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The Mechanisms and Consequences of Shrub Encroachment on the Virginia Barrier Islands

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The mechanisms and consequences of shrub encroachment on the Virginia barrier islands

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ABSTRACT

Shrub encroachment is a global phenomenon driven by direct and indirect anthropogenic influence which alters plant communities and ecosystem function. Many studies have investigated drivers and consequences of woody plant establishment, but mesic landscapes are underrepresented in the literature. My objective was to assess the mechanisms of *Morella cerifera* encroachment into coastal mesic grassland, the potential for self-reinforcement, and consequences on community composition, nutrients, and landscape productivity. I studied temperature and water microclimate modification by *Morella cerifera* presence and removal to understand ecosystem engineering and community composition changes. Additionally, I examined the influence of shrubs on surrounding grassland species traits and soils. Lastly, I assessed the macroclimatic and age drivers of shrub productivity and habitat change across a chronosequence (i.e. a space for time substitution) and over time.

As shrubs establish and form thickets, species diversity is reduced while transpiration and annual productivity increase. Shrub presence modifies microclimate, reducing extreme temperature events and variance; shrubs were 18 °C cooler in summer and 2 °C warmer in winter. Woody vegetation in arid habitats does similar microclimate modification. Removal of established thickets led to lower soil moisture coupled with a wider range of temperatures. Grass plots have a lower water table than shrub plots by ~1 cm but shrub plots had higher water table temporal variability, suggesting hydraulic lift. Clear cut soil exhibited intermediate characteristics between grass and shrub, indicating that shrub removal could revert the habitat to some grassland-like characteristics. Proximity to shrubs and supplemental N fertilization impact the adjacent temperature extremes as well as plant functional traits, demonstrating edge effects due to N addition by shrubs on adjacent grassland. While temperature is the dominant driver of shrub establishment, shrub age and water dynamics (rainfall and water table depth) exert stronger controls on shrub productivity. Rainfall is typically coupled strongly with depth to water table, however, our annual rainfall and water depth did not correlate and water table depth increased more than would be due to sea level rise, supporting the possibility that *M. cerifera* engages in hydraulic lift. Soil carbon and N increase with shrub age and were higher in shrub soils than grassland soils but many nutrients leach into water table. Shrubs in mesic habitats establish via similar mechanisms as those in drylands - microclimate modification, increased soil moisture – but result in a monospecific thicket rather than creating refugia for other species, as is common in arid habitats. I conclude that *M. cerifera* establishment and persistence has ecosystem engineering capabilities that results in notable habitat feedbacks and modifications, expanding our understanding of shrub encroachment in mesic systems.

CHAPTER ONE

Mechanisms and consequences of shrub encroachment on the Virginia barrier islands

Lauren K Wood

Introduction

Woody encroachment into grassland habitats has been documented for centuries, both in the southeastern US and globally (Tape et al 2006, Knapp et al 2008, D'Odorico et al. 2010, D'Odorico et al. 2012, Zinnert et al. 2016, Sabater et al. 2019, Charles et al. 2020, Huang et al. 2020). One primary driver of woody encroachment on a global scale is altered stress and disturbance by climate change (Medeiros and Pockman 2011, Higgins and Scheiter 2012, Sistla et al. 2013). Long-term research, along with recent modeling and empirical efforts, has found that coastal woody encroachment in the mid-Atlantic region is partially driven by warming winter temperatures and decreased freezing-induced hydraulic failure (Huang et al. 2018). The varying causes of woody expansion at a global scale highlight the importance of disentangling mechanisms of expansion in different ecosystems.

Due to position at the land-sea interface, coastal plants experience climate change presses (i.e., temperature changes, CO_2 concentration increase, precipitation regime shifts) and pulses (i.e., increased storm frequency) (Ehrenfeld 1990, Young et al. 2011). Sea level rise (SLR) and increased storm frequency and intensity can trigger plant physiological stress responses and accelerate community turnover (Hayden et al. 1995, Zinnert et al. 2016, Zinnert et al. 2019, Paudel and Battaglia 2021). Barrier islands act as the front line in the face of damaging storm currents and winds and protect >6% of mainland coastlines globally (Stutz and Pilkey, 2001). Species presence on barrier islands is limited both by the abiotic stresses inherent in proximity to the ocean and dispersal across water via wind, waves, and animals. Coastal systems protect populous, economically significant terrestrial areas. Habitats with higher topography and plant species that adopt a woody life history can more successfully buffer damage from storms; as storm intensity increases and sediments on barrier islands continue to shift, vegetation communities are likely to change as well. Woody encroachment is occurring rapidly on the northern Gulf of Mexico, coastal dune systems in the Netherlands, and on the mid-Atlantic barrier islands (Battaglia et al. 2007, Zinnert et al. 2016, Howard et al. 2018, Valdés-Correcher et al. 2018, Weaver and Armitage 2018). In each instance, the expansion is primarily driven by a different environmental parameter (nutrient input, sea level rise, grazing, or temperature).

While many species ranges are expanding and contracting in coastal habitats due to altered habitat characteristics, the shrub *Morella cerifera* (Myricaceae), or wax myrtle, is expanding into coastal grassland along the mid-Atlantic coast despite loss of island area to sea level rise and erosion (Zinnert et al. 2016). *Morella cerifera* is an evergreen shrub that has an obligate association with nitrogen-fixing Frankia in its root nodules (Sande and Young 1991, Young 1992, Wijnholds and Young 2000); these traits can enhance productivity relative to grassland species (Shiflett et al. 2017). During the summer, *M. cerifera* can maintain high rates of photosynthesis and assimilate carbon into aboveground biomass, maintained by high water movement, to the extent that it resembles the productivity of broadleaf deciduous woody species (Shiflett et al. 2014). During the winter, the evergreen leaf habit allows plants to maintain some level of photosynthetic activity, providing an advantage over dormant grassland species. During leaf-drop, which occurs year-round, nitrogen is retained in the leaves rather than resorbed before abscission (Brantley and Young 2008) potentially increasing soil nitrogen. Understanding mechanisms behind this regime shift from grassland to shrubland in coastal systems is of interest, especially as this expanding shrub has limited salt tolerance (Naumann et al. 2007, Naumann et al. 2008) and coastal areas are losing habitat to intrusion which is being boosted by shrub presence (Zinnert et al. 2016, Zinnert et al. 2019, Wood et al. 2020). Previous work shows *M. cerifera* shrub encroachment is driven by warmer winters, limiting shrub exposure to the freezing-induced hydraulic failure threshold of -15 °C (Huang et al. 2018). Shrubs also modify the microclimate on a small scale, likely enhancing establishment and growth and leading to a positive feedback (Thompson et al. 2017).

The goal of my dissertation was to investigate the mechanisms by which *M. cerifera* encroaches into grassland and the biotic and abiotic consequences of shrub establishment. I hypothesized that shrub encroachment impacts the community (including transition areas) by reducing species diversity but increases productivity and transpiration and creates self-reinforcing microclimate modification. I further hypothesized that shrub establishment will result in higher water table and enhanced soil moisture, C, and N with lower bulk density. Grassland traits and microclimate were hypothesized to be modified both by proximity to shrub and by supplemental N input. Shrub annual net primary productivity (ANPP) is hypothesized to be controlled by shrub age, temperature, and water availability.

Objectives

1) Quantify microclimate variance differences, community physiological response, and community composition in three vegetation zones: grassland, transition areas, and shrub thickets.

2) Test temperature and water table microclimate modification by *M. cerifera* and legacy influences of establishment and removal on the soil habitat, soil moisture, and recovery of vegetation through shrub removal.

3) Investigate the influence of both microclimate modification and altered N input in a factorial design of shrubs on abiotic and biotic impacts of adjacent grassland.

4) Determine the effect of macroclimate changes on ANPP using long-term data from the Hog Island chronosequence and quantify consequences of long-term shrub encroachment on N and C in the soil as well as dissolved organic carbon (DOC) and nitrogen (DON) in the freshwater table.

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CHAPTER TWO

Decreased temperature variance associated with biotic composition enhances coastal shrub encroachment

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Abstract

Regime shift from grasslands to shrub-dominated landscapes occur worldwide driven by altered land-use and climate change, affecting landscape function, biodiversity, and productivity. Warming winter temperatures are a main driver of expansion of the native, evergreen shrub, *Morella cerifera*, in coastal landscapes. Shrub establishment in these habitats alters microclimate, but little is known about seasonal differences and microclimate variance. We assessed influence of shrubs on microclimate variance, community composition, and community physiological functioning across three vegetation zones: grass, transitional, and shrub in a coastal grassland. Using a novel application of a time-series analysis, we interpret microclimatic variance modification and elucidate mechanisms of shrub encroachment at the Virginia Coast Reserve, Long-Term Ecological Research site. As shrub thickets form, diversity is reduced with little grass/forb cover, while transpiration and annual productivity increase. Shrub thickets significantly reduced temperature variance with a positive influence of one day on the next in maximum air, minimum air, and maximum ground temperature. We also show that microclimatic temperature moderation reduces summer extreme temperatures in transition areas, even before coalescence into full thickets. Encroachment of *Morella cerifera* on the Virginia barrier islands is driven by reduced local exposure to cold temperatures and enhanced by abiotic microclimatic modification and biotic physiological functioning. This shift in plant community composition from grassland to shrub thicket alters the role of barrier islands in productivity and can have impacts on the natural resilience of the islands.

Introduction

Woody vegetation is rapidly encroaching into grassland globally as a result of altered environmental drivers [1-3]. The abrupt conversion to shrub-dominated habitats includes drivers such as altered fire or grazing pressures, increased atmospheric CO_2, and altered temperature ranges [3, 4-6]. Warming temperatures associated with macroclimatic change, particularly in winter minima, can expand the range limits of cold-intolerant shrub species [3, 6, 7-8] while macroclimatic variance in precipitation enhances success of woody vegetation, particularly in the transition ecotones [9-10]. Thus, no single factor provides dominant influence on woody encroachment.

Increased annual temperatures may improve physiological functioning of encroaching species, possibly as a feedback between abiotic microclimatic conditions and shrub success to promote range expansion for woody shrubs [3, 7]. On smaller scales, encroaching woody vegetation frequently modifies microclimatic conditions resulting in intra- and interspecific facilitation [11-14]. Changes in microclimatic temperatures within shrub thickets include temperature moderation with diminished instances of extreme temperatures that can decrease productivity and cause physiological damage of xylem structures [3, 6]. In areas with warming winter temperatures, thicket-forming species that are vulnerable to cold-associated damage, such as freezing-induced embolism, are able to take advantage of fewer cold temperature extremes [1, 6, 15]. Multiple encroaching species are cold intolerant, thus a warming climate and self-reinforcing microclimatic modification allow aggressive expansion of shrubs in to historical grassland [3, 8, 11, 13, 16-18].

Regime shifts in vegetation and the resulting modified microclimate beget community changes, often leading to increased diversity with sparse canopy shrubs and decreased diversity in densely leaved canopies [13, 18-19]. These altered plant communities tend to have higher productivity and carbon assimilation and may support different faunal species. Changes in productivity increase soil nutrient assimilation and alters soil microbial communities [20]. While the drivers and stresses differ among various habitats undergoing woody encroachment, resulting community change and ecosystem consequences are similar.

Among landscapes that have experienced regime shift from grass to woody vegetation are low elevation swale/slack communities along the United States mid-Atlantic and Gulf coasts [21-26]. Coastal ecosystems in this region have reduced diversity relative to inland communities [27] with unique environmental stressors. On a large scale, wind, waves, tides, and storms result in dynamic sediment movement, sea spray, low canopy cover, and highly reflective sandy sediment, which increase irradiance. Regionally, atmospheric and oceanic climate change drivers are altering temperatures, storm regime, and sea level [25, 28]. Species diversity is restricted as a result of these environmental filters [27, 29]. For island landscapes, species diversity is additionally filtered by dispersal [26-27, 30]. The vegetation on barrier islands is further limited by the availability of freshwater and precipitation regime; barrier islands have a freshwater lens that is entirely precipitation-fed [21, 31-32]. Coastal habitats are understudied environments where vegetation dynamics present an unique opportunity to understand shrub encroachment mechanisms.

Over the past 32 years, shrub cover has increased on the Virginia barrier islands by ~40% despite an overall decrease in island area due to sea level rise and erosion (Fig. 1)[28, 33]. *Morella cerifera* (wax myrtle), a native, evergreen shrub forms thickets up to 7 m tall with increased leaf area index (LAI), increasing community productivity and nitrogen input into the system [34-35]. High productivity of shrubs, along with formation of monospecific thickets, influences carbon assimilation and nutrient input into the system. Evidence suggests *M. cerifera* has been expanding due to decreased exposure to extreme winter temperatures, which impair shrub physiological functioning [6, 13, 28]. Warmer temperatures in the winter and cooler temperatures in the summer likely enhance shrub survival and productivity. Further, experimental and modeling results demonstrate that a small increase in minimum temperature cause a shift from grassland to *M. cerifera* dominated alternative stable state [6].

Although mean temperature modification occurs between grass and shrub regime shift, temperature extremes and variance have yet to be quantified across zones including areas in transition. In order to understand the mechanism of shrub encroachment into coastal grassland, our objective was to quantify microclimate variance, community physiology, and community composition in three vegetation zones: grassland, transition areas, and shrub thickets. We hypothesize that shrub encroachment immediately impacts the community (including transition areas) by reducing species diversity, but increases productivity and transpiration. We further hypothesize that shrub canopy creates self-reinforcing microclimate modification. We use a time-series analysis as a novel way to interpret microclimatic modification to better understand the mechanisms of shrub encroachment.

Results

Species composition and physiology

Species richness was highest in grassland (6 ± 0) and transition plots (5 ± 0) (Table s2.1). Grassland had highest cover of graminoid species (86 ± 2 %), which was significantly higher than transition and shrub plots (F = 84.65, $P < 0.0001$, Table 2.1). Forb cover differed between grass (14 ± 2 %) and shrub plots (1 ± 2 %) only. Transition areas decreased grass cover (32 ± 2 %) with increased shrub cover (61 ± 2 %) compared to grassland plots. Shrub plots effectively excluded all other plant functional types, reducing species richness to 2 ± 0 and exhibiting 98 ± 2 % woody cover (Table s2.1, Table 2.1). Percent cover for species composition shifted from dominant grass, *Spartina patens*, in grasslands to shrub monoculture of *Morella cerifera* with *Baccharis halimifolia* found along the edge (Table s2.1). Coastal communities tend to have low vegetation diversity. Establishment of shrubs reduced Shannon-Weiner diversity from 1.7 ± 0.1 in grassland to 0.1 ± 0.1 in fully thicketized shrubs (F= 68.98 $P < 0.0001$, Table 2.1). Transition plots did not differ in diversity from grassland.

Despite differences in plant functional type and species composition, SLA did not differ among zones (F = 1.80, $P = 0.208$, Fig. 2.2) although there was a trend towards higher SLA in shrub plots. ANPP was highest in full shrub thickets ($\chi = 9.0$, $P = 0.0111$) and 1.4 – 1.8 x greater than transition and grass plots (Fig. 2.2). Establishment of shrubs increased transpiration (E) during spring and summer (season x zone interaction, F=9.16, $P < 0.0001$, Fig. 2.6). In spring, E was 2.5 - 9.5 x higher than transition and grass plots. In summer, shrub E was highest, with a 3 - 11 fold increase relative to transition and grass plots, respectively. Transition E trended toward being higher than grass zones by 12 and 14 mg H_2O m^{-2} s^{-1} in spring and summer respectively but was not statistically different in either season (Fig. 2.3).

Microclimate -

Shrub plots exhibited lower variance in both ground and air temperatures relative to grass and transition plots, most notably in in maximum air temperature. Shrub variance was 13.4°C and 18.2°C lower than transition and grass, respectively (shrub σ^2= 11.9, transition σ^2= 25.3, and grass σ^2= 30.1) (Fig. 2.4). Grass and transition plot maximum air temperatures were not normally distributed (K-S = 0.0073, $P < 0.001$ and K-S = 0.056, $P = 0.013$, respectively) where shrub plots were normally distributed (K-S = 0.041, $P = 0.167$). Annual temperatures in grass plots exhibited a wider range and higher variance (Fig. 2.4). Shrub plots had the lowest mean maximum air temperature throughout all of the seasons ($P \leq 0.0125$) (Fig. 2.5). Minimum ground temperatures were altered throughout all seasons as well; transition plots had consistently higher temperatures than shrub and grass. All three vegetation zones were different in summer for maximum air and ground temperature and minimum ground temperature ($P \leq 0.0125$).

Auto-regressive conditional heteroscedasticity (ARCH) showed that shrub zones exhibited a local effect of variance across maximum and minimum air temperatures as well as maximum ground temperature ($P = 0.0022$, $P = 0.0021$, $P = 0.0109$, respectively; Table 2.2). Grass zones had a marginal local effect of the temperature variance in minimum ground temperature only ($t = 2.03$, $P = 0.04$). Transition zones had a local effect in minimum air temperature only ($P = 0.03$).

Extreme warm temperatures in summer were higher in grassland plots relative to transition and shrub plots (Fig. 2.6). Shrub plots were cooler than grass in the summer by 18.4°C and the warmer in the winter by 2.5°C ($P = 0.0013$ and $P = 0.021$ respectively, Fig. 2.6). Winter and summer extreme temperatures did not differ between shrub and transition plots ($P = 0.058$, Fig. 6). Shrub zones exhibited cooler mean and maximum temperatures than transition and grass zones ($P<0.001$ for both) (Table s2.2). Annual mimimum temperatures did not differ statistically ($P=0.07$) (Table s2.2).

Discussion

Encroachment of *Morella cerifera* in coastal systems has consequences for landscape function by creating new ecological scenarios with increased shrub cover and loss of other species [33]. *Morella cerifera* rapidly expands across the landscape, creating full monospecific thickets within ~15 years after seedlings establish, and arrests succession preventing establishment of maritime forest [36]. We show that shrub presence decreases extreme temperatures and temperature variance, even in transition areas with freestanding shrubs that have not coalesced into thickets. Annual maximum temperature is lower within shrub thickets. The immediate effect of shrub presence impacts evapotranspiration, potentially creating a positive feedback with shrub growth [37]. Once shrubs coalesce into expansive thickets, they reduce biodiversity but increase productivity, a notable departure from expected biodiversity-productivity relationships [38-40].

Macroclimatic warming has been documented along the VCR over the last 32 years and associated with *M. cerifera* success [6, 28]. Here we show that microclimatic temperature moderation reduces summer extreme temperatures with shrub presence, even before coalescence into full thickets (i.e. 8.7 and 18.4 ^{o}C cooler in transition and shrub zones, respectively). The transition zone is similar to both grass and shrubs with respect to extreme winter temperatures. This expands on our previous understanding of microclimate modification by highlighting the gradual change in extreme temperatures [3, 13, 41-42].

Recent literature has demonstrated the importance of climate variance, as mean values may not fully represent climatic influence [10, 43]. Here we document through ARCH analysis that shrubs have a significant impact on temperature variance, creating self-reinforcing microclimate modification observed in both minimum and maximum temperatures. Shrubs also impact transition areas through modifying variance of minimum temperatures. As shrubs exhibit hydraulic failure when temperatures are < -15 ^{o}C [6], reduced temperature variance in winter months may be critical to the success of shrub growth. In this system, water is typically not limiting, especially in cooler, winter months [31]. Variability in temperature and water availability may have a larger role in shrub encroachment globally [9-10].

With the change from grassland to shrubland, we document 3- to 11-fold higher canopy transpiration (*E*) where shrubs are present (transition and shrub zones) relative to open grassland. This has implications for a system reliant on precipitation input [44-46]. When precipitation rate is low, shrubs access the freshwater lens, reducing groundwater, and potentially decreasing water availability for the surrounding plant communities in summer months, which can alter diversity and function [31, 47]. Most notably in summer, shrub presence increases transpiration, which may create a positive feedback wherein water evaporation accelerates evaporative cooling and decreases temperature stress, bringing microclimate closer to photosynthetic temperature optimum of 30 ^{o}C [48]. Microclimate modification within shrubs likely benefits vegetation function.

The homogenization of the landscape with *Morella* expansion is contrary to other extreme habitats, where encroaching species with open canopies provide refugia to more sensitive species [49]. In this system, transition zones reflect an amalgam between grass and shrub zones in microclimate measures and plant cover; however, our hypothesis that diversity would be reduced in these zones was not supported, and we did not find evidence of transition areas providing refugia for other species. In landscapes where encroaching shrub density is high and nitrogen is added via nitrogen fixation [34], a sharp decline in species richness is observed [50-51]. For barrier island systems, the decrease in diversity and homogenization of vegetative communities with fully formed thickets across the landscape has negative consequences by modifying sediment dynamics and reducing island migration in response to sea level rise [33].

Encroachment and altered PFT cover across the zones result in obvious structural complexity change with taller shrubs and more complex branching relative to grassland [35, 37, 51]. In many studies a standard functional trait used to assess differences in vegetative community functioning (i.e. SLA) is also observed to differ between woody and herbaceous communities [52]. In this coastal system, SLA was not statistically different among the zones despite the difference in PFT and plant forms (i.e. deciduous herbaceous vs. woody evergreen). There is a trend of lower SLA in grassland and higher SLA in shrubland. *Morella cerifera* has unique functional traits for an evergreen species that are representative of a resource acquisitive species [37, 52-53].

Identifying mechanisms of shrub encroachment in a vulnerable landscape is an important part of understanding the consequences for landscape change. Feedbacks between microclimate and transpiration alter overall landscape function change with the regime shift from grassland to shrubland. We use a novel time-series analysis to show that microclimate modification is self-reinforcing through temperature variance in plots where shrubs are present (i.e. both transition areas with free-standing shrubs and full monospecific thickets). Variance in abiotic niche constraints may play a bigger role in shrub encroachment in many habitats; ARCH may be used to better illuminate the drivers of woody expansion globally. Enhanced microhabitat and high physiological activity of the encroaching shrub likely accelerates expansion of *M. cerifera*, leading to a shrub monoculture. This expansion has consequences for ecosystem function as seen in high ANPP, but also creates new ecological scenarios that alter response of barrier islands to sea-level rise [37].

Methods

Study site

Research was conducted on Hog Island in Northampton County, VA 37°27′ N, 75°40′ W, part of the Virginia Coast Reserve (VCR), a National Science Foundation funded Long-term Ecological Research site owned and run by the Nature Conservancy. Hog Island is ~ 8 km from the Eastern Shore of Virginia, ~12 km long and undergoes less rollover and sediment erosion than smaller, lower topography islands. Between 1984 and 2016, shrub cover increased from 7% of upland to 29% on Hog Island [33] (Fig. 2.1). Shrub encroachment has been documented on many other islands in the VCR over the same timeframe[25, 33]. Due to heavy encroachment on Hog Island, we focused on the encroachment zone in the southern portion of the island as all suitable habitat, defined by elevation, has been occupied by shrubs north of our study location [29, 30, 54]. In 1984, the study area was composed of grassland, marsh, and sandy soils (i.e. bare) that may contain low vegetative cover (Fig. 2.1). By 1998, the area was fully converted to grassland with no interior marsh. Due to dispersal limitations, shrubs reached the southern portion of the island in the last decade, providing an opportunity to study the encroachment process [30]. Shrubs did not dominate the landscape until the late 1970s to early 1980s and this regime shift has been linked through experiments and models to fewer freezing events through since the 1970s [6, 23, 25]. Historical records indicate that maritime forest occurred on Hog Island in the early 1900s, however, since the 1980s, succession has been altered by development of tall monospecific shrub thickets of *M. cerifera* and lack of transition to maritime forest [29, 36]. Forested plots on other nearby islands have transitioned to dominance by *M. cerifera* (Zinnert, unpublished data). Once established, seedlings grow rapidly and shrub thickets persist on the landscape unless lost due to shoreface erosion from sea-level rise [33, 55].

Experimental design

A map of the VCR was created using ArcGIS version 10 imagery (Esri, Redlands, CA) (Fig. 2.2). Fifteen spatially disparate 5x5 m plots were established during the summer of 2014 and categorized as one of three vegetation zones: grassland, transition, and shrub zones (Fig. 2.2). Microtopography is variable at small spatial scales and plays an important role in vegetation composition due to access to the freshwater lens [32]. The range of elevation where *M. cerifera* exists is narrow[32] and grassland plots were placed at similar elevations to those of the transition and shrub plots to limit confounding factors. Further, each plot is considered independent as they are separated by geomorphic features such as interior dunes, relic marsh channeling with high salinity, and other topographic features that exclude *M. cerifera* establishment (Fig. 2.2). These geomorphic features allow for independence of all variables measured in this study. Each shrub plot was placed within thickets separated by the previously mentioned factors. Grass canopy height was ~ 0.7 m maximum, the canopy in the transition zone was ~1.5 m, and thicket canopy was > 4 m in height. Plot elevation ranged from 0.7 m to 1.6 m above sea level. Within each plot soil, surface and air temperature were measured hourly from July 2014 to September 2017 (HOBO U23-003, Onset Inc. Bourne, MA).

Species composition was quantified using the Daubenmire method at the end of the growing season [56]. Species composition was divided into plant functional type (PFT) and percent cover was normalized within each PFT to total cover. Shannon-Wiener Diversity index was calculated using the following equation:

$$H' = -\sum_{i=1}^{R} p_i \ln p_i$$

where p is the proportion of individuals of ith species.

