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Seasonal facilitative and competitive trade-offs between shrub seedlings and coastal grasses

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Abstract. Shrub expansion is occurring in grasslands globally and may be impacted by the balance of competition and facilitation with existing grasses. Along the mid-Atlantic and Gulf coasts, the native shrub *Morella cerifera* (wax myrtle) is rapidly expanding and displacing other native coastal species. Recent research suggests that much of this expansion is due to warming winter temperatures, as temperatures below -15°C kill *M. cerifera*. The objective of this project was to understand the importance of species interactions with grasses on the growth and physiology of *M. cerifera* at the seedling life stage through both field and laboratory experiments. In the field, grasses were removed around seedlings and microclimate and shrub physiology and growth were measured. Seeds and seedlings were experimentally frozen to measure the freeze tolerance at both life stages. We found that grasses provided $\sim 1.3^{\circ}\text{C}$ insulation to shrubs during winter. A freezing threshold for *M. cerifera* seedlings was experimentally found between -6°C and -11°C , but seeds remained viable after being frozen to the coldest ecologically relevant temperatures. Seedlings competed for light with grasses during warm months and grew more where grasses were clipped, revealing a trade-off between winter insulation and summer light competition. *Morella cerifera* exhibits ecosystem engineering at the seedling stage by significantly reducing summer maximum temperatures. When seedlings are very young (less than one year), grasses appear to improve germination and seedling survival. These phenomena enable rapid expansion of *M. cerifera* across the landscape and likely inform shrub expansion mechanisms in other systems. Although seedlings are small and relatively vulnerable, this life stage appears to have significant implications for ecosystem trajectory in grasslands undergoing shrub encroachment.

Key words: freeze tolerance; grassland; microclimate modification; shrub encroachment; shrub expansion; shrub seedling.

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INTRODUCTION

Shrub encroachment is often constrained by the vulnerability of young seedlings to environmental stressors (D'Odorico et al. 2012), which may be ameliorated by facilitation from existing grassland species. During the seedling stage, individuals need protection from harsh environmental conditions like excess solar radiation, heat, wind, and drought (Bruno 2000). Mature

grasses not only facilitate seedlings of their own species but also often facilitate seedlings of other species, including shrubs (Maestre et al. 2001, 2003) similar to the well-documented effects of nurse plants (Filazzola and Lortie 2014). In this way, grasses can facilitate the establishment of shrubs and trees, possibly increasing the rate of succession and changing the successional trajectory of a system, even leading to the development of novel ecosystems (Walker et al. 2003,

Angelini et al. 2011, Stinca et al. 2015). The interplay of facilitation and succession has been especially well-documented in the context of shrub expansion.

Global expansion of shrubs into grasslands has led to large changes in ecosystem diversity and function (Archer et al. 1995, Hiltbrunner et al. 2014). Many factors have been attributed to expansion such as increased winter temperatures, increased CO₂ levels, and changes in water availability (Archer et al. 1995, Morgan et al. 2007, Devaney et al. 2017, Huang et al. 2018); however, less attention is focused on the seedling life stage and possible species interactions. Shrubs that associate with nitrogen-fixing bacteria are particularly successful in nutrient-poor ecosystems (Hiltbrunner et al. 2014). Globally, it has been difficult to restore systems that have suffered invasion by shrubs and their nitrogen-fixing microbes back to a natural state due to nutrient legacy effects (Grove et al. 2015, Broadbent et al. 2017). This suggests that even in systems with encroaching native shrubs, a return to the original ecosystem state may be impossible to achieve. This has direct implications for vegetation shifts being observed in coastal grassland sites. Plants in coastal habitats experience many stresses including extreme temperatures, nutrient-poor soils, drought, salt spray, and overwash (Young et al. 2011). Because of these environmental stressors, coastal systems, especially barrier islands, are an excellent place to study ecological interactions in a stressful environment (Leatherman et al. 1979, Zinnert et al. 2017).

