densities at shallow treatments (t. ratio = -2.349, df = 34, p = 0.020) (Fig 3a). In the summer experiment shoot density had an effect with experimental week (Chi-sq = 90.021, DF = 3, p < 0.001) (Fig 3b). Throughout the experiment the shoot density observed during week 3 was significantly lower compared to week 0 (t.ratio = 3.359, DF = 37, p = 0.002), week 1 (t.ratio = 7.616, DF = 37; p < 0.001), and week 2 (t.ratio = 8.583, DF = 37, p < 0.001). Week 0 had a significantly lower density than week 1 (t.ratio = -4.292, DF = 37, p = 0.001) and week 2 (t.ratio = -5.291, DF = 37, p = < 0.001). Although not significant, in the summer there was a trend towards greater shoot densities at deep water treatments.

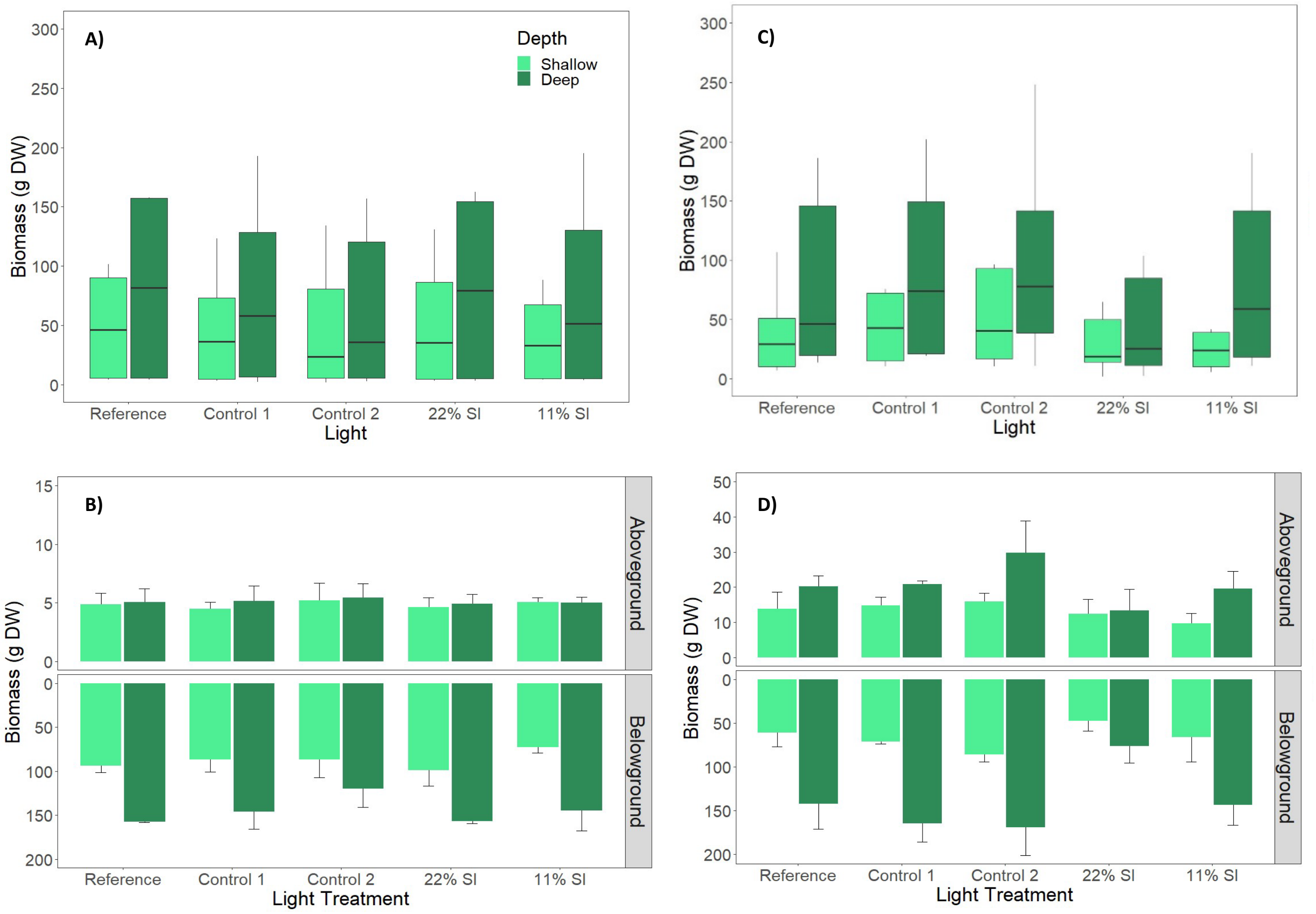

Figure 2 | Field experiment results for *H. wrightii* mean (±SE) total biomass (aboveground and belowground) during the **(A)** winter and **(B)** mean ±SE aboveground and belowground biomass during the winter at shallow and deep depths for each light treatment. Mean ±SE Total biomass during **(C)** the summer and **(B)** mean ±SE aboveground and belowground biomass during the summer at shallow and deep depths for each light treatment. Reference represents meadow biomass prior to shading (n = 4). Control 1, Control 2, 22%SI, and 11%SI represents mean ±SE biomass for each light treatment after 3 weeks of exposure to the light treatment (n = 4 per light treatment). Aboveground biomass Y-axis scale are different ranges for winter and summer figures.

