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BENTHIC MICROALGAE RESPONSE TO A WARMING CLIMATE AND SHIFT IN FOUNDATIONAL VEGETATION SPECIES IN A SALTMARSH-MANGROVE ECOTONE

By

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Abstract

The ecotone in Northeast Florida is experiencing rapid change as mangroves begin to encroach northward into saltmarsh dominated coastal wetlands. This is especially prevalent within the boundaries of the Guana Tolomato Matanzas National Estuarine Research Reserve where studies are attempting to understand the consequences of more mangroves. Although we are beginning to understand some of the implications of this shift in dominant vegetation, the true effects and the accompanying sustained climatic warming effects on the resilience of wetland habitats and their associated communities remains unknown. Primary producers such as benthic microalgae (BMA) are important food sources in both mangrove and saltmarsh communities and can provide insights into how changes can affect community dynamics. Algal community biomass and composition was measured within three different marsh vegetation types (*Batis maritima, Spartina alterniflora,* and *Avicennia germinans*) and compared to assess if vegetation is a potential driver of algal community dynamics. Warming chambers deployed on both marsh dominated and mangrove dominated plots were also used to investigate how warming could potentially drive algal community change. Abiotic conditions and vegetation structure were assessed as potential structuring forces on algal communities. Algal biomass and functional group diversity differed among vegetation types and varied by season. Abiotic factors (temperature, light) and vegetation structural properties (% cover, stem height, stem density) were correlated with patterns of algal biomass. Algae biomass and functional group diversity also responded to increased air temperature treatments in the spring with community shifts towards cyanobacterial dominance in warming treatments. These findings suggest that changes in BMA communities should be investigated further as potential bottom up drivers of community shifts resulting from the marsh to mangrove transition.

Introduction

Shifts in wetland vegetation

Coastal intertidal saltmarshes are some of the most productive systems on Earth. These ecosystems are characterized by the salt-tolerant vegetation species that colonize coastal areas and facilitate other organisms to inhabit these areas. Coastal wetland plants are considered foundation species because of their role in habitat creation, ecosystem dynamics, and development of ecological communities (Dayton 1972; Ellison *et al.* 2005; Osland *et al.* 2012). Saltmarsh grasses such as *Spartina alterniflora* are considered foundation species because they directly control tidal wetland ecosystem structure and function by regulating ecosystem dynamics, facilitating wetland community development, and providing valuable ecosystem services and habitat (Teal 1962; Bertness and Leonard 1997; Alongi 2009; Barbier *et al.* 2011; Osland *et al.* 2013).

In recent decades it has been observed that shifts in ecosystem structure have been occurring as woody plants encroach upon grasslands and wetlands globally due to temperature shifts (Saintilan and Rogers 2015). One such encroachment is the migration of mangroves into salt marsh ecosystems occurring at many tropical-temperate ecotones. This poleward migration of mangroves has been linked to a decrease in the frequency of sustained freeze events (Cavanaugh *et al.* 2013). Mangroves are limited in their range by cold temperature intolerance, so the decrease in frequency of sustained freeze events enables mangroves to expand poleward (Saintilan *et al.* 2014). Although mangroves such as *Avicennia germinans* serve as foundation species within their respective range, their structure and characteristics make them vastly different from the saltmarsh grasses they are displacing. Range shifts of mangroves into saltmarshes have been shown to result in changes of community structure (Osland *et al.* 2013), belowground processes (Coldren *et al.* 2019), and ecosystem services (Barbier *et al.* 2011), but the broader scale ecosystem changes that can result from this shift are still not well understood.

Both mangrove- and saltmarsh-dominated systems provide important services such as carbon storage, surface elevation stability, wave attenuation, reduction of erosion, and essential habitats (Barbier *et al.* 2011; Gedan *et al.* 2011; McLeod *et al.* 2011; Barreto *et al.* 2018). However, the different structures of these plants can affect ecosystem-level processes such as hydrology, sedimentation rate, and nutrient cycling (Bertness 1988; Leonard and Luther 1995; Levin and Talley 2000). Also, mangroves and saltmarshes vary in their detrital input. *Spartina* have cycles of growth and dieback where almost all annual growth is cyclically discarded. Mangroves deposit leaf litter much less frequently and can keep individual leaves for up to 17 months (Gill and Tomlinsen 1971). This difference in detrital matter deposition and quality could significantly impact food webs which are partially fueled by this detrital material (Peterson *et al.* 1985; Levin and Talley 2000; Levin *et al.* 2006). Although one study by Henry and Twilley (2013) found that differences in productivity and tissue chemistry between *Spartina* and *Avicennia* did not result in significant soil chemistry changes, they did find higher phosphorous levels in *Avicennia* dominated sediments. In addition to chemical changes, vascular marsh plants have the potential to modify the amount and quality of light reaching the sediment, thus affecting temperature (Gallagher 1971; Bertness and Hacker 1994) and algal growth (Fortes and Luning 1980; Seliskar *et al.* 2002). All these factors are important structuring mechanisms for micro-communities within the sediments that are the base of the food webs within coastal systems. Understanding how changing physical conditions will influence benthic communities within coastal marshes will be a critical step towards elucidating the broader impacts of mangrove range shifts.

In mangrove-saltmarsh ecotones, other vegetation species (e.g., rushes and succulents) can coexist, varying in distribution depending on environmental conditions. In Northeast Florida, *Batis maritima* can often be observed near mangroves or in higher elevation areas of marshes. This salt tolerant, woody succulent is found in many tropical wetlands and can form extremely dense patches (Marcone 2003). Less is known about how these secondary species influence mangrove colonization, however due to their coupled distribution with mangroves, it is important to consider the benthic structuring potential of these plants which vary drastically in structure to both marsh grasses and mangroves.

Benthic community dynamics in wetlands

The benthic microalgae (BMA) community, or as MacIntyre *et al.* (1996) refers to them, the "secret garden" of shallow water systems, consists of unicellular eukaryotic algae and cyanobacteria that grow within the upper several millimeters of illuminated sediments. The difficulty of sampling, high variability and inconspicuous appearance of BMA have contributed to their 'secret' nature and underrepresentation in current climate modeling and change studies. Despite this, recent stable isotope analyses have shown that in both salt marsh- and mangrove-dominated intertidal systems, BMA are the strongest link between primary producers and primary consumers, and likely contribute more to food webs then either saltmarsh or mangrove primary production (Couch 1988; Sullivan *et al.* 1990; Currin *et al.* 1995; Pascal *et al.* 2013; Cnudde, *et al.* 2015; Nelson *et al.* 2019; Baker *et al.* 2021). This link emphasizes the importance of understanding how changing conditions associated with shifts in primary vegetation will affect BMA biomass and community structure.

BMA biomass has been shown to vary significantly even on small spatial scales (m), displaying differences under different marsh vegetation types, suggesting a significant relationship between canopy cover and biomass (Pinckney and Zingmark 1993; Whitcraft *et al.* 2007; Kon *et al.* 2010). From a bottom-up perspective, changing physiochemical conditions associated with a marsh to mangrove transition such as light availability, temperature, elevation, inundation time, currents, and nutrient dynamics could potentially have significant effects on BMA biomass and community composition (Gallagher 1971; Luning 1980; Bertness and Hacker 1994; Seliskar *et al.* 2002). Trees, succulents, and grasses have substantially different canopy cover and could affect the photosynthetic active radiation (PAR) that reaches the sediments. Temperature is another important consideration in this process because it has been shown to be indirectly correlated with BMA biomass in the soils (Whitcraft *et al.* 2007; Sin *et al.* 2009). Sediment temperature is often linked to the amount of light that reaches the soil. Less light penetration could result in cooler soils, facilitating algal community shifts (Gallagher 1971; Bertness and Hacker 1994). Differences in elevation may also affect soil temperatures by influencing tidal inundation times. Alber and O'Connell (2019) found that elevation differences were negatively correlated with soil temperature in many marshes.

From a top-down perspective, changes in primary vegetation cover may affect the BMA community via differential grazing pressure. Johnston and Grunner (2018) found that macrofauna selectively sort between different vegetation types even at fine spatial scales. That study focused mainly on the physical drivers of this sorting; however, the additional factor of food availability could influence sorting as well. Although BMA is an important food source, benthic diatoms in particular have been identified as the most consumable BMA group with many species in these intertidal environments relying on them heavily as a food source (Pickney

2003, Wasserman *et al.* 2015*)*. Studies have shown that algal consumers can have a strong structuring force on algal communities; Wasserman *et al.* (2015) found that in the presence of consumers, diatom biomass was reduced but overall algal biomass remained fairly consistent. This finding reinforces the importance of studies that include overall community analysis in BMA methodology.

On a broader scale, mangrove range expansion is largely driven by climate-induced warming (Osland *et al.* 2013), so investigating direct effects of atmospheric temperature is another important consideration while studying wetland plant range shift implications. While some studies have shown that increased temperatures can be negatively correlated with species richness and overall BMA biomass (Sin *et al.* 2009; Whitcraft *et al.* 2007), other studies have shown that when increased temperatures are coupled with increased light availability, overall biomass can increase significantly (Laviale *et al.*2015; Werner & Matthiessen 2017). This suggests that further study is needed to understand how increased temperatures coupled with decreased light availability, due to shading from mangroves, will affect BMA biomass.

BMA communities are composed of three primary subgroups, green algae (chlorophytes), blue green algae (cyanobacteria), and diatoms, with other groups such as dinoflagellates and cryptophytes present in smaller concentrations. Variation in community composition is due, in large part, to the variable responses of each respective group to different physical, chemical, or biological conditions. Under certain conditions, it is possible for the community balance to be disturbed resulting in the rapid growth of a certain group which can lead to a bloom of that species. There are a few different types of benthic harmful algal blooms (BHABs) that are capable of harming invertebrates as well as humans, mostly through respiratory symptoms (Vila *et al.* 2016). Studies have shown that temperature can increase growth rate and biomass accumulation of many harmful algae species (Graneli *et al.* 2011; Fricke *et al.* 2018). Further, cyanobacteria exhibit higher maximum specific growth rates in higher temperatures relative to other algal groups, suggesting that in higher temperature scenarios, cyanobacteria may outcompete other algal groups (Paul *et al.* 2011). This is problematic not only because many cyanobacteria species are toxic and thrive in these higher temperatures (Paul *et al.* 2011; Davis *et al.* 2009), but also because cyanobacteria in general are less readily consumed by micro-invertebrates due to their filamentous structure (Haney, 1987). Therefore, it is vital to not only understand overall biomass in these communities but also monitor target species to track potential shifts in community composition that could lead to higher trophic level impacts or BHABs.

The goal of this study was to understand the impacts of climate-driven mangrove range expansion on estuarine ecosystems. To partially address this goal, the specific objectives of this study were first, to investigate how BMA biomass and community composition vary under different primary vegetation types, and second, to test how warming air temperatures affect those relationships. To address these objectives, this study was conducted in a saltmarsh-mangrove ecotone and sediment chlorophyll *a* was used as a proxy for BMA biomass. We hypothesized that (1) differences in plant structure and environmental conditions would result in significant variations in overall BMA biomass and community composition, and (2) warming would increase overall BMA biomass and shift communities towards more heat tolerant species such as cyanobacteria. The study also addresses the need for baseline BMA community information by investigating the BMA communities within the Northeast Florida ecotone. Being situated within an ecotone provides a unique opportunity to study the effects of temperature and primary vegetation change on biomass and community composition within a single marsh system.

Methods

Study system

The field component of this study took place within the Guana Tolomato Matanzas National Estuarine Research Reserve (GTMNERR). The Reserve covers 76,000 acres of coastal lands in Northeast Florida from Ponte Vedra Beach to Palm Coast and encompasses the northern range of Florida's black mangrove (*A. germinans*) populations (Williams *et al.* 2014). The coastal wetlands in this area are dominated by *S. alterniflora, B. maritima*, and *A. germinans* (Dix *et al.* 2021).

Multiple freeze events resulted in mass mortality of mangroves in much of Northeast Florida and the northern Gulf of Mexico in the 1980's (Stevens *et al.* 2006; Rodriguez *et al.* 2016). Since then, Northeast Florida has experienced a reduction in freeze events congruent with mangrove range expansion (Fig. 1, Cavanaugh *et al.* 2013; Dix *et al.* 2021), and has been a hotbed for mangrove range expansion studies. Projects have documented historical expansion and contraction in the area (Rodriguez *et al.* 2016), modeled expansion rates (Cavanaugh *et al.* 2015), characterized local mangrove adaptations (Dangremond and Feller 2016), studied belowground process changes (Doughty *et al.* 2016; Coldren *et al.* 2019), investigated faunal shifts (Johnston and Grunner 2018; Cannizzo *et al.* 2019), and more (Dix *et al.,* 2021). Currently, the most comprehensive study of ecosystem changes due to mangrove expansion in Northeast Florida is underway by PI Samantha Chapman of Villanova University and many collaborators. 'Warming Ecosystem Temperatures in a Florida Ecotone Experiencing Transition' (WETFEET) is funded by the National Science Foundation from August 2017 - January 2023 and was the catalyst for this project. Objectives of the WETFEET project include investigating above- and belowground effects of warming and mangrove encroachment into saltmarsh systems. Specifically, the study seeks to examine how warming can affect carbon storage, biomass accumulation, and surface elevation in these dynamic systems. In the first two years of establishment, warmed plots were found to be an average of 0.5 °C (std dev 1.2 °C) warmer than the control plots ($p < 0.05$) during daylight hours. Specifically, the half-hourly difference between warmed and control plots averaged 1.60 °C (std. dev. 1.55 °C) during the day and −0.61 °C (std. dev. 0.59) at night (Chapman *et al.* 2021). The average temperature increase projected for northeast Florida over the next 50 years (model ensemble Jacksonville) is 1.6 °C, which renders our warming scenario likely over the next 20 years (Chapman *et al.* 2021, Diem *et al.*. 2017). Soil temperatures were found to be 0.466 °C (std. dev. 0.779°C) and 0.270 °C (std. dev. 1.855°C) cooler on average within chambers in spring and summer respectively but highly variable especially at 2-cm depth (Fig 13). Salinity of soil porewater was measured using a refractometer in all plots during both sampling events and not found to be significantly different among treatments in either season.

This study was conducted within the intertidal zone at the middle of the three WETFEET sites (29°50’10.30" N 40.4 57, Fig. 2).

The middle site used for this study was the intertidal zone of a tidal creek off the Matanzas River just south of St. Augustine, FL. The site was dominated by *S. alterniflora* with pockets of *B. maritima* and some well-established *A. germinans*, representative of much of the mangrove-saltmarsh ecotone in Northeast Florida. Soils at the site were 10% organic matter and were histosol/hydrosols (Chapman *et al.* 2021). Average elevation was 0.57 m NAVD88 [North American Vertical Datum of 1988]). Tidal range averaged 1.5 m at the nearby GTMNERR water quality station (NERRS 2020). Mean high water at nearby tidal gauges averaged 0.63 m (NAVD88) and mean low water averaged −0.65 m (NAVD88, Chapman *et al. 2021*). Average precipitation over approximately 20 yrs. for the area was 132 cm/yr. (NERRS 2020). Temperatures for the study period ranged from 0–40.8 °C (Fig 3). Porewater salinity at this site ranged from 30-40 ppt (Chapman *et al.* 2021).

Experimental Design

To investigate the effect of vegetation structure and environmental conditions on BMA, communities were sampled under the three dominant vegetation types within three areas of the middle site in March 2020 (spring) and August 2020 (summer). Due to weather constraints, only two of the three areas were sampled in the summer. Three 0.33 m x 0.33 m plots were haphazardly placed within monocultures of each vegetation type in each area of the creek for a total of nine plots in each vegetation type, 27 plots total (Fig. 4). Within each plot, vegetation structural properties were evaluated, abiotic environmental conditions were measured, and sediment cores were collected for chlorophyll *a* biomass (details below). Three additional sediment cores were collected within each plot to investigate other parameters not included in the scope of this thesis (Fig. 5).

To investigate the effect of warming on BMA, we made use of WETFEET open-top passive warming chambers at the middle site.Chambers were deployed in May 2018 and consisted of 4.5-m^3 PVC frames covered with 152 µm plastic film (Green-tek). The plastic film allowed 90% of PAR to penetrate but trapped infrared radiation and interrupted convective heat loss, creating an average maximum warming effect of about 1.60 °C (Chapman *et al.* 2021, Fig 3a). Four warming chambers around mangroves and four warming chambers around marsh vegetation (a mix of *S. alterniflora* and *B. maritima*) were paired with control plots without plastic for a total of 16 plots sampled (Fig 6). Sampling took place 27 months and 34 months after plot establishment in August 2020 (summer) and March 2021 (spring), respectively.

Vegetation structure and environmental conditions

Within each plot vegetation plot, soil salinity (±1 practical salinity units [psu]) of the top 0.5 cm was measured by squeezing porewater from the sediment surface through a syringe filter onto a hand-held salinity refractometer (Whitcraft and Levin 2007). Salinity for the warming experiment was measured from established pore water wells within plots but similarly filtered and measured. Temperature at 2-cm depth was measured using an MX2300 HOBO temperature sensor at the time of sample collection for both vegetation and warming plots. The effect of shading was measured by calculating the percent of incident light reduction (%ILR). A LICOR Quantum PAR sensor was used to measure ambient light above the canopy and below the canopy simultaneously. Four measurements were averaged for each plot (Whitcraft and Levin 2007). For warming experiments, %ILR was only recorded in the summer sampling due to device malfunction.

To characterize plant structure in vegetation plots, percent cover of vegetation species was visually estimated in each plot including live and recently dead vegetation. For mangrove plots, the tallest branch was measured with a tree height pole; for other vegetation types, in each plot, the five tallest stems were measured with a flexible meter stick to establish mean canopy height (stem height). Stem density for the dominant vegetation species was determined by counting all individual stems.

Plot locations were recorded with a Trimble GPS. A Sprinter digital bar code level was used to measure elevations in the middle of each plot during the spring sampling. A deep-rod surface elevation table benchmark at the WETFEET middle site was used as a geodetic control mark to relate plot elevations to NAVD88.