Along the Virginia barrier islands, island vegetation change has been rapid, with a 40% increase in woody cover in the Virginia Coast Reserve (VCR) observed in 27 yr, predominately by one shrub species, *Morella cerifera* (wax myrtle; Zinnert et al. 2019). Due to an association with nitrogen-fixing actinobacteria, *Frankia*, (Wijnholds and Young 2000) and ability to modify microclimate at the adult stage (Thompson et al. 2017, Huang et al. 2018), *M. cerifera* rapidly expands: Within 15 yr, a grass-dominated swale can become a closed-canopy shrub thicket (Zinnert et al. 2017). Few plants exist in the understory of mature thickets, but on the edges, survival and growth of species may be facilitated by *M. cerifera* (Thompson et al. 2017) similar to its northern congener, *M. pennsylvanica*

(Shumway 2000). Previous work suggests that much of *M. cerifera* expansion on Virginia barrier islands is due to a warming of winter minimum temperatures. Grasslands rapidly convert to shrub-dominated thickets when minimum temperatures warm above a threshold of -15°C (Huang et al. 2018). Historically, these cold winter temperatures maintained grasslands by killing shrubs. Due to climate change, winter minimum temperatures colder than this threshold have occurred only four times since 1985 (Huang et al. 2018). Previous work on *M. cerifera* microclimate modification and freezing tolerance has focused on adult shrubs, leaving a gap in our knowledge of how seedlings, the leading edge of encroachment, respond to freezing temperatures.

The objective of this research was to understand the importance of species interactions with grasses on the growth and physiology of the expanding shrub, *M. cerifera*, at the seedling life stage. A field experiment was conducted manipulating grass density (clipped vs. unclipped plots) surrounding shrub seedlings, and laboratory experiments were performed to determine shrub seed and seedling freezing tolerance. It was hypothesized that grasses facilitate shrub growth through microclimate moderation. It was further hypothesized that *M. cerifera* seedlings would be less cold tolerant than adults and will have a warmer threshold (i.e., $>-15^{\circ}\text{C}$). By investigating these interactions and freezing effects, a better understanding of plant community dynamics can be attained as swale grasslands are invaded by *M. cerifera* in a changing climate.

MATERIALS AND METHODS

Field site

The VCR contains 13 barrier islands on the Eastern Shore of Virginia, is owned by The Nature Conservancy, and is part of the Long-Term Ecological Research Network (Hayden et al. 1991). The study site was the southern portion of Hog Island (37.417°N , 75.686°W), a barrier island. Shrub thickets first established in swales on the north end of the island and have been progressing rapidly southward (Young et al. 2007, Zinnert et al. 2011, Woods et al. 2019). This research focused on the leading edge of this invasion on the southern end of the island (Woods et al. 2019). The study location was dominated

by coastal grassland species including *Ammophila brevifoliosa*, *Sporobolus pumilus*, *Solidago sempervirens*, and *Andropogon* spp. with many *M. cerifera* seedlings.

Field experiment

The goal of the field experiment was to measure shrub seedling growth, ambient temperature, and light in the presence and absence of grass. In March 2018, 1 × 1 m plots were established with three treatments ($n = 10$ in each treatment): a young *M. cerifera* seedling and intact grass canopy (S), a *M. cerifera* seedling with grass clipped from the plot (SC), and intact grass canopy with all *M. cerifera* seedlings removed (G). A one seedling per m^2 density was chosen to eliminate intra-specific competition. All points were randomly chosen while controlling for environmental heterogeneity (Fig. 1). Following initial clipping in March, resprouting grasses in SC were treated with glyphosate (KnockOut, 1.92%; Globe Chemical,

Avondale Estates, Georgia, USA) to eliminate belowground competition. Any further regrowth was re-clipped as needed. To stop recolonization by grasses outside the plot, grassroots were severed at the plot edge using a shovel. Two SC plots were abandoned following complete mammal herbivory of the shrub seedlings in March and April. Replacement SC plots were established near the damaged plots in May. No further herbivory occurred.