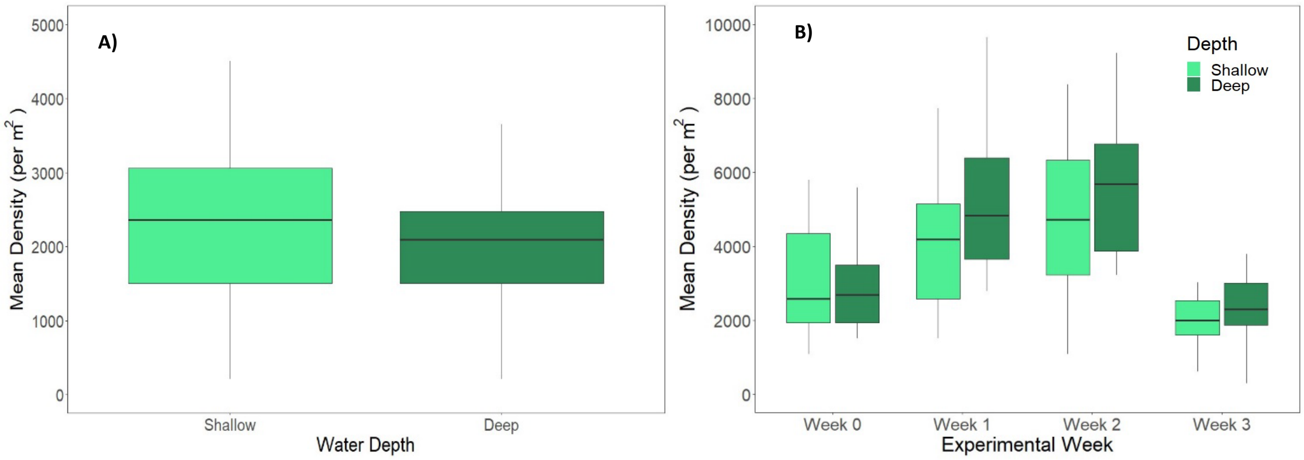

Figure 3 | Field experiment results for *H. wrightii* mean (±SE) shoot density (per m^2) during the **(A)** winter density for shallow and deep treatments and summer **(B)** shallow and deep treatments during each experimental week treatments. Y-axis scale are different ranges for winter and summer figures.

Table 1 | Field experiment results for *H. wrightii* mean shoot density and standard deviation during the summer and winter for depth and experiment week.

	Summer	**Winter**
Depth		
Shallow	3529.0 ± 1907.5	2393.3 ± 978.1
Deep	4019.0 ± 2016.9	2030.6 ± 774.5
Experiment Week		
Week 0	3013.1 ± 1314.1	2456.1 ± 884.5
Week 1	4710.9 ± 2048.8	2375.6 ± 810.7
Week 2	5230.0 ± 1923.5	2100.4 ± 1126.7
Week 3	2152.4 ± 830.2	1915.8 ± 630.5

Leaf Length

In the winter *H. wrightii* leaf length was impacted by depth (Chi-sq = 5.069, DF = 1, p = 0.0244) and time (Chi-sq = 13.639, DF = 3, p = 0.0034). *H. wrightii* leaf lengths were significantly longer at shallow treatments (t.ratio = -2.255, DF = 34, p = 0.026) (Fig 4a). Across all depths, leaf length decreased significantly by week 2 (t.ratio = 3.570, DF = 34, p = 0.003) and continued to decrease in length through week 3 (t.ratio = -2.622, DF = 34, p = 0.049) (Fig 4a; Table 2). In the summer *H. wrightii* leaf length was impacted by depth (Chi-sq = 33.846, DF = 1, p < 0.001) where *H. wrightii* leaf lengths were significantly longer at deep depths (t.ratio = 5.827, DF = 37, p < 0.001) (Fig 4b; Table 2). Leaf lengths were also impacted by light treatment (Chi-sq = 9.923, DF = 3, p = 0.019). The 11% SI light treatment had longer leaves than the controls or 22% SI light treatments (t.ratio = 2.703, DF = 37, p = 0.047).

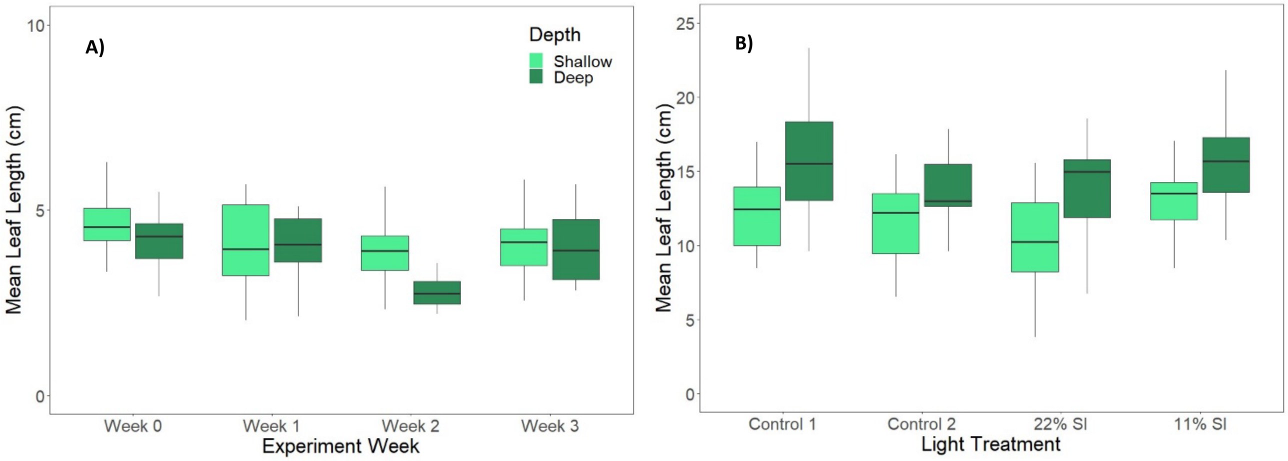

Figure 4 | Field experiment results for *H. wrightii* mean (±SE) leaf length (cm) during the **(A)** winter for shallow and deep treatments for each experiment week. **(B)** Summer mean leaf length for shallow and deep light treatments. Y-axis scale are different ranges for winter and summer figures.

Table 2 | Field experiment results for *H. wrightii* mean leaf length (cm) and standard deviation during the summer and winter for depth and experiment week.

	Summer	**Winter**
Depth		
Shallow	11.8 ± 2.8	4.2 ± 1.0
Deep	14.8 ± 3.2	3.8 ± 1.1
Experiment Week		
Week 0	12.8 ± 3.2	4.3 ± 0.9
Week 1	13.2 ± 3.4	4.1 ± 1.0
Week 2	14.7 ± 3.1	3.5 ± 1.2
Week 3	12.6 ± 3.4	4.1 ± 1.0

C:N Ratios

In the winter *H. wrightii* belowground C:N ratio had an effect with experimental week (Chi-sq = 19.8794, DF = 3, p = 0.0001). Belowground C:N ratio were highest during experiment week 3 compared to experimental week 0 (t.ratio = -3.890, DF = 34, p = 0.001), week 1 (t.ratio = -2.619, DF = 34, p = 0.040), and week 2 (t.ratio = -3.831, DF = 34, p = 0.0010) (Fig 5a). In the summer *H. wrightii* aboveground C:N ratio was affected by light treatment (Chi-sq = 14.441, DF = 3, p = 0.0024). Aboveground material had lower C:N ratio in the 11% SI treatment compared to controls (control 1 t.ratio = 3.034, DF = 37, p = 0.0177; control 2 t.ratio = 2.680, DF = 37, p = 0.0420) and 22%SI had a lower aboveground C:N ratio than control 1 (t.ratio = 2.648, DF = 37, p = 0.0420) (Fig 5b).