Specific leaf area (SLA) of the two most predominant species per plot was assessed in 2016 by getting dry weight of 2 cm^2 leaf area for woody species and a linear portion of leaf for graminoid species of at least 4 cm long. Community-weighted SLA was calculated using up to the two most abundant species, accounting for species that represent >10% in each plot using the following equation:

$$\sum_{i=1}^{R} p_i \, t_i$$

where *p* is the normalized relative contribution of the species and *t* is the species SLA.

Stomatal conductance (Decagon Devices SC-1, Pullman, WA) was measured on north facing, sun-exposed branches of three to five plants of the two most prevalent species in each zone over three seasons. Stomatal conductance (g_s) was measured during spring, summer, and autumn of 2017 on the same day between 10:00 – 14:00 in all plots and restricted to sunny days (PPFD > 1300 $\mu mol\ m^{-2}\ s^{-1}$) to minimize confounding factors. Leaf area index (LAI) (Li-Cor 2200 C) was assessed annually at the end of the 2017 growing season. Leaf-level transpiration (*E*) was derived from g_s multiplied by daily vapor pressure deficit. Canopy *E* was calculated using up to the two most abundant species in each plot using the following equation:

$$\sum_{i=1}^{R} p_i \, t_i \times LAI$$

where *p* is the normalized relative contribution of the species and *t* is the leaf-level transpiration, multiplied by LAI to get canopy-wide transpiration [57-58].

Shrub biomass was collected by randomly selecting and clipping 50 shoots of the current year’s growth around the north side of the shrub crowns. Shoots were then dried at 60 °C for 72 hours. Annual net primary productivity (ANPP) was derived using the allometric equation for juvenile *M. cerifera* shrubs [59] :

$$ANPP = 707 + (31 * g\ shoot^{-1})$$

To estimate ANPP of herbaceous species, aboveground biomass in 10 x 100 cm plots was harvested at peak biomass. Biomass was dried and weighed as described above.

Statistical analysis

One-way analysis of variance (ANOVA) was used to determine differences in extreme temperature events of the 10% warmest temperatures in the summer and coolest temperatures in the winter, PFT, and SLA. ANPP across vegetation zones was analyzed using non-parametric Kruskal Wallace test due to unequal variances. Mean maximum temperature and canopy transpiration (*E*) were analyzed using two-way ANOVA among seasons and zones. Maximum detrended air temperature was tested for normal distribution using a Kolmogorov-Smirnov (K-S) test. Due to unequal variance and lack of normality, Kruskal Wallace test was used to compare among zones within seasons for maximum, minimum air and ground temperatures. A Bonferonni correction was applied to account for multiple comparisons. To compare across zones, temperature data were detrended to remove seasonal variance. Autoregressive conditional heteroskedasticity (ARCH) test of daily mean temperatures addressed temperature variance relative to local microclimate over time. This test, derived from economics, is a time series analysis to test for variance differences among zones; positive ARCH will exhibit high variance leading to high variance of the next day as described in Seekell et al. (2012) [60]. This is a novel approach to analyzing microclimate data.

Acknowledgements

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Additional information

Author contributions

LKW assisted with design of experiment, performed experiments, analyzed data, and wrote the manuscript. SH analyzed data and assisted with writing. JCZ conceived and designed the experiment and assisted with data analysis and writing. All authors reviewed the manuscript.

Competing interests

The author(s) declare no competing interests.

Figure Legends

Fig. 2.1. Shrub cover has expanded on Hog Island, Virginia since 1984 represented in red. Data are from Zinnert et al. [33]. The black box denotes the study area. Photos of shrub cover on the southern portion of the island from November 2017 provided by Julie C. Zinnert.

Fig. 2.2. Specific leaf area (SLA; left) and annual net primary productivity (ANPP; right) across the three vegetation zones. Values represent mean ± SE. The asterisk represents significant differences using a post-hoc Tukey Test.

Fig. 2.3. Two-way ANOVA of seasonal canopy E (mmol H_2O m^{-2} s^{-1}) across zones. Values represent mean ± SE. Letters indicate differences among season and zone based on Tukey post-hoc test.

Fig. 2.4. Frequency distribution of detrended maximum air temperature for grass, transition and shrub zones from Hog Island, Virginia.

Fig. 2.5. Mean maximum and minimum air and ground temperatures across the grass, transition, and shrub zones divided into season. Bars represent mean ± SE. Letter codes denote significant differences with Tukey test within each season among zones.

Fig. 2.6. The 10% most extreme temperatures during the winter (black) and summer (white). Values represent mean ± SE. Letters represent statistical differences by one-way ANOVAs and post-hoc Tukey tests.

Fig. 2.7. Study location of Hog Island, Virginia. Map produced using OpenStreetMaps from the ArcGIS basemap, permitted under an open license for academic and commercial use, created by Lauren K. Wood Approximate plot locations of vegetation types are overlaid on high resolution 2013 imagery [61]. Representative images of grass, transition and shrub zones. Photos provided by Lauren K. Wood.

Table 2.1 Plant functional type % cover using the Daubenmire method and Shannon-Wiener biodiversity across zones on Hog Island, VA. Values represent mean ± SE. Letters represent differences among zones according to the Tukey post-hoc test.

Variable	Grass zone	Transition zone	Shrub zone
Graminoid cover	86.1 ± 2.2^{a}	32.4 ± 2.2^{b}	0.9 ± 2.2^{c}
Forb cover	13.9 ± 2.4^{a}	6.7 ± 2.4^{ab}	1.0 ± 2.4^{b}
Woody cover	0 ± 0^{a}	60.9 ± 2.3^{b}	98.1 ± 2.3^{c}
H' Diversity	1.7 ± 0.1^{a}	1.6 ± 0.1^{a}	0.1 ± 0.1^{b}

Table 2.2 Autoregressive conditional heteroskedasticity results for maximum and minimum temperatures in grass, transition, and shrub zones on Hog Island, VA. Significant values in bold indicate local control of one day on the next.

Zone	Max air	Min air	Max ground	Min ground
Grass	*t*= 1.04, *P* = 0.2987	*t*= 0.54, *P* = 0.5894	*t*= 0.92, *P* = 0.3570	*t*= 2.03, *P* = 0.0425
Transition	*t*= 0.89, *P* = 0.3733	*t*= 2.24, ***P* = 0.0254**	*t*= 0.96, *P* = 0.3376	*t*= 2.01, *P* = 0.0448
Shrub	*t*= 2.98, ***P* = 0.0029**	*t*= 2.30, ***P* = 0.0217**	*t*= 2.35, ***P* = 0.0190**	*t*= 1.95, *P* = 0.0511

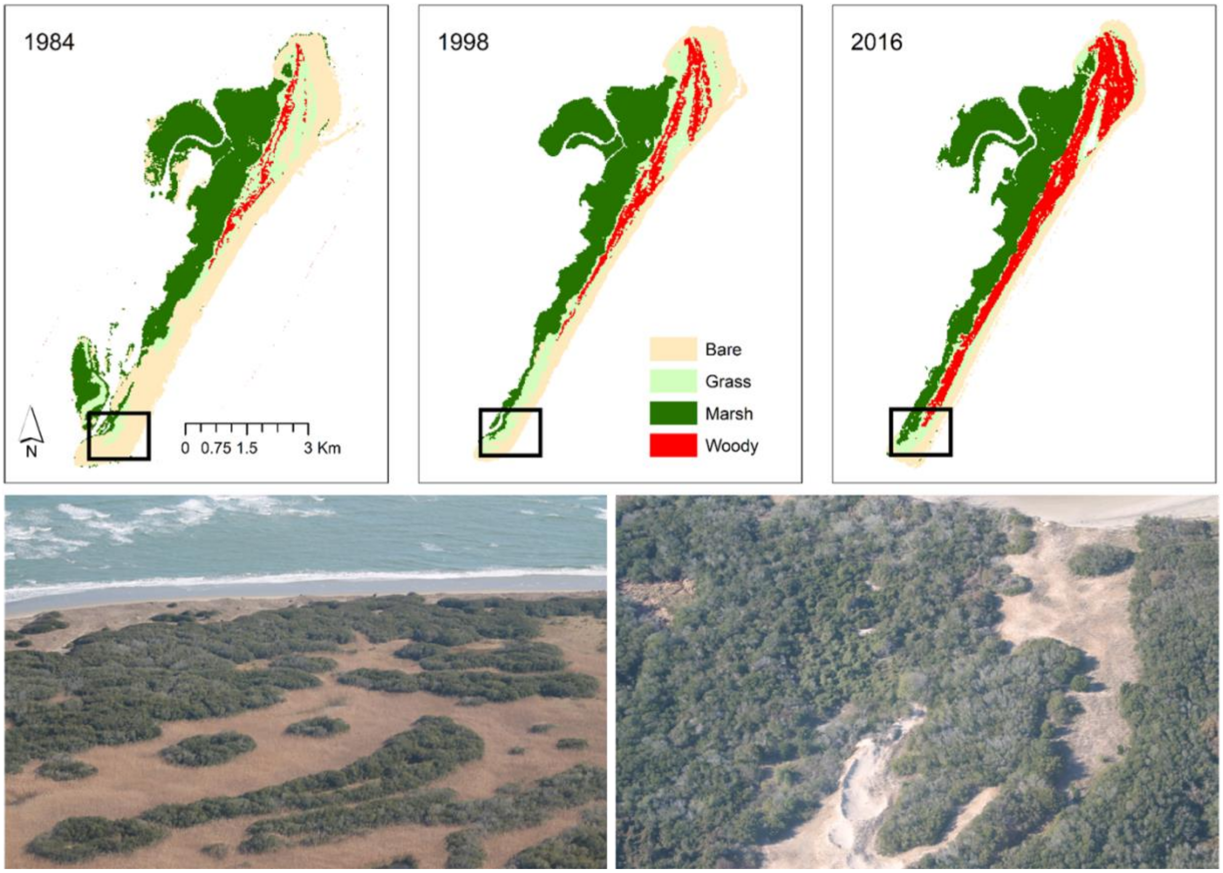


Figure 2.1

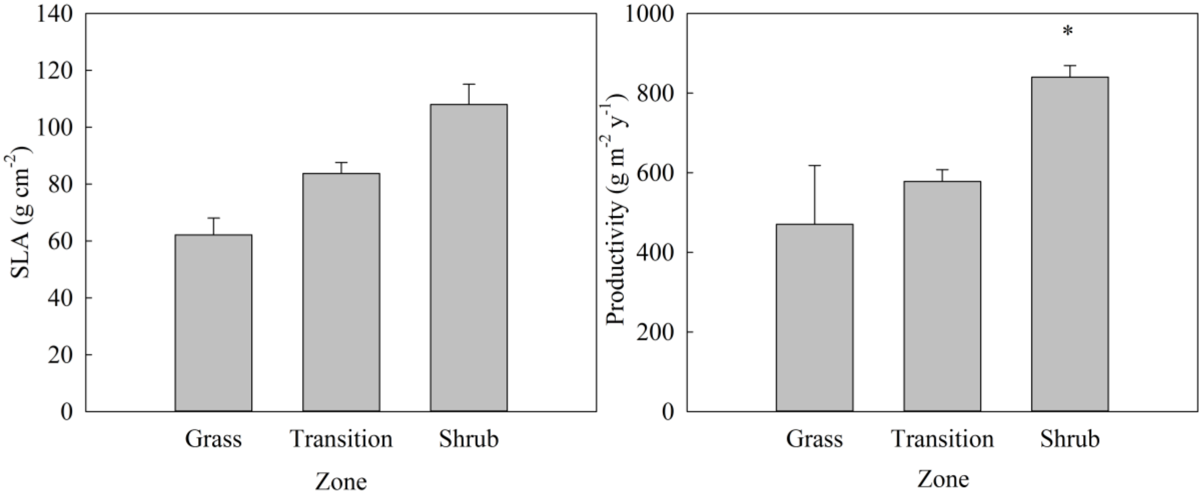

SLA (g cm-2)
140
120
100
80
60
40
20
0
Grass
Transition
Shrub
Zone
Productivity (g m-2 y-1)
1000
800
600
400
200
0
*
Grass
Transition
Shrub
Zone

Figure 2.2

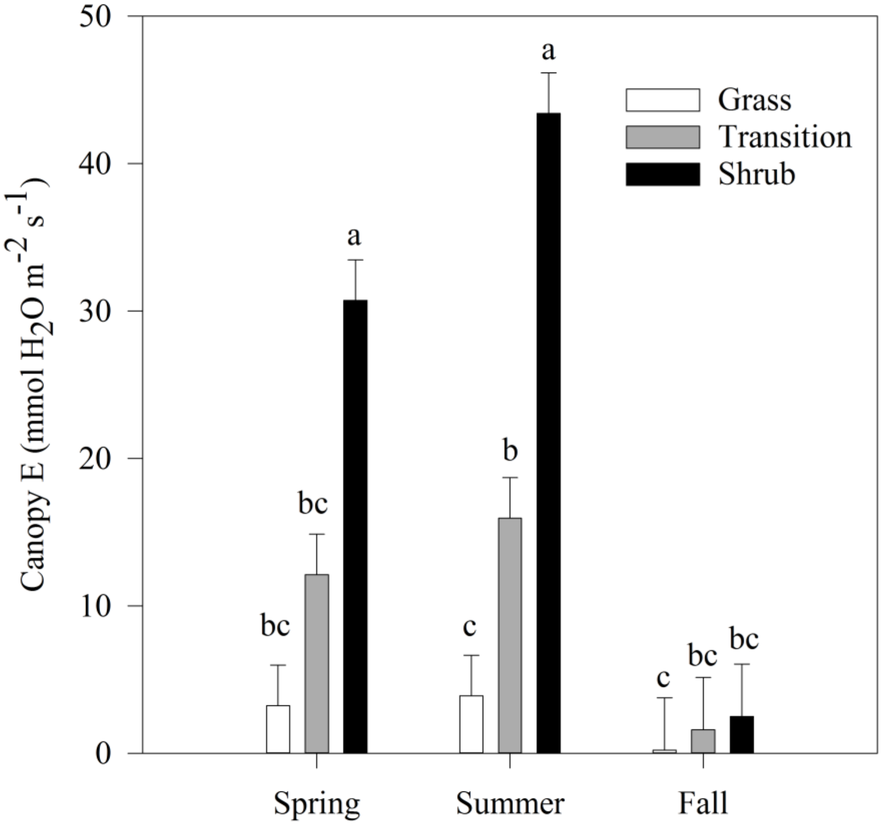

50
40
30
20
10
0
Canopy E (mmol H_2O m^{-2} s^{-1})
Grass
Transition
Shrub
bc
bc
a
c
b
a
c
bc
bc
Spring
Summer
Fall

Figure 2.3

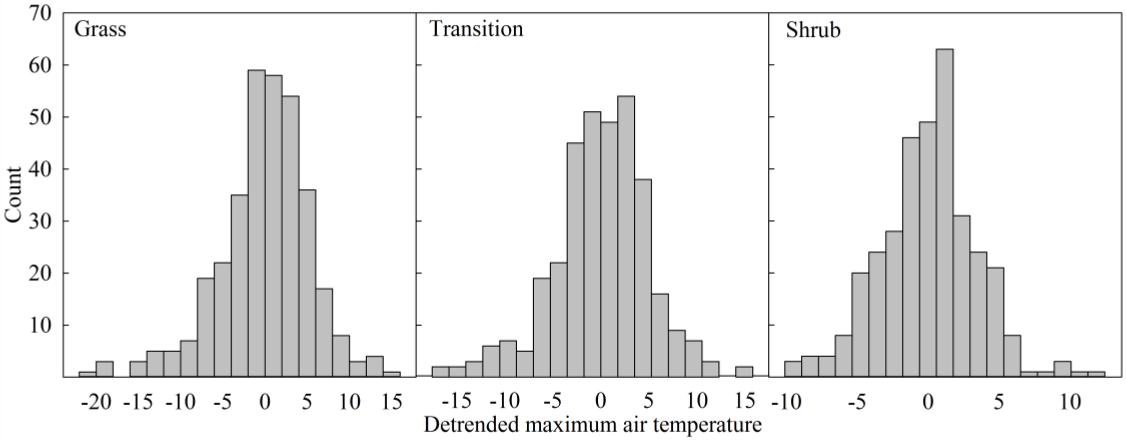
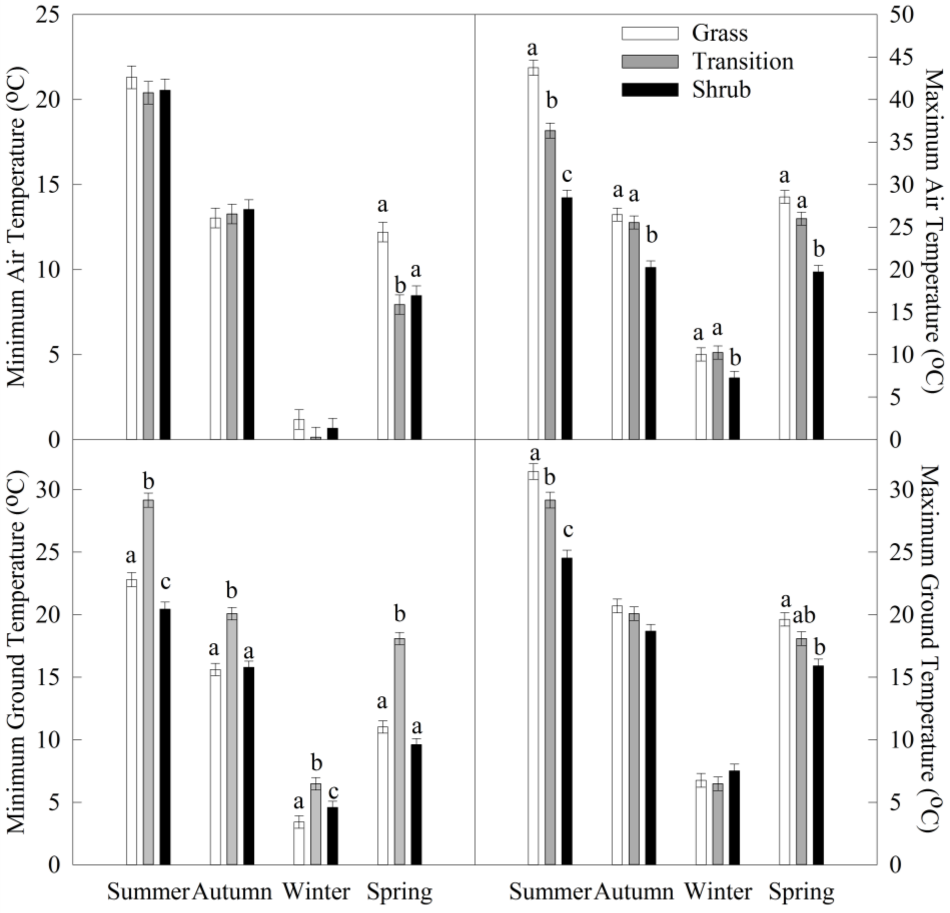

Grass
Transition
Shrub
Count
70
60
50
40
30
20
10
-20 -15 -10 -5 0 5 10 15
-15 -10 -5 0 5 10 15
-10 -5 0 5 10
Detrended maximum air temperature

Figure 2.4



Grass
Transition
Shrub
Minimum Air Temperature (oC)
Maximum Air Temperature (oC)
Minimum Ground Temperature (oC)
Maximum Ground Temperature (oC)
0
5
10
15
20
25
30
35
40
45
50
Summer
Autumn
Winter
Spring
a
b
c
ab

Figure 2.5

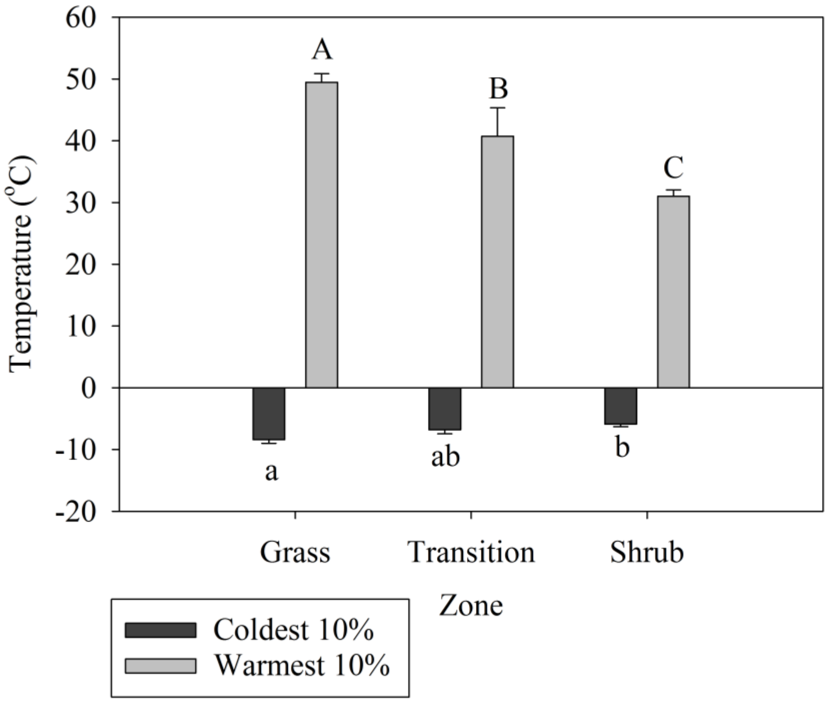

60
50
40
30
20
10
0
-10
-20
Temperature (°C)
A
B
C
a
ab
b
Grass
Transition
Shrub
Zone
Coldest 10%
Warmest 10%

Figure 2.6

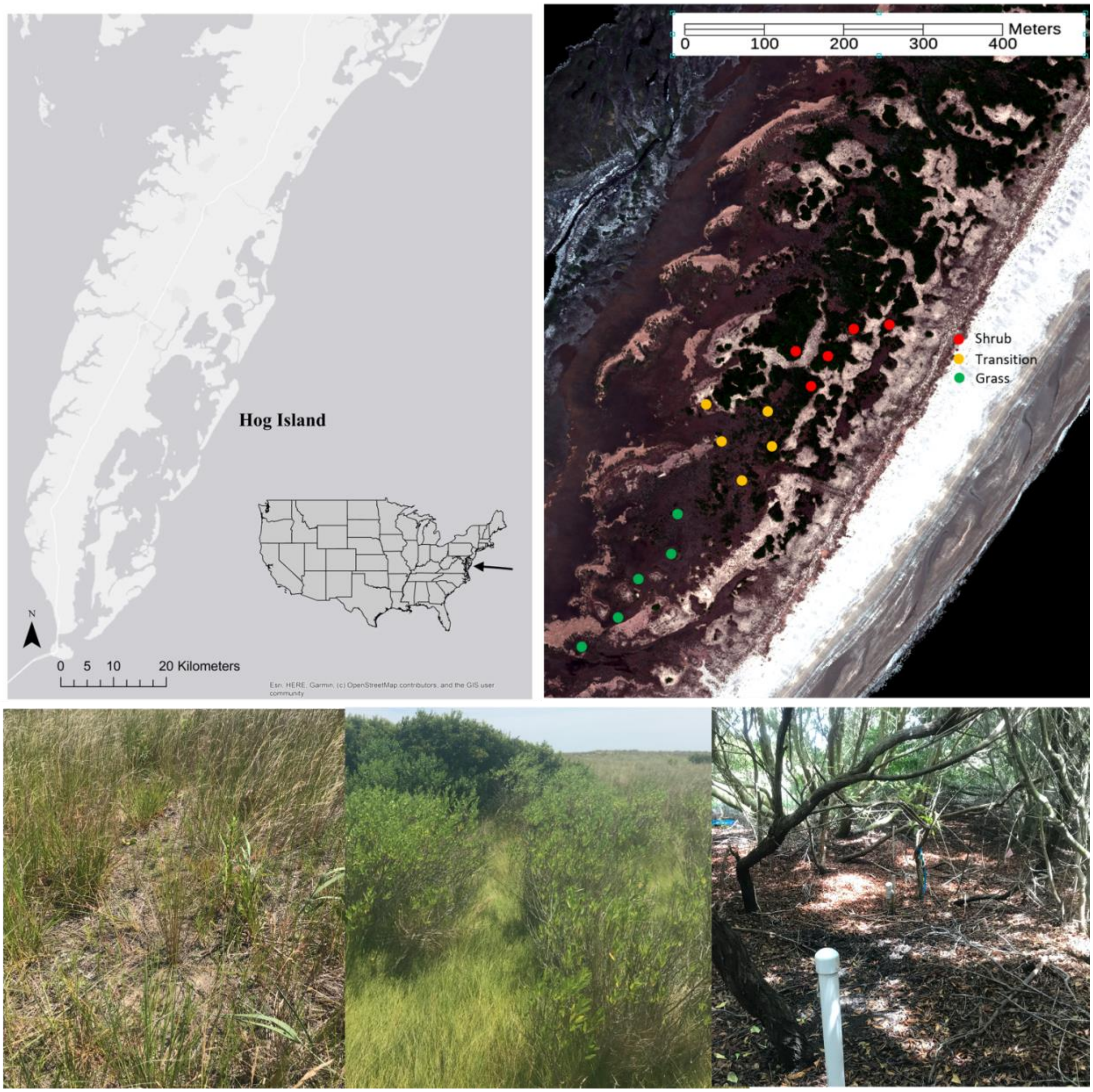

Hog Island
N
0 5 10 20 Kilometers
Esri, HERE, Garmin, (c) OpenStreetMap contributors, and the GIS user community
0 100 200 300 400 Meters
Shrub
Transition
Grass

Figure 2.7

Table s2.1 Mean, maximum, and minimum ± SE of annual air temperature among vegetation zones on Hog Island, VA. A Kruskal-Wallis test was performed among zones, Tukey post-hoc results are indicated by different letters ($p<0.05$).