For warming experiment, we measured and recorded air temperatures and relative humidity at 30-minute intervals in a subset of warmed and control plots using Hobo data loggers (Onset, Bourne, Massachusetts). Specifically, we used Hobo MX2302 temperature and relative humidity loggers with RS3-B solar radiation shields anchored at 1-m height halfway between the center of the plot and side wall (Chapman *et al.* 2021*)*. Soil temperatures were measured at 2- and 10-cm depths using Hobo data loggers MX2300.

BMA biomass and community composition

BMA biomass is often estimated as chlorophyll *a* content per unit of sediment (Grinham *et al.* 2007). In all vegetation and warming plots, sediment was collected in four cores using 60-ml syringes with the tips cut off. Cores were stored in a dark cooler on ice and transported back to the lab where all subsequent procedures were conducted in a dark room. The top 0.5 cm from each of the four cores from each plot were homogenized, then subsampled for chlorophyll *a* analysis (Fig. 5). Samples were extracted in 100% acetone for 24 hours (Southwell *et al.* 2017). Chlorophyll *a* concentrations (corrected for pheophytin) were determined fluorometrically using EPA method 445 with a Turner Trilogy fluorometer and compared against standard curve.

Algal biomass and relative abundance of functional groups were determined fluorometrically *in situ* using the BBE Moldenke BenthoTorch. The BenthoTorch uses the *in vivo* fluorescence of algal cell pigments to estimate abundance of green algae, blue-green algae (cyanobacteria), and diatoms. For algae differentiation, the BenthoTorch uses seven LEDs for fluorescence excitation. The LEDs emit light at three selected wavelengths (470 nm, 525 nm, 610 nm). The device then measures fluorescence-emission around 685 nm, which is widely accepted as a measure of chlorophyll content of algae in aquatic systems (Beutler *et al.* 2002; Grinham *et al.* 2007), and calculates a correction for reflectance of sediments. Since algae of the same division contain a similar composition of photosynthetic pigments, their fluorescence excitation spectrums are significantly different (Beutler *et al.* 2000; Beutler *et al.* 2002) but retain a fixed emission wavelength at 680nm. This is because fluorescence is emitted mainly by the chlorophyll *a* of the photosystem II antenna system, which consists of the evolutionarily conserved chlorophyll *a* core antenna and species-dependent peripheral antennae. This structure makes it possible to differentiate divisions of algae by their fluorescence excitation spectrum (Aberle *et al.* 2006; Beutler *et al.* 2002). The BenthoTorch is a novel method for measuring algal biomass and functional group diversity *in situ* and relatively few studies have applied this method in muddy intertidal environments with variable sediment photic zones. BenthoTorch measurements were collected within each vegetation and warming plot.

Data Analysis

Data from vegetation and warming plots were analyzed separately using univariate analyses in SPSS V26.0. Prior to all univariate analyses, assumptions of normality and equal variances were tested, and values were transformed as necessary. For the vegetation questions, three-way Analysis of Variance (ANOVA) was used to test for differences among means of environmental, vegetation, and biomass parameters based on the factors of Season (spring/summer), Area (1, 2, 3), Vegetation Type (*B. maritima, A. germinans, S. alterniflora*), and all interaction terms. Where appropriate ($p > 0.25$, Underwood 1997), the Area factor was pooled to increase sample size and the analysis rerun as a two-way ANOVA with factors of Season, Vegetation Type, and their interaction. Significant interactions and differences were followed up with appropriate simple main effects analyses and Tukey's B post hoc with Bonferroni adjustment for multiple tests applied.

For the warming experiment, environmental parameters (% ILR, temperature, and salinity), and biomass (in situ, extracted) were investigated for differences among means using a two-way ANOVA with the factors of Season (summer, spring), Treatment (*Avicennia* warmed, *Avicennia* control, Marsh warmed, Marsh control), and their interaction. Significant interactions and differences were followed up with appropriate simple main effects analyses and Tukey's B post hoc with Bonferroni adjustment for multiple tests applied.

Multivariate analyses were performed in PRIMER (PRIMER 7.0.20 Quest Research Limited, Auckland New Zealand) to compare BMA community composition among vegetation types (Whitcraft *et al.* 2007), environmental conditions and vegetation structural properties among vegetation types, and BMA community composition among warming treatments. To compare community composition among vegetation types, a three-way crossed Analysis of Similarity (ANOSIM) was performed on the Bray Curtis similarities of square root transformed algal functional group biomass values. For environmental parameters, data were normalized, then Euclidean distances were calculated and used to run a three-way crossed ANOSIM. The three-way ANOSIM approach allows for no difference in one factor while having significant differences in other factors (Whitcraft *et al.* 2007). Significant factors were then combined and rerun as one-way ANOSIM with pairwise analysis to determine significant groups. If factor was not significance but had an R value close to one, the factor was still retained in one way ANOSIM. R values represent the degree of segregation among groups with values closer to one representing a greater degree of separation. Significance is a product of the number of permutations so factors with low replication (season) are more likely to have insignificant results even with complete segregation of groups (Clarke *et al.,* 2014). To compare relationships between vegetation structural properties and BMA community composition, a stepwise regression analysis of all six factors was run in PRIMER (BEST) to determine the best fit factors. For the warming experiment, a three-way crossed ANOSIM was run on the Bray Curtis similarities of square root transformed algal functional group biomass values and followed up with combined factor one-way ANOSIM with pairwise comparisons for significant groups.

The hypotheses proposed for this investigation are based on the expectation that the structure of foundational vegetation in tidal wetlands has influence over BMA biomass and composition. This study is not designed to elucidate the mechanisms of influence, but environmental and vegetation characteristics measured simultaneously with BMA can help explain some of the variability observed in BMA biomass. To assess these patterns more fully, relationships among all variables were explored using non-parametric Spearman's rank correlation analysis within individual seasons and combined.

Results

Environmental conditions and vegetation structure

All investigated parameters (temperature, percent incident light radiation (%ILR), salinity, percent cover, stem height, and stem density) varied among vegetation types and seasons. Soil temperatures were lower on average under *A. germinans* than either *S. alterniflora* or *B. maritima* in spring ($F_{2,66} = 13.140$, $p < .000$, Fig. 7a), but temperatures were not significantly different among vegetation types in summer ($F_{2,66} = 2.631$, $p = .08$, Fig. 7b). Soil temperatures were consistently higher in the summer than in the spring ($p < .001$, Fig. 7). Mean light transmission (%ILR) was not significantly different among seasons but within sampling areas, %ILR was different among vegetation types ($F_{2,59} = 43.695$, $p < .000$; $F_{2,59} = 13.307$, $p < .000$). In both seasons, %ILR was lower under *A. germinans* than under *S. alterniflora* and *B. maritima* (Fig. 7c, d).

All other parameters discussed here had insignificant Area effects ($p > .25$) so the factor was pooled. Salinities were found to be higher on average in spring than in summer, and they showed significant differences among vegetation types in summer ($F_{2,25} = 8.629$, $p = .001$); soils under *B. maritima* had the lowest salinities, soils under *A. germinans* had the highest, and soils under *S. alterniflora* had intermediate salinities ($p < .025$, Fig. 7e, f).

Vegetation cover was significantly higher overall in spring compared to summer ($F_{1,30} = 6.65$, $p = 0.015$), and values were significantly different among vegetation types ($F_{1,30} = 22.78$, $p < .000$) in both seasons. *A. germinans* had the highest percent cover, followed by *B. maritima*, then *S. alterniflora* ($p < .025$, Fig. 8a, b). Mean stem heights and stem densities were both significantly different among vegetation types ($F_{2,30} = 316.284$, $p < .000$; $F_{2,30} = 30.671$ $p < .000$, respectively). *A. germinans* stem heights were highest on average, followed by much lower heights of *S. alterniflora* and shortest heights of *B. maritima* in both spring and summer ($p < .025$, Fig 8c, d). Stem densities were highest on average in *A. germinans* with *S. alterniflora* and *B. maritima* having similarly lower densities ($p < .025$, Fig. 8e, f).

Overall environmental conditions were significantly different among vegetation types (ANOSIM, $R = 0.745$, $p = .001$, S. Fig. 1c). Differences in overall environmental conditions among areas and seasons were both found to be non-significant. However, $R = 1.00$ for Season, which suggests complete segregation of groups, so the low significance ($p = 0.10$) was likely due to low permutation replication rather than true biological irrelevance. Therefore, the factors of

Season and Vegetation Type were combined and reinvestigated with a one-way ANOSIM. Analysis returned significant effects of season and vegetation type on overall environmental conditions ($R=0.745$, $p = .001$). Follow-up pairwise comparisons and bootstrap means plots showed high amounts of segregation between groups ($R>.748$, $p = .001$, S. Table 1, Fig. 9). However, environmental conditions under *B. maritima* and *A. germinans* appear to have been similar overall (S. Table 4, Fig. 9).

BMA biomass and community composition

BMA biomass measured as chlorophyll *a* (Chla) in mg/m^2 both *in situ* and extracted showed similar patterns and responses to both season and vegetation. Both biomass measures showed no significant effect of sampling area, so the data were pooled for all areas. Extracted Chla was significantly higher in spring compared to summer under all three vegetation types (*B. maritima* $F_{1,28} = 27.833$, $p < .000$; *A. germinans* $F_{1,28} = 10.597$, $p = .003$; *S. alterniflora* $F_{1,28} = 53.282$, $p < .000$). Extracted Chla was significantly different among vegetation types in both spring and summer ($F_{2,28} = 16.487$, $p < .000$; $F_{2,28} = 3.356$, $p = .049$ respectively), but differences were dependent on season (S x V interaction $F_{2,28} = 4.111$, $p = 0.027$). On average, Chla was lowest under *Avecennia* in both spring and summer ($p < .025$ Fig 10a, b).

Total *in situ* Chla was significantly different among vegetation types in spring but not in summer ($F_{2,66} = 26.552$, $p < .000$; $F_{2,66} = 3.061$, $p = .054$) and was higher in spring compared to summer (*B. maritima* $F_{2,66} = 28.197$, $p < .000$; *A. germinans* $F_{2,66} = 120.152$, $p < .000$; *S. alterniflora* $F_{2,66} = 130.96$, $p < .000$). In spring, *in situ* Chla was lowest under *A. germinans*with *B. maritima* having intermediate values that were similar to both *A. germinans* and *S.alterniflora* ($F_{2,66} = 26.552$ $p < .000$, Figure 10c).

Algal functional group assemblages were found to be diatom dominated in all three vegetation types in both seasons with cyanobacteria as the second most abundant group and green algae present in low amounts (Fig. 11). Algal assemblages were significantly different among vegetation types (ANOSIM R = .436, p = .001, S. Fig 2c). Season was not found to have a significant effect on algal assemblage (p = .10) however the R statistic of R=1 suggested that season did have a relevant effect, so the factor of season was retained for further analysis. In the follow up one-way ANOSIM both season and Vegetation were found to significantly effect algal functional group assemblage (R = 0.54, p = .001). Pairwise comparisons and a metric MDS plot of bootstrapped means showed significant differences in algal assemblages except for beneath *Avicennia* and *Spartina* canopies in the summer (Fig. 12b). *Batis* also appeared to maintain a similar algal assemblage in both spring and summer (R = .181, Fig 11b). Season also was shown to significantly influence algal assemblages especially under *Avicennia* and *Spartina* canopies (S. Table 2).

Relationships between BMA and environmental and vegetation parameters

When data from both seasons were combined, temperature was negatively correlated with BMA biomass measured as both *in situ* and extracted Chla (Table 1). This overall finding was different from the observed significant positive relationship between temperature and extracted Chla within each season (Table 2, 3). *In situ* Chla was not correlated with temperature within seasons (Table 2,3).

Overall, light transmission (%ILR) was positively correlated with both *in situ* and extracted Chla (Table 1). This trend was consistent within individual seasons and when seasons were combined, although %ILR explained much more of the variance within season than overall

(Table 2, 3). Salinity showed no significant relationship with algal biomass overall or within seasons (Table 1, 2, 3). Percent cover did not show a significant relationship with algal biomass overall or within summer but did show significant negative relationships with both *in situ* and extracted Chla in spring (Table 2). Stem height was negatively correlated with both *in situ* and extracted Chla overall and had similar trends within each season (Tables 1, 2, 3). Stem density was negatively correlated with extracted Chla overall and within both seasons (Tables 1, 2, 3), but was only significantly negatively correlated with *in situ* Chla in summer (Table 3).

Due to novelty of the BenthoTorch deviceit was important to investigate correlations between extracted Chla measured with the acidification method (commonly applied method), *in situ* Chla, and the algal functional group outputs that the device produces. Total *in situ* Chla, which was calculated as the sum of all three algal functional group biomass values, was strongly positively correlated to extracted Chla (R=.744, p<.01). *In situ* cyanobacteria Chla had the strongest positive correlation with extracted Chla (R=.807, p<.01), but diatoms also showed a significant positive correlation with extracted Chla (R=.616, p<,01). Green algae Chla values were not found to be correlated with extracted Chla values, but this was expected due to the overall low abundance and high variability of Green algae in the study system. Although the BenthoTorch had a strong correlation with extracted Chla values, the values were much lower from the BenthoTorch (Fig. 10, note different y-axis scales).

Overall vegetation structural properties were significantly related to algal functional group assemblages ($\bar{\bar{\bar{R}}}$=0.326, p=.01). Stepwise regression was run within seasons due to the large effect of season on parameters. Within season, the vegetation structure properties that best related to BMA assemblages were percent cover, stem height, and stem density (R=0.326).

Warming Response

For the warming portion of the project environmental correlations were not the main objective so environmental parameters of temperature, %ILR, salinity were the only values recorded within treatment plots. %ILR was only collected in summer due to issues with the device during the second sampling. Environmental parameters in warming experiment plots were not significantly different among treatments and no interactions were observed (Fig 14, Fig 15 Table 4). Soil temperature was significantly higher on average in the summer ($F_{1,56}$=497.606, P<.000).

In situ Chla differed among treatments but differences were dependent on season (significant S x T interaction $F_{3,56}$=3.7 P=0.017) with significant warming effects only observed in spring ($F_{3,56}$=9.662 P<.000; Fig. 16a, b). Extracted Chla was similar among all treatments and seasons ($F_{3,24}$=1.75 P=.184); however, patterns in spring followed patterns of *in situ* Chla and power for the experiment was low due to low replication and high variability (power=.189, Fig 16b, c).

Algal community assemblages were significantly related to warming treatment (R=0.396, p=.001, S. Fig 3c). Metric MDS plot of bootstrapped averages showed that community assemblages in the spring warming treatments (marsh and *Avicennia)* appeared segregated from other groups (Fig. 17), although multivariate analysis returned only significant segregation for Spring Marsh warming assemblage. Importantly, it was observed that the *Avicennia* warmed treatment showed a similar shift in cyanobacterial relative abundance to the Marsh warmed treatment. In spring warmed treatments (marsh and *Avicennia)*, the relative abundance of cyanobacteria was double the abundance in controls (Fig 18d). Although the assemblage for *Avicennia* warming treatment was not statistically segregated (S. Table 3), a doubling of relative abundance in one algal functional group is a biologically important note.

Discussion

We found support for our first hypothesis that biomass and community composition of BMA are different under different vegetation types. Our comprehensive investigation of plant structure and environmental conditions also indicated that plant structural properties and differences in abiotic conditions underneath plant communities influence BMA biomass and functional group assemblages. These findings are similar to other studies that indicate that BMA are highly influenced by temperature (de Jonge *et al.* 2012; Kwon *et al.* 2018), light intensity (Whitney and Darley, 1983; Pinckney & Zingmark 1993), tide exposure (Pinckney and Zingmark, 1991; Brotas *et al.* 2003), and vegetation (Pinckney and Zingmark, 1993a; Kon *et al.* 2010, Whitcraft *et al.* 2007). Although supported by much of the existing literature, these findings are important and novel because they are specific to the three dominate coastal wetland species in the Northeast Florida ecotone and have implications for understanding ecosystem impacts of a warming climate. Also, these are among the first reported estimates of BMA biomass in Northeast Florida.

Northeast Florida has experienced a loss of at least 1,190 ha of saltmarsh due to the encroachment of mangroves from 1990 to 2014 (Dix *et al.* 2021). Using our biomass estimates and the conservative conversion of 40 g Chla/g C (Lorenzen 1968), this vegetation loss potentially equates to a loss of 11,285 g C/yr due to decreases in algal biomass under mangrove canopies. With more saltmarsh losses expected as mangrove encroachment continues, it will be increasingly important to quantify what the resulting loss in primary production means for the system and how warming-induced cyanobacteria abundance can impact that dynamic.

According to a comprehensive review of published BMA data by Kwon *et al.* (2020), there are very few studies that have investigated BMA biomass patterns extensively under mangrove canopies. In fact, the only referenced mangrove data in that study came from their own investigations that occurred in the Eastern hemisphere including data from Cambodia and Australia (Kwon *et al.* 2020). Their study reports differing results to the data presented here suggesting that there is little difference in overall biomass between mangrove and saltmarsh habitats. However, the reported annual averages for mangroves (90.8 mg Chla m^{-2}) in Kwon *et al.* (2020) were much higher than those observed in this study (51.3 ± 22.14 extracted mg Chla m^{-2}). BMA biomass is extremely variable at both local and global scales so establishing local baselines, especially in changing systems like the ecotone of Northeast Florida is important.

Global annual average biomass values for saltmarshes reported by Kwon *et al.* (2020) were 86.5 mg Chla m^{-2}, which were similar to those reported from studies in Southeast U.S. *Spartina* marshes (90 mg Chla m^{-2}; Sullivan & Moncrieff 1988; Pinckney and Zingmark 1993a, 1993b) and the extracted values from this study (89.2 ± 43.3 mg Chla m^{-2}).