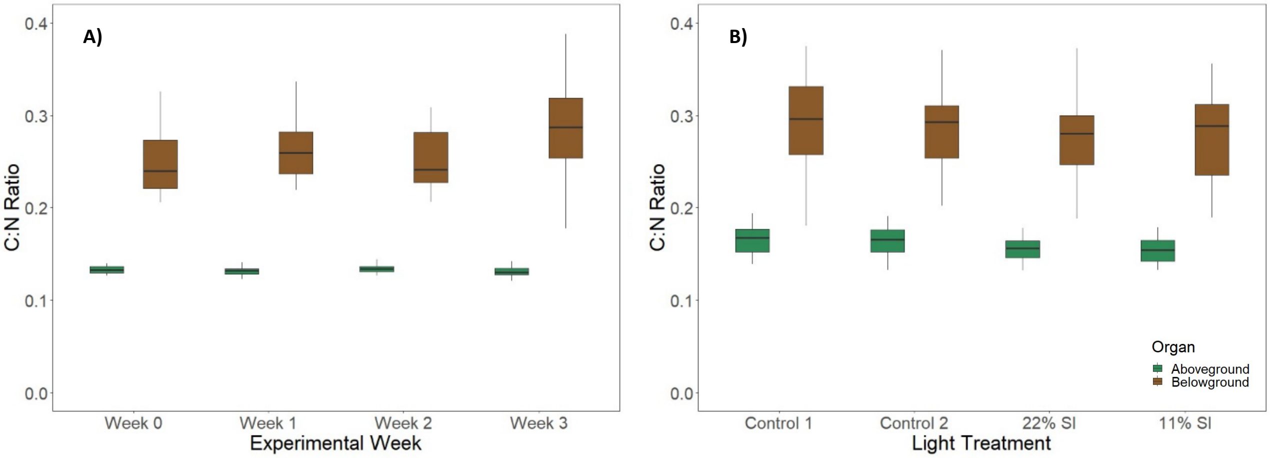

Figure 5 | Field experiment results for *H. wrightii* aboveground (green) and belowground (brown) mean (±SE) C:N ratio during the **(A)** winter for each experiment week and **(B)** summer for each light treatment.

Abiotic Measures:

During the winter experiment period water temperatures ranged 4.0 – 14.1°C with a mean of was 10.7 ± 1.8°C (mean ± SD) (Figure 6). During the summer experiment period water temperatures ranged 23.3 – 32.9°C with a mean of 28.0 ± 1.5°C (mean ± SD) (Fig 6). During the summer experiment there was an observable decrease in water temperature on and proceeding experiment day 16 (31 August 2023) when hurricane Idalia passed over the region producing 17-25cm of rainfall and 1.2m storm surge (NWS 2023).

During the winter, the daily percent difference in PAR between shallow ambient and deep ambient treatments ranged -12.4 – 22.3% (Fig 7; Table 3). Deep ambient plots received an average of 73% more light compared to deep 22% SI plots and 85% more light than deep 11% plots. Shallow ambient plots received an average of 44% more light than the shallow 22% SI plots. During the winter daily percent difference in PAR between shallow ambient and deep ambient treatments ranged 34 – 80% (Fig 7; Table 3). Deep ambient plots received an average of 63% more light compared to deep 22% SI plots and 88% more light than deep 11% SI plots.