DS1921 iButton data-loggers (Thermochron, Baulkham Hills, New South Wales, Australia) were installed in all plots to measure temperature 5 cm above the ground every 2 h from 18 February to 7 August 2018. Daily and monthly minimum and maximum temperatures were calculated from these temperature data. The 10% coldest and hottest temperatures of all records were also calculated.

In each plot with a seedling (treatments S and SC), the height, maximum crown diameter,

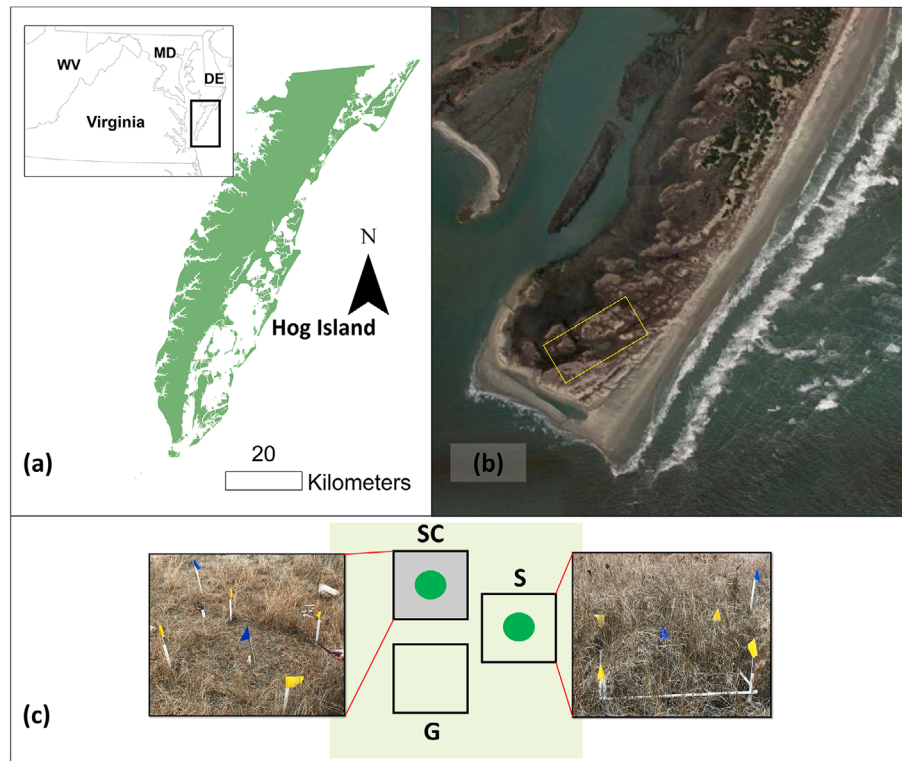


Fig. 1. (a) Location of study site, Hog Island, within the state of Virginia and on the Delmarva Peninsula. (b) Location of study swale on the southern tip of Hog Island. (c) The three treatments: grasses only (G), *Morella cerifera* seedling with grasses clipped (SC), and *M. cerifera* seedling with grasses intact (S).

diameter perpendicular to the maximum diameter, and number of stems of the *M. cerifera* were measured in March, May, June, and August. Crown area was calculated by Eq. 1.

$$\text{canopy area} = \pi \times \text{maximum crown diameter} \times \text{perpendicular diameter} \quad (1)$$

Percent change in height and canopy area was calculated by Eq. 2, where initial measurement was the first measurement of that time period (i.e., May) and the final the last (i.e., June).

$$\text{percent change} = \left(\frac{\text{final measurement} - \text{initial measurement}}{\text{initial measurement}} \right) \times 100 \quad (2)$$

In October, Hurricane Michael caused salt spray and minor overwash on the south end of Hog Island. The storm was followed by a lengthy low-pressure system which caused extensive salt spray and saltwater flooding. By the following spring, only one shrub seedling survived, and thus, final biomass was not measured.