Temperature measure	Grass zone	Transition zone	Shrub zone	H, p value
Mean	18.7 ± 0.5 ^{a}	16.8 ± 0.5 ^{a}	14.2 ± 0.4 ^{b}	30.3, $P<0.001$
Max	26.1 ± 0.8 ^{a}	23.8 ± 0.6 ^{a}	18.3 ± 0.5 ^{b}	62.1, $P<0.001$
Min	11.3 ± 0.5 ^{a}	9.8 ± 0.5 ^{a}	10.2 ± 0.5 ^{a}	5.4, $P=0.07$

Table s2.2 Mean ± SE of species percent cover across vegetation zones on Hog Island, VA

Species	Grass zone	Transition zone	Shrub zone
Morella cerifera	0 ± 0	76 ± 6	93 ± 3
Baccharis halimifolia	0 ± 0	4 ± 3	4 ± 3
Spartina patens	72 ± 6	62 ± 8	0 ± 0
Setaria parviflora	5 ± 3	11±7.2	0 ± 0
Solidago sempervirens	1 ± 1	1 ± 1	0 ± 0
Ammophila breviligulata	4 ± 3	0 ± 0	0 ± 0
Aster lanceolata	2 ± 1	0 ± 0	0 ± 0
Festuca geniuclate	0 ± 0	1 ± 1	0 ± 0

CHAPTER THREE

Ecosystem engineering of a native encroaching shrub

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Abstract

Shrubs are encroaching and acting as ecosystem engineers in many grasslands globally. Many studies have examined *in situ* microclimate in relation to shrub encroachment, but few studies have experimentally tested the effect of shrub expansion on ecologically critical abiotic factors. In this study, we evaluate the ecosystem engineering capacity of *Morella cerifera* by comparing temperature, water table depth, and soil properties in experimental clear-cut, shrub thicket, and grassland plots. Temperature variation was reduced by shrubs, while shrub removal increased the range of temperatures and created a temperature habitat more similar to that of the bare sand. Clear-cut plots had the lowest water table and driest soils at all depths, followed by grassland, and shrub plots had greater temporal variability within the water table than either grass or clear-cut, suggesting hydraulic lift. Shrubs have a lasting impact on soil bulk density through their effects on annual leaf detritus production, which is not immediately reversed when shrubs are removed along with the associated leaf-litter input. Ammonium and nitrate, assessed through ion-exchange, exhibits variability in concentration, likely due to leaching or rapid use by N-limited plants. There was higher total %N and %C in the shrub plot soil. Soil $\delta^{15}N$ was within the range of atmospheric fixation in all plots. Both shrub encroachment and removal create altered microhabitats within a stressful environment. Shrub removal creates a dry, warm environment where different vegetation re-colonizes. Understanding the local effects of shrub encroachment and removal and longer-term consequences of establishment contributes to modeling shrub growth under various climate scenarios and predicting barrier island response to sea-level rise.

Keywords: Shrub encroachment, ecosystem engineer, soil nitrogen, microclimate, water table

Manuscript highlights:

- Shrubs engineer habitat through temperature and soil water modification
- Shrub removal creates habitat that has variable temperature and dry soil
- Soil is wetter under shrubs with higher nitrogen content

Introduction

Shrub encroachment into previously grass-dominated landscapes is a global regime shift driven by climate and land-use change (Tape and others 2006, Ratajczak and others 2017, Huang and others 2018, Sabater and others 2020, Scharnagl and others 2019). In many cases, encroaching shrubs act as ecosystem engineers, modifying the microhabitat to better facilitate shrub productivity and expansion (D'Odorico and others 2010, Wood and others 2020). Climate change presses and pulses, such as increased temperatures and altered storm frequency, along with land-use change, drive shifts in vegetation communities toward woody vegetation (Brunsell and others 2017, Guo and others 2017, Ratajczak and others 2017, Huang and others 2018, Huang and others 2020). Vegetation regime shifts, whether from sudden natural disaster, anthropogenic development, or establishment of invasive species, alter the immediate habitat, including temperature, air movement, and water vapor (Pielke and others 1998, Bonan 2008, Thompson and others 2017). The change in microclimate can alter physiological functioning of the current vegetation, creating a feedback to further modify microclimate (D'Odorico and others 2010, Geissler and others 2019, Wallace and Baltzer 2020, Wood and others 2020). Productivity increases with new vegetation composition, though it is precipitation dependent; annual net primary productivity (ANPP) in xeric systems or dry periods is less likely to increase relative to mesic sites (Knapp and others 2007, Aguilar and others 2012, Rolo and others 2016).

Shrub removal typically increases species diversity (Berlow and others 2003, Chun and Choi 2012, Ding and Eldridge 2019, Liu and others 2019, Sundqvist and others 2020) and may be related to accompanying abiotic changes. Removal of encroaching woody vegetation, both mechanically or via prescribed burning, can help restore species to pre-encroached vegetation (Williams and others 2017) and increase infiltration of precipitation into soil while decreasing erosion (Williams and others 2019). Natural occurrences of dieback can result in a temporary change in vegetation but many of the encroaching shrubs are effective at re-sprouting (Ladwig and others 2019). While many papers look at the effectiveness shrub removal protocols and management practices on restoring plant communities (Bestelmeyer and others 2018, Watson and others 2019, Williams and others 2020), very few address microclimate differences with shrub removal.

Removal of woody vegetation in drylands increases temperature and decreases growing season, biomass and evapotranspiration (Marchesini and others 2015). Much of the research on removal of encroaching woody vegetation occurs in arid and sub-alpine landscapes, with fewer studies in temperate, mesic systems, which often respond differently to abiotic factors (Jackson and others 2002, Ariza and Tielbörger 2011, Metz and others 2020). Soil moisture and distance to water table can be transformed by shrub presence and removal (Ansley and others 2018). Additional litterfall input from shrubs and fine root turnover increases water holding capacity while removal can decrease drawdown of the water table (Stevenson and Day 1996, Dzikiti and others 2013, Hanslin and Kollmann 2016, Ding and Eldridge 2019, Li and others 2018, Tölgyesi and others 2020). Shrub encroachment into mesic systems can lead to increased water use and, in the case of *Salix* species encroaching into Florida, can dry up a wetland (Budny and Benscoter 2016). Mesic habitats may be encroached by plants with high transpiration, followed by dry down, but with little change in precipitation input, the soil moisture does not decrease to the point of limiting plant productivity (Sabater and others 2020). Drier climates often have increased soil moisture as a consequence of shrub encroachment (Price and Morgan 2008) but can prolong the effects of drought due to higher water usage and need for greater recharge by precipitation (Hao and others 2019).

In addition to altered vegetative composition and microclimate effects, shrub encroachment can also lead to altered soil microbial and physical properties (Liao and others 2008, Van der Putten and others 2013, Collins and others 2016, Collins and others 2019, Kinnebrew and others 2020, Liu and others 2020). Change in dominant woody species can alter soil properties such as organic matter, phosphorus, and N in many habitats (Hughes and Enslow 2005, Medina-Villar and others 2015). Nutrient stocks in soil are often increased with change from forb and graminoid-dominated habitats to increased woody vegetation (Pinheiro and others 2016, Zhou and others 2018).

Coastal systems are particularly susceptible to the pressures of climate change as plant communities and ecological interactions are affected by both atmospheric (i.e. temperature and precipitation) and oceanic (i.e. sea level rise and storm damage) drivers (Ezer and others 2013, Pivovaroff and others 2015, Charles and others 2020). Along the Virginia coastline, warming climate is resulting in expansion of native shrubs into previously grassland dominated communities (Huang and others 2018). This is also seen along the US Atlantic and Gulf coasts (Devaney and others 2017, Charles and others 2020). The Virginia barrier islands have undergone rapid shrub encroachment over the past 32 years, despite a loss in land area (Zinnert and others 2016, Zinnert and others 2019). *Morella cerifera* is a native, evergreen shrub with an obligate association with N-fixing *Frankia* (Wijnholds and Young 2000). Expansion of *M. cerifera* is primarily driven by increasing winter minimum temperatures, reducing cold-induced damage, and enhanced microclimate (Thompson and others 2017, Huang and others 2018, Sinclair and others 2020). On Hog Island, VA, *M. cerifera* establishes in swales behind 1.5 – 2 m tall primary dunes where they are mostly protected from salt intrusion and have access to the freshwater table (Young and others 2011, Zinnert and others 2019).

Altered nutrient and water availability with coastal shrub encroachment contributes to changes in vegetation composition, with reduced biodiversity, and reinforced vegetation regime shift (Linders and others 2019, Wood and others 2020). Establishment of *M. cerifera* results in higher ANPP, which decreases as thickets age, and leaf litter increases the carbon and N pool in the soil (Brantley and Young 2008). Sandy soils, as in coastal systems, are typically characterized by rapid nutrient leaching and fast water drainage, even following precipitation input as there is little water retention in sandy soil unsaturated by the water table (Hayden and others 1995, Chen and others 2006, Zhou and Zhao 2019). On barrier islands, woody species rely on precipitation inputs to the freshwater lens and shrub growth is closely related to precipitation and water table depth (Aguilar and others 2012, Masterson and others 2014). While shrubs in dry locations can contribute to desertification reversal, in more mesic sites woody expansion can increase water loss to atmosphere (Maestre and others 2009, Budny and Benscoter 2016). Sea level rise leads to temporarily altered salinity in the freshwater lens through inundation, resulting in changed plant composition, but the little work has been done to understand influence of encroaching shrubs on depth to the freshwater lens (Ogurcak and others 2019).

In this study we experimentally tested the ecosystem engineering of a native encroaching shrub, *Morella cerifera*, into coastal grassland, the legacy influences of shrub establishment on the habitat, and recovery of vegetation through shrub removal. Specifically, we investigated the influence of shrub presence and shrub removal on microclimate (i.e. temperature and water table depth), soil characteristics, and vegetation composition relative to unencroached grassland. We hypothesized that the increased canopy cover of shrub decreases depth to water table, increases soil moisture and, soil N and C content, and soil inorganic N content, and moderates temperatures relative to grassland and areas where shrubs have been removed.

Methods

Study site:

Research was conducted on Hog Island in Northampton County, VA (37°27′ N, 75°40′ W), part of the Virginia Coast Reserve, a National Science Foundation funded Long-Term Ecological Research site owned and run by the Nature Conservancy. Hog Island is ~ 8 km from the Eastern Shore peninsula of Virginia, ~12 km long and undergoes less rollover and sediment erosion than smaller, lower topography islands (Zinnert and others 2019). The shrub encroachment on Hog Island has been well documented (Zinnert and others 2016, Huang and others 2018, Woods and others 2019, Zinnert and others 2019, Wood and others 2020) and thus presents the opportunity to experimentally test facets of shrub ecosystem engineering.

Experimental design

Three vegetation zones were experimentally established across the landscape: grass, shrub, and shrub removal (hereafter "clear-cut") zones (Fig 3.1). In 2015, 5 x 5 m separate plots were established in un-encroached grassland and fully formed thickets (n=5). In early spring 2016, 10 x 10 m plots were clear-cut of fully mature shrubs, wherein 5 x 5 m plots were established with a surrounding 2.5 m buffer zone (n=3). Resprouts were cut back annually to maintain plots as clear-cut. To ensure independence of plots, microtopography was similar within all plots. Microtopography plays a limiting role in vegetation composition due to access to the freshwater lens (Masterson and others 2014); it is variable at small spatial scales creating microhabitats where higher elevation areas have limited water availability. The range of elevation where *M. cerifera* exists is narrow and grassland plots were placed at similar elevations to those of the shrub and clear-cut plots to limit confounding factors. Further, each plot is considered statistically independent as they are separated by geomorphic features such as interior dunes still present after beach accretion, relic marsh channeling with high salinity, features that exclude *M. cerifera* establishment (Fig 3.1). These geomorphic features allow for independence of all variables measured in this study. In the clear-cut plots, the top layer of non-decomposed leaf litter was raked and removed to remove the maximum amount of shrub influence and make the clear-cut plots as similar to grassland plots as possible to best test microclimate. Roots were not exhumed to minimize plot soil disturbance. Precipitation data was acquired from NOAA NCEI datasets and summarized by month from June 2016 through July 2018 (provided by the NOAA/ESRL Physical Sciences Laboratory, Boulder Colorado from their web site at https://www.psd.noaa.gov/).

Each plot included ground and air temperature sensors (U23-002, OnSet, Bourne MA). These sensors took hourly measurements from December 2016 until June 2018 and were downloaded 3-6 times annually. Water table sensors (U20L-04, OnSet, Bourne, MA) were installed in each vegetation zone (n=3) and monitored from June 2016 - June 2018 and downloaded at the same time as the temperature sensors. Water table depth is tightly tied to precipitation (Eeman and others 2010). Precipitation across the study period shows a mesic habitat with high seasonal variability (Fig 3.2). The maximum monthly rain over the study period was 243 mm, the minimum is 11 mm, and the monthly mean is 87 mm (Fig 3.2).

To get a snapshot of zone soil moisture and bulk density, to obtain samples for elemental analysis, soil cores were collected in 2018 from 3 plots in each vegetation zone (3.2 cm diameter, 30 cm depth). Cores were separated into 10 cm depth increments, placed into a plastic bag and processed immediately upon returning from the field. Cores were weighed to get water mass before and after drying at 105°C for 72 hours. Bulk density was calculated by dividing dry weight by volume. Soil subsamples were sent to Cornell Isotope Lab in order to get elemental %C, %N, and $\delta^{15}N$.

Ion-exchange resin bags (IERBs) were used to assess ammonium (NH_4) and nitrate (NO_3) within the soil over the 2018 growing season (Lajtha 1988). PVC collars (2.5 cm radius, 7.8 cm height) containing resin beads were placed in the soil in April 2018 so the top of the PVC collar was level with the soil surface; charged N molecules were extracted and analyzed using methodology of Nave and Lemoine (2009). IERB bags were collected in September 2018, rinsed with deionized water, and resins were removed from the bag. Resins were extracted in a specimen cup using 30 ml of 2M KCl overnight on a shaking table. The extractant was filtered through a 0.45 um Durapore filter (3M Corporation, Minneapolis, MN) using a vacuum to speed the process. The resulting solution was analyzed for NO_3 and NH_4 content using a microplate reader following the protocol in Sims and others (1995).

Species composition was assessed in late summer 2017 and 2019 using the Daubenmire method (Daubenmire 1959), which allows greater than 100% cover to account for canopy layers. Using this method will often lead to over 100% cover, accounting for upright plant growth strategies. Vegetation composition was normalized to a percentage of total cover. Due to weather constraints, species composition was not documented in 2018.

Statistics:

Temperature was examined annually and compared among zones. The warmest 10% of summer days and coldest 10% of winter days were compared among zones using 1-way ANOVA to assess extreme temperatures. To determine the effect zone has on water table measurements, a repeated measures mixed model was employed to measure zone while controlling for the month in which the measurement occurred. To examine differences in variance among the three zones, a preliminary model was fit the data for each site in each zone in order to detrend the data and adjust for trend, season, and local temporal dependence. Predictions from the model were subtracted from the raw data to create a detrended series for each zone and site. Pairwise tests for variances among the three zones were computed as an overall comparison. The same analysis was run seasonally to determine the variability among the zones within each quarterly season. For soil parameters derived from cores (i.e., relative soil moisture, bulk density, and isotopes), two-way ANOVA was conducted using depth and zone; significant results are reported in the text. A two-way ANOVA was run on species composition data to compare among zones and years. Significant interactions in two-way ANOVAs were followed by a post-hoc Tukey test. When interactions were not significant, main effects comparisons were analyzed using Tukey test. Statistical analysis was done using SAS 9.4 for the repeated-measures mixed model and Sigma plot for ANOVA and non-parametric alternatives.

Results

Shrub encroachment and removal both produce altered microclimates with different vegetation composition than un-encroached grassland. Annual minimum air and ground temperatures were cooler in clear-cut plots than in grass and shrub plots (F = 25.8 $P < 0.0002$ and F = 9.8 $P < 0.0001$, respectively, table 3.1). Annual mean air temperatures did not differ between grass and clear-cut but both were warmer than shrub plots (F = 9.7 $P < 0.005$, table 3.1). Annual mean ground temperatures did not differ among grassland, shrub, or clear-cut zones. Annual maximum air temperatures were significantly warmer in clear-cut plots than shrub plots by ~14 ºC (F = 9.3, $P = 0.005$) with grass plots exhibiting an intermediate (table 3.1). Annual maximum ground temperatures in clear-cut plots were warmer than those of grass and shrub plots by 13 ºC and 11 ºC, respectively (F = 21.2, $P < 0.0001$, table 3.1). The warmest summer temperatures were coolest in shrub plots for ground and air temperatures (F = 30.1, $P > 0.0001$; F = 111.9, $P > 0.0001$, respectively, Fig 3.3). Clear-cut plots had the warmest air extremes, ~ 14°C warmer than grass and 30°C warmer than shrub ($P < 0.0001$). Extreme ground temperatures in winter were warmest in clear-cut plots by 7°C (F = 328.7, $P > 0.0001$) and extreme winter air temperatures were different among all three zones (F = 46.2, $P > 0.0001$, Fig 3.3). Shrubs had the warmest winter air temperatures and clear-cut plots had cooler winter air temperatures than grass and shrub by ~ 3 and 7°C, respectively.

Water table depth varied by zone; the deepest water table seasonally was consistently in clear-cut plots and the shallowest was in shrub plots (F = 21.9 $P = 0.001$, Fig 3.4). Grass plots had a deeper water table than shrub plots by ~ 1 cm (Fig 3.4). Detrended variability in water table depth for the entire timescale showed significant differences among all three zones with shrub plots exhibiting the most variability, and grass plots had the lowest (table 3.2). Seasonally, detrended variability was highest in shrub plots across all seasons (table 3.2).

Soil chemical and physical properties differed occasionally among zones and depth. Summer soil moisture exhibited a significant interaction between zone and depth (F = 6.1 $P < 0.003$). Shrub soils had > 4-fold higher water content in shallow soils (0-10 cm) than grass and clear-cut plots; clear-cut soils were driest (Fig 3.5). Bulk density exhibited an interaction between zone and depth (F = 4.0, $P = 0.018$). Grass and clear-cut plots did not differ with depth, but shrub plots had lower bulk density at 0-10 cm, compared to 21-30 cm (Appendix 1). Soil % N differed by zone (F = 5.1, $P = 0.018$) and depth (F = 20.1, $P < 0.001$) and was highest in shrub soils with clear-cut soils being an intermediate. Across zones, % N was highest in the first 10 cm (Appendix 3.1). The same trend was seen in soil % C (zone: F = 5.3, $P = 0.02$; depth: F = 12.7, P

< 0.001, Appendix 3.1). Soil $\delta^{15}N$ were all within the range of -1 to 1 and differences were only seen among zones with grassland and clear-cut $\delta^{15}N$ differing (F = 4.8, *P* = 0.02, Appendix 3.1). Soil NO_3 and NH_4 showed no differences but exhibited high variability among zones (H = 2.2 *P* = 0.5 and F = 1.4, *P* = 0.3, respectively, Appendix 3.1).

Vegetation composition was dissimilar among zones during 2017 (table 3.3). The shrub, *Morella cerifera*, dominated shrub plots (100 % cover), while the C4 grass, *Spartina patens*, dominated grass plots (~47 % cover), and the C3 grass, *Ammophila breviligulata*, was prevalent in clear-cuts (~37% cover) (table 3.3). Overall species richness was low, ranging from 2-12, but the shrub plots had 7-10 fewer species than the grass and clear-cut plots, respectively (table 3.3). Clear-cut plots transitioned from *A. breviligulata* dominated to *S. patens* dominated by 2019. Total species cover, which accounts for different canopy layers of plants, increased in clear-cut plots in 2019 but the increase was not statistically different (table 3.3).

Discussion

Globally, shrubs are expanding into grasslands and altering habitat microclimate, including local hydrology. The objective of this study was to test microclimate modification and legacy influence by shrubs, through removal. Along the mid-Atlantic coast, shrub expansion due to warming climate affects local and ecosystem level processes through microclimate modification, exclusion of grasses, and creates a shrub monoculture. Shrubs also have landscape level consequences by limiting natural sediment redistribution processes, and thus the response of barrier islands to sea-level rise (Zinnert and others 2019, Reeves and others in prep).

In our temperate mesic environment, both establishment and removal of shrubs modifies soil and microclimatic properties, engineering a different habitat from that of the historical grassland, resulting in dissimilar vegetation composition. Shrub removal supports recolonization of grasses and forbs in many habitats (Berlow and others 2003, Williams and others 2017, Ding and Eldridge 2019, Watson and others 2019), but the success of restoration depends a great deal on shrub size and removal method. Shrubs moderated air and ground temperatures. Shrub removal caused extreme temperatures and higher variation, with microclimatic temperatures similar to those of sparsely vegetated sand (Sinclair and others 2020). A wide range of extreme temperatures likely contributes to the establishment of different species than un-encroached grassland, leading to novel grassland composition. Fragmented vegetative landscapes lead to cooler temperatures in the winter where vegetation is sparser due to altered edge effects (Latimer and Zuckerberg 2017) while the physical characteristics of the exposed darker soil after shrub removal can potentially account for warmer summer temperatures. Grassland plots tended to have warmer temperatures than shrubs and minima were not as cold as the clear-cut plots.

In addition to microclimate modification, shrubs increased mean and variability of water table depth. Clear-cut plots consistently had the lowest water table (-1.03 m, ~13cm lower than shrub plots), likely due to extreme temperatures and exposure of the dark soils to full sun (Tudoroiu and others 2018), and the lowest variability in water table depth. Shrub plots exhibited greatest variability across all seasons, agreeing with findings of water table fluctuation over shorter timescales in arid riparian habitats (Yue and others 2016). Variability in water table depth is supported by much higher transpiration in shrubs (> 20 mmol H_2O m^{-2} s^{-1}) relative to grassland, as well as the ability to transpire year-round due to the evergreen nature and high efficiency of water movement and use compared to other woody evergreens (Shiflett and others 2014, Shiflett and others 2017, Wood and others 2020). Despite higher variability, the water table was shallowest in shrub plots, but only by ~1cm (annually) compared to grassland plots. Rooting depth for grasses and shrubs is concentrated in the upper 20 cm of soil (Conn and Day 1993), thus a difference of 1 cm at ~ 90 cm depth could affect access by plants to the freshwater lens. Exposure of darker soil from decomposed leaf litter in clear-cut plots to sunlight likely facilitates early warming in spring (Hinzman and others 1991) which may affect seedling establishment.

Although water table depth was highly variable in shrub plots, the enclosed canopies of shrubs temper soil moisture loss and subsequent removal can accelerate it (Liu and others 2020). Vegetation colonized clear-cuts after shrub removal, but exposed soil resulted in increased evaporation from soils and thus low soil moisture (Ács 2003). This is contrary to findings of increased soil moisture post-removal in semi-arid and montane habitats (Fan and others 2018). Sandy coastal soils drain water very quickly, with organic matter playing an influential role in water retention (Rawls and others 2003), thus vegetative cover and leaf litter input can vastly improve soil moisture. Grassland plots, with comparatively less shading than shrub plots, had an intermediate level of soil moisture.

Shrub plots had the highest soil moisture than all other plots, likely due to increased boundary layer with shrub vegetation and decreased sun flecks through the dense canopy (Ács 2003, Brantley and Young 2008). At 11-20 cm depth, there was reduced soil water content in shrubs which is likely due to dense fine roots in the upper 20 cm of soil (Conn and Day 1993) and high water use by shrubs (Shao and others 1995, Aguilar and others 2012, Wood and others 2020). With regards to bulk density, shrub plots were the least compact, grasses were the most compact, and clear-cut plots an intermediary. Shrub plots have continued input of leaf litter annually (Brantley and Young 2008), maintaining a high organic matter content which promotes lower bulk density and higher soil moisture. This suggests that over time, shrub removal could revert back to pre-encroachment soil characteristics.

Lack of differences among zones in bioavailable N (NO_3 and NH_4) was unexpected given the N-fixation symbiosis and high N content of *M. cerifera* litter (Brantley and Young 2008, Turpin-Jelfs and others 2019). Movement of bioavailable N across the landscape over time could account for the lack of difference among zones as IERBs measure soil N species over time while the other soil measures were from a spot measurement. High variation in bioavailable N within grass and clear-cut plots could mask differences among zones; however, coastal soils are unique due to the low water and nutrient holding capacity which may further complicate soil dynamics. Soil $\delta^{15}N$ values among all zones were -1 to 1, indicating the potential for N-fixing bacteria within the rhizosphere of grassland species leading to increased $\delta^{15}N$ within those soils (Teal and others 1979, Bagwell and others 2001, Yousuf and others 2014, Craine and others 2015, Brown and Zinnert 2018). Shrubs in this study were relatively young (<30 years old) (Woods and others

2019) and may not have built up sufficient N in the soil despite significant input of leaf litter (Brantley and Young, 2008). Shrub plots had higher C and N in soil than grass plots, which was more evident in shallow soils, and is similar to other studies of encroachment effects on soil (Collins and others 2016); clear-cuts exhibit similar soil C and N to grassland and shrubland indicating an intermediary habitat with the potential to revert back to grassland characteristics. Sandy soils may also quickly leach nutrient input out of the soil into the groundwater (Osman 2018, Li and others 2018, Huang and Hartemink 2020, Zinnert unpublished data) and moved via groundwater flow. The availability of soil N can also be influenced by the decomposition rates, which can be lower in areas that are frequently saturated (Smith and Day 2017) – thus the shallowness of the water table in *M. cerifera* habitat could affect the amount of N held in soil.