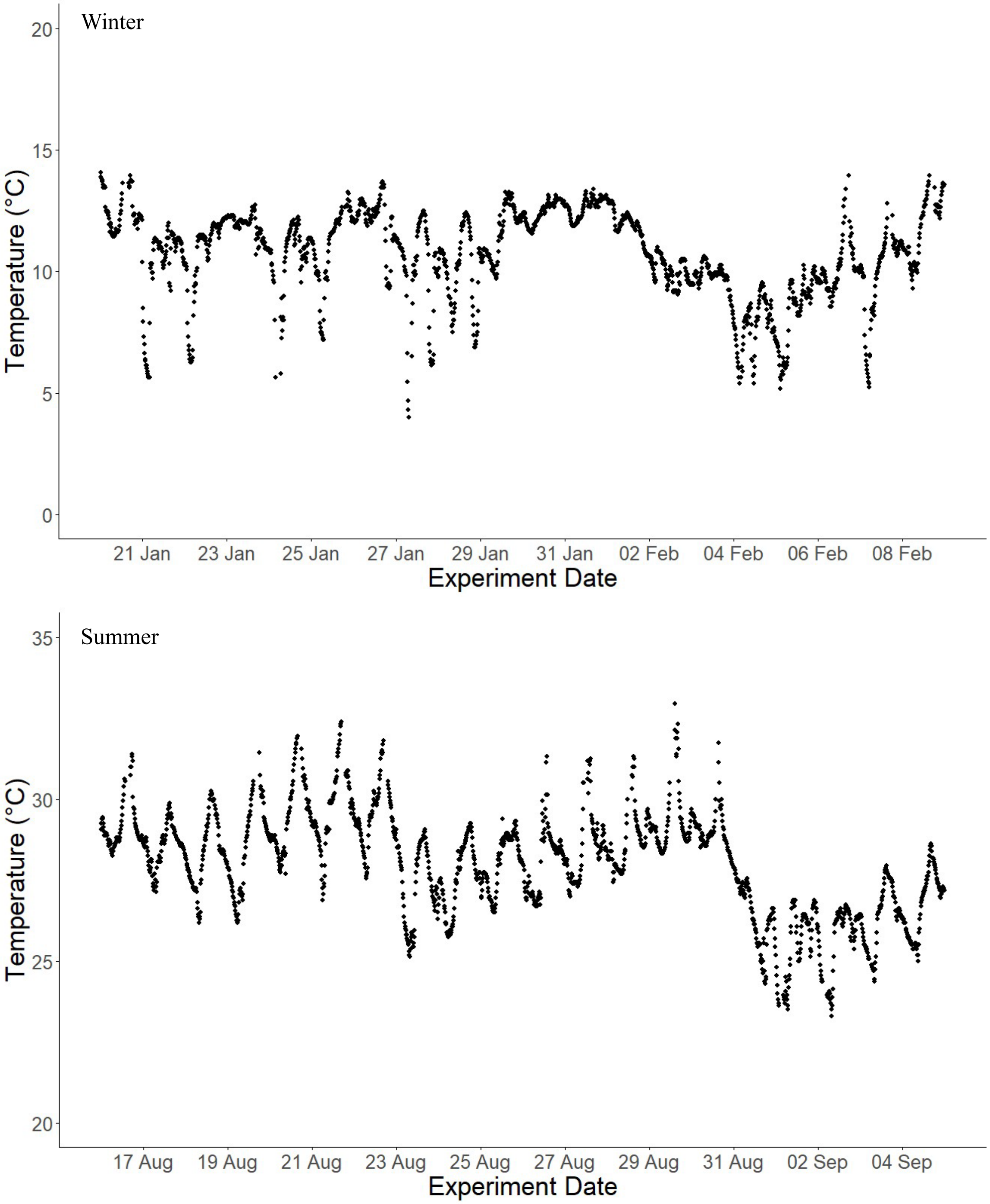

Figure 6 | Daily water temperature at Middle Marsh during the winter (top) and summer (bottom) field experiment. Y-axis scale are different ranges for winter and summer figures.

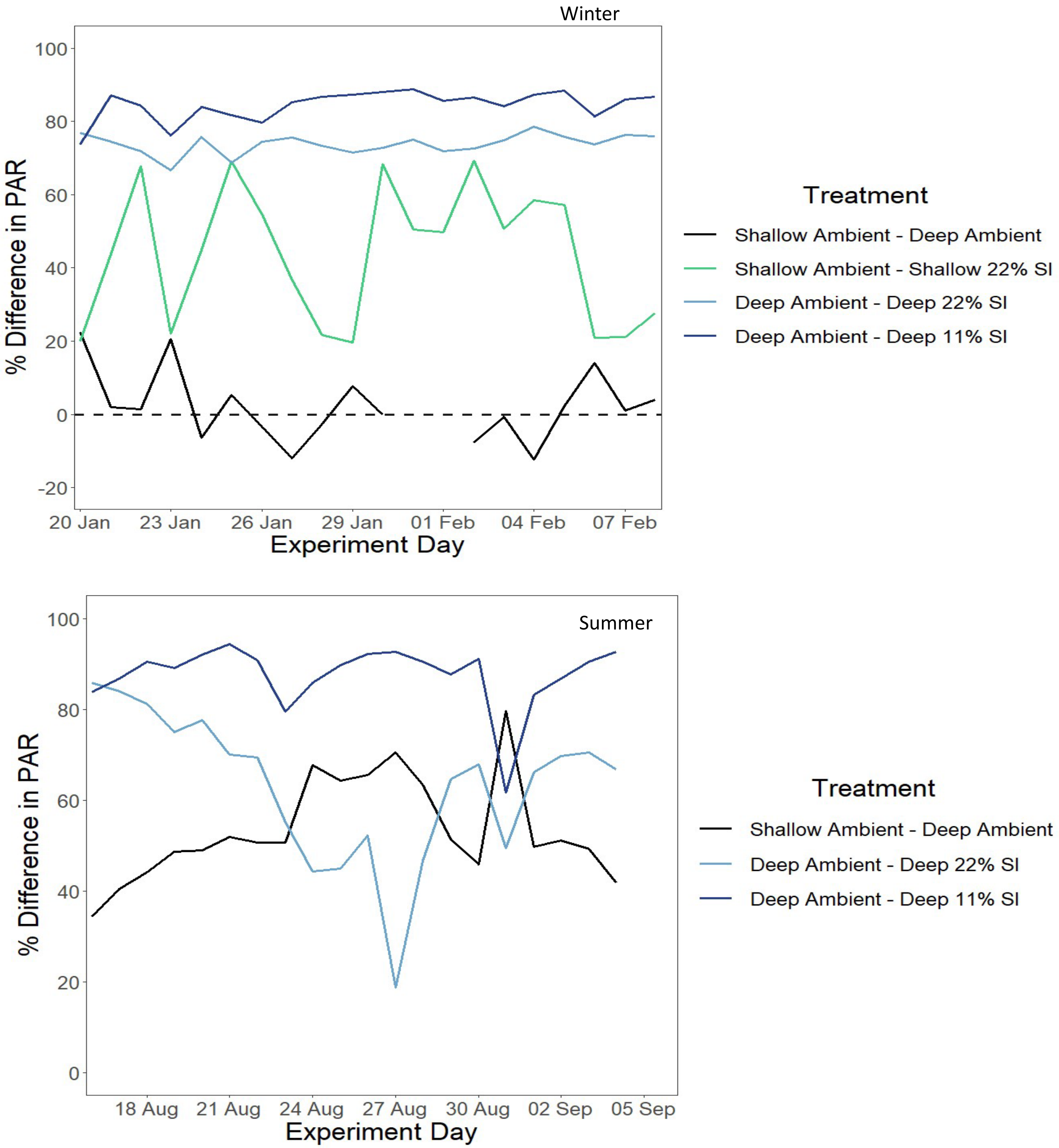

Figure 7 | Winter (top) and summer (bottom) daily percent difference in PAR (µmol m^2 s^1) for: shallow ambient - deep ambient, deep ambient – deep 22% SI, deep ambient – deep 11% SI, and shallow ambient - shallow 22% SI (winter only).

Table 3| Comparison of light conditions over the 3 -week experiment represented by % difference in mean PAR. Depth to the bottom was 0.5m for shallow treatments and 1m for deep treatments during mean low tide. Values are mean ± standard deviation. NAs are due to loss of PAR sensor.