The differences between mangrove and marsh biomass observed in this study were consistent with a recent study by Walker *et al.* (2019), which presented the first set of known average and annual variation values for BMA biomass in Northeast Florida. Walker *et al.* (2019) reported an average annual standing stock of 25 mg Chla m^{-2} in saltmarsh plots and approximately 13 mg Chla m^{-2} in mangrove plots, lower than other Southeast U.S. marsh systems (Pinckney and Zingmark 1993a, 1993b, Sullivan & Moncrieff 1988) and those seen in this study. The low values observed by Walker *et al.* (2019) are likely due to the *in situ* measurement of Chla using the BBE BenthoTorch which was found in this study to produce lower overall Chla values than traditional extracted fluorescence methodology. The methodology of Walker *et al.* (2020) also involved sampling along creek edges whereas our samples were taken from within the marsh platform. Despite these differences in methodology, we maintain that our observations of relatively lower BMA biomass under mangroves are consistent with the patterns observed by Walker *et al.* (2019) and biologically relevant.

The overall mechanisms behind algal biomass differences are complex and beyond the scope of this study, however, we did observe certain trends relating plant structure, light, and temperature to algal biomass. Specifically, low light conditions within mangrove canopies were correlated with lower biomass values within season. Our findings that plant structural properties alter BMA assemblages provide support for other work showing that vegetation can influence BMA (Pinckney and Zingmark, 1993a; Kon *et al.* 2010, Whitcraft *et al.* 2007). Light is especially important for these photosynthesizing organisms and can impact productivity. Although productivity was not directly investigated in this study it is noted that available light levels under mangrove canopies were well below the published values of the minimum light intensity at which BMA photosynthesis saturates, usually between 300 to 500 μmol photons m^{-2} s^{-1} (Pinckney & Zingmark 1991, 1993). By applying our %ILR values to daily PAR values from a nearby weather station (GTMPCMET, NERRS 2020), it was observed that in both spring and summer values of ILR did not reach saturation levels underneath *Avicennia* canopies. Beneath *Batis*, saturation levels were not reached in spring and beneath *Spartina* saturation levels were consistently met in both seasons.

The observed segregation of algal functional group assemblage also supports our hypothesis that not only are overall biomass values different under different vegetation types, but the community assemblage is different as well. This assemblage level shift holds important implications for the health of the system (Fleeger *et al.* 2017) and warrants further analysis using more comprehensive techniques such as morphology or DNA assessment. Relative biomass abundance values for the different algal groups suggest that the system investigated is dominated by diatoms overall, but levels of cyanobacteria were higher in both marsh plants (Fig 8c, d). This assemblage data was also consistent with that found in Walker *et al. (*2020) which showed slightly higher cyanobacteria Chla values in saltmarsh plots compared to mangrove plots although that study did not find significantly different results. This bodes well for future intertidal wetlands in a mangrove dominated scenario and their potential resilience against harmful algal blooms. The primary drivers of observed trends in multiple correlation analysis were the three vegetation characteristics that were investigated (% cover, stem height, stem density); suggesting that plant structural properties rather than resulting microclimate changes investigated within the scope of this study are more influential on BMA community. It is noted that community composition and biomass of BMA also respond strongly to differences in nutrients (Stutes *et al.* 2006, Davis *et al.* 2009, Pascal *et al.* 2013, Mueller *et al.* 2016). However, nutrient differences associated with different vegetation types were outside the scope of this study and are not well described in current literature.

There is some evidence to support our hypothesis that air temperature warming would increase overall algal biomass and shift community dominance towards more heat tolerant species like cyanobacteria. Although significant treatment effects were only observed in the spring sampling, the spring results showed a significant difference in total *in situ* chlorophyll with averages higher in warming treatments when compared to controls. Although not statistically significant, likely due to low replication, extracted Chla values also showed a similar trend (Fig 13d). These data are consistent with studies that showed, when coupled with increased irradiance, higher temperatures can increase Chla (Laviale *et al.* 2015; Werner & Matthiessen, 2017). This increase in Chla biomass, due to warming, may offset some of the reductions in productivity that we expect as a result of mangrove encroachment.

Control plots showed constant Chla values in situ over the course of the study. This is likely due to the vegetation composition of the plots. In the vegetation portion of this project it was observed that *Batis and Avicennia* BMA communities displayed low seasonal variance when compared to *Spartina.* In the warming experiment the established marsh treatment plots were characterized as mixed marsh but were often dominated by *Batis* with *Spartina* present in lower amounts. Although the lower threshold of Chla values was not affected by season due to the nature of the communities under their respective vegetation types, we suggest that the stronger upper threshold response of BMA to warming treatment observed in spring sampling is connected to peak algal biomass typically occurring in late winter or early spring (Sullivan and Currin 2002). In the summer, plant canopies (especially marsh canopies) were observed to be denser and have higher percent cover in all treatments. This summer canopy induced light limitation, although apparently not significant enough to reduce Chla values seasonally, could have prevented BMA from taking advantage of any warming benefits in the summer. Studies have shown that warming response is strongest only when algae also have access to sufficient light (Laviale *et al.* 2015; Werner & Matthiessen, 2017). Additionally, algal growth rates do not display a linear relationship with temperature. Instead they show a relationship where after surpassing a maximum threshold growth rates will drop off and decline (Paul *et al.* 2011), due to this pattern some studies have even shown a negative relationship between temperature and overall biomass and species richness (Whitcraft *et al.* 2007; Sin *et al.*. 2009). Although it was not directly measured in this study, it is possible that the high temperatures within the marsh in the summer could have had the overall BMA community already operating past its upper threshold and thus further warming effects were not observed. Also, previous literature supports that low levels of BMA biomass is often observed in summer which could lead to dilution of any observable patterns (Sullivan and Currin 2002; Walker *et al.* 2020). Although there was an element if seasonality to the warming treatment effect, we maintain that the observed increase in Chla in spring confirms our hypothesis.

In addition to overall Chla trends there was an especially strong response of cyanobacteria to warming treatments in the spring. The response of cyanobacteria without a coupled decrease in other algal groups suggests that there is not necessarily a competitively exclusive relationship between BMA groups. This is consistent with other studies in Gulf coast marshes which found that competition between diatoms, filamentous cyanobacteria and green algae appear to be unimportant (Sullivan and Currin 2002). This lack of competition suggests increases in cyanobacterial dominance are unlikely to affect overall levels of readily consumable algal biomass such as that contributed by diatoms. These findings support our hypothesis of increased cyanobacterial dominance due to cyanobacteria in general tending to thrive in high heat, high irradiance, and high salinity environments due to their increased tolerance to extreme conditions compared to diatoms (Sullivan and Currin 2002, Paul *et al.* 2011). **T**his increase in cyanobacterial dominance under warming conditions suggests that intertidal systems may be at higher risk of cyanobacteria BHABs under projected climate conditions.

Alternative explanations of increased biomass in warming treatments could suggest that observed treatment effects could be due to shading of soils from chambers rather than air temperature warming within them. This explanation holds some weight because BMA have been shown to expand their chloroplasts to receive more light as a physiological photo-acclimation response which leads to increased sediment Chla values (Fisher *et al.* 1998). However, we argue that experimental artifacts are not the most likely driver of observed biomass increases because shading experiments have shown either a decrease or no effect of shading alone on Chla values and have also provided little evidence for shading alone being responsible for community shifts (Sullivan and Currin 2002). Also, *in situ* measurement of PAR within the chambers show no significant differences between control and warmed plots. Finally, shading effects and the trend of ILR in the vegetation portion of this study suggest that there is a positive relationship between light and Chla which would not support the hypothesis of increased Chla and cyanobacteria in chambers due to shading effects.

Another alternative hypothesis for the observed increase in biomass and shift toward cyanobacterial dominance could be that the slight cooling effect of chambers could be driving observed community trends. We maintain that air temperature warming rather than the slight soil temperature cooling effect within chambers is the primary driver of observed patterns. We maintain this conclusion for two reasons. First the scale of soil temperature cooling (0.88-1.91 °C average max) is much lower than that of air temperature warming (3.2 °C average max). And also, the existing body of literature and our own within season trends suggest that increases in temperature rather than decreases are associated with higher Chla values (Laviale *et al.* 2015; Werner & Matthiessen, 2017). Additionally, BMA occupy the surface sediments which are highly influenced by air temperatures and are extremely variable.

“Climate change” has been coined by many and has become a ubiquitous phrase that takes a broad range of global changes and boils them down into one term. However, climate change is not just one thing it is a broad sweep of changes that are expected to occur, and indeed are already occurring, as a result of increases in anthropogenically introduced atmospheric carbon. These changes don’t happen in isolation and often can have interactive effects that can compound implications and create feedback loops. The encroachment of mangroves into saltmarshes is one such situation that has a multifaceted slant. There is a growing body of research describing potential impacts of mangrove encroachment, some positive and some not so positive (Kelleway *et al.* 2017). The research presented here adds to this body of knowledge by describing a potential decrease of available biomass to upper trophic levels in the form of reduction in BMA biomass. This could have serious implications for the overall productivity in these systems and deserves further study. A potential factor that may offset these observed decreases in productivity is predicted climate warming which we suggest is likely to increase overall algal biomass. Although warming may not be sufficient to completely offset vegetation caused decreases in biomass, the silver lining of this scenario is that as mangroves continue to become more dominant these mangrove communities may become less vulnerable to the shifts towards cyanobacterial dominance observed because of warming. As the pattern of mangrove encroachment continues to unfold it will be important to examine the succession effects of this process and establish baselines further ahead of the encroachment front to document changes as they occur. Taking an integrative holistic approach to this change is the only way we can begin to understand the broad range of global implications associated with these extremely dynamic coastal systems.

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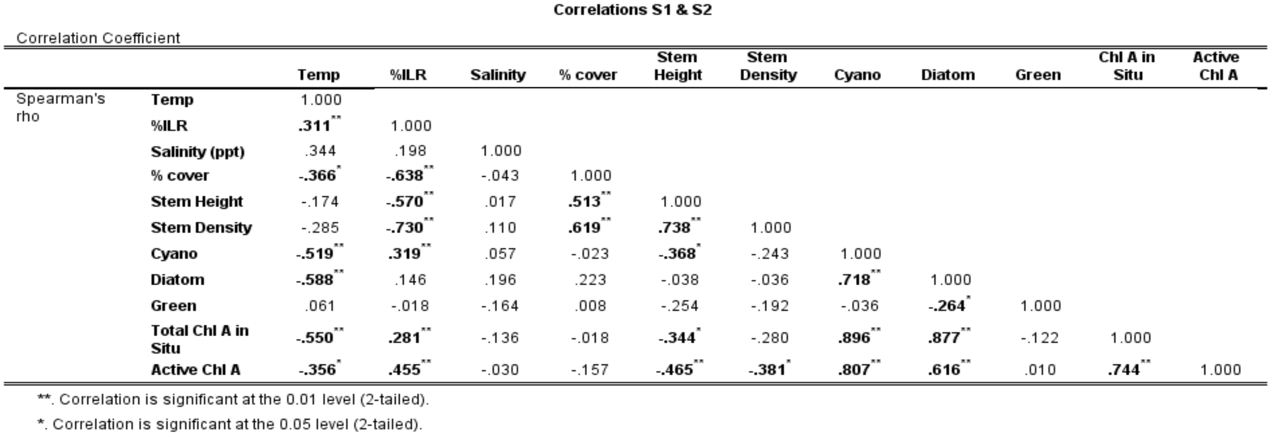
Tables and Figures



Correlations S1 & S2

Correlation Coefficient

		Temp	%ILR	Salinity	% cover	Stem Height	Stem Density	Cyano	Diatom	Green	Chl A in Situ	Active Chl A
Spearman's rho	**Temp**	1.000										
	%ILR	**.311****	1.000									
	Salinity (ppt)	.344	.198	1.000								
	% cover	**-.366***	**-.638****	-.043	1.000							
	Stem Height	-.174	**-.570****	.017	**.513****	1.000						
	Stem Density	-.285	**-.730****	.110	**.619****	**.738****	1.000					
	Cyano	**-.519****	**.319****	.057	-.023	**-.368***	-.243	1.000				
	Diatom	**-.588****	.146	.196	.223	-.038	-.036	**.718****	1.000			
	Green	.061	-.018	-.164	.008	-.254	-.192	-.036	**-.264***	1.000		
	Total Chl A in Situ	**-.550****	**.281****	-.136	-.018	**-.344***	-.280	**.896****	**.877****	-.122	1.000	
	Active Chl A	**-.356***	**.455****	-.030	-.157	**-.465****	**-.381***	**.807****	**.616****	.010	**.744****	1.000

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

Table 1 Spearmans rank correlation table for all pairwise combinations of biomass variables (Cyano, Diatom, Green, Chla in situ, Active Chlas), vegitation variables (percent cover, stem height, stem density), and abiotic condition variables (temperature, percent incident light radiation, salinity) combined between spring and summer samplings. R values presented for each pairwise combination and R-values with '*', and '**' are significant at P<0.05, and P<0.01 respectively

.

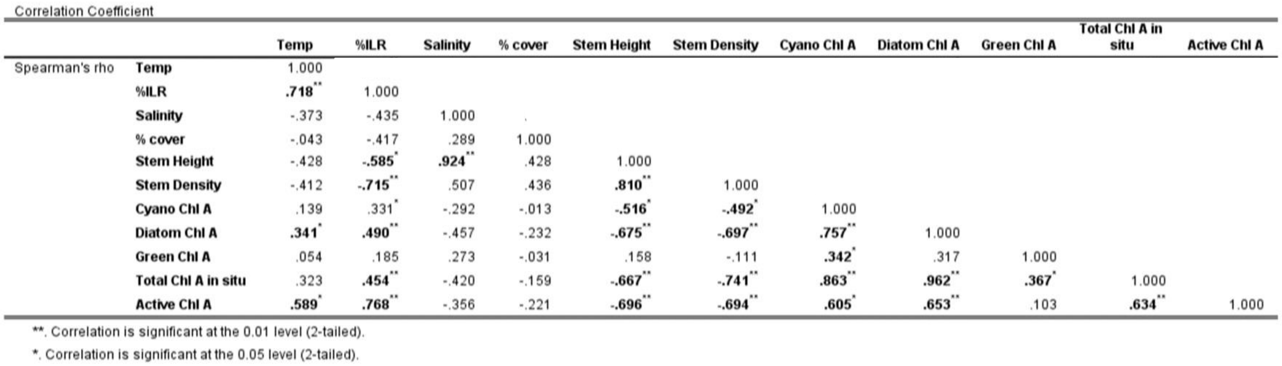
Correlation Coefficient

		Temp	%ILR	Salinity	% cover	Stem Height	Stem Density	Cyano Chl A	Diatom Chl A	Green Chl A	Total Chl A in situ	Active Chl A
Spearman's rho	**Temp**	1.000										
	%ILR	.632**	1.000									
	Salinity	.500**	.318	1.000								
	% cover	-.424*	-.786**	-.141	1.000							
	Stem Height	-.385*	-.488**	-.097	.586**	1.000						
	Stem Density	-.474*	-.691**	.007	.676**	.669**	1.000					
	Cyano Chl A	.177	.532**	-.018	-.574**	-.430*	-.405*	1.000				
	Diatom Chl A	.120	.116	.124	.125	.371	.181	.149	1.000			
	Green Chl A	-.042	-.193	-.240	-.030	-.511**	-.256	-.031	-.562**	1.000		
	Total Chl A in Situ	.166	.385**	-.073	-.440*	-.245	-.296	.756**	.628**	-.218	1.000	
	Active Chl A	.444*	.720**	-.117	-.641**	-.558**	-.582**	.646**	.061	-.014	.502**	1.000

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

Table 2 Spearmans rank correlation table for all pairwise combinations of biomass variables (Cyano, Diatom, Green, Chla in situ, Active Chlas), vegitation variables (percent cover, stem height, stem density), and abiotic condition variables (temperature, percent incident light radiation, salinity) for Spring sampling. R-values with ‘*’, and ‘**’ are significant at P<0.05, and P<0.01 respectively.



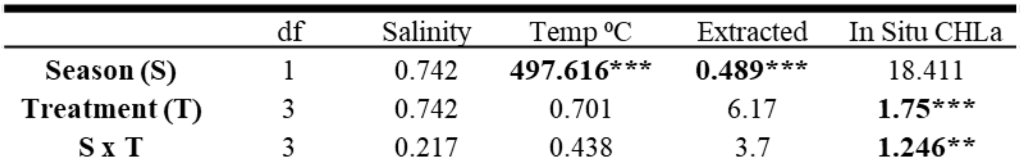
Correlation Coefficient

		Temp	%ILR	Salinity	% cover	Stem Height	Stem Density	Cyano Chl A	Diatom Chl A	Green Chl A	Total Chl A in situ	Active Chl A
Spearman's rho	**Temp**	1.000										
	%ILR	**.718****	1.000									
	Salinity	-.373	-.435	1.000								
	% cover	-.043	-.417	.289	1.000							
	Stem Height	-.428	**-.585***	**.924****	.428	1.000						
	Stem Density	-.412	**-.715****	.507	.436	**.810****	1.000					
	Cyano Chl A	.139	.331*	-.292	-.013	**-.516***	**-.492***	1.000				
	Diatom Chl A	**.341***	**.490****	-.457	-.232	**-.675****	**-.697****	**.757****	1.000			
	Green Chl A	.054	.185	.273	-.031	.158	-.111	**.342***	.317	1.000		
	Total Chl A in situ	.323	**.454****	-.420	-.159	**-.667****	**-.741****	**.863****	**.962****	**.367***	1.000	
	Active Chl A	**.589***	**.768****	-.356	-.221	**-.696****	**-.694****	**.605***	**.653****	.103	**.634****	1.000

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

Table 3 Spearmans rank correlation table for all pairwise combinations of biomass variables (Cyano, Diatom, Green, Chla in situ, Active Chlas), vegitation variables (percent cover, stem height, stem density), and abiotic condition variables (temperature, percent incident light radiation, salinity) for Summer sampling. R values presented for each pairwise combination and R-values with ‘*’, and ‘**’ are significant at P<0.05, and P<0.01 respectively.



	df	Salinity	Temp ºC	Extracted	In Situ CHLa
Season (S)	1	0.742	**497.616*****	**0.489*****	18.411
Treatment (T)	3	0.742	0.701	6.17	**1.75*****
S x T	3	0.217	0.438	3.7	**1.246****

Table 4 Results of two-way analysis of variance testing the main and interactive effects of Season (S) and Treatment (T) on *In Situ* Chla, Extracted Chla, temperature, and salinity. Degrees pf freedom (df) and F-values are presented for each factor and response variable. F-values with '*', '**' and '***' are significant at $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively.