The range of extreme temperatures coupled with low soil moisture in the clear-cut plots acts as an environmental filter, presents challenges for establishment of vegetation. Sandy soils tend to drain very quickly and thus the access to water can be very limiting with little addition of water-holding organic matter. Physically, soil characteristics in clear-cut plots have recovered from shrub encroachment/removal and are more similar to grassland, but vegetation remains different three growing seasons following removal. Clear-cut plots have differed in cover and dominant vegetation, suggesting that recovery is impacted by the removal of such a prevalent ecosystem engineer. The clear-cut plots had a greater prominence of the C3 dune building grass, *Ammophila breviligulata.* This dune building grass is more prevalent on dune crests and tend to establish in the extreme environment of bare or sparsely vegetated sand. *Spartina patens* is the most common grass in unencroached grassland, though *A. breviligulata* could have established within the clear-cuts facilitated by excess N, depleting the soil to a similar N content to grassland (Day and others 2018). Clear-cut plots increased *S. patens* cover by 2019, but total cover was higher than unencroached grasslands. Studies in other habitats show removal does not alter vegetation composition (Liu and others 2019) but tends to promote increased diversity in mesic areas such as ours (Ding and Eldridge 2019). In comparison with shrub plots, clear-cuts can only increase in species diversity or richness from a monoculture.

Shrub encroachment on the Virginia barrier islands results in an engineered microhabitat where temperature is moderated, the water table fluctuates more, and soil characteristics are altered through annual leaf litter input and increased shading by shrubs relative to grassland (Fig 3.6). By removing shrubs, we could both test the microclimate modification and understand any legacy effects on the microhabitat – shrub removal created a novel habitat with a wide range of extreme temperatures, deeper water table, and drier soil, allowing for grass and forb vegetation to eventually colonize, but in differing amounts relative to un-encroached grassland. This unique microhabitat reflects potential edge effects encroaching *M. cerifera*, wherein the shrubs facilitate establishment of seedlings and other species within a refuge habitat in an otherwise harsh environment. These expanding shrubs affect the abiotic and biotic habitat on a smaller-scale and understanding the local impacts of shrub encroachment contributes to modeling shrub growth (Huang and others 2018, Huang and others 2020) and predicting barrier islands response to sea-level rise under future climate scenarios (Zinnert et al. 2019).

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Figure 3.1. A map of the approximate locations of plots on Hog Island, VA (Google Earth Pro 2013 imagery).

Figure 3.2. Monthly precipitation from the VCR Hog Island meteorological station from June 2016 to July 2018 (*DOC/NOAA/NESDIS/NCDC*). The line represents the mean monthly precipitation across the time scale.

Figure 3.3. The 10% warmest summer temperatures and coldest winter temperatures among the zones ±SE. Letters represent post-hoc comparisons via Tukey test, where differing letters exhibit statistical differences. Capitalized and lowercase letters indicate which measurements are compared – i.e. maximum air temperatures are only compared to maximum air temperatures among zones

Figure 3.4. Histograms of daily mean water table depth by zone and annual mean water table depth.

Figure 3.5. Relative soil moisture among zones and depths. A two-way ANOVA was run with a post-hoc Tukey test to compare among depths and vegetation types. Differing letters indicate differences in % moisture as indicated by the Tukey test.

Figure 3.6. A conceptual framework showing where microclimate modification fits into the bigger consequences of shrub encroachment.

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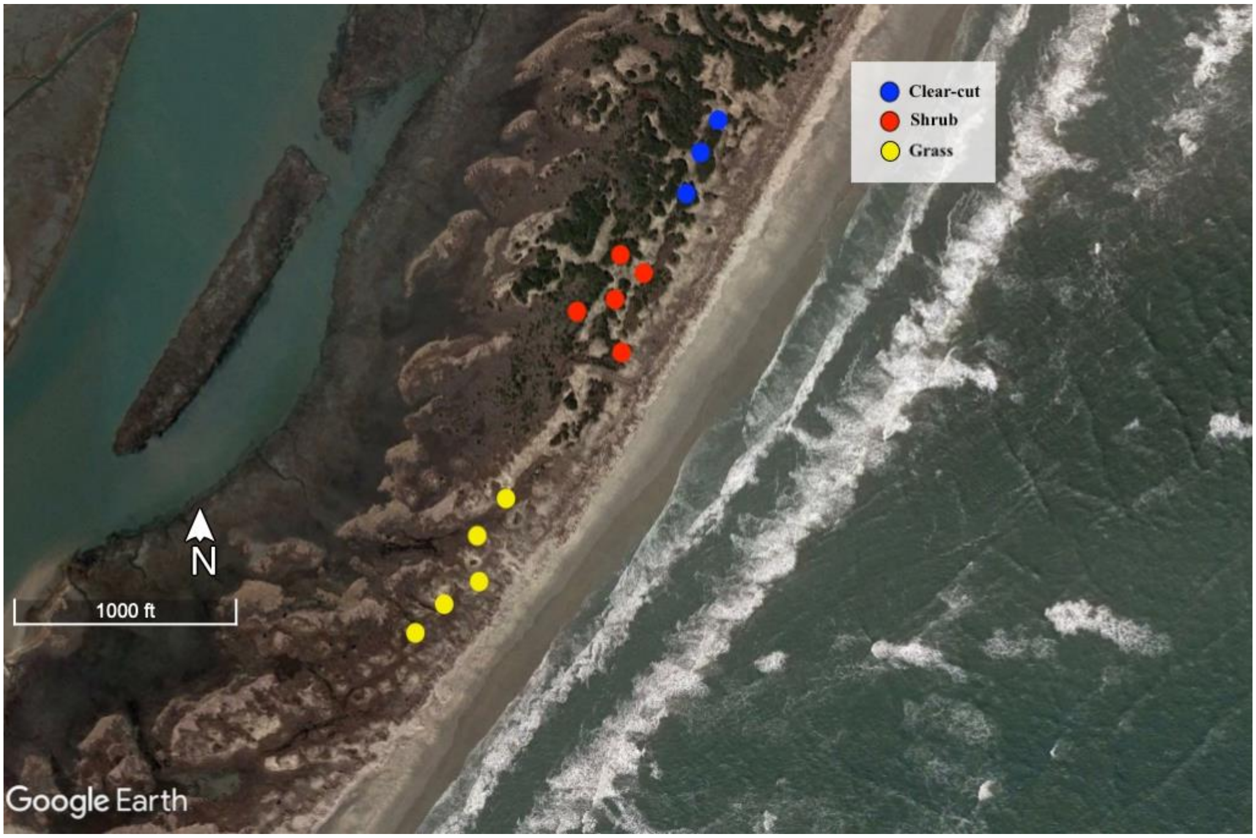
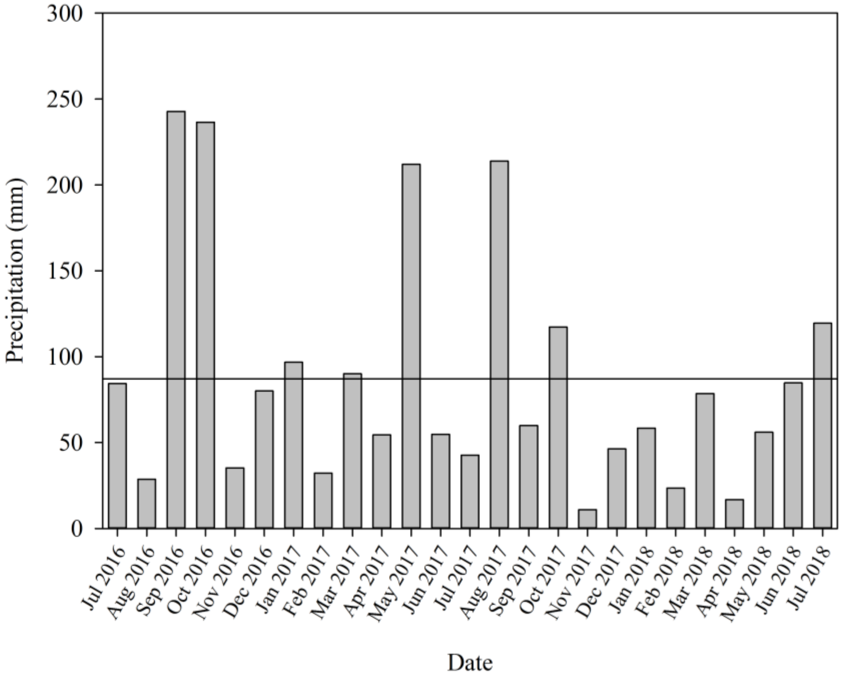


Figure 3.1



300
250
200
150
100
50
0
Precipitation (mm)
Jul 2016
Aug 2016
Sep 2016
Oct 2016
Nov 2016
Dec 2016
Jan 2017
Feb 2017
Mar 2017
Apr 2017
May 2017
Jun 2017
Jul 2017
Aug 2017
Sep 2017
Oct 2017
Nov 2017
Dec 2017
Jan 2018
Feb 2018
Mar 2018
Apr 2018
May 2018
Jun 2018
Jul 2018
Date

Figure 3.2

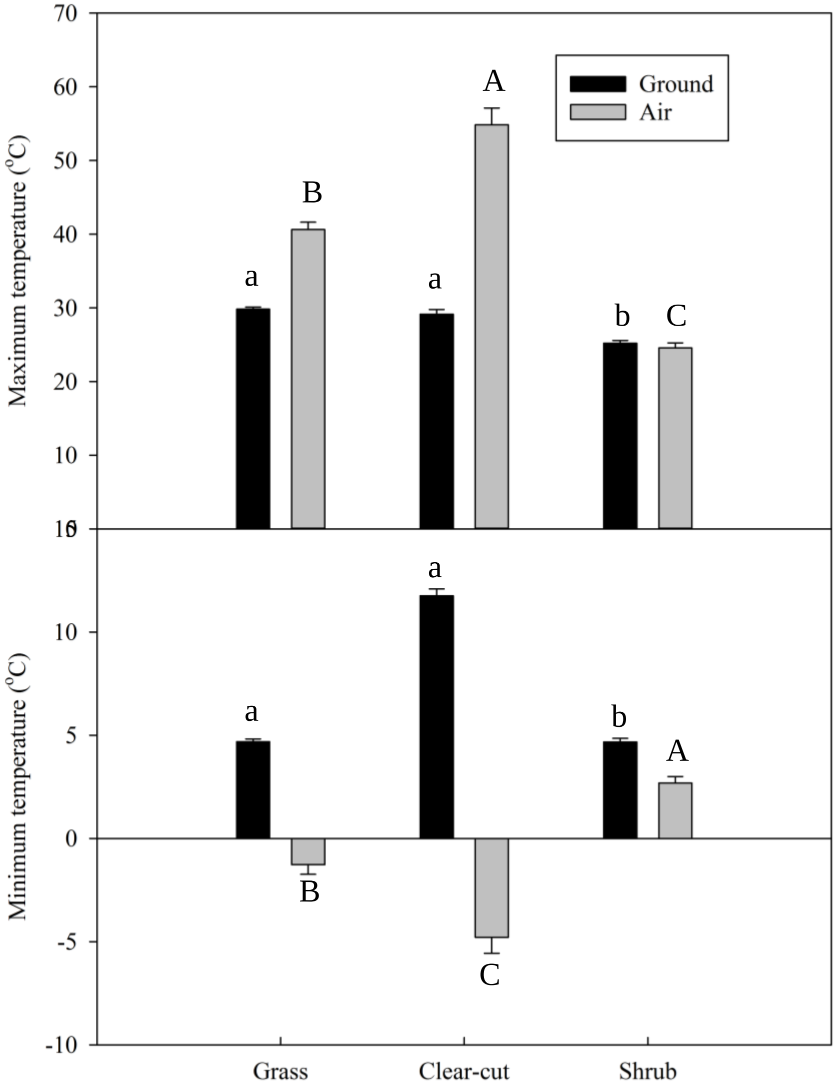

70
60
50
40
30
20
10
0
15
10
5
0
-5
-10
Maximum temperature (°C)
Minimum temperature (°C)
Ground
Air
A
B
a
a
b
C
a
a
b
A
B
C
Grass
Clear-cut
Shrub

Figure 3.3

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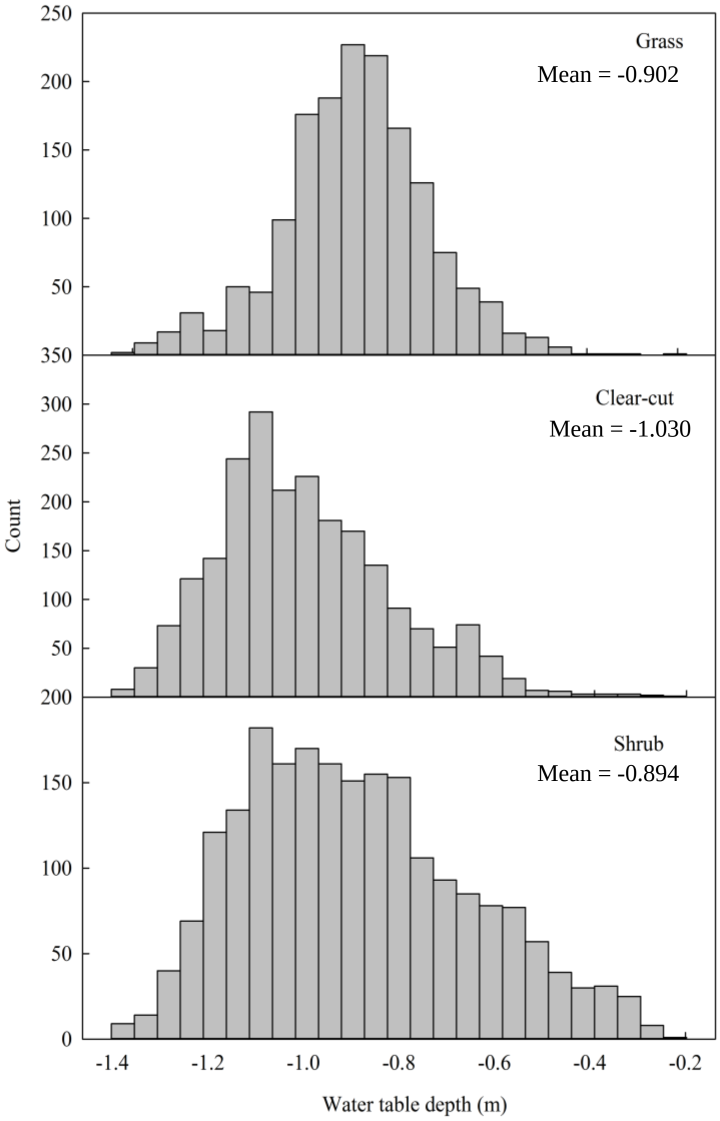
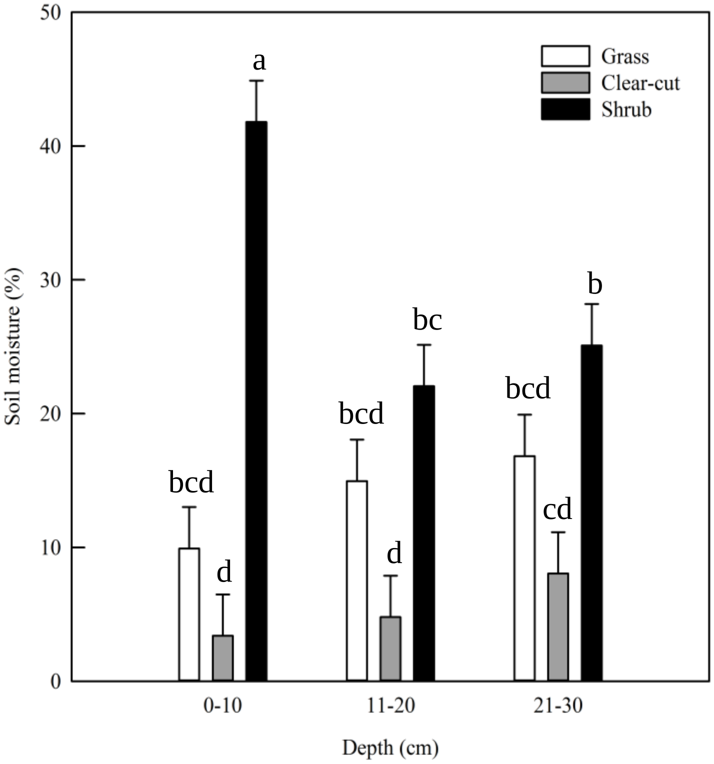


Grass
Mean = -0.902
Clear-cut
Mean = -1.030
Shrub
Mean = -0.894
Count
250
200
150
100
50
350
300
250
200
150
100
50
200
150
100
50
0
-1.4
-1.2
-1.0
-0.8
-0.6
-0.4
-0.2
Water table depth (m)

Figure 3.4



Soil moisture (%)
0
10
20
30
40
50
0-10
11-20
21-30
Depth (cm)
Grass
Clear-cut
Shrub
bcd
d
a
bcd
d
bc
bcd
cd
b

Figure 3.5

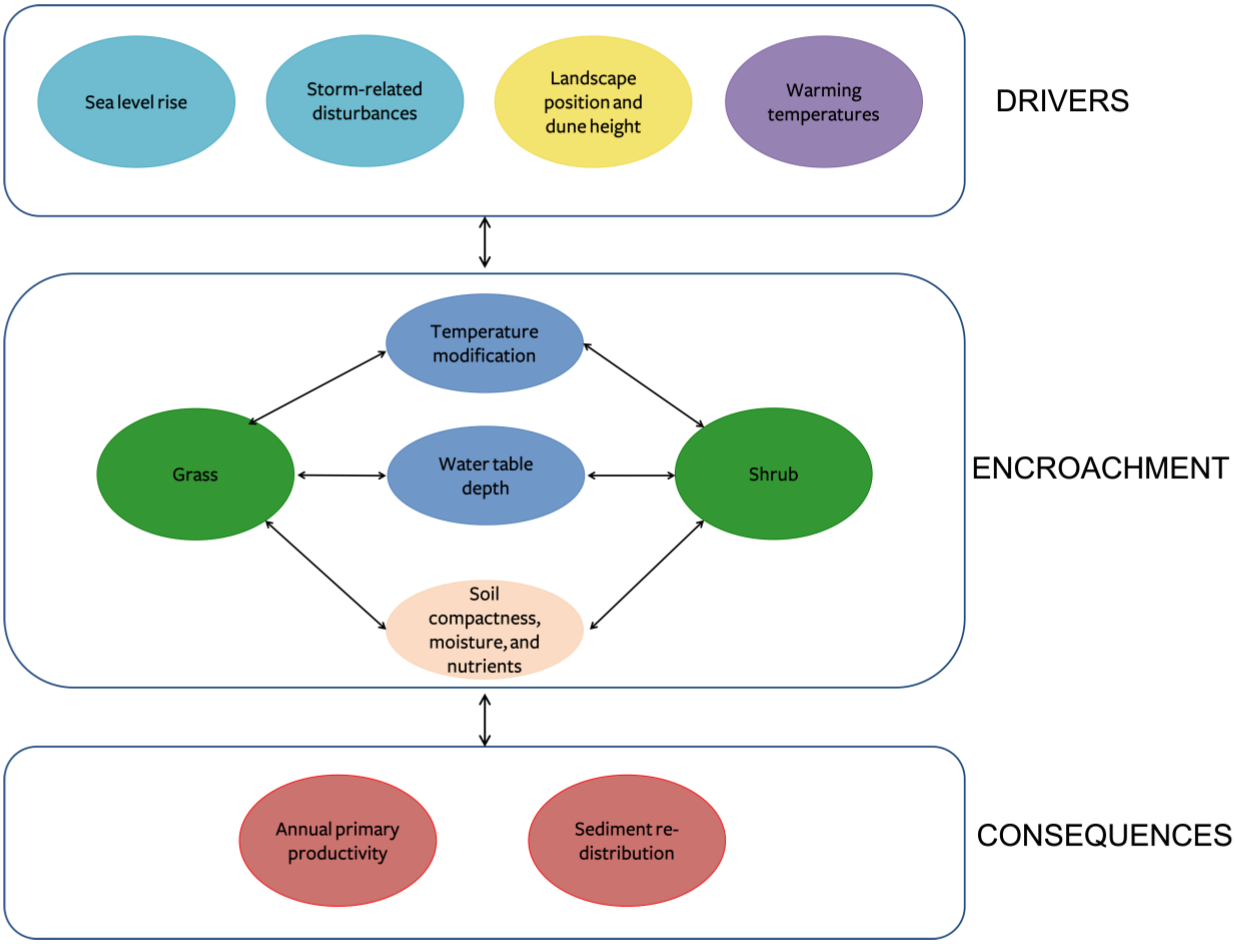

Sea level rise
Storm-related disturbances
Landscape position and dune height
Warming temperatures
DRIVERS
Temperature modification
Grass
Water table depth
Shrub
Soil compactness, moisture, and nutrients
ENCROACHMENT
Annual primary productivity
Sediment re-distribution
CONSEQUENCES

Figure 3.6

Table 3.1. Annual air (white) and ground (grey) temperature among zones from July 2017 – June 2018 ±SE when most sensors were recording consistently. Letters indicate differences among zones using a post-hoc Tukey test

Temperature measure	Grass	Clear-cut	Shrub
Minimum	4.8 ± 0.4 ^{a}	1.1 ± 0.5 ^{b}	5.9 ± 0.4 ^{a}
	9.6 ± 1.3 ^{a}	0.6 ± 1.5 ^{b}	7. 8 ± 1.1 ^{a}
Mean	16.0 ± 0.3 ^{a}	16.1 ± 0.4 ^{a}	14.5 ± 0.3 ^{b}
	14.3 ± 1.1	16.5 ± 1.3	13.7 ± 0.9
Maximum	36.0 ± 2.0 ^{ab}	42.9 ± 2.6 ^{a}	28.8 ± 2.03 ^{b}
	21.9 ± 1.4 ^{b}	33.0 ± 1.6 ^{a}	20.4 ± 1.2 ^{b}

Table 3.2. Seasonal detrended variance in water table among zones. Letter codes represent differences among zones

	Grass	Clear-cut	Shrub
All	0.0730[a]	0.0779[b]	0.0862[c]
Winter	0.0818[a]	0.0837[a]	0.0920[b]
Spring	0.0733[a]	0.0723[a]	0.0861[b]
Summer	0.0562[a]	0.0679[b]	0.0778[c]
Fall	0.0772[a]	0.0861[b]	0.0875[b]

Table 3.3. Dominant four species, cover, and species richness for 2017 (white rows) and 2019 (grey rows) among zones and species richness ± SE. Species cover was not acquired in 2018 due to weather limitations. Differing letters indicate statistical differences among vegetation zones within the year, identified by post-hoc Tukey tests.

Species	Grass	Clear-cut	Shrub
Morella cerifera	0 ± 0^{b}	0 ± 0^{b}	97.5 ± 0^{a}
	0 ± 0^{b}	0 ± 0^{b}	97.5 ± 0^{a}
Spartina patens	47.0 ± 9.5^{a}	10.0 ± 5.0^{b}	0 ± 0^{b}
	54.2 ± 0.6^{a}	62.5 ± 0^{a}	0 ± 0^{b}
Ammophila breviligulata	1 ± 0.6^{b}	37.5 ± 0^{a}	0 ± 0^{b}
	0 ± 0^{b}	25.8 ± 11.7^{a}	0 ± 0^{b}
Solidago sempervirens	2.5 ± 0^{a}	1.7 ± 0.8^{a}	0.5 ± 0.5^{b}
	1.7 ± 0.8^{a}	1.7 ± 0.8^{a}	0 ± 0^{b}
Total cover	94.5 ± 5.5	82.5± 10.2	100 ± 0
	95.9 ± 2.2	100 ± 0	100 ± 0
Species richness	10.8 ± 1.4^{a}	9.3 ± 1.5^{a}	2 ± 0.8^{b}
	8.7 ± 0.3^{a}	12 ± 2.0^{a}	1.2 ± 0.2^{b}

Appendix 3.1. Soil characteristics among zones ± SE. Letter differences in super script indicate differences among zones and depths, identified using a Tukey test on a significant interaction. Differing letters at the top of a soil characteristic indicates differences by vegetation type and differences along the side indicates differences among soil depth. Bulk density, N content, and C content were run as 2-way ANOVAs, NH_4+, NO_3- were run as 1-way ANOVAs. $\delta\ ^{15}N$ was run as a 2-way ANOVA but showed no difference among depths.