Treatment	Mean % Difference in Winter PAR	Mean % Difference in Summer PAR
Shallow Ambient - Deep Ambient	-1.84 ± 14.82	53.52 ± 11.38
Shallow Ambient – Shallow 22%	43.7 ± 18.6	na
Shallow Ambient – Shallow 11%	na	na
Deep Ambient - Deep 22% SI	73.44 ± 4.27	63.04 ± 16.47
Deep Ambient - Deep 11% SI	84.49 ± 2.92	87.59 ± 7.12

Lab Experiment

SAV Metrics:

The lab experiment showed no detectable effects from light treatment or experiment week. Percent change in photosynthetic efficiency in the 22% SI and 11% SI treatments trended towards increasing throughout the experiment (Fig 8). Total biomass was driven by the weight of the belowground biomass and belowground biomass was always greater than aboveground biomass (Fig 9). Mean leaf length did not have an effect with light treatment of experiment week (Fig 10). Belowground C:N rations in 22% SI and 11% SI treatments trended towards increasing throughout the experiment while belowground ratios decreased in the ambient treatment (Fig 11).

Abiotic Measures:

Over the 3-week winter lab experiment period (04 February 2023 – 24 February 2023) water temperatures showed a range from 10.1 – 11.8°C. Mean water temperature during the winter experiment period was 10.8 ± 0.4 (mean ± SD). PAR values for the ambient treatment ranged 6.6 – 8.2 µmol, 22% SI ranged from 1.6 – 1.9µmol and 11% SI range 0.6 – 0.8µmol (Figure 12). Mean PAR during the experiment for ambient was 7.3 ± 0.5 (mean ± SD), 22% SI was 1.8 ± 0.1, and 11% SI was 0.7 ± 0.1.

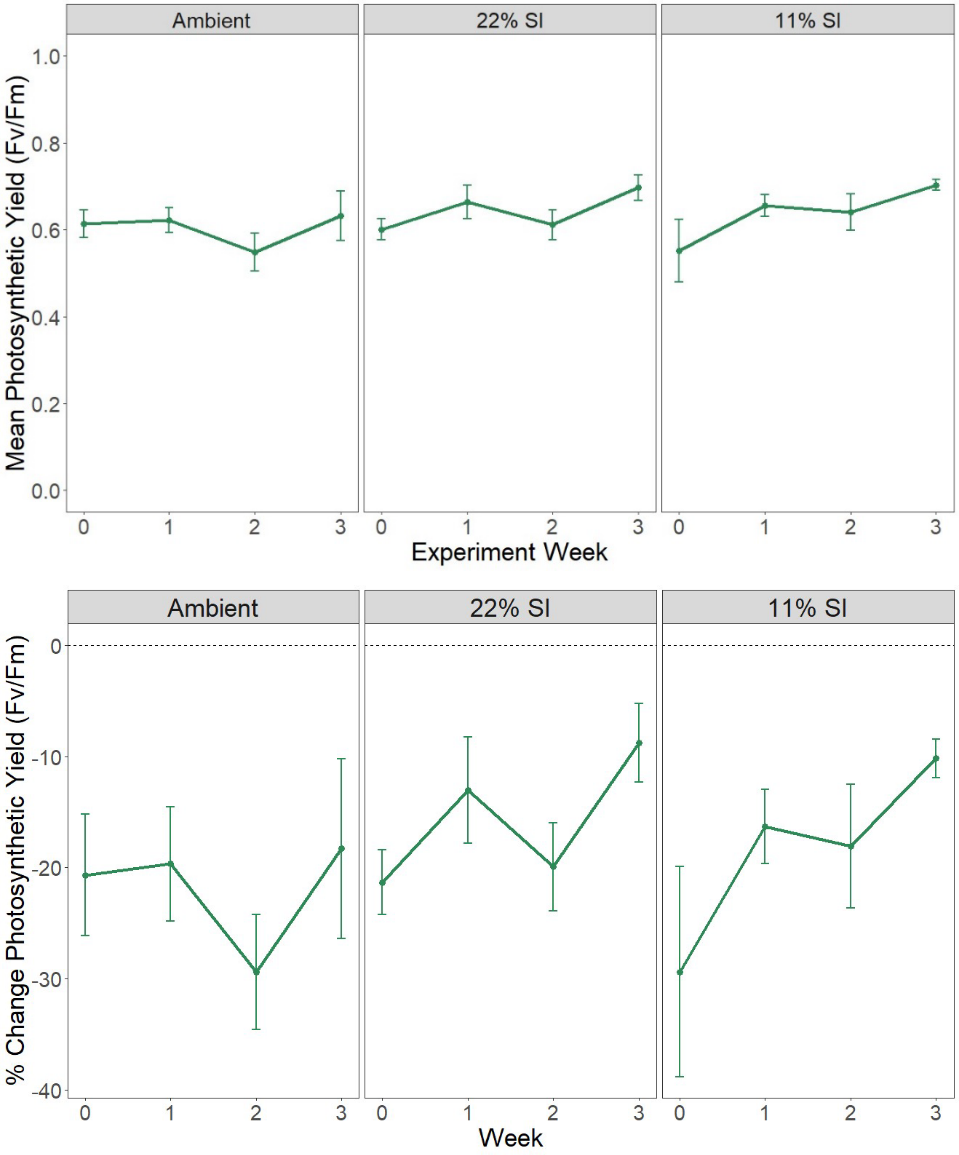

Figure 8 | Winter lab experiment results for **(A)** Weekly mean photosynthetic yield (Fm/FV) for each light treatment during each experimental week and **(B)** Mean (±SE) percent change in photosynthetic efficiency (F_v/F_m) over the 3-week experiment.