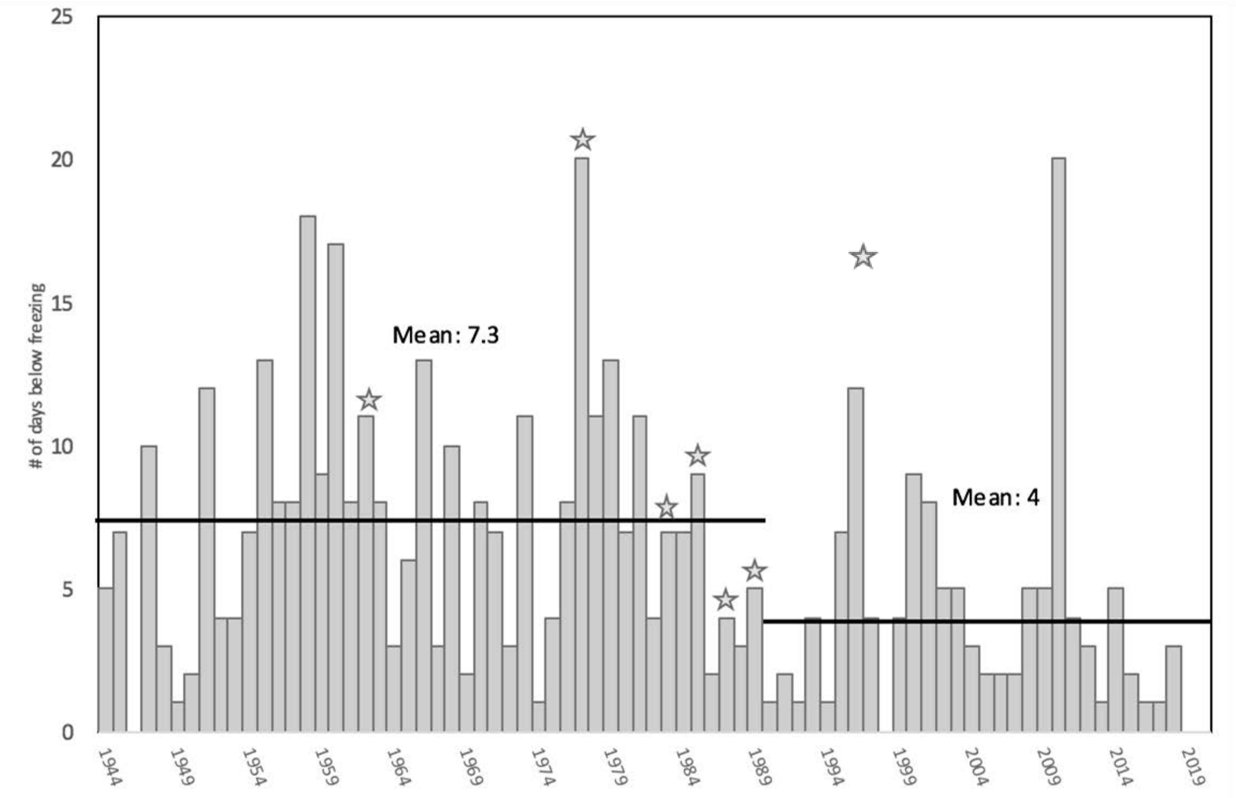


Figure 1 Number of days per year with a mean temperature below freezing (0 ºC) in St. Augustine, Florida (National Climatic Data Center, 2019). Horizontal black lines represent mean number of days before and after 1989, which marks the most recent catastrophic frost-induced *A. germinans* dieback in Florida. Stars represent recorded die-off events of *A. germinans* by Stevens *et al.* (2006). Graph adapted from, Henry and Twilley (2011).

Figure 2 Map indicating study site location. Map on the left indicates all three WETFEET project sites with pins. Sites are located within the bounds of the GTMNERR in Northeast Florida. Map on the right shows the middle WETFEET project site which is a small creek with patchy distribution of mature A. germinans, S. alterniflora, and B. maritima. Location of chambers within the creek is denoted by pin.

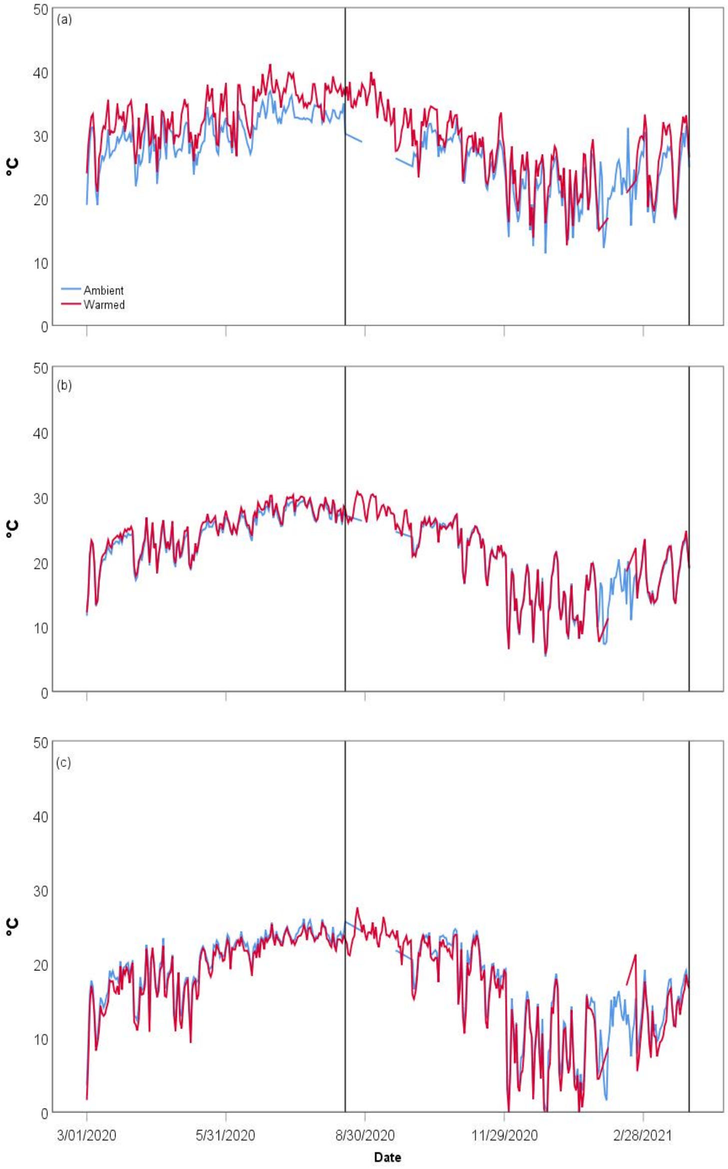


Figure 3 Warming experiment temperature graphs showing maximum daily air temperatures (a), mean daily air temperatures (b) and minimum daily air temperatures (c) in warmed and control plots over time. Black vertical lines signify sampling dates.

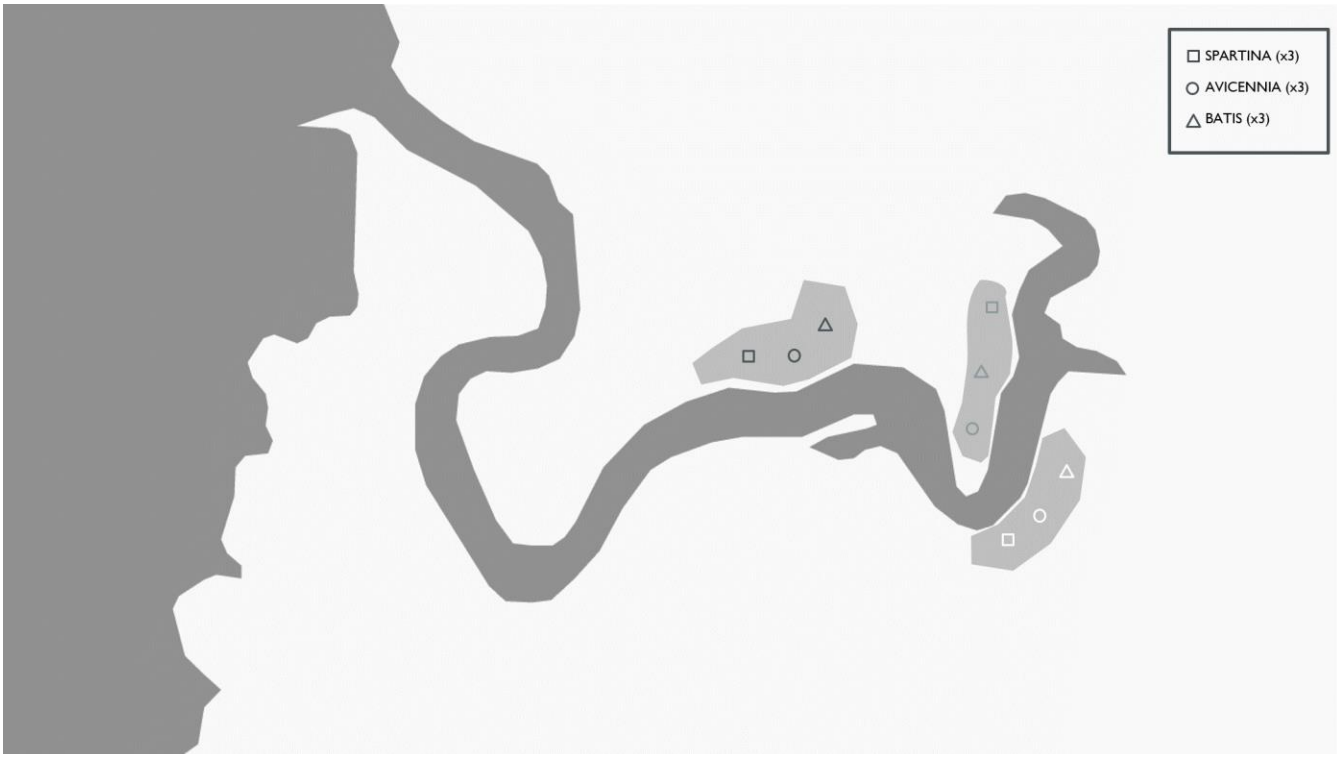


Figure 4 Schematic of vegetation sampling design. Note: only areas 1 and 2 (white and grey shapes) were sampled in summer.

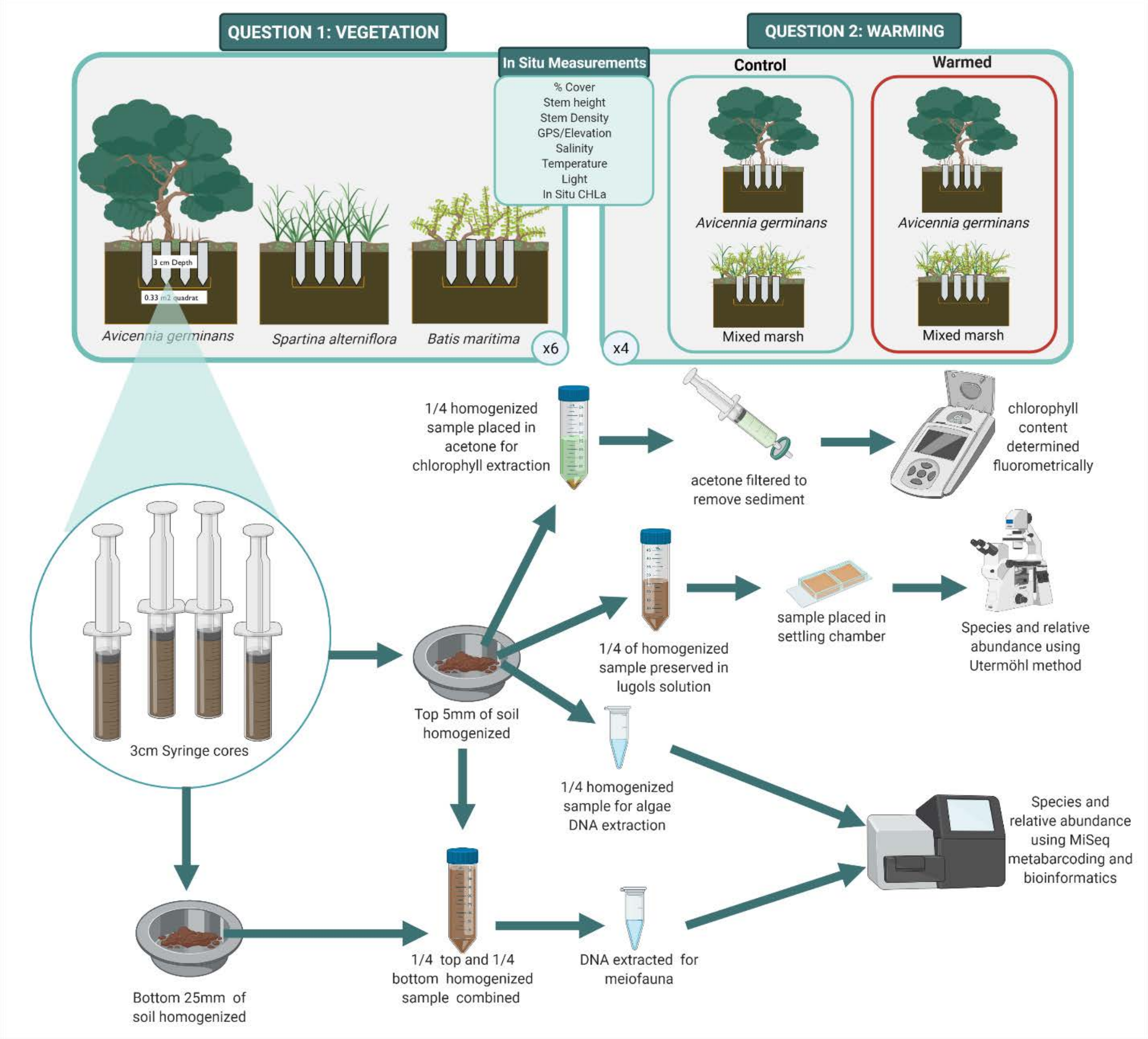


Figure 5 Schematic showing experimental design for vegetation and warming experiments. Note: only *in situ* parameters and extracted chlorophyll are discussed as part of the scope of this thesis.