Soil characteristic		Grass	Clear-cut	Shrub
Bulk density				
	0-10cm	2.00 ± 0.03^{ab}	1.74 ± 0.13^{ab}	1.28 ± 0.06^{b}
	11-20cm	2.08 ± 0.11^{a}	2.14 ± 0.12^{a}	1.66 ± 0.28^{ab}
	21-30cm	2.08 ± 0.05^{a}	2.17 ± 0.06^{a}	2.35 ± 0.18^{a}
N (g m^{-2})				
	0-10cm	0.03 ± 0.01^{bc}	0.05 ± 0.01^{ab}	0.09 ± 0.01^{a}
	11-20cm	0.01 ± 0.01^{c}	0.02 ± 0.01^{bc}	0.03 ± 0.01^{bc}
	21-30cm	0.02 ± 0.01^{bc}	0.02 ± 0.01^{bc}	0.02 ± 0.01^{bc}
$\delta^{15}N$		b	a	ab
		0.44 ± 0.2	-0.42 ± 0.2	0.28 ± 0.2
C (g m^{-2})		a	ab	b
A	0-10cm	0.15 ± 0.07	0.45 ± 0.07	0.48 ± 0.07
B	11-20cm	0.07 ± 0.07	0.10 ± 0.07	0.14 ± 0.07
B	21-30cm	0.06 ± 0.07	0.08 ± 0.07	0.21 ± 0.07
NH_4+		7.92 ± 5.4	9.04 ± 2.4	5.08 ±.2.2
NO_3-		0.31 ± 0.3	4.17 ± 4.2	2.82 ± 0.8

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CHAPTER FOUR

Woody encroachment impacts plant traits on shrub edge

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Abstract

Background:

Shrub encroachment is occurring globally and impacts the immediate habitat. Although shrub establishment is known to influence landscape function and biodiversity, it is important to investigate effects of shrub on adjacent grasslands. On the mid-Atlantic barrier islands, nitrogen (N)-fixing *Morella cerifera* is encroaching into suitable habitat, altering species growth form and microclimate. The objective of this study is to identify ways in which *M. cerifera* influences functional traits of surrounding grassland at different distances, both with microclimate modification and with additional N. We established plots at 3 different distances from shrubs – 0m, 50m, and 100m – and applied slow-release ammonium nitrate fertilizer to half of the plots. Temperature, grassland functional traits, and grassland plant tissue isotopes were monitored.

Results:

Ecosystem engineering by shrubs through distance and N input influenced microclimatic seasonal mean temperatures and temperature extremes. Proximity to shrubs and N addition moderated extreme temperature events by 1-6 °C. Height and aboveground biomass were greater adjacent to shrubs and with N addition. Stem density, specific leaf area, specific root length, belowground biomass, and tissue isotopes did not differ among distances, though stem density doubled with N.

Conclusions:

Shrub canopy temperature modification in early encroachment is greater during winter and summer months, bringing local temperatures closer to 30 °C, the photosynthetic optimum for *M. cerifera*. N addition had a similar influence on microclimate as close proximity to shrubs. The influence on neighboring grassland species is limited to height, density, and aboveground biomass. Supplemental N likely contributes to the ecosystem engineering by *M. cerifera* indirectly through stimulating grassland species growth. Influence of shrubs on biotic and abiotic factors may be restricted to adjacent communities or require a longer timeframe to produce measurable differences in a wider area.

Additional index words: shrub encroachment, edge effects, grasslands, microclimate, tissue isotopes

Introduction

Encroachment of woody vegetation into grassland is a global phenomenon with site-specific drivers and consequences (Briggs, Hoch, and Johnson, 2002; Kremer *et al.*, 2014; Stevens *et al.*, 2016; Yu *et al.*, 2017; Rudgers *et al.*, 2018; Charles *et al.*, 2020). In many landscapes, encroaching shrubs act as ecosystem engineers, altering the microclimate, soil characteristics, and water availability for surrounding vegetation (Angelini *et al.*, 2011; D'Odorico *et al.*, 2012; Thompson, Zinnert, and Young, 2016; Wood, Hays, and Zinnert, 2020; Wood, Hays, and Zinnert, in review). Shrubs decrease extreme temperatures within the microhabitat and increase soil moisture (Wood, Hays, and Zinnert, in review). Many shrubs alter light availability to neighboring species as well (Alday *et al.*, 2014; Shiflett, Zinnert, and Young, 2014; Dohn *et al.*, 2017). These minor modifications to microclimate can add up and lead to cumulative landscape change, including higher annual net primary productivity (ANPP) and carbon assimilation, change in landscape water availability, and altered landscape function (Nadal-Romero *et al.*, 2018; Ward *et al.*, 2018; Zinnert *et al.*, 2019; Reeves *et al.*, in prep).

Along with alteration of temperature, light, and water availability in the habitat, shrub encroachment can lead to changes in soil characteristics (Hanslin and Kollman, 2016). Organic matter from leaf litter and fine root turnover is often higher in shrub-encroached habitats leading to lower bulk density (Brantley and Young 2008; Wood, Hays, and Zinnert, 2020). Nitrogen input from leaf-drop can be particularly influential in vegetative communities, often resulting from or enhancing encroachment of woody vegetation (Brantley and Young, 2008; Kurten *et al.*, 2008; Dangremond *et al.*, 2020). Soil carbon and organic matter increases in shrub habitats as well (Lunstrum and Chen, 2014; Charles *et al.*, 2020; Dahl, Daalgard, and Bork, 2020; Kinnebrew *et al.*, 2020; Wood, Young, and Zinnert, in prep). Additionally, increased leaf-litter input develops the water holding capacity of the soil, which alters microbial decomposition rate (Sørenson *et al.*, 2019; Wood, Hays, and Zinnert, in review)

Shrub encroachment can have long-term impacts on functional traits of the plant community, driven by input of resources by shrubs or decreased resources access due to shrub use. In stressful environments, surrounding species invest in acquisitive traits (Utaile *et al.*, 2020) or resource conservation traits (Geissler *et al.*, 2019). Limited light and increased nutrients can contribute to increased grassland species height and biomass initially (Saixiyala *et al.*, 2017) before outcompeting grassland species (Randle, Stevens, and Midgley, 2018; Dahl, Dalgaard, and Bork, 2020; Katz and Stavi 2020).

Coastal systems present a unique opportunity to study shrub encroachment in a stressful environment. Plant species in coastal habitats are subject to presses (e.g., sea level rise, SLR) and pulses (e.g., increased storm frequency) of climate change, along with high salinity, quick draining soil, and high winds. The mid-Atlantic coast has a higher rate of SLR (3.4 ± 0.5 mm yr^{-1}, Piecuch *et al.*, 2018), making the vegetation of the east coast of United States more vulnerable to that climatic stressor. Freshwater access in island habitats is limited to the precipitation-fed freshwater lens which can become saline with storm surge inundation (Kiflai *et al.*, 2020), impacting plant physiological success and community structure (Tolliver, Martin, and Young, 1997; Paudel and Battaglia, 2021). Coastal species in sandy soil have additional nutrient limitation owing to swift leaching of nutrients (Ehrenfeld, 1990; Young *et al.*, 2011). When coastal species are released from N and P limitation, they are able to be more productive (Zhang *et al.*, 2015; Brown and Zinnert, 2021).

Barrier islands act as the front line in the face of damaging storm currents and winds and protect >6% of mainland coastlines globally (Stutz and Pilkey, 2001). Our site, the Virginia

Coast Reserve Long-Term Ecological Research Site (VCR), is undeveloped, having been owned by The Nature Conservancy since the 1980s, and thus represents a landscape apart from direct anthropogenic influence, allowing us to investigate impacts of shrub encroachment without direct human disruption. Shrub expansion has been well-documented on the VCR, where cover has increased ~40% over 32 years despite a loss in land mass to erosion and sea level rise (Zinnert *et al*., 2016; Huang *et al.*, 2018). Evidence suggests that shrub establishment and thicketization increases the rate of erosion of sediment from the barrier islands, decreasing the likelihood of island rollover (Zinnert *et al*., 2019; Reeves *et al*., in prep). The encroaching species, *Morella cerifera*, is native, evergreen, and has an association with N-fixing bacteria, releasing it from the nitrogen limitation that is common in sand-based soils (Wijnholds and Young, 2000; Young *et al.*, 2007; Brown and Zinnert, 2021). Warming temperatures allow for range expansion of *M. cerifera* (Huang *et al*., 2018; Sinclair, Woods, and Zinnert, 2020) while microclimate modification and precipitation help maintain shrub presence and high productivity (Aguilar *et al*., 2012; Thompson, Zinnert, and Young, 2017; Wood, Hays, Zinnert, 2020; Wood, Hays, Zinnert, in review). Barrier island landscapes have quick-draining soil and limited nutrients; N-rich leaf-litter input from *M. cerifera* has the potential to both enhance soil moisture (Wood, Hays, and Zinnert, in review) and increase nutrient content via leaf drop and higher decomposition (Brantley and Young, 2008; Graziani and Day, 2015), creating islands of fertility and influencing the neighboring plant community. While *M. cerifera* is expanding in cover, the shrub perimeter increases, creating the potential for shrub edge effects influencing the surrounding grassland community and soil characteristics increases as well (Table 4.1) (Huang et al., 2021). The combination of temperature microclimate modification, water availability changes, and nutrient supplementation by shrubs could impact the adjacent grassland traits and habitat during the early stages of encroachment.

The purpose of this study was to investigate the influence of both microclimate modification and altered N input in a factorial design by shrubs on abiotic and biotic impacts of adjacent grassland. We established nitrogen (N) fertilized and non-fertilized plots (C) at 0 m, 50 m, and 100 m from shrub edge. Our hypotheses are:

> H1: Distance from shrub edge will influence microclimate and grass traits. Grassland plots at 0 m from *M. cerifera* will have cooler temperatures in the summer, taller plants, greater biomass, and higher stem density with higher tissue N content than plots at 50 and 100 m.
>
> H2: Nitrogen fertilization plots will have cooler temperatures in the summer, taller plants, greater biomass, and higher stem density, similarly to 0 m plots.
>
> H3: There will be an interaction with nitrogen input and microclimate modification by proximity to shrubs. Control plots at 100 m will have shorter plants, warmer summer temperature, and less nitrogen content in plant tissues relative to nitrogen plots at 100 m.

This study will contribute to our understanding of shrub encroachment influence on vital coastal grassland species and enhance our understanding of community dynamics on critical barrier islands landscapes.

Methods

Study site

Hog Island is part of the VCR LTER chain of 16 undeveloped barrier islands off the coast of Virginia (Figure 4.1). The landscape is made up of alternating dunes and swales, low lying areas protected by dunes, where *M. cerifera* establishes. Young (<20 yr), encroaching shrubs are prevalent on the southern portion of Hog Island, where we established the experiment (37.417° N, 75.686° W). Microtopography plays an important role in community composition and plant resource acquisition; sites were chosen to minimize confounding factors among treatments by maintaining equivalent elevation among plots.

Shrub perimeter

Based on imagery used in Huang et al. (2021), we calculated shrub perimeter from 1986, 1994, and 2013. Full methods are described in Huang et al. (2021), but briefly summarized here. Shrub cover was assessed from georectified aerial photography (USGS Earth Explorer) and hyperspectral imagery on 5 Jul 1986 (color infrared), 20 Mar 1994 (RGB), and 26 May 2013, (48 band hyperspectral; USACE-TEC & JALBTCX, 2018). Regions of interest (ROI) were selected in each year for shrub cover using the bands available in each image (ENVI 5.5.3, LH3 Harris Geospatial) based on geo-rectified aerial photography, and field surveyed woody thickets of known age using a Trimble Geo-XT GPS unit. Supervised classifications were performed using the maximum likelihood method. Resulting shrub cover was exported to ArcGIS 10.7 (ESRI) and then exported to FRAGSTATS 4.2 for spatial pattern analysis. Perimeter and area of each shrub patch were calculated.

Experimental design

Transects were established in March 2018 with plots at three distances from a *M. cerifera* shrub: 0 m, 50 m, and 100 m behind the primary dune and parallel to the beach (Figure 4.1). These distances account for varying degrees of influence of the focal shrub in microclimate and N input. At each distance, two 50 x 50 cm plots were established for control and annual addition of slow-release ammonium nitrate at 10 g m^{-2} y^{-1} to represent N input by *M. cerifera* leaf litter (Brantley and Young, 2008). Plots were fertilized annually from 2018 – 2020.

Ground temperature was measured every two hours using ibuttons (Thermochron, Baulkham Hills, New South Wales, Australia) from April 2018 through February 2020, with a gap due to accessibility of the field site. Annual grass and forb height and density were measured at the height of the growing season in 2019. Root tissue from soil cores (3.75 cm in diameter, 20 cm deep) (used for specific root length, SRL), leaves from the most prominent species for specific leaf area (SLA), above, and belowground biomass were collected in the summer 2019. Leaf samples were put in bags with a damp paper towel and stored in a cooler to prevent tissue breakdown in the field and ensure an accurate leaf scan. Soil samples were kept in bags in the cooler as well and were sieved upon returning from the field using 3.35 mm,1.00 mm, and 500 µm sieves to separate roots from soil. Roots from cores and leaves were scanned to obtain SRL and SLA using WinRhizo software (Regent Instruments, Quebec, Canada). Samples were dried for 72 h at 60 ◦C, weighed, and then ground using a Wiley Mini-Mill and sent to the Cornell Isotope lab (COIL, https://cobsil.cornell.edu/) for carbon, nitrogen, $\delta^{13}C$, and $\delta^{15}N$ analysis to assess influence of *M. cerifera* and additional nitrogen fertilizations on leaf and root tissue construction.

Aboveground biomass was collected in 2019 from a 10 x 10 cm area for each distance and treatment. Biomass was dried for 72 h at 60 ◦C and weighed.

Statistics

Variables that did not meet assumptions were transformed as needed. Seasonal mean temperatures were compared among distances and between treatments using two-way ANOVAs. Significant interactions were followed by a post-hoc Tukey test. When interactions were not significant, main effects comparisons were analyzed using Tukey test. Extreme temperatures were identified as the 10% warmest and coldest temperatures for each plot and analyzed using a two-way ANOVAs. When variables had unequal variance or were not normally distributed and could not be rectified with data transformations, they were compared using Kruskal Wallace for distance and Mann-Whitney for N treatment. Stem density, SLA, aboveground biomass, root biomass, SRL were compared using two-way ANOVAs.

Results

Morella cerifera is expanding in cover on Hog Island, increasing the shrub perimeter over time (table 4.1). The edge effects of shrub encroachment increased 4 - 10 km per year over ~30 years, increasing the ecosystem engineering influence and microclimate modification potential across Hog Island (table 4.1)

Distance and N treatment had seasonal influence on mean temperature. Nitrogen input had a cooling effect in the summer (U = 1246851.5, P = 0.014) and a warming effect in the winter (U = 922818, *P* < 0.001) (Figure 4.2). Adjacent to shrubs (0 m), plots were cooler in the summer by 1-1.5 ^{o}C (H = 253.1. *P*<0.001) but were not significantly warmer in the winter (H = 5.83.3. *P*=0.06) (Figure 4.2). Spring and fall are more transitional seasons but still exhibited influence by N treatment. Fall temperature were 2 ^{o}C warmer in N plots than C plots (U = 892647.5, *P*<0.001) and spring temperatures were 0.4 ^{o}C cooler in N plots than C plots (U = 689985, *P*<0.001) (Figure 4.2). In fall, microclimate was modified by *M. cerifera* in 0 m plots relative to 50 m plots but not 100 m plots (H = 15.3. *P*<0.001). Similarly, spring temperatures were cooler in 0 m plots than 50 m plots and 100 m plots (H = 16.8. *P* < 0.001).

Extreme temperatures, the 10% warmest and 10% coolest days, exhibit differences by treatment and distances. N plots had ~3 °C cooler maximum temperatures than C plots. Extreme cold temperatures were warmer in the 0 m plots by ~1°C (H = 49.7, $P < 0.001$) (Figure 4.3). *Morella cerifera* modified extreme temperatures in 0 m plots – extreme warm temperatures were cooler in 0 m plots than 50 and 100 m plots by ~6 °C (H = 191.7, $P < 0.001$) (Figure 4.3).

Grassland plants were 7.5 – 13 cm taller near shrubs but exhibited substantial variation at 100 m from the shrub edge (H = 13.72, $P = 0.001$) (Figure 4.4). Plants were 6 cm taller when treated with N (Figure 4.4). There was no interaction between distance and N treatment in stem density but distance (F = 4.9, $P=0.009$) and N treatment (F = 25.2, $P<0.001$) had significant effects (Supplementary Table 4.1, Figure 4.5). By distance, 0 m plots had 2-3 more stems per 0.25 m^2 than 50 and 100 m plots (Figure 4.5). N treatments produced plots with 2-5 more stems per 0.25 m^2 than C plots (Figure 4.5). SLA did not differ among distances or nitrogen treatments (Supplementary Table 4.2) (Figure 4.6). Aboveground biomass was 3 x higher in 0 m plots than 50 and 100 m plots (F = 5.9, $P= 0.003$) and N treatment plots at all distances had twice as much biomass as C plots (F = 21, $P<0.001$) (Figure 4.7), but there was no interaction (F= 1.6, $P= 0.2$).

Belowground biomass was greater in 0 m plots than 50 m plots by 0.01 g 0.25 m^{-2}, 100 m plots did not differ from either distance (Figure 4.8). There was no difference between belowground biomass for the control and N plots. SRL did not differ among distances or N treatments (Supplementary Table 4.3) (Figure 4.9). Leaf %C, %N, $\delta^{13}C$, $\delta^{15}N$ also showed no differences among distances or N treatments ($P> 0.05$) (Table 4.2).

Discussion

Shrub expansion is impacting grassland landscapes globally, altering temperature, light, and nutrients, impacting the productivity and characteristics of the neighboring grassland species (Hughes and Denslow, 2005; Price and Morgan, 2008; Alday *et al.*, 2014; Guo *et al.*, 2017, Havrilchak and Schedlbaur, 2018). Nutrient addition in coastal systems altered traits relating to competition and resource acquisition, like height and biomass (Brown and Zinnert, 2021). *Morella cerifera* expansion on the Virginia barrier islands is well-studied on a local scale. This paper sought to expand our understanding of shrub encroachment influence in a larger area and how different ecosystem alterations by shrubs may impact grassland traits.

Shrub influence among distances was mostly limited to the area immediately surrounding the shrub. Summer and spring mean temperatures are cooler adjacent to shrubs, bringing the mean temperatures closer to the photosynthetic optimum of 30°C (Young, 1992). The range of extreme temperatures immediately surrounding shrubs was greatly reduced, as expected, further supporting *M. cerifera* as an ecosystem engineer (Thompson, Zinnert and Young, 2017; Wood, Hays and Zinnert, 2020; Wood, Hays, and Zinnert, in review). Plants were taller and denser when adjacent to shrubs, resulting in higher aboveground biomass, demonstrating that *M. cerifera* ecosystem engineering creates a more productive grassland on the edge of its canopy. Ecosystem engineering by shrub edge effects is increasing with accelerated expansion resulting in increased shrub perimeter (Huang *et al.*, 2021).

Supplemental N input also influences seasonal mean temperatures – summer mean temperatures were cooler and winter mean temperatures were warmer in N plots, indicating that along with shading and evaporative cooling, additional N from *M. cerifera* leaf litter influences temperatures (Brantley and Young, 2008). Extreme temperatures were also mitigated in N plots where N addition limited extreme warm temperatures in summer and cold temperatures in winter. Additional N input into the system is a mechanism by which *M. cerifera* contributes to temperature microclimate modification. Added N also resulted in taller plants, denser vegetation, and greater aboveground biomass. Root biomass did not differ between treatments.

SLA showed no differences among distances or between N treatments consistent with previous nutrient additions (Brown and Zinnert, 2021). Aboveground biomass was observed after 2 years of fertilization and by treatment. The dramatic increase in the N plots at 0 m (~4 x the C plots) potentially concealed the combined influence of N input and microclimate modification on adjacent plant growth. SRL had no differences among distance or between N treatments and also exhibited a lot of variability primarily in the 100 m plots. This could represent increased allocation into acquisitive plant traits (Lozano *et al.*, 2019; Sharma *et al.*, 2021) and could be measurable after several more years of fertilization.

In this study, the modified temperatures, coupled with soil differences, may have led to taller grassland plants adjacent to shrubs. The response of grassland traits to N addition was limited to height, stem density, and aboveground biomass; all had a difference by distance as well which suggests either a secondary role of N influence on traits or a cumulative effect of shrub soil modification on grassland traits (Piyasinghe, Gunatilake, and Madawala, 2019). Grasses and forbs may take a bigger role in facilitating shrub expansion on the barrier islands, providing modified habitats in which seedlings can survive, particularly in wetter, shadier habitats (Velasco and Becerra, 2020). Grasses provide a microclimate buffer for *M. cerifera* seedlings of ~1.3 ºC in winter, supporting seedling survival (Sinclair, Woods, and Zinnert, 2020). Given that grass height and stem density were increased by proximity to a shrub and were greater with N addition, mature shrubs may play an indirect role in seedling survival through grassland traits that we cannot capture without a longer-term experiment.

Soil alteration by *M. cerifera* is well documented on the mid-Atlantic barrier islands (Brantley and Young, 2010; Wood, Hays, and Zinnert, in review) and additional nutrient input into this system has notable and long-lasting impacts on decomposition and plant traits (Day *et al.*, 2004; Brown and Zinnert, 2021). The lack of differences among leaf and root %C and %N was unexpected given the high productivity and N-rich leaf litter input of shrubs (Brantley and Young, 2008; Wood, Hays, Zinnert, 2020). The expected range of tissue δ^{15}N for atmospheric N fixation is -1 to 1 (Craine *et al*., 2015) and although many of our tissue measures fall outside of that range, they all hover close enough to not be significantly different. This indicates that the source of tissue N is likely a combination of fixation by anaerobic *Frankia* and aerobic soil diazotrophs (Smercina *et al.*, 2019), which produce δ^{15}N ~ -2.5 ‰. Soil δ^{15}N may also be impacted by denitrification, given sufficient NO_3 availability through *Frankia* and *M. cerifera* supplementation (Inglett, Rivera-Monroy, and Wozniak, 2011).

This study was designed with transects at distance from a focal shrub, parallel to shore, but the distance to shrubs perpendicular to the shore was not accounted for and may have influenced variability in grassland traits. Constraints of the *in situ* plant spatial distribution could be overcome through extensive shrub removal or using new areas of encroachment on other islands with little surrounding shrub cover. Additional data, including distance to perpendicular shrubs, species composition, inorganic soil N, seed predation, and seed germination, would enhance our understanding of how *M. cerifera* impacts the local community dynamics. Seed germination and predation were attempted in the beginnings of this study, but due to low seed viability were not included in this paper.

Shrub expansion is occurring across many different coastal system, and the expansion of a N-fixing species in low nutrient soils has landscape level consequences. This is the first paper to examine the influence of shrubs on the grassland edge, which is important to account for due to increased shrub perimeter over time (Huang, *et al*., in press). Because we know *M. cerifera* is still increasing in cover, understanding the immediate impact of establishment on the habitat and neighboring species will help predict changes in species, community traits, and nutrient dynamics. This study demonstrates the influence of the shrub edge, possibly through increased available N to the surrounding grassland, resulting in plants that exhibit competitive traits. These data can inform models and management for resilience to climate change events.

Conclusions

Woody encroachment is a complex process with a variety of mechanisms and consequences. Woody encroachment in coastal habitats further complicate the story due to unique stresses and dynamic habitat processes. Understanding the influence of shrubs on the adjacent habitat at different timepoints after establishment and on different scales creates a fuller picture of encroachment and the distance at which shrubs can impact neighboring species. Our study suggests that shortly after establishment, coastal shrubs primarily affect the individuals bordering the shrub edge and supplemental N from shrubs plays a secondary role in microclimate modification and altered functional traits. A longer period of establishment may be required to influence species at greater distances.

Acknowledgements: Thank you to Philip Tuley for his contribution of image processing in Huang et al. 2021, this added invaluable insight to the changes in shrub influence over time. This work was funded in part by the National Science Foundation Long-Term Ecological Research grants (DEB- 1237733 and DEB-1832221) and by the College of Humanities and Sciences at Virginia Commonwealth University, awarded to Dr. Zinnert.

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Figure 4.1. A map showing the location of Hog Island (red box and white arrow, top) and the experimental set up (bottom). Grey boxes represent N treated plots and black boxes are control (C) plots (Google Earth, earth.google.com/web/).

Figure 4.2. Seasonal mean temperatures ± SE among distances and treatments. Each season had a difference among zones and between N treatments, differing letters indicate statistical differences via Mann-Whitney U statistic or asterisks indicate differences among N treatments via Kruskal Wallis H statistic.

Figure 4.3. 10% warmest and 10% coolest temperatures ± SE among distances and between N treatments. Both differed by distance and by N treatment, according to Mann-Whitney and Kruskal Wallis tests; differing letters indicate statistical differences via Mann-Whitney U statistic or asterisks indicate differences among N treatments via Kruskal Wallis H statistic.

Figure 4.4. Plant height ± SE among distances (A) and nitrogen treatments (B). Differing letters indicate statistical differences via post-hoc Mann-Whitney comparisons among distances or asterisks indicate differences among N treatments via Kruskal-Wallis test.

Figure 4.5. Stem density ± SE among distance and N treatments. Differing letters indicate statistical differences via post-hoc Tukey tests or asterisks indicate differences among N treatments.

Figure 4.6. SLA ± SE among distances and treatments showed no difference. There is notable variability in the 0 m C plots.

Figure 4.7. Aboveground biomass ± SE among distances. Differing letters indicate statistical differences via post-hoc Tukey test comparisons or asterisks indicate differences among N treatments.

Figure 4.8. Mean root biomass ± SE among distances. Differing letters indicate statistical differences among distance using a post-hoc comparisons. There were no differences among N treatments.

Figure 4.9. Specific root length (SRL) ± SE showed no differences among distance or between N treatments.