Historical weather

For historical climate reference, daily high and low temperatures were obtained from the Painter, Virginia meteorological station from December 1955 until February 2019 (*National Oceanic and Atmospheric Association* 2019). Daily minimum and maximum temperatures were calculated.

Freezing experiment: seeds

To assess germination success of *M. cerifera* seeds under freezes of different duration and temperature, seeds were collected on Hog Island, Virginia, USA. Seeds were scarified, covered in a paper towel, and placed in twelve Petri dishes, 250 seeds per dish. Petri dishes were randomly assigned to three different temperature treatments, 4°C, -5°C, and -16°C for four different time durations, 1, 4, 8, and 12 weeks. Upon completion of the freezing experiment, each treatment combination was allowed to germinate in a growth chamber with a 14-h photoperiod and a 25:20°C temperature regime at a photon flux density of 400 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$.

Fifty seeds of each treatment combination were placed in 6 × 6 × 3 clam shell plastic containers with perforations in the bottom and filled with a 3:1 sand: soil mixture. Seeds were placed in 10

columns and five rows on top of the sand: soil mixture. There were five replicates of each treatment combination. Seeds were watered, and germination was recorded every five days with a Hoagland solution used to approximate nutrients in the natural soil environment (Hoagland and Arnon 1950). When a seed germinated, it was removed from the container. A seed that contained a radicle of 2 mm was counted as germinated. Seeds were treated and observed for 50 d.

Freezing experiment: seedlings

The purpose of the seedling freezing experiment was to quantify seedling survival and physiological health after freezes of different temperatures. *Morella cerifera* seeds were scarified and germinated in early September 2018 and transplanted to 15-cm pots in October, two per pot. All were grown in the VCU glasshouse for nine weeks. Seedlings were transplanted into nine-centimeter pots, one per pot, and moved to an environmental chamber (Model #E15; Conviron, Winnipeg, Manitoba, Canada) with 14 h of sunlight and kept at 25:20°C. After nine weeks, randomly chosen seedlings were moved to a walk-in refrigerator and kept at 4°C to mimic wintertime temperatures ($n = 35$). Grow lights (GRO-LUX Wide Spectrum F-40; LEDVANCE, Wilmington, Massachusetts, USA) were placed above the seedlings and LED construction lights to the sides (Craftsman 2000 Lumens LED Work Light; Craftsman, New Britain, Connecticut, USA; and Utilitech LED Construction Light; Utilitech, West Lawn, Pennsylvania, USA) delivering $\sim 400 \text{ mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. A subset of seedlings remained in the 25:20°C environmental chamber to serve as a warm control ($n = 7$). Daily light duration was reduced to 10 h to simulate winter light conditions in January. Throughout the experiment, all seedling pots sat in a pan of water to reduce water stress.

Seedlings were randomly assigned to freezing and control treatments. Freezing treatments were -20°C, -15°C, -11°C, and -6°C, and control treatments were 4°C and 25°C ($n = 7$). Prior to freezing, height was recorded with a ruler and general health was noted. Dark-adapted chlorophyll fluorescence (i.e., F_v/F_m) was measured on the 4th fully expanded leaf using a MINI-PAM-II photosynthesis yield analyzer (Walz Company, Effeltrich, Germany).

To simulate an early morning winter freezing event, seedlings were placed in a chest freezer (Model FFFC09M1RW; Frigidaire, Charlotte, North Carolina, USA) that had been allowed to warm to 0°C. The target freezing temperature was achieved and then maintained for 180 min ($\pm 1^\circ\text{C}$) following Huang et al. (2018) and Medeiros and Pockman (2011). After this time, the freezer was allowed to warm back to 0°C, and the seedlings were removed. Immediately following removal from the freezer, general health was noted, number of green, necrotic, and curled leaves was recorded, and F_v/F_m was measured. Leaf disks were excised from the youngest fully expanded leaves to measure electrolyte leakage described below.