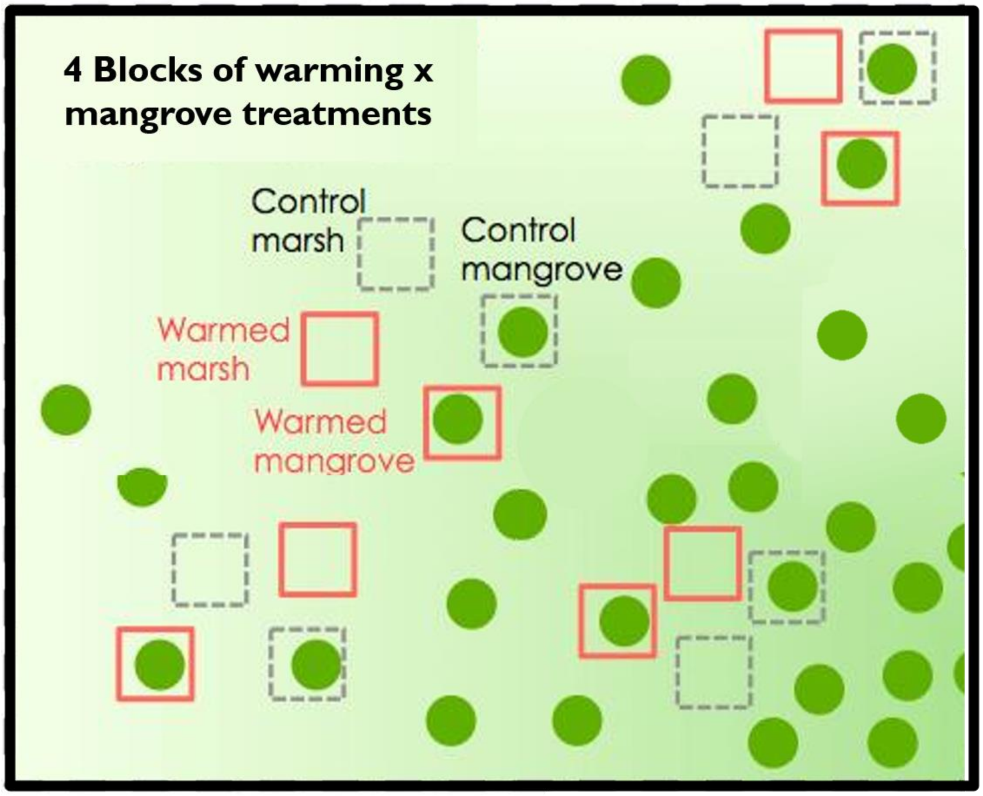


Figure 6 Schematic of warming sampling design

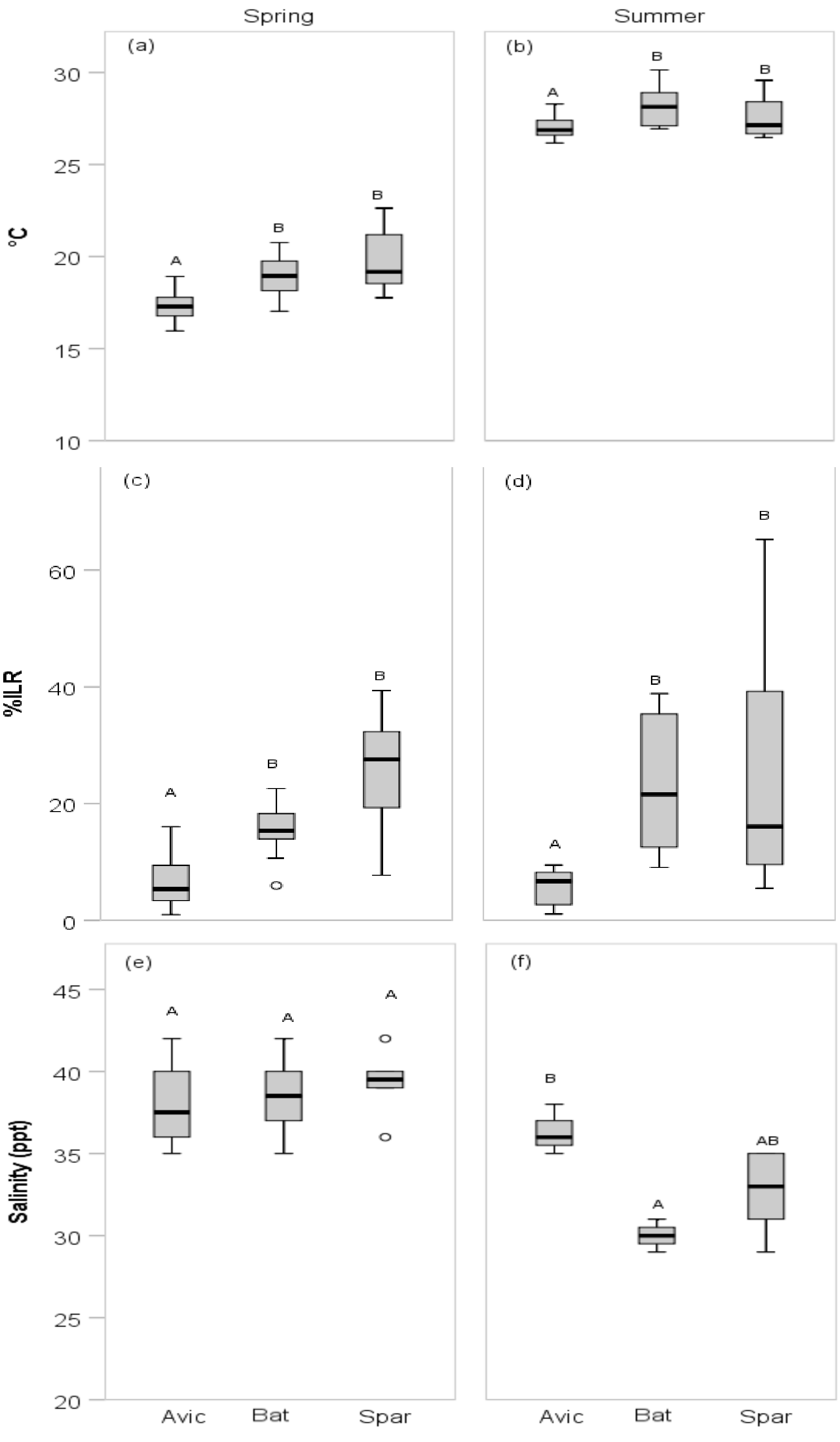


Figure 7 Temperature (a,b), percent incident light radiation (c,d), and salinity (e, f). under three vegetation types *Avicennia (*Avic), *Batis (*Bat), *Spartina (*Spar) in each season (spring left, summer right). Significant groups determined by two-way ANOVA with follow up Tukey's-B post-hoc denoted by capital letters. Dark lines in box and whisker plots represent the median values.

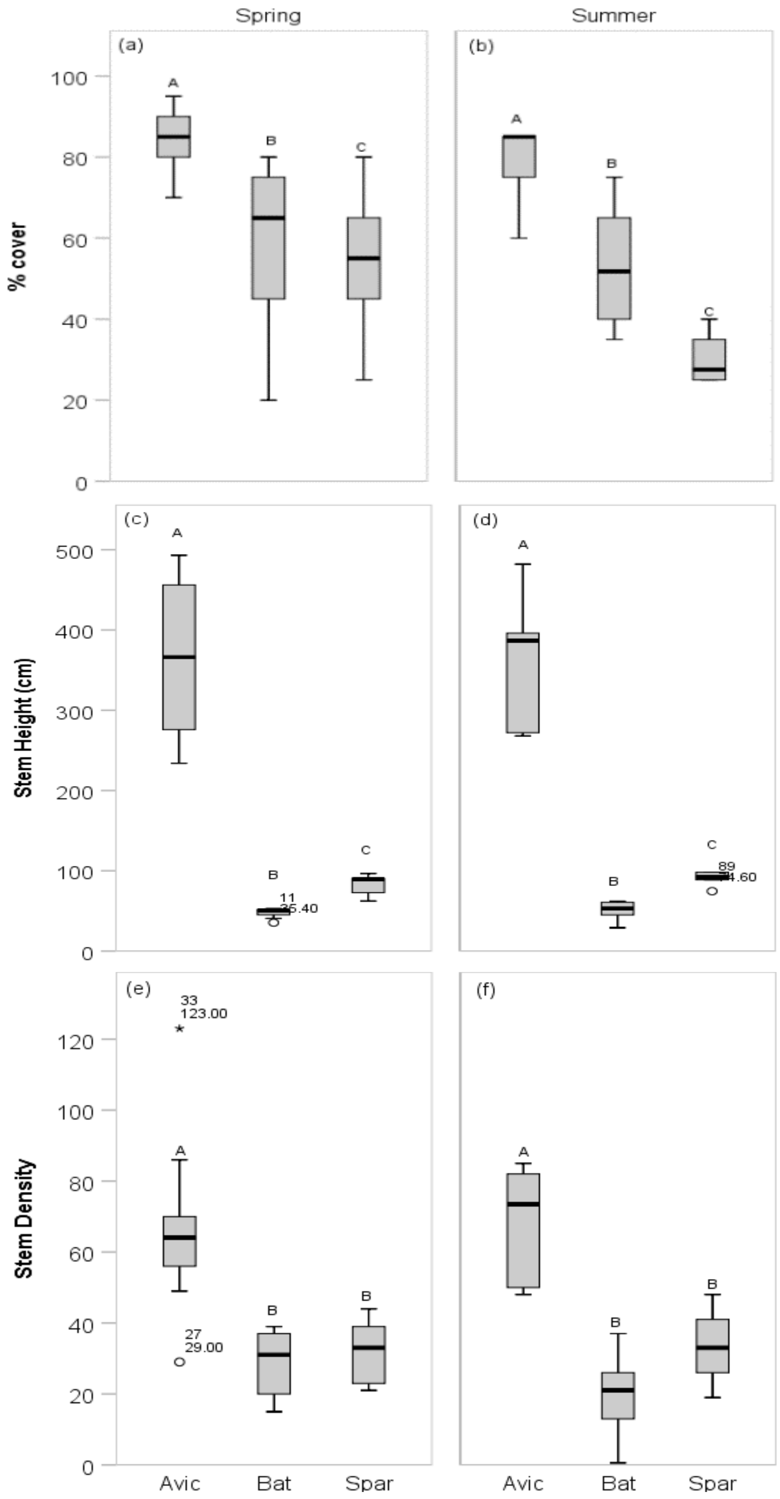


Figure 8 Percent cover (a,b), stem height (c,d), and stem density (e, f) under three vegetation types *Avicennia (*Avic), *Batis (*Bat), *Spartina (*Spar) in each season (spring left, summer right). Significant groups determined by 2 way ANOVA with follow up tukey's-B post hoc denoted by capital letters. Dark lines in box and whisker plots represent the median values.

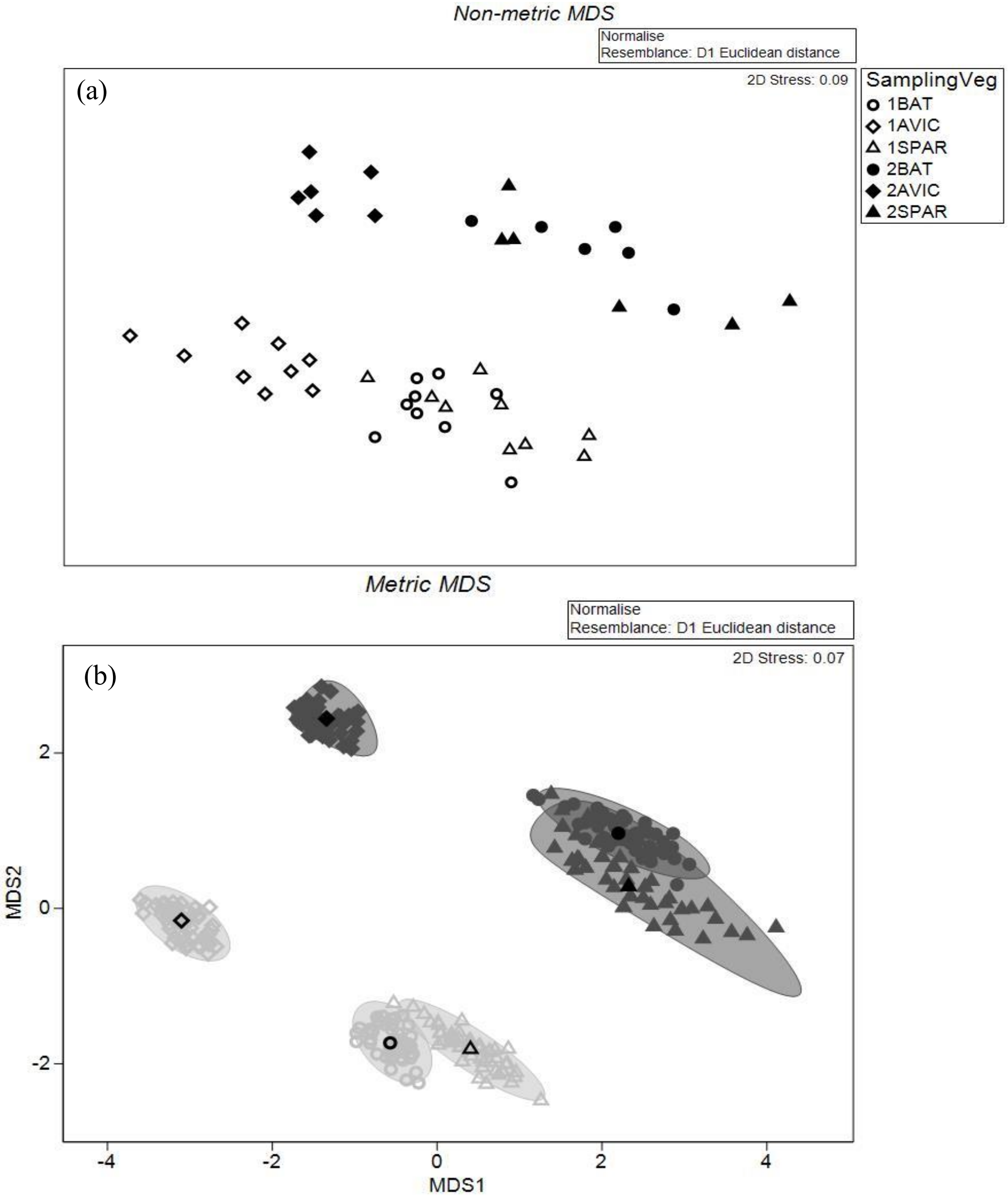


Figure 9 (a) Non-metric MDS plot showing all distances between environmental and vegetation parameters from areas dominated by *Avicennia, Batis, and Spartina.* Spring samples are denoted by open shapes and summer samples are denoted by solid shapes. (b) Metric MDS plot showing bootstrap mean distributions for distances between environmental and vegetation parameters samples from areas dominated by *Avicennia, Batis, and Spartina.* Spring samples are denoted by open shapes and summer samples are denoted by solid shapes.

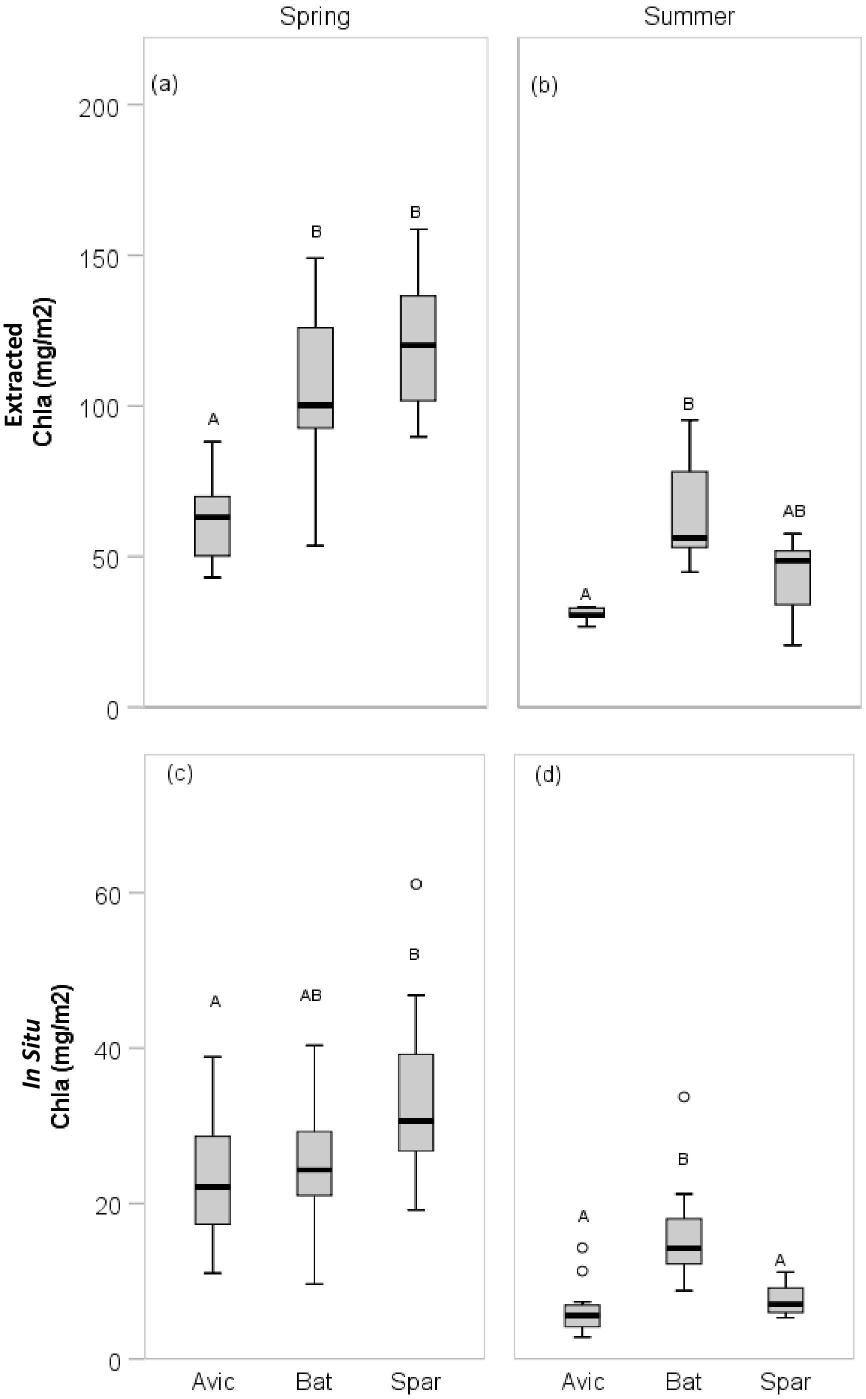


Figure 10 Algal biomass reported in mg/m^2 of Chlorophyll A extracted (a, b) and *in situ* (c, d). for three vegetation types *Avicennia (*Avic), *Batis (*Bat), *Spartina (*Spar) in each season (spring left, summer right). Significant groups determined by 2 way ANOVA with follow up tukey's-B post hoc denoted by capital letters. Dark lines in box and whisker plots represent median values.

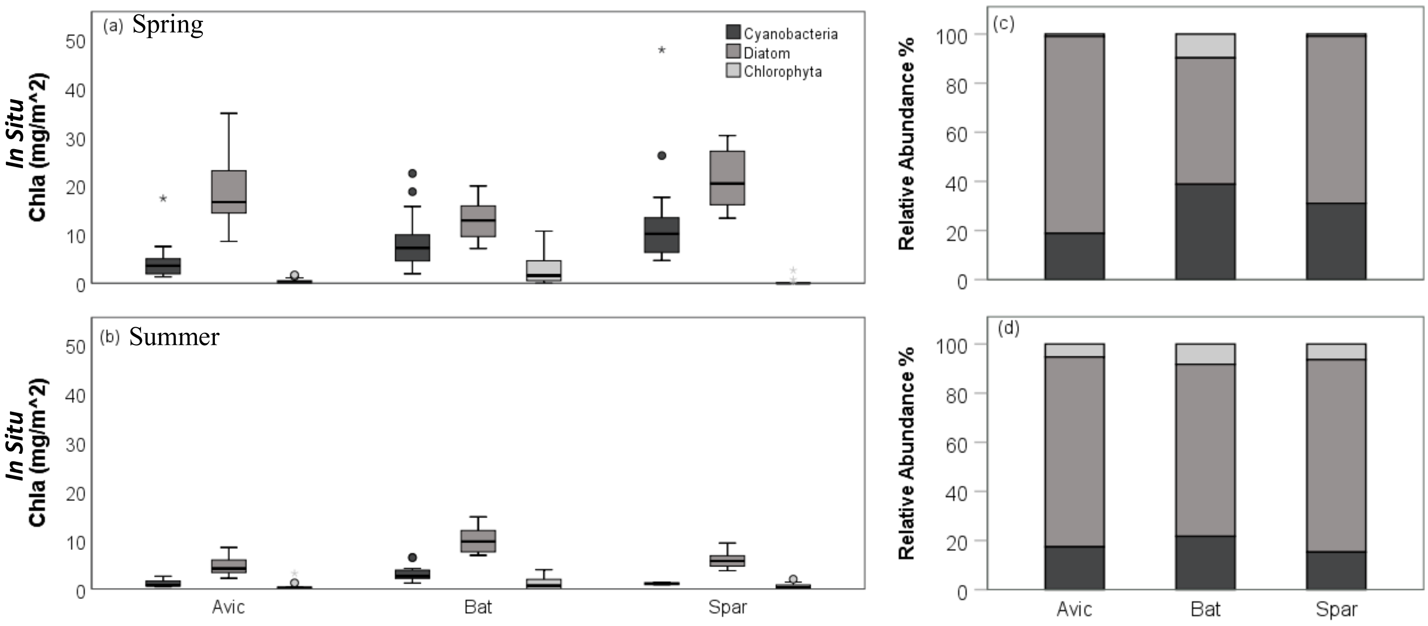


Figure 11 (a, b) *In situ* algal biomass of each algae group cyanobacteria, diatoms, and chlorophyta within each vegetation type in each season (spring a, summer b). (c, d) Stacked bar graphs of relative abundance percent for three algal functional groups within each vegetation types. Spring(c), and Summer(d) presented separately.