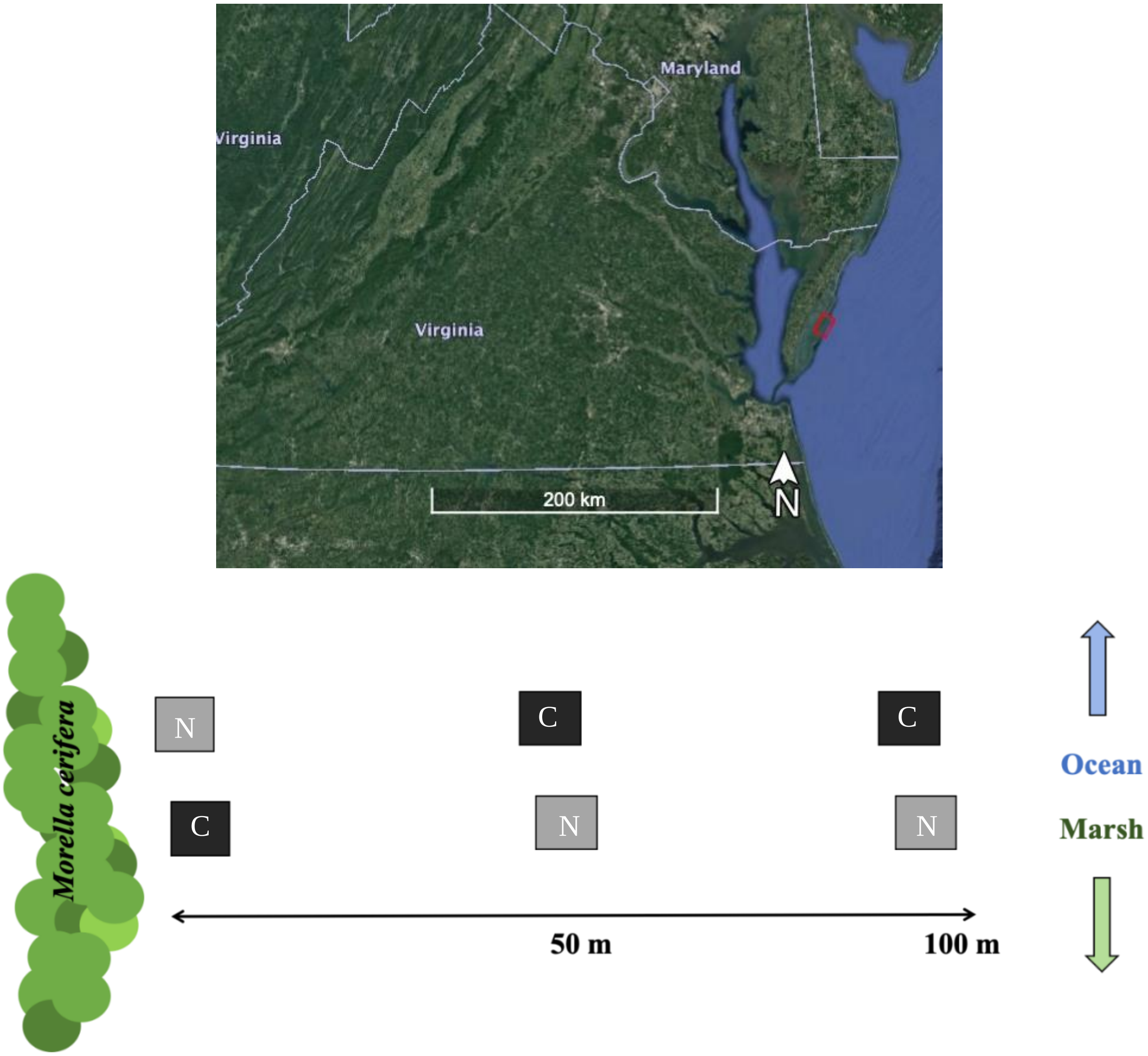
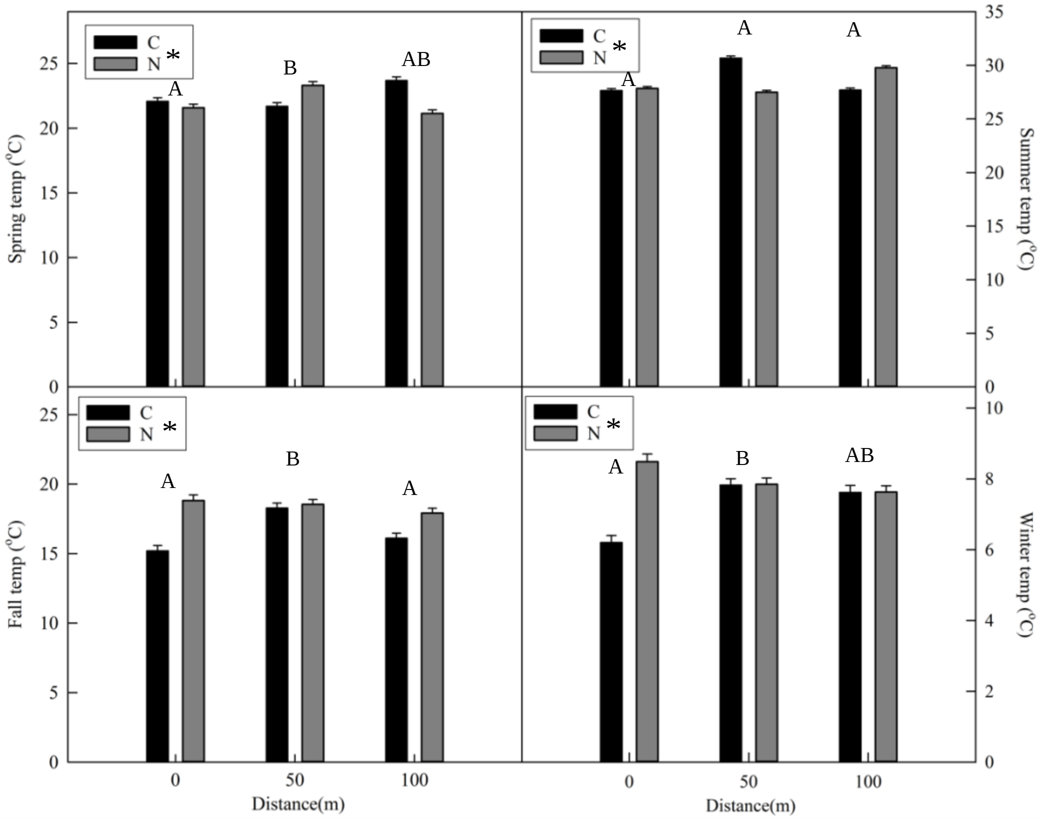

Maryland
Virginia
Virginia
200 km
N
Morella cerifera
N
C
C
C
N
N
Ocean
Marsh
50 m
100 m

Figure 4.1



C
N *
Spring temp (°C)
Summer temp (°C)
Fall temp (°C)
Winter temp (°C)
A
B
AB
A
A
A
A
B
A
A
B
AB
0
5
10
15
20
25
30
35
2
4
6
8
50
100
Distance(m)

Figure 4.2

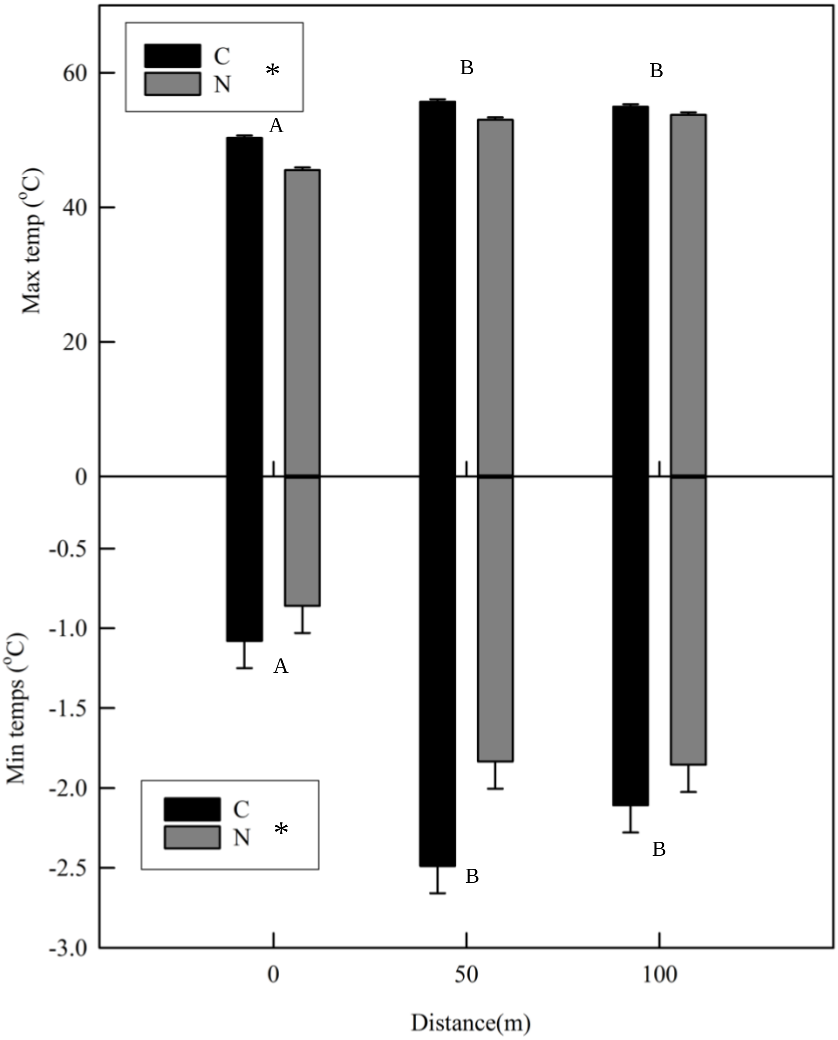
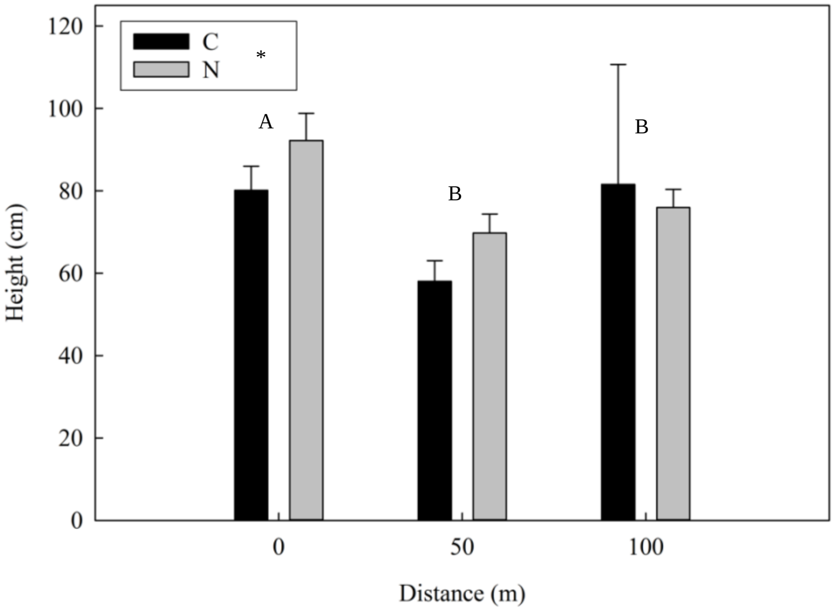

C
N
*
Max temp (oC)
60
40
20
0
A
B
B
-0.5
-1.0
-1.5
-2.0
-2.5
-3.0
Min temps (oC)
A
B
B
C
N
*
0
50
100
Distance(m)

Figure 4.3



120
100
80
60
40
20
0
Height (cm)
C
N
*
A
B
B
0
50
100
Distance (m)

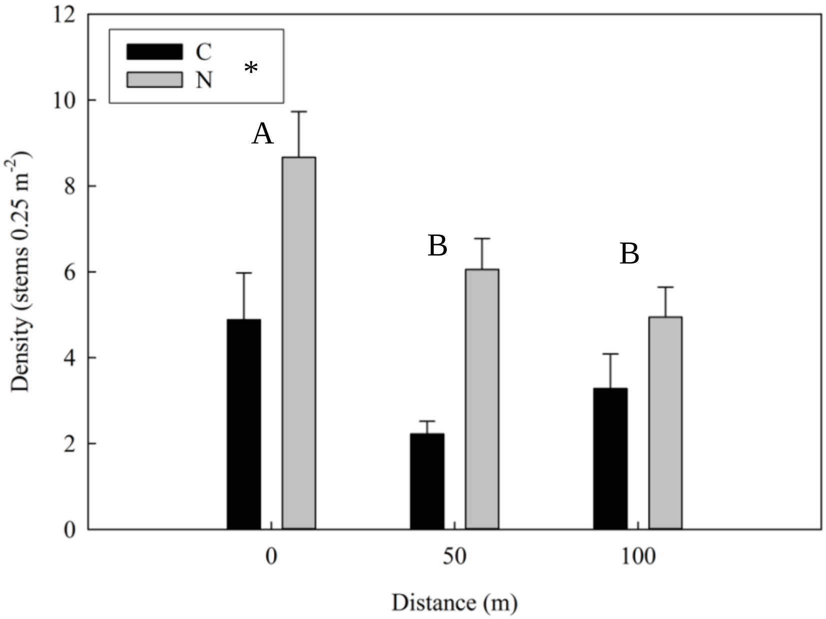


Figure 4.5

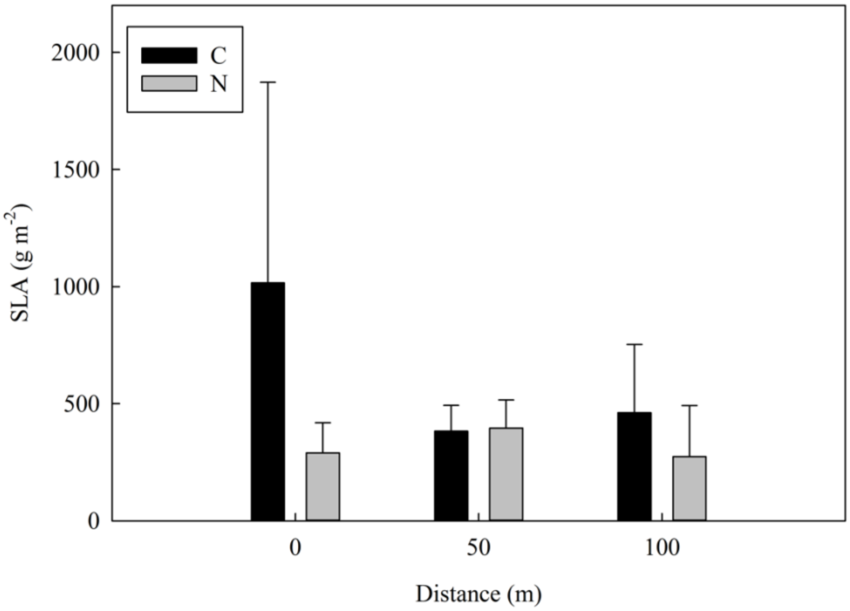
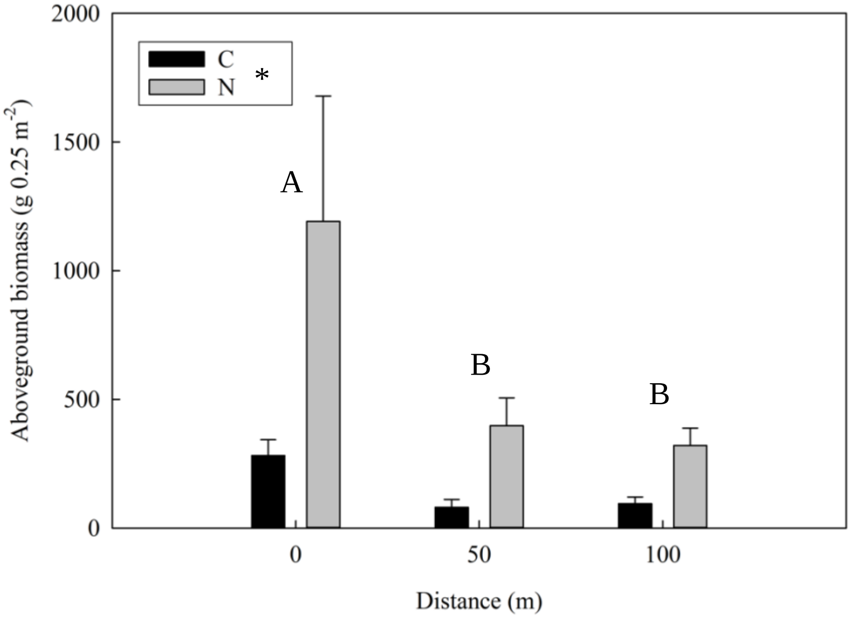
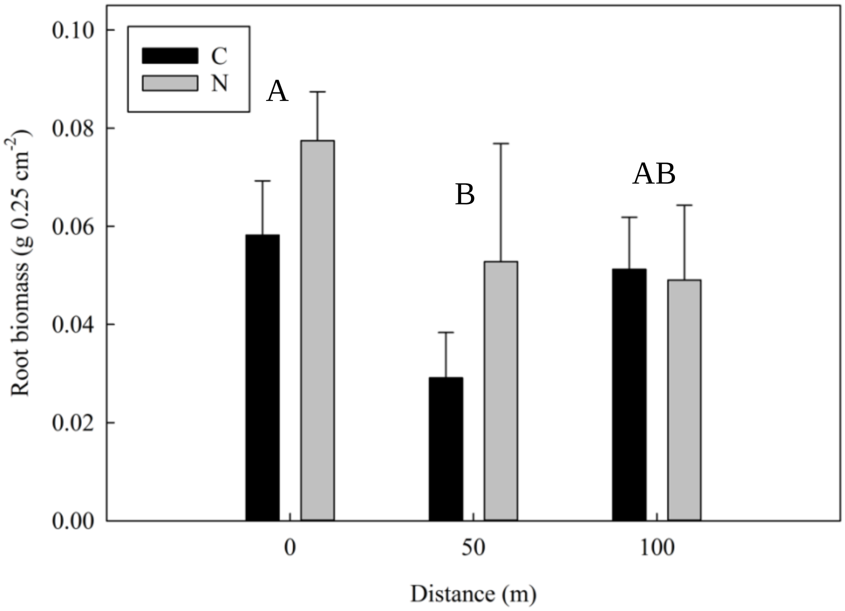


Figure 4.6



2000
1500
1000
500
0
Aboveground biomass (g 0.25 m^{-2})
C
N
*
A
B
B
0
50
100
Distance (m)

Figure 4.7


C
N
A
B
AB
0.10
0.08
0.06
0.04
0.02
0.00
Root biomass (g 0.25 cm^{-2})
0
50
100
Distance (m)

.Figure 4.8

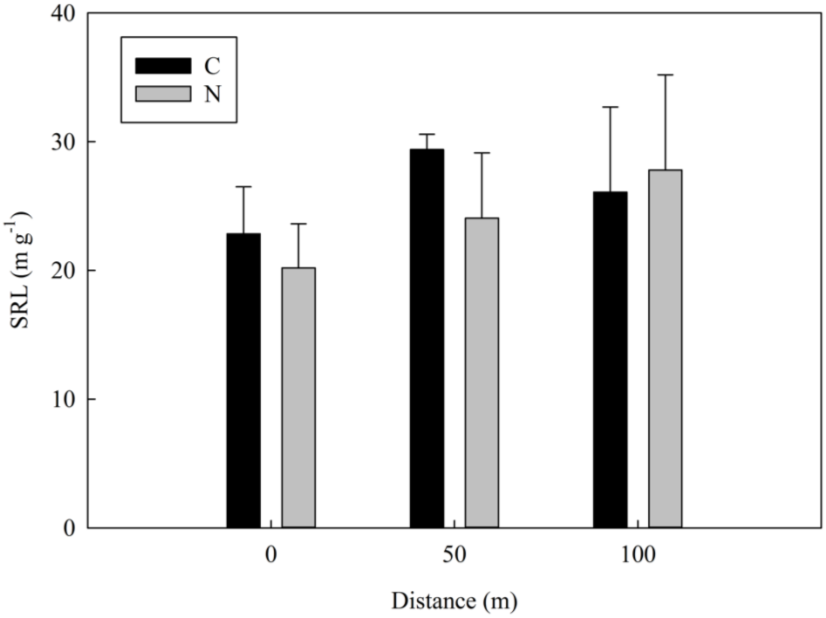


Figure 4.9

Table 4.1. Perimeter and cover of shrubs over 27 years. Increase perimeter creates increased edge effects.

Year	**Perimeter (km)**	**Area (ha)**
1986	120.1	84.9
1994	308.3	119.9
2013	400.5	313.2

Table 4.2. %N, %C, δ13C, and δ15N from roots and leaves of plants from the transects after 2 years of fertilization. 0 m plots are in white, 50 m from the shrub are in light grey, and 100 m from the shrub are in dark grey. No statistical differences among distances or between N treatments were found.

Measure	Control leaf	Nitrogen leaf	Control root	Nitrogen root
%C	37.4 ± 4.5	44.7 ± 4.5	47.2 ± 1.0	46.2 ± 1.0
	44.7 ± 4.5	38.2 ± 4.5	44.6 ± 1.0	47.4 ± 1.0
	39.5 ± 4.5	43.5 ± 4.5	44.4 ± 1.0	46.5 ± 1.0
%N	1.7 ± 0.3	1.9 ± 0.3	1.01 ± 0.1	1.0 ± 0.1
	1.4 ± 0.3	1.2 ± 0.3	0.8 ± 0.1	0.7 ± 0.1
	1.3 ± 0.3	1.3 ± 0.3	1.0 ± 0.1	0.7 ± 0.1
$\delta^{13}C$	-26.6 ± 2.9	-21.6 ± 2.9	-22.8 ± 2.1	-21.0 ± 2.1
	-17.2 ± 2.9	-18.3 ± 2.9	-16.7 ± 2.1	-23.5 ± 2.1
	-20.9 ± 2.9	-21.2 ± 2.9	-20.4 ± 2.1	-16.6 ± 2.1
$\delta^{15}N$	-2.2 ± 0.9	-1.2 ± 0.9	-2.2 ± 0.7	-2.0 ± 0.7
	-1.7 ± 0.9	0.6 ± 0.9	-2.1 ± 0.7	-1.2 ± 0.7
	-2.7 ± 0.9	1.5 ± 0.9	-2.4 ± 0.7	-0.2 ± 0.7

Supplementary table 4.1. Two-way ANOVA table for density among distances and N treatments.

Source of Variation	**DF**	**SS**	**MS**	**F**	**P**
Distance	2	3.871	1.936	4.351	0.016
Treatment	1	9.414	9.414	21.161	<0.001
Distance x Treatment	2	0.511	0.255	0.574	0.565
Residual	94	41.817	0.445		
Total	99	55.726	0.563		

Supplementary table 4.2. Two-way ANOVA table for SLA among distances and N treatments.

Source of Variation	**DF**	**SS**	**MS**	**F**	**P**
Distance	2	0.920	0.460	0.308	0.741
Treatment	1	1.436	1.436	0.961	0.346
Distance x Treatment	2	0.729	0.364	0.244	0.787
Residual	12	17.929	1.494		
Total	17	21.013	1.236		

Supplementary table 4.3 Two-way ANOVA table for SRL among distances and N treatments.

Source of Variation	**DF**	**SS**	**MS**	**F**	**P**
Distance	2	1.445	0.722	0.435	0.651
Treatment	1	0.758	0.758	0.456	0.505
Distance x Treatment	2	2.000	1.000	0.602	0.554
Residual	29	48.172	1.661		
Total	34	52.417	1.542		

CHAPTER FIVE

Shrub age and water dynamics influence primary productivity, carbon, and nitrogen stocks in a coastal environment

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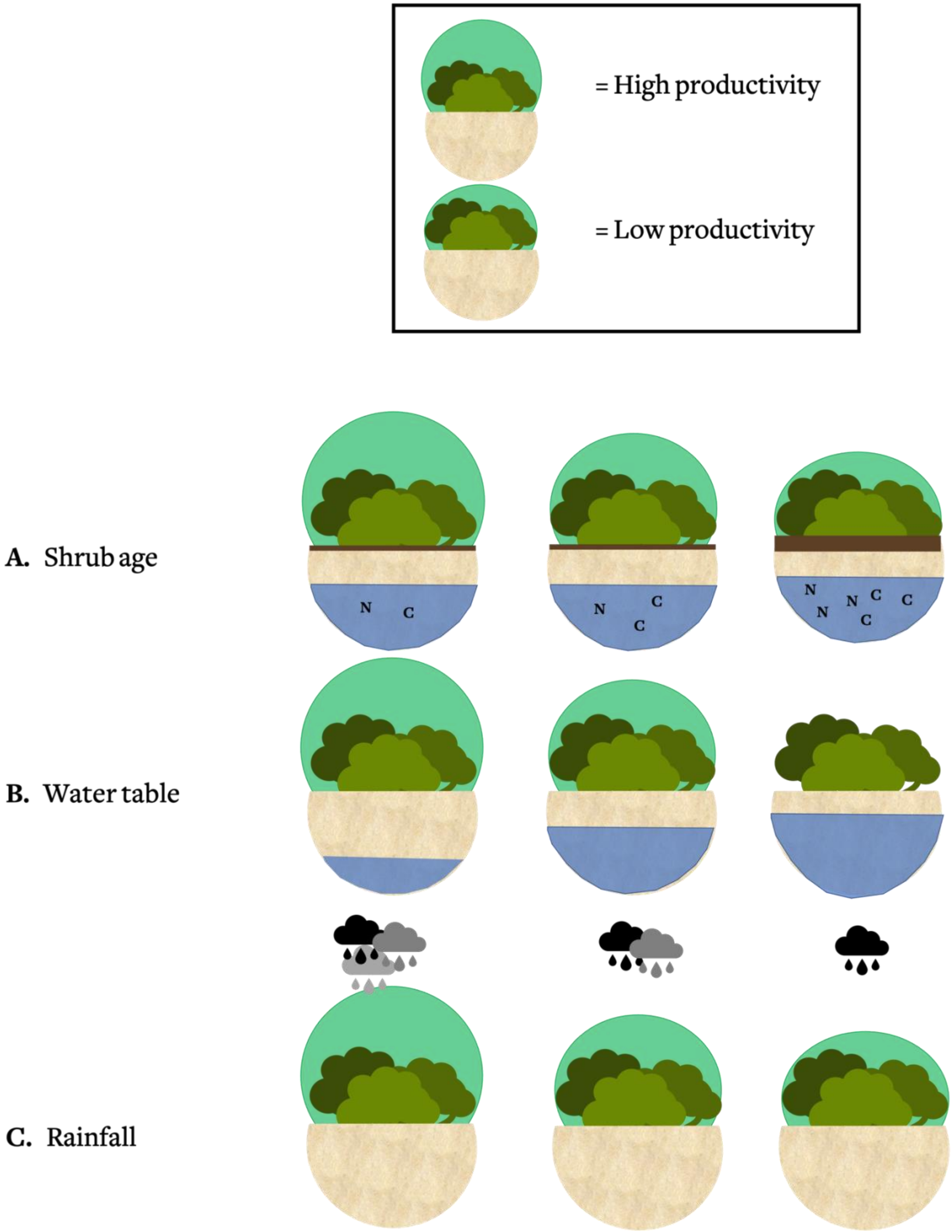
Keywords: shrub encroachment, primary productivity, barrier island, macroclimate, chronosequence

Formatted for Science of the Total Environment, in prep.