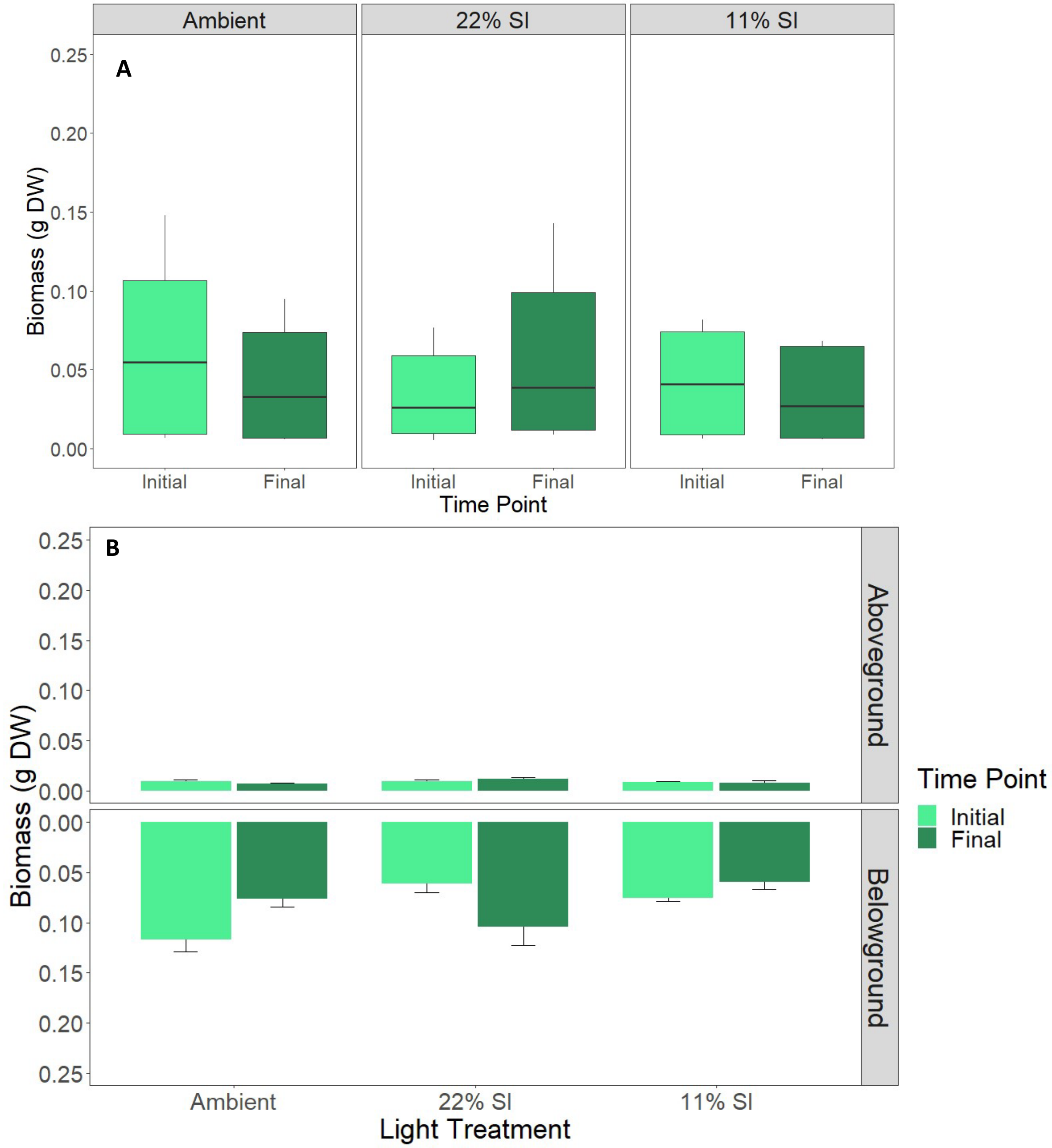

Figure 9 | Winter lab experiment results for **(A)** mean (±SE) total *H. wrightii* biomass (aboveground + belowground) and **(B)** mean (±SE) *H. wrightii* aboveground and belowground biomass for each light treatment. Initial represents the biomass after 2 weeks of acclimation to the laboratory tanks prior to shading (n = 4). Final represents biomass for each light treatment after 2 weeks of tank acclimation and 3 weeks of exposure to the light treatment (n = 4 per light treatment).

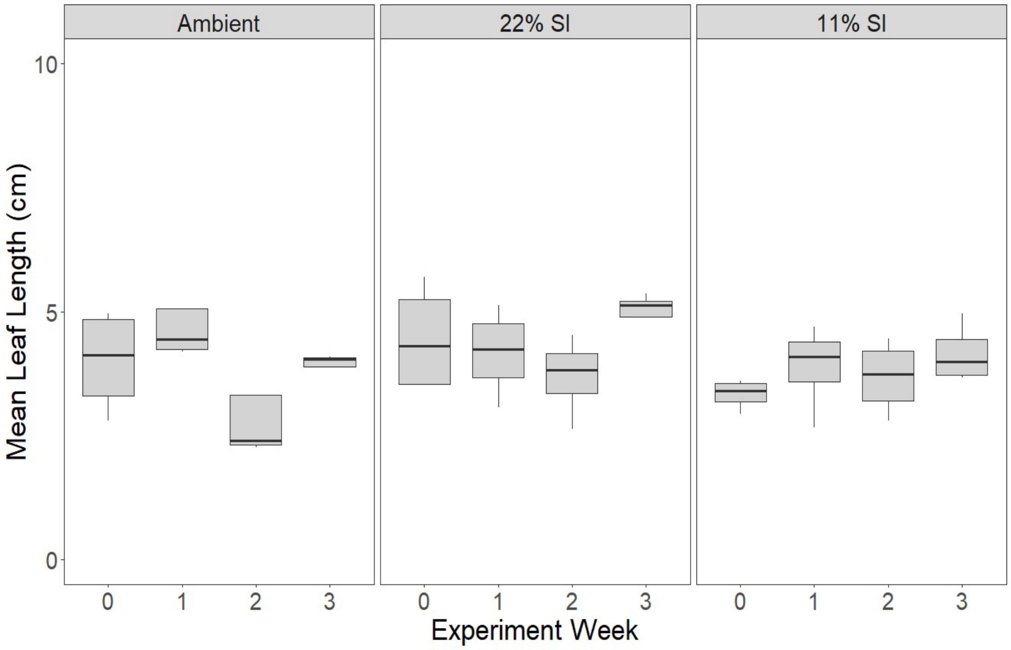

Figure 10 | Winter lab experiment results for mean (±SE) leaf length for each experiment week.