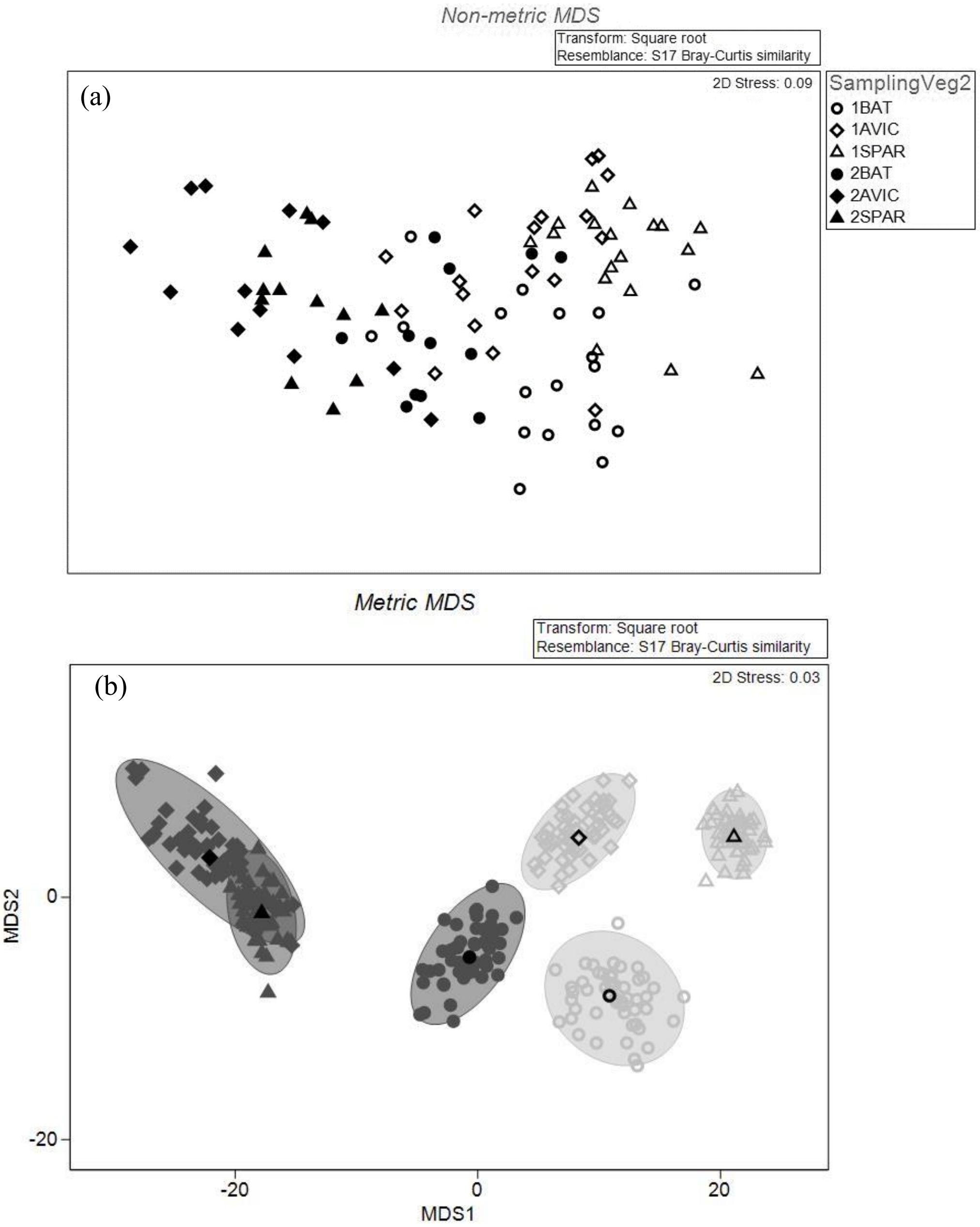


Figure 12 (a) Non-metric MDS plot showing all distances between algal functional group biomass samples from areas dominated by *Avicennia, Batis, and Spartina.* Spring samples are denoted by open shapes and summer samples are denoted by solid shapes. (b) Metric MDS plot showing bootstrap mean distributions for distances between algal functional group biomass samples from areas dominated by *Avicennia, Batis, and Spartina.* Spring samples are denoted by open shapes and summer samples are denoted by solid shapes.

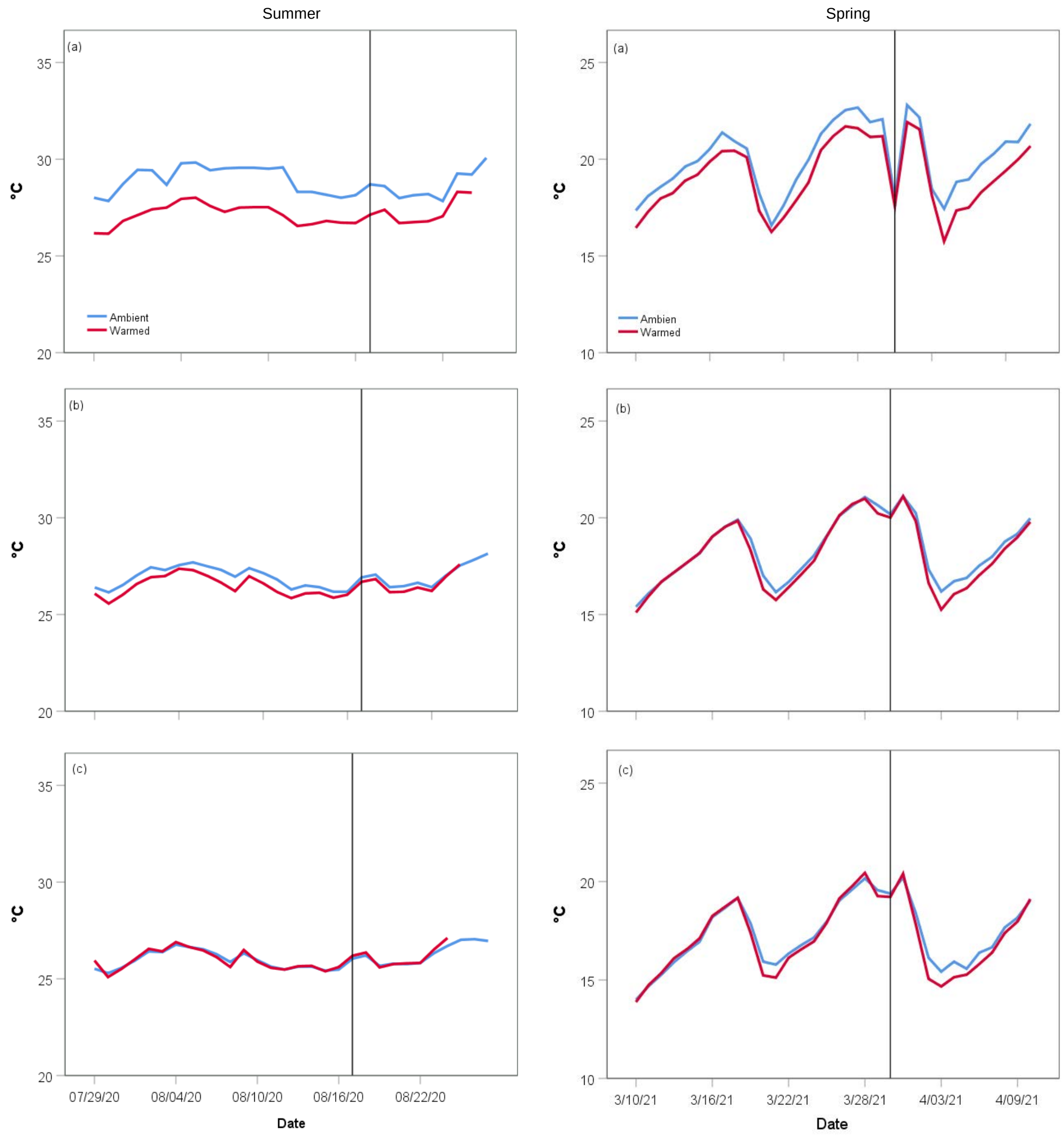


Figure 13 Warming experiment temperature graphs showing maximum daily soil temperatures (a), mean daily soil temperatures (b) and minimum daily soil temperatures (c) in warmed and control plots over thirty-day intervals surrounding sampling dates. Black vertical lines signify sampling dates.

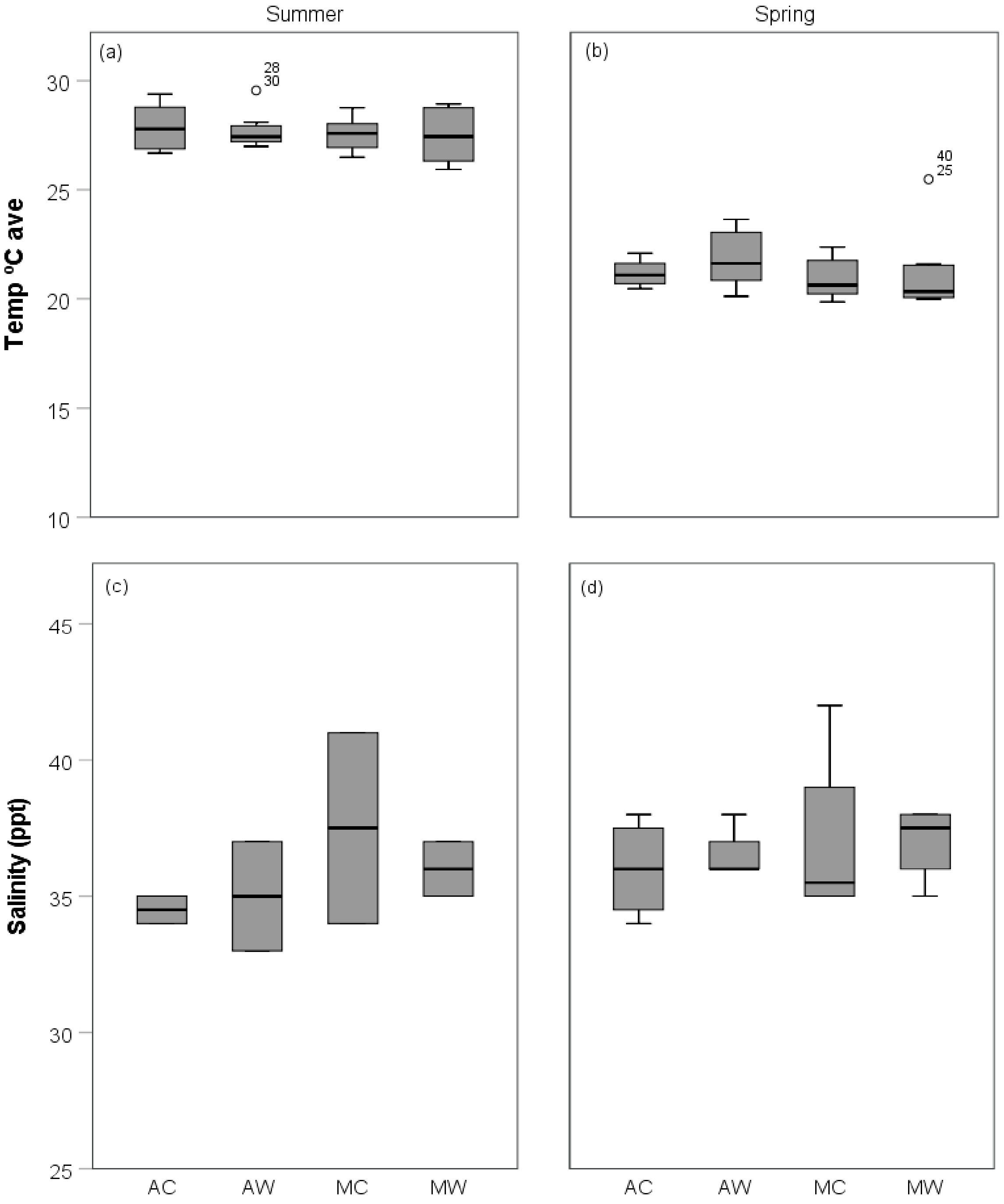


Figure 14 Abiotic parameters temperature (a, b) and salinity (a, b) measured in treatment plots for warming experiment in summer (left) and spring (right). Dark line in box and whisker plot represents median values.

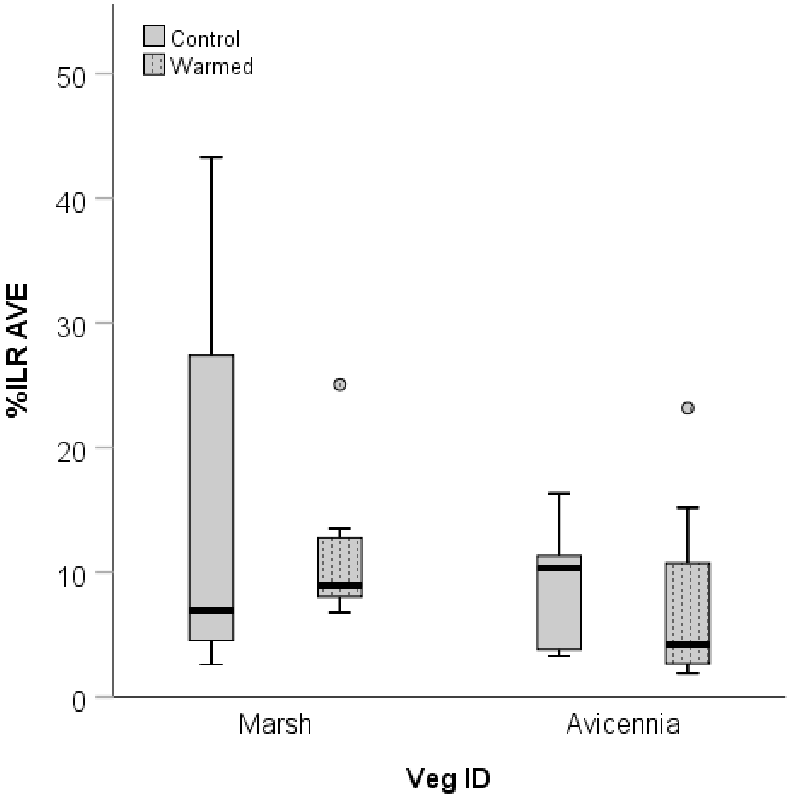


Figure 15 Incident light radiation measured in control (solid) and warmed (dotted) treatments during summer warming experiment sampling. Dark line in box and whisker plots represent median values.

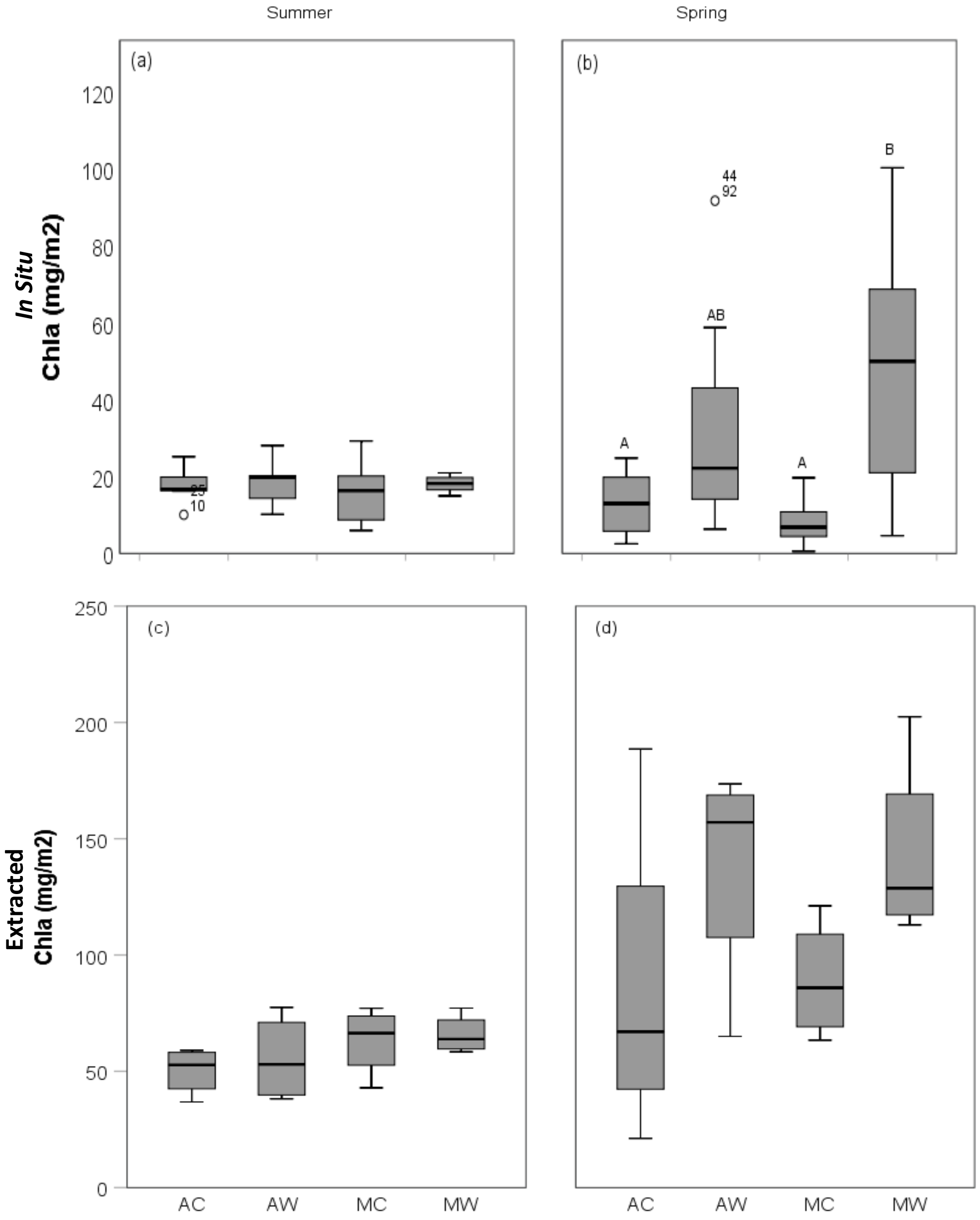


Figure 16 Algal biomass *in situ* (a, b) and extracted (c, d) measured in treatment plots for warming experiment in summer (left) and spring (right). Significant groups from 2 way ANOVA with follow up post hoc denoted by capital letters.