Abstract

Drivers of shrub productivity over time and associated landscape impacts of encroachment in mesic and coastal habitats are under-represented in the literature. Long-term (1990-2007) productivity was measured for a native, expanding, nitrogen-fixing shrub, *Morella cerifera*, coupled with macroclimate drivers to identify meteorological controls on annual net primary production (ANPP) across a barrier island chronosequence of shrub age. Soil and groundwater nutrient properties were compared with un-encroached grassland soil to evaluate impacts of vegetation on nutrient dynamics. We hypothesized that temperature, precipitation, and shrub age would be strong predictors of ANPP. We expected that shrub establishment would increase soil nitrogen (N) and carbon (C), but with some loss to groundwater due to leaching from sandy soils. Results demonstrated that shrub ANPP declined with age at the same rate among all thickets, regardless of shrub age, but there was variability from year to year. When climate variables were included in models, shrub age, precipitation, and water table depth were consistent predictors of ANPP, whereas temperature had no effect. Water table depth decreased over time, reducing ANPP. This may be due to both rising sea-level and feedbacks with shrub establishment and evapotranspiration. Soil N and C increased with shrub age and were higher than grassland; however, groundwater analysis indicates loss of N and C to groundwater. Our results demonstrate that complex interactions between shrub age and hydrological properties highlight the contribution of island productivity to coastal carbon how long-term shrub encroachment affects soil characteristics.

Graphical abstract:



Introduction

Shrubs are encroaching into grasslands globally resulting in altered landscapes and ecosystem function (Tape et al., 2006; Ratajczak et al., 2017; Huang et al., 2018; Sabater et al., 2019; Scharnagl et al., 2019; Huang et al, 2020). Site-specific drivers include land-use change, altered grazing and fire regimes, warming temperatures, and changes in frequency and amount of precipitation (Huang et al., 2018; Malfasi et al., 2020; Sulwiński et al., 2020; Tormo et al., 2020). Climate change forecasts predict more variable precipitation patterns, including amount and frequency of rainfall and storm events (Liu et al. 2017), which may enhance vegetation change rates and productivity (Gherardi and Sala 2015). Expansion of shrubs into grasslands typically leads to increased primary productivity of the plant community (Knapp et al., 2008; Havrilchak et al., 2018; Higginbottom and Symeonakis, 2020). Plants on islands are particularly vulnerable to changes in water availability as they rely on a precipitation-fed freshwater lens in the soil, which is sensitive to inundation and drought (Aguilar et al., 2012; Kiflai et al., 2020).

Encroaching woody vegetation may act as ecosystem engineers, modifying the microclimate to reduce mortality, increase productivity, and accelerate expansion (D'Odorico et al., 2010; He et al., 2015; Wood et al., 2020). Water use dynamics can be dramatically altered by a shift in dominant plant functional type as demonstrated in arid systems (Medeiros and Pockman, 2011; Iqbal et al., 2021). Woody species tend to have higher productivity and thus use more water, altering water availability for surrounding species (Winkler et al., 2019; Peguero-Pina et al., 2020). Increased N, P, and K input from leaf litter can accumulate over time and create nutrient rich areas of soil or "islands of fertility" with older vegetation (Brantley and Young, 2008; McCulley and Jackson, 2012; Farella et al., 2020; Zhou et al., 2021). Small-scale microhabitat changes eventually lead to cumulative landscape change, including increased productivity and carbon assimilation, altered landscape hydraulics and function (Eldridge et al., 2011; Nadal-Romero et al., 2018; Ward et al., 2018; Zinnert et al., 2019).

Coastal systems are vulnerable to the pressures and pulses of climate change as vegetative communities are affected by both macroclimatic (i.e. temperature and precipitation) and oceanic (i.e. sea level rise and storm surge/inundation) drivers (Ezer et al., 2013; Pivovaroff et al., 2015; Charles et al., 2020). Barrier islands make up a small portion of global coastlines, but are abundant along the Atlantic Ocean, protecting significant areas of economic and ecological value (Stutz and Pilkey, 2001, Feagin et al., 2015; Hanley et al., 2020). Along the US Atlantic and Gulf coasts, woody cover, primarily *Morella cerifera,* has been expanding (Battaglia et al., 2007; Zinnert et al., 2019). On the Virginia barrier islands, expansion of this salt sensitive shrub occurred despite a decrease in island surface area (Zinnert et al 2016). The main driver of *M. cerifera* expansion is warming winter temperatures in the region, 0.06 °C year $^{-1}$ since the 1950s (Huang et al., 2018; Goldstein et al., 2018).

Morella cerifera is a native, evergreen shrub that has an association with a nitrogen-fixing actinomycete, *Frankia*, in root nodules (Young 1992; Brantley and Young, 2010). *Morella* establishes in low-lying elevations behind foredunes (i.e. swales) that are ~1.5 – 2 m in height, where individuals are protected from salt spray/intrusion by storm surge and have access to the freshwater lens (Young et al. 2011; Woods et al. 2019). Adequate access to bioavailable nitrogen (N) via N-fixing bacterial root nodules, in an otherwise nitrogen limited habitat, allows for high productivity and development of dense, closed canopy thickets (Shiflett and Young, 2010; Shiflett et al., 2013, 2014; Zinnert et al., 2016). Although *M. cerifera* influences on microclimate and species composition have been quantified on a small scale (Thompson et al. 2017; Wood et al. 2020), effects of annual macroclimatic patterns on primary productivity over longer timeframes have not been documented.

Many studies rely on chronosequences (i.e. space for time substitutions) to investigate succession of plant communities, diversity, plant productivity, soil nutrients, soil microbial activity, and fungal communities (Springsteen et al., 2009; Hollingsworth et al., 2010; Blaser et al., 2014; Cava et al., 2017; Yang et al., 2019; Li et al., 2020). These are valuable opportunities to investigate long-term data in a more compressed time scale. Chronosequences occur in areas with regular disturbance or landscape change, such as fire, overwash, flooding, restoration, or sand accretion (Brantley and Young, 2010; Hollingsworth et al., 2010; Pellis et al., 2019; Laskar et al., 2021). On Hog Island, Virginia, sand accretion occurred on the north end of the island at ~5 m year $^{-1}$ since the late 1800s, establishing a chronosequence of alternate dune-swale complexes of progressively younger soils from the bayside to the ocean side (Figure 1) (Hayden et al., 1991; Shiflett et al., 2014). This phenomenon allows us to study differences among shrub and soil ages and drivers of productivity by age over time.

Our study determined the effect of macroclimate changes on annual net primary productivity (ANPP) using long-term data (1990-2007) from the Hog Island chronosequence on the Virginia barrier islands. We quantified consequences of long-term shrub encroachment on N and C in the soil for the top 50 cm, as well as dissolved organic carbon (DOC) and nitrogen (DON) in the freshwater table. We hypothesized that temperature and hydrological variables (i.e. water table depth and precipitation) would be influential controls on ANPP due to photosynthetic temperature optimum for *M. cerifera* and known reliance on the soil freshwater lens (Young et al., 2007; Aguilar et al., 2012). We further hypothesized that soil N and C are highest under older thickets at all soil depths, due to longer timeframe of litter input, but groundwater DOC and DON will be more variable due to potential hydrological movement.

Methods

Study site:

This study was performed on Hog Island, part of the Virginia Coast Reserve (VCR) Long-term Ecological Research network (LTER), a barrier island located ~10 km east of the Eastern Shore of Virginia, USA (37°270 N, 75°400 W) (Figure 5.1). Remote sensing analyses have documented landscape change, including sand accretion on the north end of Hog Island, as well as subsequent establishment of *Morella cerifera* shrub thickets (Young et al. 2007, Zinnert et al. 2016). Using this information, we identified a chronosequence of shrub thicket age. The four thicket ages were designated, from ocean side of the island to mainland side, colonizing shrub (CS, ~20 yr), young shrub (YS, ~30 yr), mid-island (MS, ~40 yr), and bayside (BS, ~60 yr). Shrub thickets are all established behind protective primary dunes and do not differ in stress.

Productivity:

Beginning in 1990, annual aboveground biomass was harvested from the current-year growth, which are differentiated by stem color (Young, 2007), 75 shoots from shrubs of each thicket across the chronosequence. Bias was avoided by selecting 50 shoots total from each thicket via systematically selecting every third shoot. Each shoot was dried at 80 ºC for 48 hours and weighed. Mean annual growth was input into allometric equations to estimate annual net primary productivity (ANPP) derived from Brantley (2005). Due to constraints of site access, ANPP for the *M. cerifera* thickets was collected in 1990, 1992-2007.

Climate:

Hourly temperature and windspeed data were acquired from the VCR database for both Hog Island and Oyster, VA based on data availability (Porter et al., 2021). Water table depth data were obtained from a known shrub thicket on Hog Island, VA (Porter et al., 2020). Temperature, wind speed, and water table depth were summarized to mean, minima, and maxima for each year. Temperature data were further summarized to number of days above 29.5 °C to account for the photosynthetic optimum of *M. cerifera* (Young 1992). Precipitation was quantified as an annual sum.

Soil characteristics:

Soils were collected haphazardly across the chronosequence and in unencroached grassland (GR) in 2017 (n=3) using an aluminum tube inserted to a depth of 50 cm and separated into 10 cm sections. Each section of known volume was dried at 105°C for 72 hours and weighed to get bulk density. Subsamples from each 10 cm depth were sent to Cornell Isotope Lab (COIL, https://cobsil.cornell.edu/) for isotopic analysis of C, N, $\delta^{13}C$, and $\delta^{15}N$ to assess influence of long-term encroachment on isotopic soil contents.

Groundwater:

Groundwater was collected in 2018 (n=6) in BS, YS, and GR plots. Temporary wells were dug using a soil auger. Wells breached the freshwater lens and were allowed to fill with groundwater, which was hand pumped into ~275 mL bottles. Bottles were kept on ice in the field and frozen until further processing. Groundwater samples were shipped frozen on dry ice and processed by the University of Georgia Agricultural and Environmental Services Lab (http://aesl.ces.uga.edu/) for dissolved organic carbon (DOC) and dissolved organic nitrogen (DON).

Statistics:

Variables were log transformed as needed to satisfy parametric assumptions. Linear regressions were performed to determine trends in meteorological data over time. In order to assess ANPP differences among thickets over time, ANCOVA was used with year as a covariate and a Tukey *post hoc* test to quantify differences among thickets. To determine the influence of environmental drivers over time on ANPP, we ran a backward stepwise regression using thicket age, days above 29.5°C, annual maximum wind speed, annual mean, maximum and minimum water table depth, mean, maximum and minimum temperature, and annual sum and daily mean precipitation. We picked best-fit models based on low AIC, high R^2, and low RMSE. Terms with $P < 0.05$ were included in the top models. To assess the expected relationship between precipitation and water table depth, a Pearson correlation was performed. Soil variables were compared among thicket age and soil depth with two-way ANOVA. Significant interactions were followed by a post-hoc Tukey test. When interactions were not significant, main effects comparisons were analyzed using Tukey test. DON and DOC were log-transformed and compared using one-way ANOVAs.

Results

Many of the macroclimatic measures showed high variability but there were clear trends across the timeframe of the study. Mean temperature increased across the timeframe ($\beta = 0.16$, $r^2 = 0.21$, $P < 0.001$) (Figure 5.2). Maximum temperature increased 0.32 °C annually and minimum temperature did not have a significant regression ($P = 0.02$ and $P = 0.27$, respectively). Mean and maximum water table depth increased significantly over time ($\beta = 1.4$ and 3.0, $r^2 = 0.37$ and 0.39, respectively, $P < 0.001$ for both); water table became closer to the soil surface (Figure 5.2). In contrast, annual precipitation was variable over time but did not exhibit a trend ($r^2 = 0.04$, $P = 0.14$) (Figure 5.2). There was no correlation between annual precipitation and mean water table depth ($r = 0.09$, $P = 0.55$) indicating other influences on water table depth.

Across the whole timeframe, younger *M. cerifera* thickets (CS and YS) had significantly higher ANPP than the MS and BS thickets, with BS exhibiting significantly lower values than MS (Figure 5.3). Year and thicket age influenced ANPP ($P < 0.001$ for both) with no interaction, indicating that as thickets age, production decreased – YS had 1176 g m^{-2} y^{-1} higher ANPP than BS in 1990 and by 2007 had 270 g m^{-2} y^{-1} higher ANPP than BS. In 2007, CS, the youngest thicket had only 429 g m^{-2} $y\text{-}^{1}$ higher ANPP than BS (Figure 5.3).

The variation observed in ANPP across all sites over time indicates possible environmental drivers influencing ANPP rather than shrub age being the only influence, thus we tested several parameters (i.e. temperature, rainfall, water table depth). The top 3 models in the stepwise regression accounted for 73-74% of variation and included strong influences by thicket age and hydrological characteristics. The best fit model with the lowest AIC had five variables – thicket (all), thicket successional stage (MS,YS,CS), max water table depth, min water table depth, and mean precipitation ($P < 0.05$, RMSE = 0.20, AIC = -9.6, adj. $R^2 = 0.74$) (Table 5.1).

ANPP declined with thicket age and higher water table maximum and minimum (Table 5.1). Mean precipitation increased ANPP (Table 5.1). The second best-fit model included mean water table depth rather than maximum and minimum water table depth but otherwise maintained the same terms as the (Table 5.1). Temperature variables were not retained in the model selection.

Some soil measures differed by depth and by thicket age. Bulk density did not differ among thicket ages or between thickets and grass. Other soil characteristics were influenced by shrub presence, thicket age, and depth. The oldest soils associated with BS thickets showed increased nutrient accumulation in multiple ways. N content of soils differed by depth (F = 6.2, $P<0.001$) and thicket (F = 2.7, $P = 0.042$), but there was no interaction (Figure 5.4). The lowest N content was found at the 20-30 cm and 30-40 cm depths; GR N was lowest by 2.7 g m^{-2} (Figure 5.4). Soil C content followed a similar trend (depth F =5.9 and $P<0.001$, thicket F = 6.4 and $P<0.001$); soil C at 20-30 was lower than 0-10 cm by ~260 g m^{-2} and 30-40 cm was lower by 320 g m-2. GR C was 150 - 216 g m^{-2} lower than the BS, MS, and YS thickets while CS thickets had intermediate C levels (Figure 5.4). There was a gradient in mean values of C and N of oldest thickets to grassland, indicating that it takes time for the high litter input to accumulate, break down, and influence soil values. Soil $\delta^{15}N$ in GR soils was significantly lower than shrub soils of all ages but did not differ by depth (Figure 5.5, $P<0.001$). The trend in $\delta^{13}C$ is more complicated – grass soils tended to have the highest $\delta^{13}C$ (the least negative), followed by BS soils, and the plots at the center of the island tended to have more negative $\delta^{13}C$ (Figure s5.1).

DON in the water table was higher under BS than YS or GR (Figure 5.6). There was high variation in DOC under older thickets, which only differed from DOC in GR but was not statistically different than DOC in YS water table (Figure 5.6).

Discussion

Shrub encroachment in coastal systems has local consequences – microclimate modification and changes in soil moisture and community composition (Thompson et al., 2017; Wood et al, 2020; Wood et al., in review). Our study sought to better understand the landscape-scale impact of shrub expansion on carbon dynamics and to identify meteorological inputs that influence shrub productivity. Shrub encroachment results from a variety of drivers and controls on productivity, depending on the system and the species (D'Odorico, et al. 2012; Huang, et al., 2018; Malfasi et al., 2020; Sulwiński et al., 2020; Tormo et al., 2020). The chronosequence in our system offers a unique opportunity to better understand potential drivers of shrub productivity and ecological consequences of encroachment in a mesic system. With warming winters driving shrub cover expansion (Huang et al., 2018) and predicted changes in rainfall, we expected vegetation that is reliant on the precipitation-fed freshwater lens to be sensitive to macroclimatic water dynamics and temperature extremes.

Hydrology, in the form of both precipitation and depth to the water table, influenced ANPP; higher precipitation resulted in greater ANPP, whereas water table depth became closer to the surface over time and was associated with a small decrease in ANPP. Wetter soil typically favors shrub establishment over grasses (Gremer et al., 2018) and in our system, shrubs establish only in low-lying swales where water table access is greater (Young 1992), but they are sensitive to flooding (Tolliver et al., 1997). Sea level rise (SLR) is high in the mid-Atlantic, with evidence that it is accelerating (Sallenger et al., 2012). While SLR likely impacts the depth to the freshwater lens, the mid-Atlantic rate of SLR (3.4 ± 0.5 mm yr^{-1}) (Piecuch et al., 2018) is lower than the increase in mean and maximum water table depth (14 and 30 mm y^{-1}). Coupled with the low correlation between precipitation and water table depth, this further supports the likelihood that *M. cerifera* is potentially capable of hydraulic lift and microclimate modification due to high levels of transpiration relative to grasses (Wood et al., 2020; Wood et al., in review). Higher water table, due to shrub expansion, is similar to findings in other systems (Hashim et al., 2019). With increasing variability in recharging of the water table and potential salt intrusion by more frequent and intense storms, the water table will likely continue to play an important role in landscape primary productivity (Groeneveld, 2008; Bryan et al. 2016; Geißler et al. 2019; Kiflai et al., 2020; Holdrege et al., 2021). Precipitation contributed ANPP controls, supporting previous work in normalized difference vegetation index (NDVI) during wet and dry years in our system (Aguilar et al. 2012), while woody vegetation cover in other systems exhibit resistance to drought, particularly in dryer habitats (Medeiros and Pockman, 2011; Xu et al., 2018; Winkler et al., 2019; Peguero-Piña et al., 2020). As precipitation amount and frequency shifts with climate change, the impact on ANPP of woody vegetation in mesic environments will likely intensify (Liu et al., 2017; Xu et al., 2018).

Temperature plays a role in expansion and establishment of shrub cover in many ecosystems, and macroclimatic warming since 1950s is attributed *M. cerifera* expansion in our system (D'Odorico et al. 2010; Osland et al., 2013; Gabler et al., 2017; Huang et al. 2018), but it did not correlate with ANPP over a 17 year timescale. *Morella cerifera* productivity is less sensitive to extreme temperatures because of microclimate modification (Thompson et al., 2017; Sinclair et al., 2020; Wood et al., 2020). The decrease in extreme temperatures by *M. cerifera* structure and physiology keeps the temperature closer to the photosynthetic optimum of 30 ºC (Young 1992). Other systems have increased shrub productivity with warming with little impact by drought (Filella et al., 2004). The biggest influence on ANPP across the chronosequence is thicket age, supporting previous research looking at the decrease in shrub physiological activity with age (Shiflett et al., 2014). Many other systems and species do not exhibit decreased productivity with age (Rundel and Parsons, 1979; Hughes et al., 2006).

The soil characteristics are altered over time of shrub establishment; the oldest soil had higher N and C content than CS and GR indicating that N and C accumulates over time (Raciti et al., 2011; Lunstrum and Chen, 2014; Throop and Lajtha, 2018; Lan et al., 2021). Despite this accumulation, it is still markedly lower than forested habitats (Bell et al., 2021). However, DON and DOC were much higher in the oldest thicket, indicating leaching into groundwater, thus, soil N and C may not be reliable indicators in sandy systems. As N and C accumulate in the freshwater lens, there is an increased likelihood that the nutrients may move throughout the water table and enrich other parts of the landscape (Hefting et al., 2004; Muwamba et al., 2018). This may be especially important in the relatively nutrient poor, sandy soils on barrier islands. Some mesic habitats vary in woody expansion influence on N and C; soil C is lost due to increased microbial activity and N is accumulated from litter input (McCulley and Jackson, 2012). Other mesic grasslands exhibit notable increase in belowground C and no change in N with older woody vegetation (Ward et al., 2018; Charles et al., 2020), partially due to decreased soil biomass breakdown rates. There is a depression in both soil C and N at the 20 cm – 40 cm depth. This zone of the soil could experience variation in water table saturation which could facilitate more leaching of nutrients or an altered microbial community (Hashim et al., 2019; Xia et al., 2020).

In addition to increased N, shrub presence, regardless of age, alters the soil δ^{15}N. The newest grasslands on the ocean side of the established shrub thickets show a different amount of atmospheric fixed N than the thickets suggesting a different N source or rates of cycling. Soil δ^{13}C across the chronosequence shows more δ^{13}C depleted soils from the MS thicket to the YS thicket, indicating greater influence of C3 plants, like *M. cerifera*, in the center of the island, though the influence of decomposition by microbial communities further complicates this trend (Fernandez et al., 2003; Wang et al., 2021). The less negative $\delta^{13}C$ values on the ocean and bay side of the island likely indicates more leaf litter input by C4 species such as *Spartina alterniflora* and *Spartina patens* in the adjacent marsh. Similar to leaching of N and C in the groundwater, other compounds may also mobilize through hydrological flow.

While mesic shrub establishment in our system is largely driven by warming temperatures (Huang et al. 2018), productivity is principally associated with shrub age and water availability. The rate at which shrubs decrease ANPP with age is similar regardless of shrub age or year. Water availability influence on mesic productivity is understudied but plays a significant role; precipitation increases ANPP while higher water table decreases ANPP. Water table depth is influenced by SLR but also modified by potential shrub hydraulic lift (Wood et al., in review), further complicating the relationship between shrub presence and water table depth. The soil nutrient dynamics of shrub encroachment are complex because of nutrient leaching and the variability of water table depth over time. Soil N and C accumulate with shrub age, but isotopes likely exhibit fluctuations due to leaching and microbial activity (Li et al., 2020). Coastal habitats have additional soil nutrient flux due to additional loss of N and C from the habitat via erosion (Martínez et al., 2019); sediment movement through erosion is increased by shrub establishment in our system (Reeves et al., in prep). As shrubs are globally expanding into suitable habitat at unprecedented rates (Zinnert et al., 2016; Huang et al., 2020) these environmental alterations have big impacts in in carbon assimilation and water dynamics of mesic systems.

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Figure 5.1. Hog Island chronosequence showing thickets decreasing in age from left to right: bayside (BS), mid-island (MS), young (YS), colonizing (CS).

Figure 5.2. Annual precipitation (ppt) over the study timeframe exhibited no significant regression (A). Mean temperature increased slightly over study timeframe (B), . Mean water table depth (C) and maximum water table depth (D) both increased significantly in 17 years.

Figure 5.3. Mean adjusted ANPP (g m^{-2} y^{-1}) for all thicket ages across whole time scale (A). Differing letters indicate statistical differences via a post-hoc Tukey test. ANPP among thickets over time (bottom frame).

Figure 5.4. Soil N (A and B) and soil C (C and D) among soil depths and zones. Different letters indicate statistically significant differences using a Tukey post-hoc analysis. BS = bayside, MS = mid-island shrub, YS = young shrub, CS = colonizing shrub, GR = grassland.

Figure 5.5 δ15N did not differ across soil depth but did differ among zones. *** is $p< 0.001$ according to a post-hoc Tukey test. BS = bayside, MS = mid-island shrub, YS = young shrub, CS = colonizing shrub, GR = grassland.

Figure 5.6. DON and DOC from water table in bayside (BS), young shrub (YS), and grassland (GR) plots. Different letters indicate statistical differences using a post-hoc Tukey test.

Figure s5.1. Soil δ13C measurements among thickets and depths. There was an interaction between thicket age (including GR) and depth (F = 2.03, $p = 0.03$). BS = bayside, MS = mid-island shrub, YS = young shrub, CS = colonizing shrub, GR = grassland.