One day and one week after freezing, survival and general health were noted, number of green, necrotic, and curled leaves was recorded, and F_v/F_m was measured for each seedling. After one week, seedlings were harvested, and aboveground fresh mass was recorded. Aboveground samples were dried at 70°C for 72 h, and dry mass was recorded. Leaf disks from electrolyte leakage measurements were dried and added back to the biomass of plants from which they were removed.

Electrolyte leakage.—Electrolyte leakage, how much cellular fluid leaks into leaf interstitial fluid following rupture of cell walls, is often used to evaluate severity of freeze damage. To measure this, thirteen leaf disks (1.27 cm or 0.5" diameter) were excised from the youngest fully expanded leaves following post-freeze measurements ($n = 3$ due to destructive measurements). Fresh weight of leaf disks was recorded on a micro-scale, to estimate final biomass including removed leaf disks. The leaf disks were rinsed three times in DI water for two to three min each time (Jungklang et al. 2017). Leaf disks were placed into 15 mL of DI water in a 50-mL Thermo Scientific Nunc Conical Centrifuge Tube (Thermo Fisher Scientific, Waltham, Massachusetts, USA; Jungklang et al. 2017) and shaken at 50 rpm for 24 h (Boorse et al. 1998). Conductivity was measured using a conductivity probe (Oakton Benchtop Con 700 Meter with a $K = 1.0$ probe, Oakton Instruments, Vernon Hills, Illinois). The tubes were autoclaved at 121°C for 15 min and shaken for 24 h at 50 rpm, after which maximum conductivity was measured. Relative conductivity was calculated by dividing

initial conductivity by maximum conductivity; those with higher relative conductivity represent greater cellular damage (Boorse et al. 1998).

Statistical analyses

Parametric tests including one- and two-way analyses of variance (ANOVAs) were used when appropriate followed by a Tukey post hoc test. When normality assumptions were violated, a Kruskal-Wallis test was used. Nonparametric test results were compared post hoc using a Nemenyi test or Fisher's least significant difference (based on sample size). For tests with only two treatments and heterogeneous variances, Welch's t -test was employed. A mixed-effects model was used to analyze differences in F_v/F_m among freeze treatments and sampling time with plant ID as a random effect using R package lme4 (Bates et al. 2015). Importance of model components was tested using pairwise likelihood ratio tests. Post hoc comparisons were performed using estimated marginal means, a least squares mean method over a reference grid. All analyses were tested at the $\alpha = 0.05$ level and conducted in R (R Core Team 2017).

RESULTS

Field experiment

Microclimate modification.—There was no significant interaction of month and treatment for monthly maximum temperature ($F_{12, 184} = 1.58$, $P = 0.09$), but main effects differed (month: $F_{6, 184} = 361.90$, $P < 0.05$; treatment: $F_{2, 184} = 38.79$, $P < 0.05$). All treatments significantly differed from one another. Interestingly, both treatments with intact grass canopies (G and S) had higher maximum temperatures than plots where grasses were clipped (SC; Fig. 2a). Monthly minimum temperatures showed an interaction of treatment and month ($F_{12, 184} = 2.83$, $P < 0.05$; Fig. 2b). In the coldest month, March, there was a significant treatment effect ($F_{2, 26} = 10.69$, $P < 0.05$) where SC plots had minimum temperatures that were 1.4°C and 1.2°C colder than G and S plots, respectively.