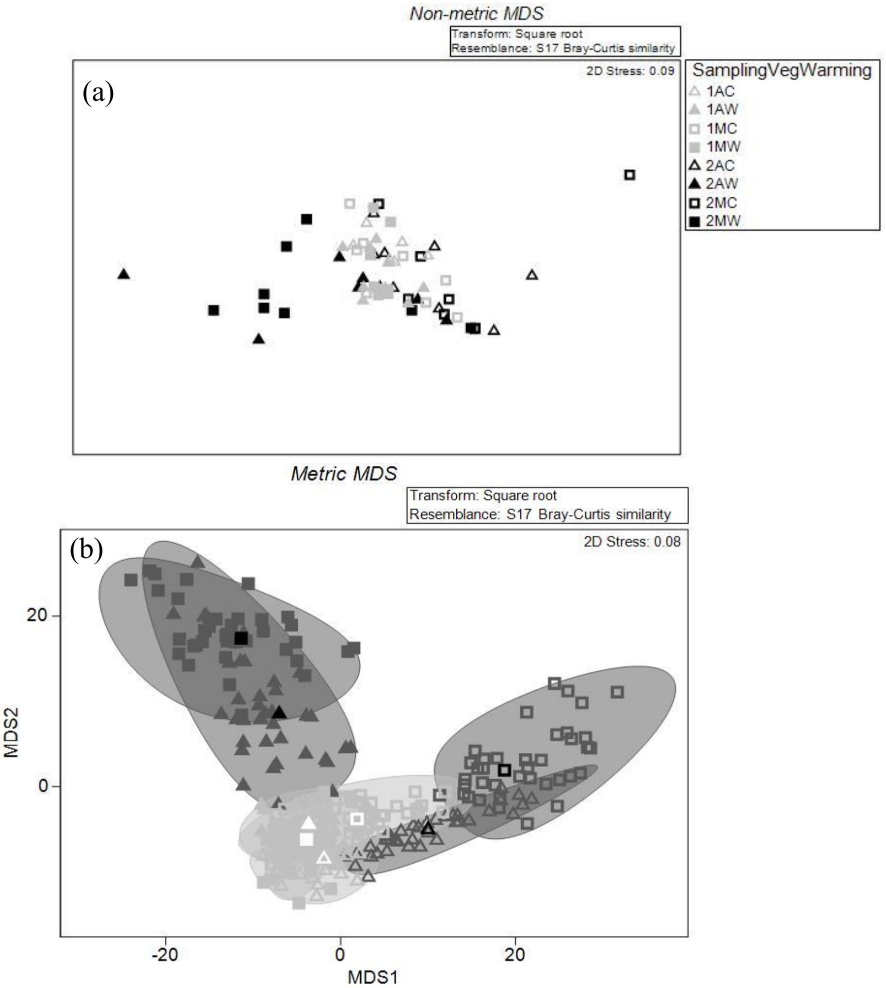


Figure 17 (a) Non-metric MDS plot showing all distances between algal functional group biomass samples from warming treatments (Avicennia warmed, Avicennia control, marsh warm, marsh control). Summer samples (1) are denoted by light grey shapes and spring samples (2) are denoted by black shapes. Open shapes signify control treatments and solid shapes indicate warmed treatments. (b) Metric MDS plot showing bootstrap mean distributions for distances between algal functional group biomass samples from warming treatments (Avicennia warmed, Avicennia control, marsh warm, marsh control). Summer samples (1) are denoted by light grey shapes and spring samples (2) are denoted by black shapes. Open shapes signify control treatments and solid shapes indicate warmed treatments

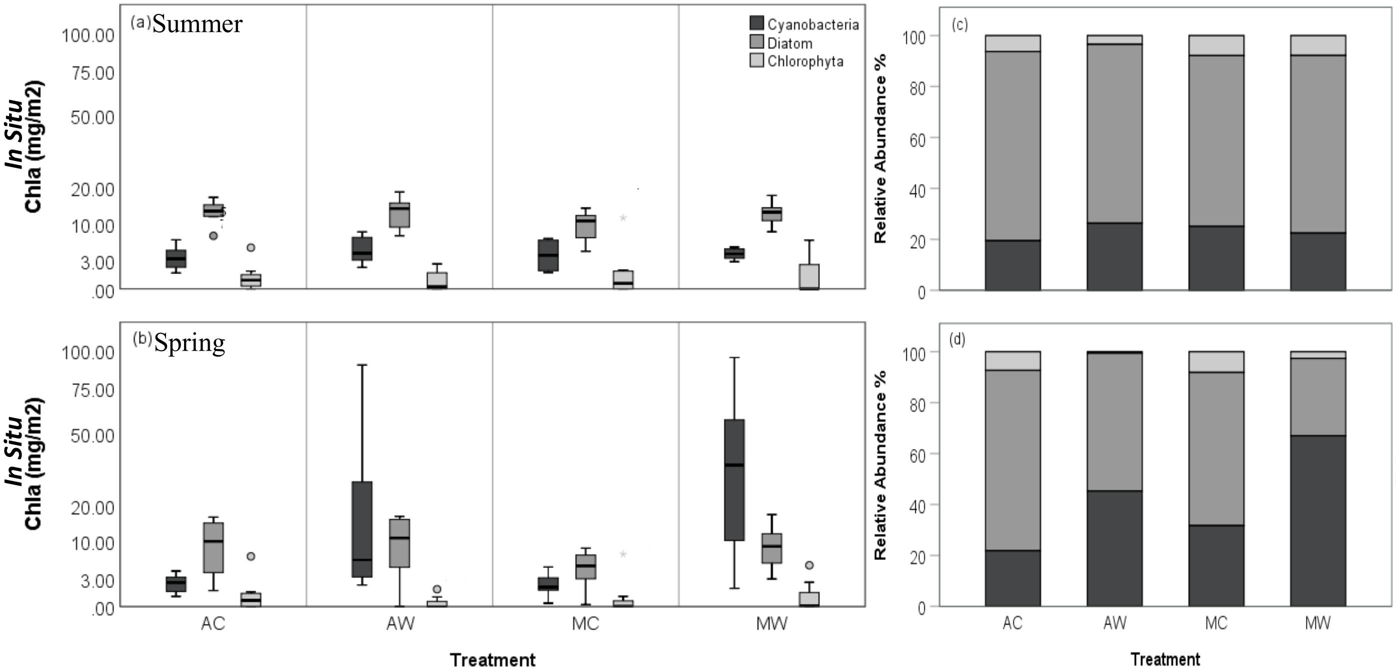


Figure 18 (a, b) Algal biomass reported in mg/m^2 of chlorophyll A within each season summer (a), and spring(b), for algae functional groups cyanobacteria, diatom, and chlorophyta in each of the four warming experiment treatments: Avicennia control (AC), Avicennia warmed (AW), marsh control (MC) and marsh warmed (MW). (c, d) Average relative abundances for algae functional groups in summer (c) and spring (d) in each of the four warming experiment treatments.

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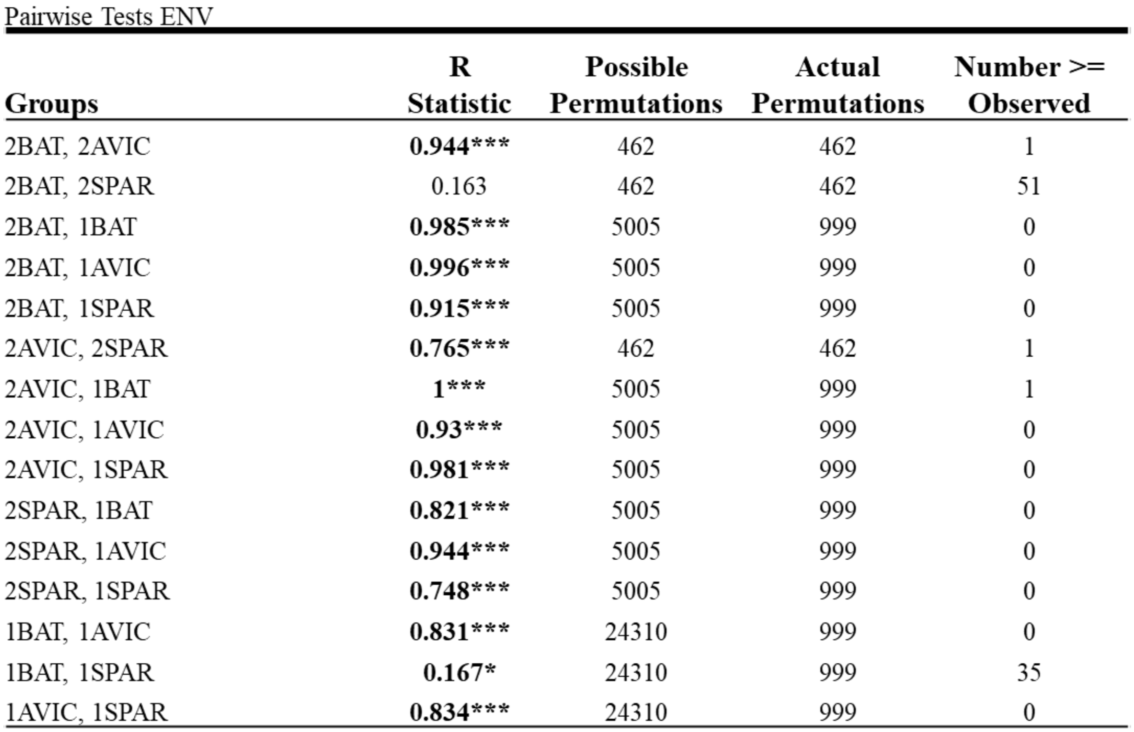
Supplementary tables and figures



Pairwise Tests ENV

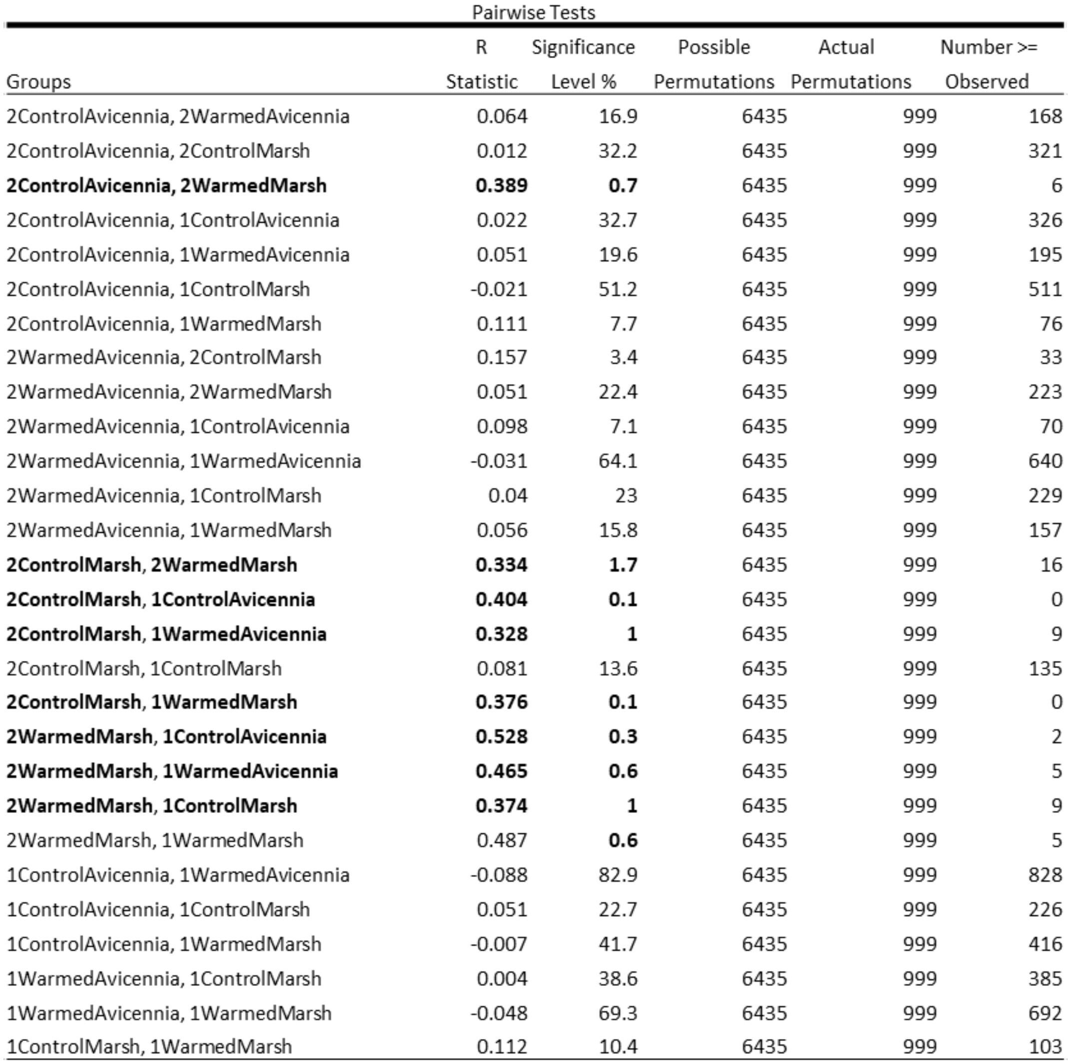
Groups	R Statistic	Possible Permutations	Actual Permutations	Number >= Observed
2BAT, 2AVIC	**0.944*****	462	462	1
2BAT, 2SPAR	0.163	462	462	51
2BAT, 1BAT	**0.985*****	5005	999	0
2BAT, 1AVIC	**0.996*****	5005	999	0
2BAT, 1SPAR	**0.915*****	5005	999	0
2AVIC, 2SPAR	**0.765*****	462	462	1
2AVIC, 1BAT	**1*****	5005	999	1
2AVIC, 1AVIC	**0.93*****	5005	999	0
2AVIC, 1SPAR	**0.981*****	5005	999	0
2SPAR, 1BAT	**0.821*****	5005	999	0
2SPAR, 1AVIC	**0.944*****	5005	999	0
2SPAR, 1SPAR	**0.748*****	5005	999	0
1BAT, 1AVIC	**0.831*****	24310	999	0
1BAT, 1SPAR	**0.167***	24310	999	35
1AVIC, 1SPAR	**0.834*****	24310	999	0

S. Table 1 Results of pairwise tests from one way analysis of similarity of environmental parameters on the combined factor of season and vegetation type. All pairwise combinations of variables are presented along with the observed R statistic, possible number of permutations, the actual permutations investigated and the number of observed permutations with R values above that obtained from observed data. R-values with ‘*’, ‘**’ and ‘***’ are significant at $P<0.05$, $P<0.01$, and $P<0.001$, respectively.

Pairwise Tests Groups

Groups	R Statistic	Possible Permutations	Actual Permutations	Number >= Observed
2BAT, 2AVIC	**0.546*****	1352078	999	0
2BAT, 2SPAR	**0.539*****	1352078	999	0
2BAT, 1BAT	**0.181****	86493225	999	8
2BAT, 1AVIC	**0.219****	86493225	999	2
2BAT, 1SPAR	**0.707*****	86493225	999	0
2AVIC, 2SPAR	**0.122***	1352078	999	28
2AVIC, 1BAT	**0.73******	86493225	999	0
2AVIC, 1AVIC	**0.76*****	86493225	999	0
2AVIC, 1SPAR	**0.971*****	86493225	999	0
2SPAR, 1BAT	**0.734*****	86493225	999	0
2SPAR, 1AVIC	**0.71*****	86493225	999	0
2SPAR, 1SPAR	**0.99*****	86493225	999	0
1BAT, 1AVIC	**0.275*****	Very large	999	0
1BAT, 1SPAR	**0.417*****	Very large	999	0
1AVIC, 1SPAR	**0.26*****	Very large	999	0

S. Table 2 Results of pairwise tests from one way analysis of similarity of algal functional group biomass for the combined factor of season and vegetation type. All pairwise combinations of variables are presented along with the observed R statistic, possible number of permutations, the actual permutations investigated and the number of observed permutations with R values above that obtained from observed data. R-values with '*', '**' and '***' are significant at $P<0.05$, $P<0.01$, and $P<0.001$, respectively.