BS
MS
YS
CS
Google Earth
Image USDA Farm Service Agency
3000 ft
N

Figure 5.1

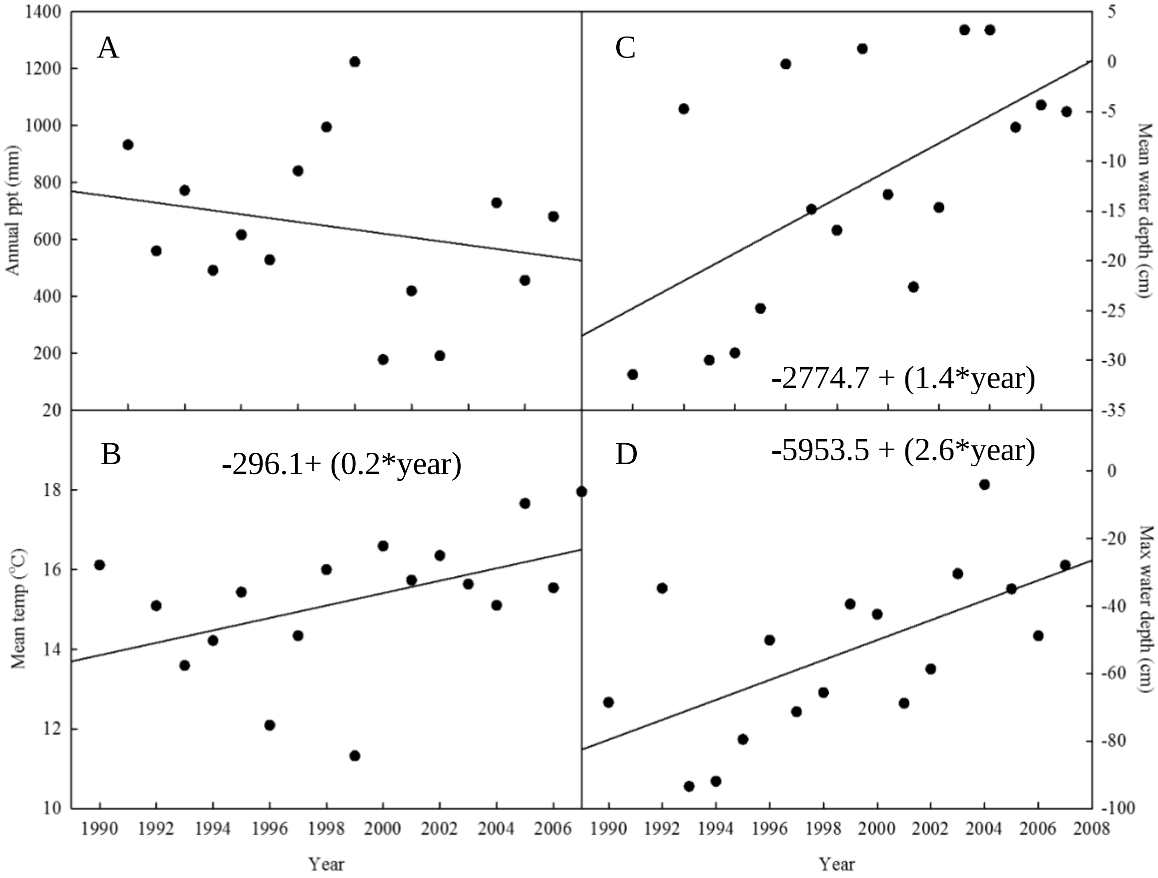

A
B
C
D
-296.1+ (0.2*year)
-2774.7 + (1.4*year)
-5953.5 + (2.6*year)
Annual ppt (mm)
1400
1200
1000
800
600
400
200
20
Mean temp (°C)
18
16
14
12
10
Mean water depth (cm)
5
0
-5
-10
-15
-20
-25
-30
-35
Max water depth (cm)
0
-20
-40
-60
-80
-100
1990
1992
1994
1996
1998
2000
2002
2004
2006
2008
Year
Year

Figure 5.2

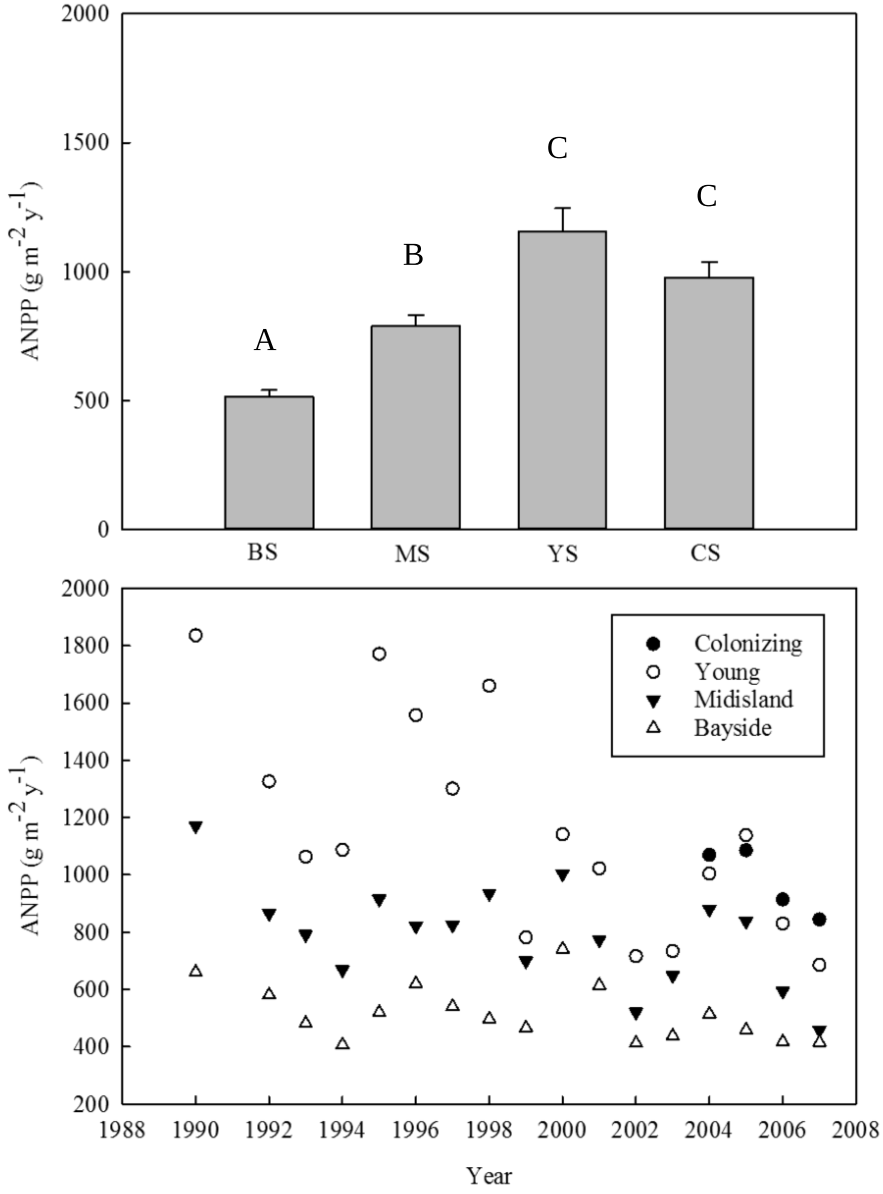

ANPP (g m^{-2} y^{-1})
2000
1500
1000
500
0
A
B
C
C
BS
MS
YS
CS
ANPP (g m^{-2} y^{-1})
2000
1800
1600
1400
1200
1000
800
600
400
200
● Colonizing
○ Young
▼ Midisland
△ Bayside
1988
1990
1992
1994
1996
1998
2000
2002
2004
2006
2008
Year

Figure 5.3

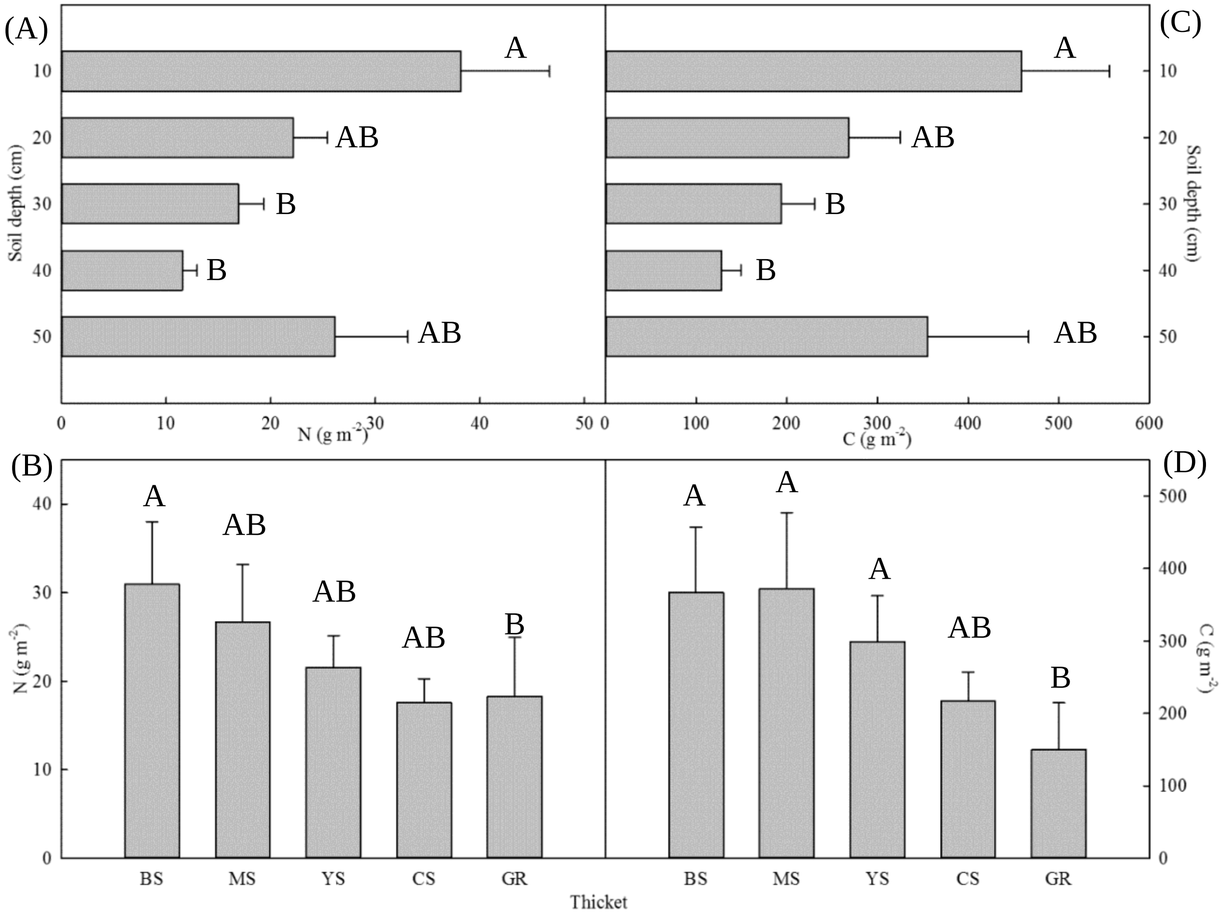

(A)
(B)
(C)
(D)
Soil depth (cm)
10
20
30
40
50
A
AB
B
B
AB
N (g m^{-2})
0
10
20
30
40
50
C (g m^{-2})
0
100
200
300
400
500
600
A
AB
B
B
AB
N (g m^{-2})
0
10
20
30
40
A
AB
AB
AB
B
BS
MS
YS
CS
GR
Thicket
C (g m^{-2})
0
100
200
300
400
500
A
A
A
AB
B

Figure 5.4

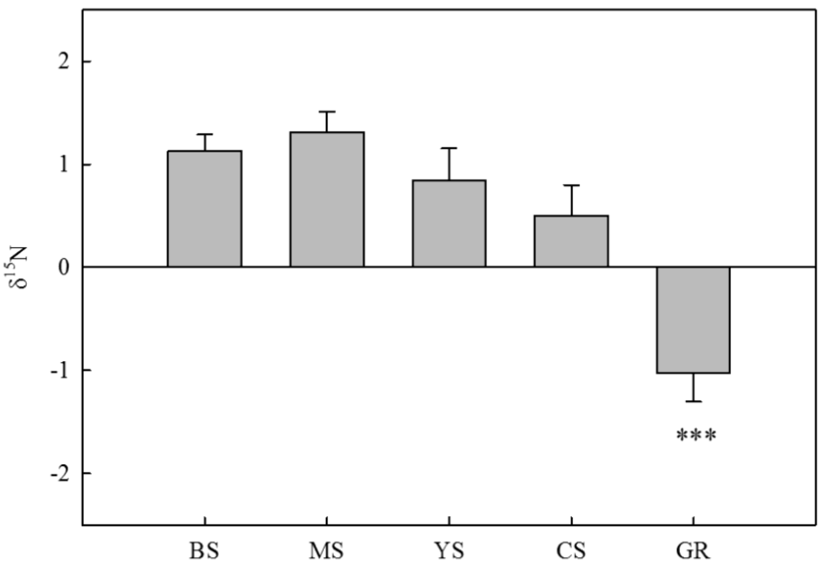
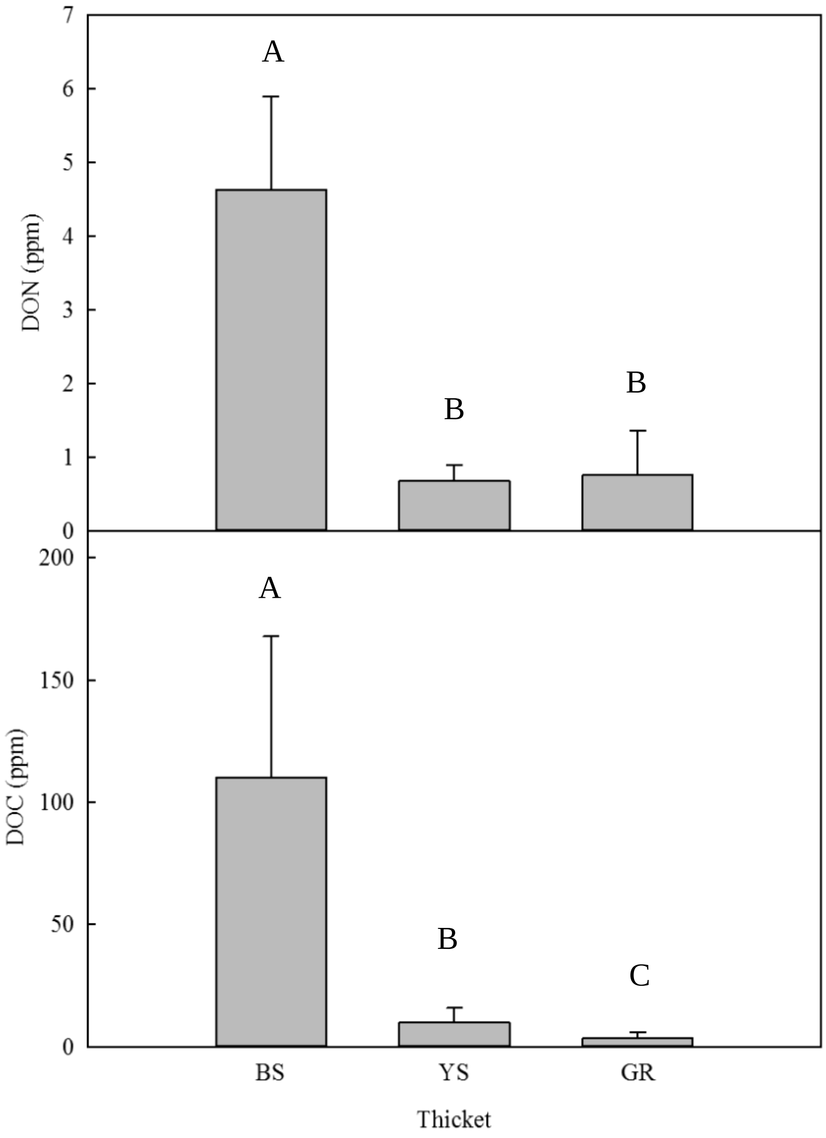


Figure 5.5



DON (ppm)
0
1
2
3
4
5
6
7
A
B
B
DOC (ppm)
0
50
100
150
200
A
B
C
BS
YS
GR
Thicket

Figure 5.6

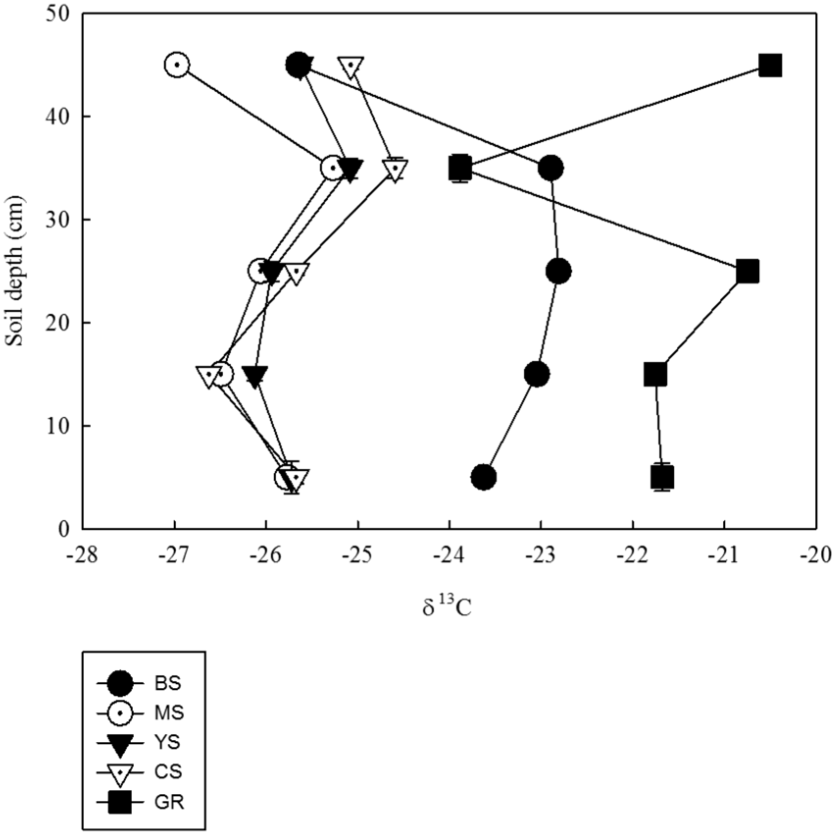

50
40
30
20
10
0
Soil depth (cm)
-28
-27
-26
-25
-24
-23
-22
-21
-20
δ 13C
BS
MS
YS
CS
GR

Figure s5.1

Table 5.1. Top stepwise regression results using AIC, r2, p-values, and RSME.

Terms	ß	p-value	RSME	AIC	r^2
Thicket (all)	-0.29	<0.0001	0.20	-9.58	0.74
Thicket (MS,YS,CS)	-0.17	<0.0001			
Max water depth	-0.01	0.0003			
Min water depth	-0.01	0.0087			
Mean precipitation	4.08	<0.0001			
Thicket (all)	-0.29	<0.0001	0.20	-9.53	0.73
Thicket (MS,YS,CS)	-0.18	<0.0001			
Mean water depth	-0.01	<0.0001			
Mean precipitation	3.44	<0.0001			
Thicket (all)	-0.30	<0.0001	0.20	-8.86	0.73
Thicket (MS,YS,CS)	-0.18	<0.0001			
Max water depth	-0.01	0.0008			
Mean precipitation	3.60	<0.0001			

CHAPTER SIX

Conclusions and significance of research

Lauren K. Wood

Woody vegetation expansion into grassland has been documented for decades globally, driven by climatic and other anthropogenic forces and displacing historical grassland species (D'Odorico et al. 2010, Higgins and Sheiter 2012, Ratajczak et al. 2017, Linders et al. 2019, Ding et al. 2020). Encroaching shrubs act a ecosystem engineers, changing temperature microclimate and soil nutrients, which can in turn affect the surrounding grassland species (McCulley and Jackson 2008, Saixiyala et al. 2017, Charels et al. 2020, Sovie et al. 2021, Zinnert et al. 2021). Much of the research surrounding shrub encroachment is focused in dryland areas (D'Odorico et al. 2010, Medeiros and Pockman 2011, Marchesini et al. 2015, Gremer et al. 2018, Geißler et al. 2019, Tölgyesi et al 2020, Lan et al. 2021) but few have investigated mechanisms of woody encroachment in mesic systems (Zinnert et al. 2016).

Often, woody vegetation acts on the habitat to modify the microclimate (D'Odorico et al. 2012, Devaney et al. 2017, Guo et al. 2017, Huang et al. 2020). Many encroaching shrubs are cold-sensitive and partially driven by warming winter temperatures (Tape et al. 2006, Huang et al. 2018), thus, feedbacks between shrubs and microclimate can help accelerate encroachment and prevent cold-induced hydraulic failure (Medeiros and Pockman 2011, Huang et al. 2018). While some studies examined microclimate modification of *M. cerifera,* they have been observational and limited to shrub edge (Thompson et al. 2017).

Shrub establishment also alters the soil nutrient and water dynamics, which further influences the surrounding habitat. Woody plant establishment can increase carbon (Chun and Choi 2012, Lunstrum and Chen 2014, Ward et al. 2018, Charles et al. 2020). Change in soil N content with shrub encroachment is more case specific, increasing in some systems (Springsteen et al. 2010, Dahl et al. 2020, Kinnebrew et al 2020) and not changing or decreasing in others (Nadal-Romero et al 2018). In our system, *M. cerifera* resorbs little N from its leaves before leaf drop (Brantley and Young 2008) and produces a dense litter layer, adding N and C into soil annually (Brantley and Young 2010). With the local temperature modification and soil amendment by leaf litter, *M. cerifera* can act as an ecosystem engineer in quick-draining sand. The objective of my research was to observe microclimate modification and variance through the transition of grassland to shrub thicket, document change in dominant species and species richness, test ecosystem engineering on temperature and water microclimate through removal, parse out edge effects of shrub establishment on surrounding species traits, and examine drivers of productiivity and long-term impacts of *M. cerifera* encroachment on the landscape.

Shrub expansion on the mid-Atlantic coast results in a drop in species diversity and an increase in primary producitivity relative to grassland (Chapter 2, Wood et al. 2020). Seasonal transpiration rates were increased by shrub presence in transition plots and was highest in fully-formed thickets, likely contributing to the cooling of extreme temperatures in summer (Chapter 2). Microclimate variance was decreased with shrubs and shrub plots exhibited greater temporal control on temperature variance in maximum and minimum air temperature as well as maximum ground temperature (Chapter 2, 3). Extreme temperatures were modified in shrub thickets - thickets were cooler in the summer and warmer in the winter relative to grassland, transitional zones, or removal plots (Chapter 2, 3, 4). Removal of shrubs created a dry habitat, creating a habitat that is re-colonized by many species that thrive in bare sand (Chapter 3, Sinclair et al.

2020, Wood et al. in review). Shrub removal increase the range of temperature extremes, further limiting which species can establish (Latimer and Zuckerberg 2017, Chapter 3). Temperature modification was limited to the immediate habitat unless there was N fertilization, a proxy for N supplementation by *M. cerifera* leaf drop (Chapter 4). This is an indirect modification of grassland traits fertlization, wherein N addition increases grass height and biomass, leading to altered temperatures (Brown and Zinnert 2021, Chapter 4).

In relation to grassland and clear-cut, shrubs increase soil moisture and height of the water table, while also increasing water table depth variability (Chapter 3). Soil moisture is enhanced by organic matter input from shrubs and water table variability is likely a consequence of the increased transpiration by shrubs and high ETR (Chapter 2, Shiflett et al 2014). The nearness of the water table to the surface in shrub plots, coupled with high water use and water table depth variability, suggests hydraulic life by *M. cerifera* (Chapter 3). In addition to shrub age, annual net primary productivity (ANPP) is controlled by water dynamics of the system (Chapter 5). Precipitation has a strong positive correlation with ANPP while higher water table leads to a decrease in ANPP (Chapter 5); this, coupled with the increase in water table depth at a rate 5 - 9 times that of SLR in this region (Piecuch et al. 2018), hydraulic lift by shrubs may play a large role in success of shrubs and ANPP (Chapter 5). Although soil N and C increase under shrubs, accumulating over time (Chapter 3, 5), NO_3- and NH_4+ in soil exhibited a lot of variability, likely due to leaching or quick uptake by N-limited coastal plants (Chapter 3). Ground water under older shrubs had significantly higher dissolved organic N and dissolved organic C than young shrubs or grasslands, suggesting that leaching over time can accumulate and move within the fresh water lens (Chapter 5). Additional N input by shrubs did not alter the plant tissue N or $\delta^{15}N$, which remained relatively constant across all species and treatments

(Chapter 4).

My research supports the hypothesis that shrub establishment is bolstered by microclimate modification, both temperature and water availability, and can impact the species cover, traits, and habitat over time. Encroachment excludes other species, leading to a monospecific thicket, and increases landscape productivity. *Morella cerifera* establishment is driven by increased winter temperatures but the rates of ANPP are more sensitive to precipitation and water table depth. Longer term establishment of shrubs increases nutrient content of soil through leaf drop, but productivity declines with shrub age. Research covering many different time and spatial scales is important to gain insight into the local and landscape-wide consequences of shrub encroachment.

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Vita:

Lauren Kathleen Wood was born to Mary Beth and Bert Wood on August 17, 1988 in Mount Airy, NC. Lauren graduated from North Carolina School of Science and Mathematics in Durham, NC in 2006 and incorporated farming, grape growing, wine making, and a nursing assistant certification into her undergraduate work. They went on to receive an AS from Surry Community College in 2011 and a BS in Ecology, Environmental, and Evolutionary Biology from Appalachian State University in 2013. Lauren's MS work focused on the ecophysiological functioning of *Abies fraseri* across and elevation gradient in the southern Appalachian mountains. Lauren received a PhD in Integrative Life Sciences with a focus on coastal plant ecology in 2021, despite a global pandemic. She will go on to an ecophysiologically-focused post doctoral position with Dr. Sparkle Malone at Florida International University working in the Everglades.