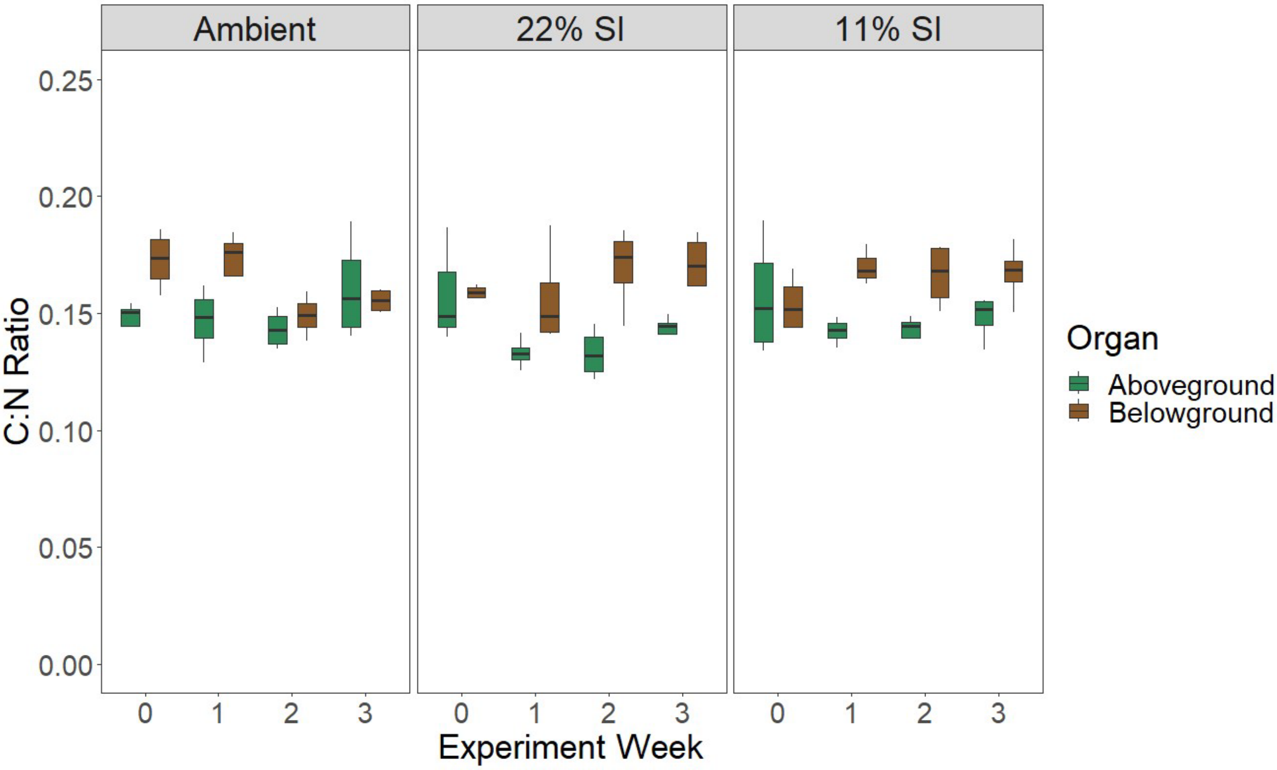

Figure 11 | Winter lab experiment results for mean (±SE) C:N ratios in aboveground and belowground material for the experiment.

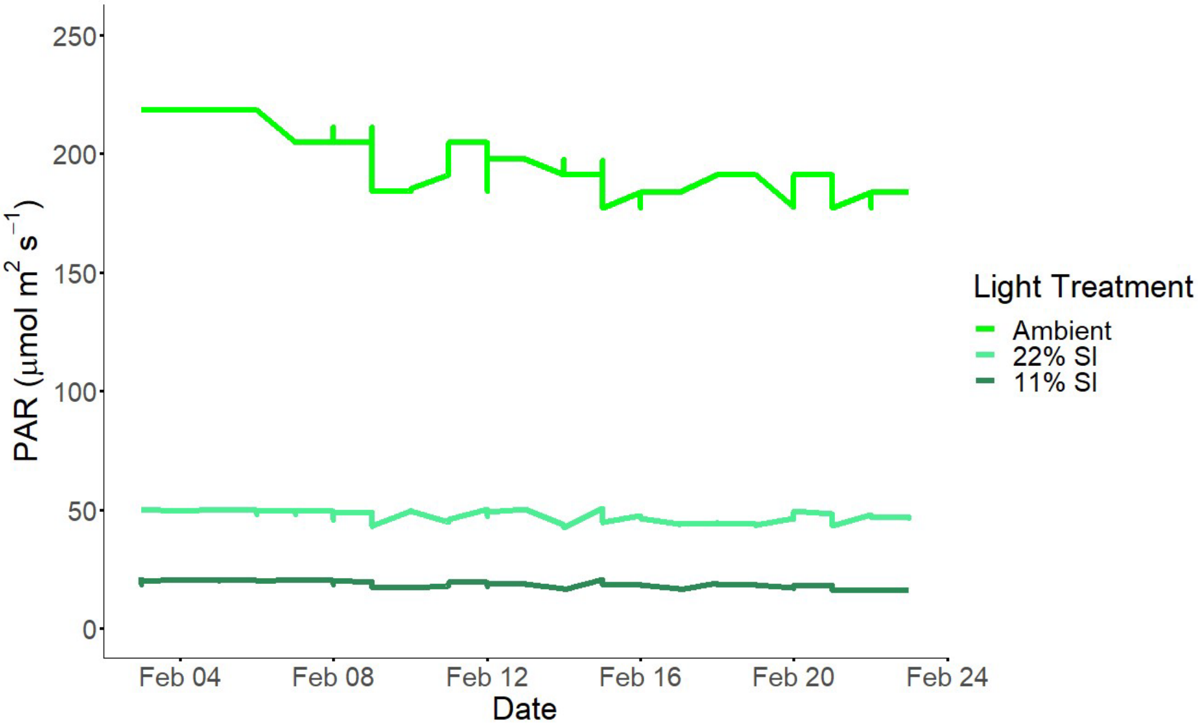

Figure 12 | Winter lab experiment daylight PAR (μmol m^2 s^{-1}) collected every 15 minutes for each light treatment.

Discussion:

Is H. wrightii limited by light or water temperature?

This study examined the structural and physiological response of *H. wrightii* to light limitation under optimal and cold-stress water temperatures. There was no direct effect of light reduction (22% SI, 11% SI) on *H. wrightii* structural metrics compared to ambient conditions in either shallow or deep depths during the summer or winter experiments. However, there were seasonal difference in ambient light availability with depth, as *H. wrightii* received on average 53% more light at shallow treatments (<1 m) compared to deep (>1m) during the summer months. During the winter, when water temperatures are stressful for *H. wrightii*, both shallow and deep depth ambient treatments received comparable amounts of light. The lack of measurable responses in *H. wrightii* to additional light reduction at the deep edge of the meadow indicate that current *in situ* light conditions at depths > 1m are already limiting. Therefore, CHPP recommended light restrictions of 22% SI at depths ≤ 1.7 m may not be adequate for growth of *H. wrightii* at the deep edge of NC high salinity SAV meadows.