Seedling growth

Total percent growth did not differ between treatments for height ($t_{16} = -0.11$, $P = 0.91$) but did differ for canopy area ($t_{9.42} = -2.61$,

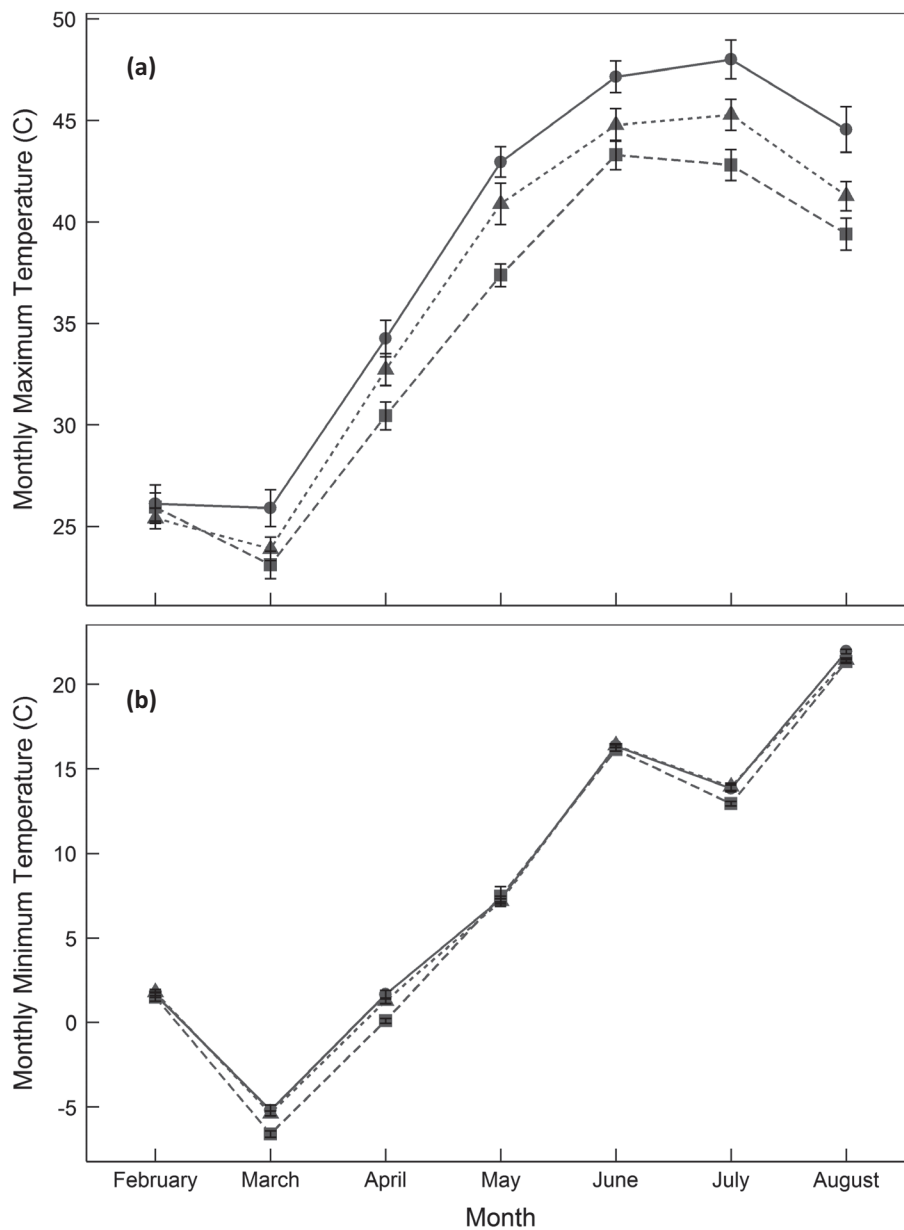


Fig. 2. Mean monthly maximum (a) and minimum (b) temperature (\pm standard error) for each treatment, 5 cm above the ground. Treatments are denoted as circles (G), triangles (S), and squares (SC). All treatments had significantly different maximum temperatures. For minimum temperatures, an interaction of month and treatment was present. Asterisks show months where SC was significantly less than other treatments. In the coldest month, March, SC had the lowest minimum temperature.