Pairwise Tests

Groups	R Statistic	Significance Level %	Possible Permutations	Actual Permutations	Number >= Observed
2ControlAvicennia, 2WarmedAvicennia	0.064	16.9	6435	999	168
2ControlAvicennia, 2ControlMarsh	0.012	32.2	6435	999	321
2ControlAvicennia, 2WarmedMarsh	**0.389**	**0.7**	6435	999	6
2ControlAvicennia, 1ControlAvicennia	0.022	32.7	6435	999	326
2ControlAvicennia, 1WarmedAvicennia	0.051	19.6	6435	999	195
2ControlAvicennia, 1ControlMarsh	-0.021	51.2	6435	999	511
2ControlAvicennia, 1WarmedMarsh	0.111	7.7	6435	999	76
2WarmedAvicennia, 2ControlMarsh	0.157	3.4	6435	999	33
2WarmedAvicennia, 2WarmedMarsh	0.051	22.4	6435	999	223
2WarmedAvicennia, 1ControlAvicennia	0.098	7.1	6435	999	70
2WarmedAvicennia, 1WarmedAvicennia	-0.031	64.1	6435	999	640
2WarmedAvicennia, 1ControlMarsh	0.04	23	6435	999	229
2WarmedAvicennia, 1WarmedMarsh	0.056	15.8	6435	999	157
2ControlMarsh, 2WarmedMarsh	**0.334**	**1.7**	6435	999	16
2ControlMarsh, 1ControlAvicennia	**0.404**	**0.1**	6435	999	0
2ControlMarsh, 1WarmedAvicennia	**0.328**	**1**	6435	999	9
2ControlMarsh, 1ControlMarsh	0.081	13.6	6435	999	135
2ControlMarsh, 1WarmedMarsh	**0.376**	**0.1**	6435	999	0
2WarmedMarsh, 1ControlAvicennia	**0.528**	**0.3**	6435	999	2
2WarmedMarsh, 1WarmedAvicennia	**0.465**	**0.6**	6435	999	5
2WarmedMarsh, 1ControlMarsh	**0.374**	**1**	6435	999	9
2WarmedMarsh, 1WarmedMarsh	0.487	**0.6**	6435	999	5
1ControlAvicennia, 1WarmedAvicennia	-0.088	82.9	6435	999	828
1ControlAvicennia, 1ControlMarsh	0.051	22.7	6435	999	226
1ControlAvicennia, 1WarmedMarsh	-0.007	41.7	6435	999	416
1WarmedAvicennia, 1ControlMarsh	0.004	38.6	6435	999	385
1WarmedAvicennia, 1WarmedMarsh	-0.048	69.3	6435	999	692
1ControlMarsh, 1WarmedMarsh	0.112	10.4	6435	999	103

S. Table 3 Results of pairwise tests from one way analysis of similarity of algal functional group biomass for the warming experiment combined factor of season and treatment. All pairwise combinations of variables are presented along with the observed R statistic, possible number of permutations, the actual permutations investigated and the number of observed permutations with R values above that obtained from observed data. R-values with ‘*’, ‘**’ and ‘***’ are significant at P<0.05, P<0.01, and P<0.001, respectively.

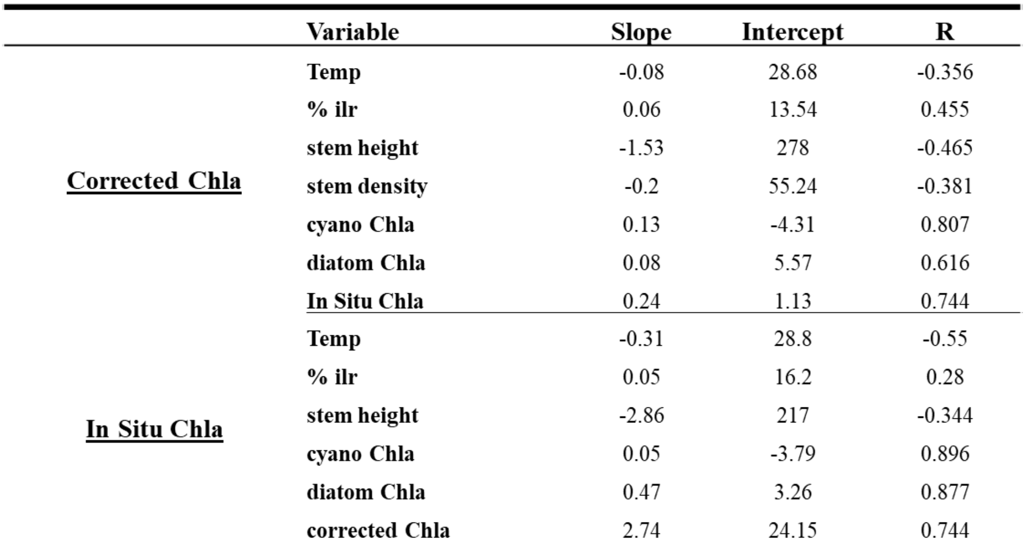
S. Table 4 Results of three-way analysis of variance testing the main and interactive effects of Sampling date (S), Vegetation type (V) and Area within creek (A) on *in Situ* chlorophyll *a* (Chla), extracted Chla, temperature, percent incident light radiation (%ILR), salinity, percent cover, stem density, and stem height. Degrees of freedom (df) and F-values are presented for each factor and response variable. F-values with '*', '**' and '***' are significant at $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively.

Dependent Variable	*df*	***In Situ* Chla**	**Chla**	**Temp**	**%Ilr**	**Salinity**	**% cover**	**Stem density**	**Stem height**
Sampling (S)	1	**402.031***	55.349	39.411	0.006	11.012	1.675	5.57	130.547
Veg (V)	2	4.583	9.142	**37.154***	6.614	1.005	15.236	12.269	**72.313***
Area (A)	1	0	2.543	0	0.664	0.014	0.576	0.051	2.007
S x V	2	**19.132****	12.713	1.587	7.923	26.069	2.624	16.584	0.173
S x B	1	1.048	4.294	15.594	**76.955****	7.471	5.048	1.04	0.02
V x B	2	6.453	4.279	0.161	**22.357***	11.443	1.891	20.198	4.375
S x V x B	2	0.648	0.509	**4.38***	0.338	0.432	1.013	0.121	1.932

S. Table 5 results of two-way analysis of variance testing the main and interactive effects of Season (S) and Vegetation type (V) on *In Situ* Chla, Extracted Chla, Salinity, Temperature, Percent cover, Stem density, and Stem height. Degrees pf freedom (df) and F-values are presented for each factor and response variable. F-values with ‘*’, ‘**’ and ‘***’ are significant at p < 0.05, p < 0.01, and p < 0.001, respectively

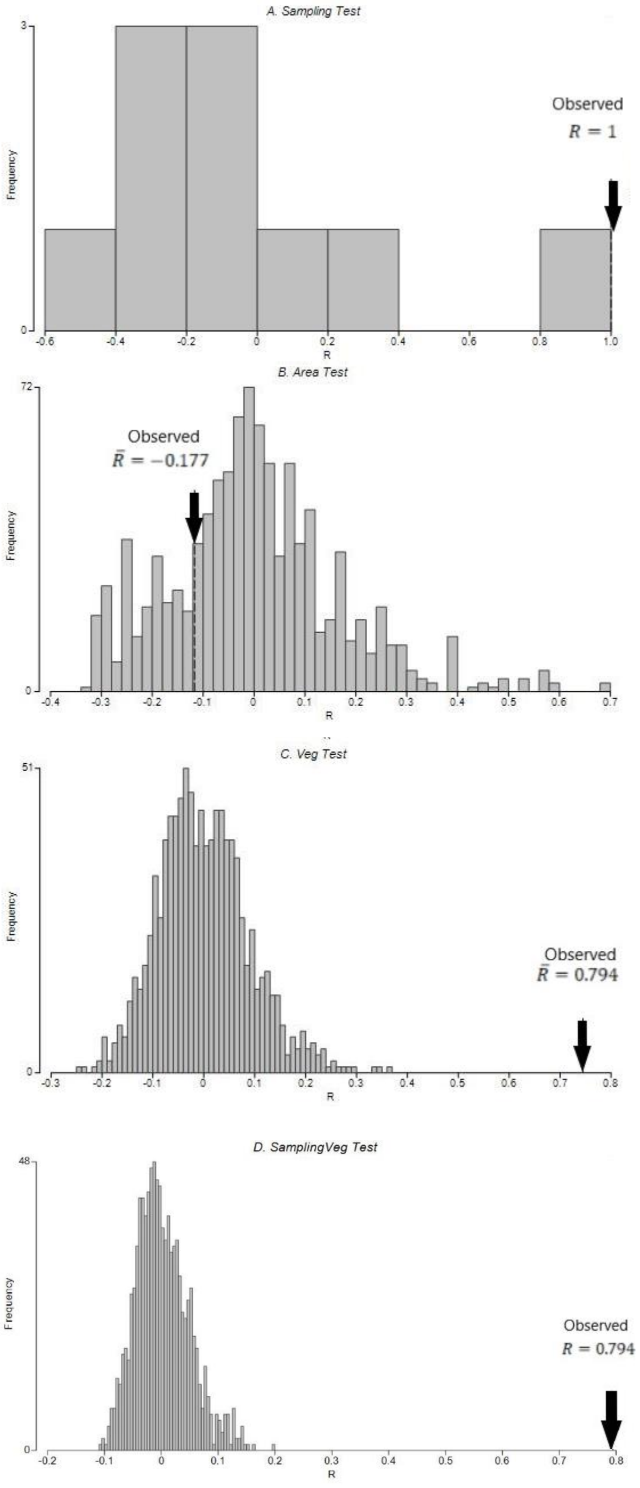
Dependent Variable	df	***In Situ* Chla**	**Chla**	**Salinity**	**Temp**	**% cover**	**Stem density**	**Stem height**
Season(S)	1	**256.045*** **	**83.493*** **	**47.173*** **	**1282.921*** **	**6.685** **	0.717	2.546
Veg (V)	2	**17.981*** **	**14.509*** **	**5.044** **	**12.459*** **	**22.78*** **	**30.671*** **	**316.284*** **
S x V	2	**11.632*** **	**4.111** **	**6.293*** **	**3.312* **	2.075	2.053	0.173

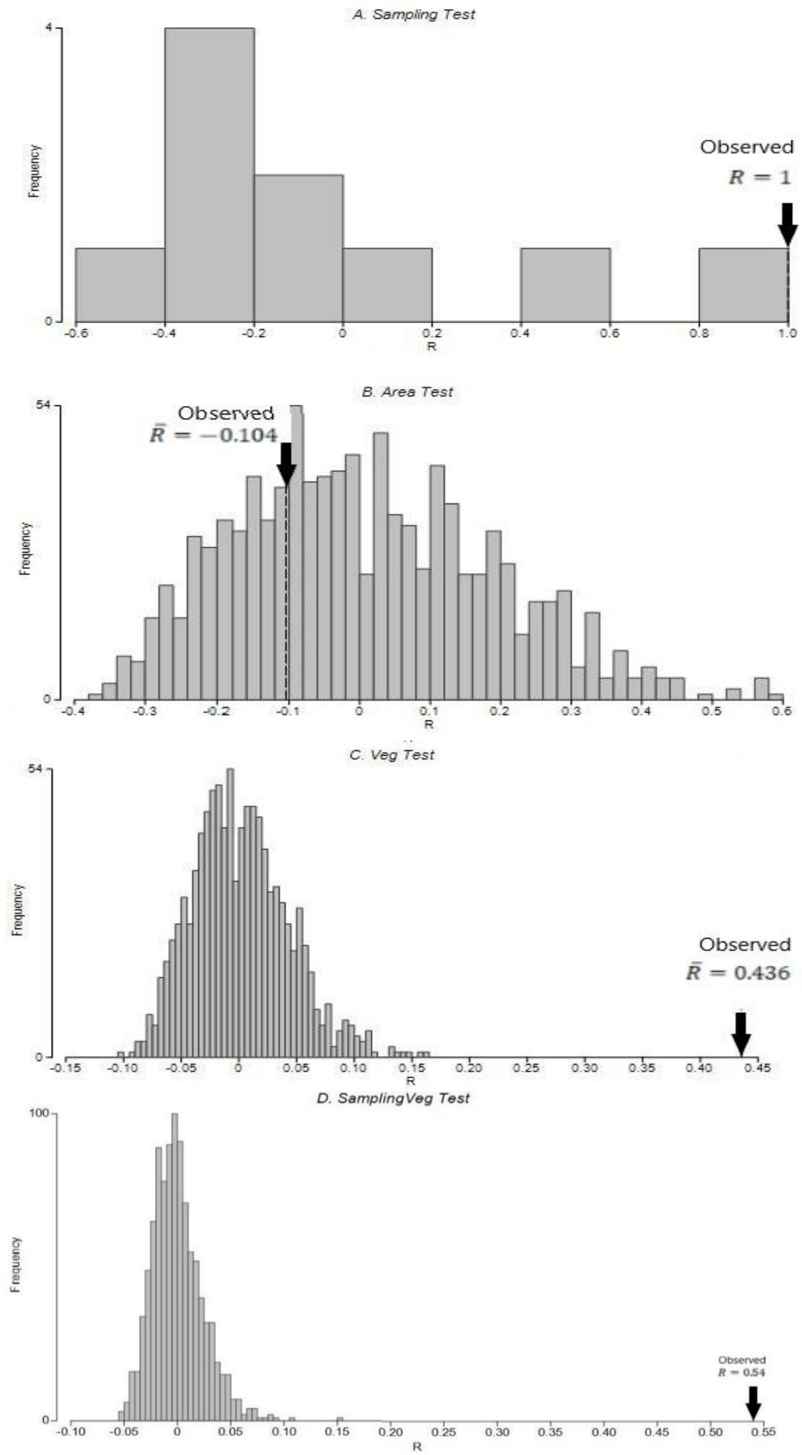
S. Table 6 The relationship between algal biomass, measured as extracted and *in situ* chlorophyll *a* (Chla), and abiotic and vegetation parameters across all vegetation types.



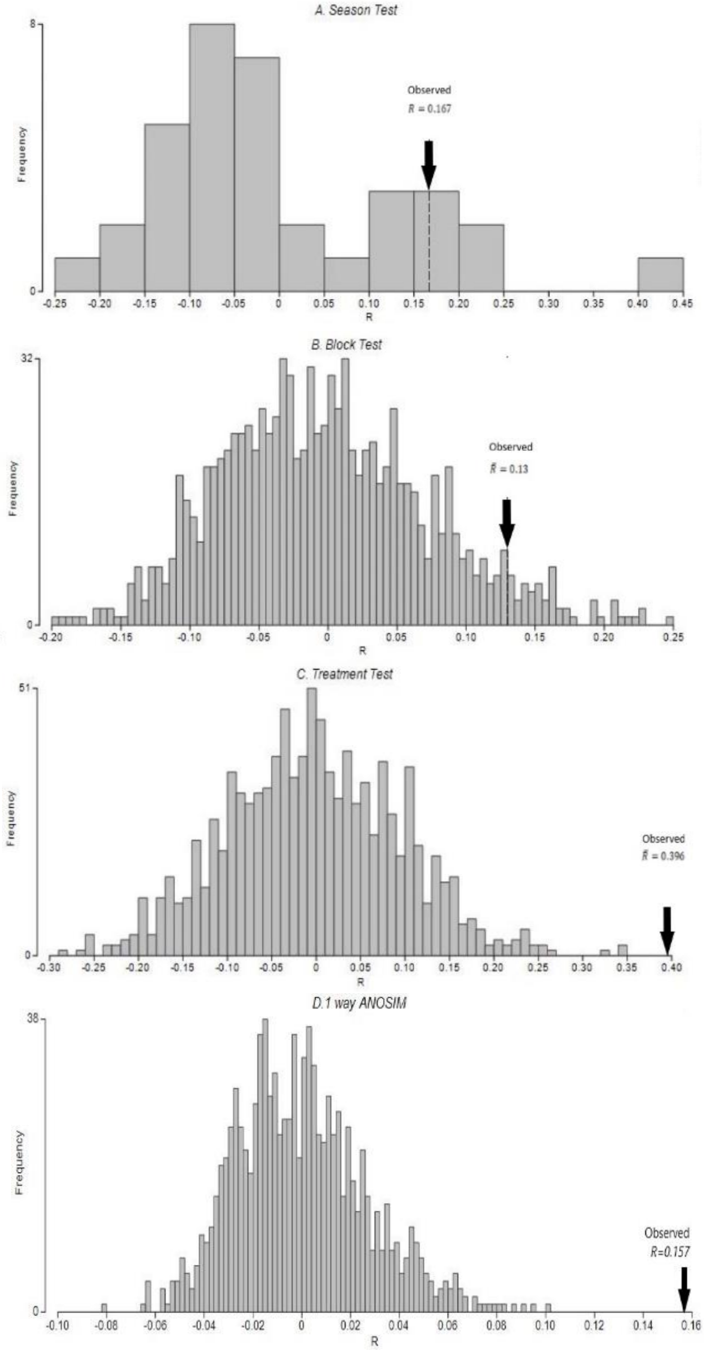
	Variable	Slope	Intercept	R
Corrected Chla	**Temp**	-0.08	28.68	-0.356
	% ilr	0.06	13.54	0.455
	stem height	-1.53	278	-0.465
	stem density	-0.2	55.24	-0.381
	cyano Chla	0.13	-4.31	0.807
	diatom Chla	0.08	5.57	0.616
	In Situ Chla	0.24	1.13	0.744
In Situ Chla	**Temp**	-0.31	28.8	-0.55
	% ilr	0.05	16.2	0.28
	stem height	-2.86	217	-0.344
	cyano Chla	0.05	-3.79	0.896
	diatom Chla	0.47	3.26	0.877
	corrected Chla	2.74	24.15	0.744

S. Figure 1 Vegetation permutation distributions for three way crossed analysis of similarity of environmental variables for factors of (Season a, Area b, and Vegetation type c). Observed R values for season, area, vegetation type denoted by arrow with value displayed on graph (R=1, $\bar{R}$ = -0.177, $\bar{R}$= 0.794 respectively) (d). Observed permutation distribution for one way analysis of variance for combined factor of sampling and vegitation type. Observed R value (R=0.794) displayed on graph with arrow.





S. Figure 2 Vegetation Permutation distributions for three way crossed analysis of similarity of algal functional groups for factors of (Season a, Area b, and Vegitation type c). Observed R values for season, area veg denoted by arrow with value displayed on graph (R=1, $\overline{R}$ = -0.104, $\overline{R}$= 0.436 respectively) (d). Observed permutation distribution for one way analysis of variance for combined factor of sampling and vegitation type. Observed R value (R=0.54) displayed on graph with arrow.



S. Figure 1 Warming experiment permutation distributions for thee way crossed analysis of similarity of algal functional groups for factors of (Season a, Block b, and Treatment c). Observed R values for season, block and treatment denoted by arrow with value displayed on graph (R=.167, $\bar{R}$ = -0.13, $\bar{R}$= 0.396 respectively) (d). Observed permutation distribution for one way analysis of variance for combined factor of sampling and Treatment. Observed R value (R=0.157) displayed on graph with arrow.