Minimal responses to light limitation were seen in *H. wrightii* physiological and morphological metrics at the deep edge of the meadow. Physiological differences in C:N ratio among light treatments suggest that *H. wrightii* was showing a response to light limitation only at the lowest light treatment. In the summer 11% SI plots had the lowest aboveground C:N ratios indicating nitrogen is high. This indicates *H. wrightii* is increasing the chlorophyll concentration or undergoing growth potentially to capture more light when ambient light was reduced to 11% SI (Dennison and Alberte 1982 and 1985; Ralph et al. 2007). Nitrogen could also increase from uptake from the surrounding environment, however nutrients in the water column and sediments are typically low (Hemminga and Duarte 2000). In the summer water temperatures are optimal but the seasonal decline in water clarity is exposing *H. wrightii* at deep depths (>1m) to light limitation. As a result, *H. wrightii* at deeper depths may not be able to expand to new areas even when *Z. marina* declines resulting in greater potential habitat loss.

The limited capability of *H. wrightii* expansion at deeper depths was supported by morphological response data. Morphological differences in leaf length between shallow and deep treatments suggest *H. wrightii* at deep (>1m) treatments is light limited in the summer under ambient light. To meet photosynthesis requirements in low light conditions plants elongate leaves and increase chlorophyll content to maximize the amount of light that can be captured (Dennison and Alberte 1982; Ralph et al. 2007). During the summer experiment *H. wrightii* at deep treatments had longer leaves than shoots found at shallow depths. However, this may have also been in response to a deeper water column providing more vertical space for the shoots to grow (Olivé et al. 2013). If the longer leaves were only related to the depth of the water column and light was not limiting at deeper depths, then *H. wrightii* leaf elongation would have been observed in the reduced light treatments throughout the course of the experiment as *H. wrightii* leaf elongation rates during August and September in Back Sound average 6.44 ± 0.08 mm day^{-1} (Jarvis et al. 2022). However, no changes in leaf length were observed. Therefore, the lack of leaf elongation in the additional light reduction treatments at the deeper depths also supports the hypothesis that *H. wrightii* was light limited in water depths > 1m.

In the winter the amount of ambient light is comparable at shallow and deep treatments, however *H. wrightii* does not expand to fill gaps left by seasonal declines in *Z. marina* due to cold temperature stress. When water temperatures are below 10°C *H. wrightii* photosynthetic efficiency is reduced by 25% compared to optimal temperatures (Stevenson et al., *submitted*). While the plants are still photosynthesizing, the reduced rate, even at optimal light levels, indicates that temperature stress may limit the ability of *H. wrightii* to grow during the winter months in North Carolina. In the winter experiment deep depths had a lower shot density and small average leaf length despite no difference in light levels between shallow and deep treatments. When plants are metabolically stressed, they reduce the leaf length and shoot density to decrease the maintenance costs (Fourqurean and Zieman 1991; Collier at al. 2009). *H. wrightii* at deep depths could be experiencing stress in the summer from light limitation and stress in the winter from cold water temperatures. When plants decrease shoot density and leaf length energy from the aboveground leaf material can be stored in the belowground (Alcoverra et al. 2001). During the winter *H. wrightii* had greater belowground biomass at deep depths. *H. wrightii* is possibly storing energy in the belowground material to persist through the period of cold water temperature (Touchettee and Burkholder 2000). During the winter experiment *H. wrightii* had higher belowground C:N ratios during week 3 of the experiment indicating nitrogen as decreasing. By week 3 in the experiment *H. wrightii* at the SAV sites had been experiencing cold temperatures for 1-2 months (Jarvis Lab data). The week 3 timepoint could have been the initial detectable response of *H. wrightii* to cold water temperatures given C:N ratios respond on the scale of weeks. Week 2 and week 3 of the experiment *H. wrightii* had significantly shorter shots. Since there were no additional effects from light treatments with experiment week it is possible *H. wrightii* was responding to exposure to prolonged cold temperatures throughout the winter.

Throughout the summer and winter field experiment there were limited impacts from the additional shading applied to *H. wrightii*. The most pronounced effects were with water depth and winter water temperatures with minimal effects from light treatment. It is likely that effects with depth were due to the differences in ambient light conditions *H. wrightii* experiences in the summer. At shallow depths (< 1m) there were no impacts from additional shading possibly indicating *H. wrightii* is receiving adequate ambient light for growth and survival at the suggested 22% SI threshold suggested by the CHPP. At deep depths (> 1m) it is possible *H. wrightii* is experiencing light limitation under current ambient light conditions. Therefore, when plants were exposed to additional light reduction they did not have the capacity to amplify their stress response further (Ostrowski et al. 2022). When *H. wrightii* is exposed to light reduction under cold temperature conditions the plants are managing thermal stress and additional effects of light limitation did not have a detectable impact.

Summary

Both light and temperature may be limiting *H. wrightii* expansion into habitat formally colonized by *Z. marina*. During summer optimal water temperatures for *H. wrightii,* light is limiting at depths > 1 m. Therefore, despite the greater thermal tolerance of *H. wrightii* compared to temperate *Z. marina,* the predicted increase in water temperatures for coastal North Carolina may not result in greater expansion of *H. wrightii* if water clarity is not improved. While light levels are adequate for *H. wrightii* growth and survival during the colder winter months, thermal stress restricts photosynthetic efficiency resulting in limited growth and expansion during this period. Combined, low light availability at depths >1 m during the summer and cold temperature stress during the winter restrict the ability of *H. wrightii* to replace *Z. marina* in NC coastal habitats. As a result, CHPP recommended light restrictions of 22% SI at depths ≤ 1.7 m, while sufficient at depths < 1m, may not be adequate to support expansion of *H. wrightii* at the deep edge of NC high salinity SAV meadows. Improvements in water clarity may be required before high salinity SAV can expand to previously recorded maximum depths of up to 2 m.

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