$P = 0.03$; Fig. 3). When growth was split between sampling periods, percent height growth and percent canopy area growth of shrub seedlings both showed a significant interaction of treatment and time period ($F_{2,52} = 10.49$, $P < 0.05$;

$F_{2,52} = 3.69$, $P = 0.03$, respectively). Percent height change differed between S and SC from June to August as SC plots grew taller more rapidly than did S plots but did not differ between treatments before June (Fig. 4a). From

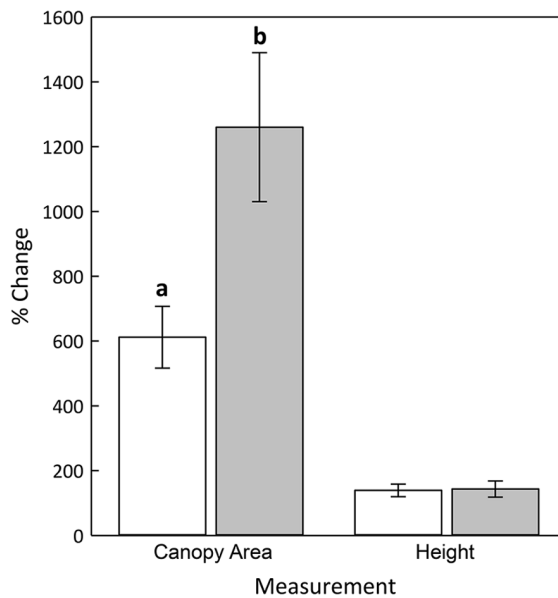


Fig. 3. Mean total percent change in canopy area and height (\pm standard error) from March until August. Height change did not differ, but SC seedlings grew about twice as much in canopy area as S seedlings. Letters show significantly different groups. S is white; SC is gray.

March until June, canopy area growth was very similar between S and SC, but SC seedlings increased significantly more than S seedlings from June to August (Fig. 4b).

Freezing experiment: seeds

There was no significant interaction in germination of *M. cerifera* seeds at different treatment combinations of freeze duration and intensity ($F_{11,48} = 1.83$, $P = 0.16$). There was a significant decline in germination at -16°C ($F_{11,48} = 6.96$, $P = 0.002$; Table 1), but no difference by freeze duration ($F_{11,48} = 1.85$, $P = 0.11$).

Freezing experiment: seedlings

Freeze treatment was an important predictor of photosystem function as estimated by F_v/F_m (Table 2). A threshold appears between -6°C and -11°C , below which frozen leaves and stems do not recover (Fig. 5). Controls and -6°C were able to maintain photosynthetic function following treatment. The colder freeze treatments (-11°C , -15°C , and -20°C) showed some delay in the effects of damage but by a week after all

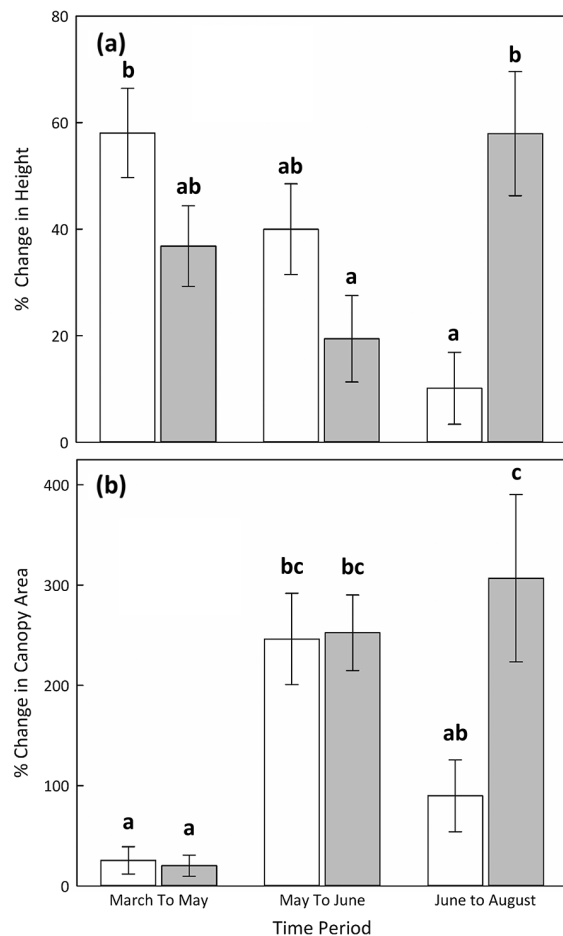


Fig. 4. Mean percent change in height (a) and canopy area (b) (\pm standard error) over two- to three-month intervals shows treatments did not differ in height or canopy area growth until after June and that SC had greater percent growth of canopy area during the summer than S. Letters denote significant differences among groups. S is white; SC is gray.

were severely photoinhibited ($F_v/F_m < 0.52$). Relative conductivity differed among freeze treatments ($X_5^2 = 15.74$, $P < 0.05$). Relative conductivity was greater in colder freeze treatments (-11°C , -15°C , and -20°C), as freezing damage led to more electrolyte leakage. The threshold estimated by this method was also between -6°C and -11°C (Fig. 6).

Freeze temperature had a significant effect on number of necrotic and curled leaves ($X^2 = 112.95$, $P < 0.05$; $X^2 = 69.85$, $P < 0.05$, respectively). There were very few necrotic leaves in any of the treatments until one week

Table 1. Mean \pm standard error for germination among *Morella cerifera* seeds at a different temperature.

Temperature (°C)	No. seeds germinated
4	24 \pm 7 ^{ab}
-5	27 \pm 5 ^a
-16	21 \pm 4 ^b

Note: Letter codes denote results of Tukey's post hoc tests.

after freezing, and only in plants frozen to $\leq -11^\circ\text{C}$. Number of curled leaves increased with colder freezes and with time after the freeze event; though all seedlings exposed to 4°C or colder had some curled leaves. Freeze treatment had a significant effect on number of green leaves and total leaves ($X^2 = 246.19$, $P < 0.05$; $X^2 = 37.32$, $P = 0.01$, respectively). The 25°C treatment had more green leaves and more leaves total at all time steps than other treatments. All treatments exposed to cold had fewer total leaves and lost very few leaves after freezing, as leaves became necrotic but did not drop. Plants frozen to $\leq -6^\circ\text{C}$ had fewer green leaves a week after freezing. Final biomass did not differ among treatments ($F_{5,12} = 1.53$, $P = 0.25$).

Extreme temperatures

From year to year, average winter minimum temperatures have increased at the VCR (Goldstein et al. 2018). Temperature events colder than -11°C , a conservative estimate of the seedling freeze threshold, have occurred 44 times since 1985 at the Painter, Virginia, meteorological station. Accounting for the 1.3°C insulation provided by grasses, only $20 < -12.3^\circ\text{C}$ events have occurred (Fig. 7). During the field experiment, only one temperature event occurred below -11°C (-12.8°C) in Painter, Virginia, but no plot

iButtons showed minimum temperatures $< -7.5^\circ\text{C}$. Temperatures $> 50^\circ\text{C}$ occurred 13 times during the study but never in a shrub plot and mostly in one grass plot. *M. cerifera* can maintain photosynthesis up to 50°C (Young 1992).

DISCUSSION

Species interactions that govern shrub expansion are complex and include both competition and facilitation. Little work has been performed on how species interactions of shrub seedlings affect transition from grassland to shrubland with a few notable exceptions (i.e., Berlow et al. 2002, Ward and Esler 2011). *Morella cerifera* encroachment on the Virginia barrier islands provides an understanding of seedling–grass interactions. Coastal systems share similarities to other ecosystems being encroached (i.e., arid and Arctic) as soils are low in resources (e.g., nutrients and freshwater) and high in abiotic stressors (e.g., flooding, saline soils, sand abrasion, high light levels, and soil temperatures) creating challenges for germination and establishment (Ehrenfeld 1990). Under such conditions, competition for resources may be secondary to facilitation when neighbors act to buffer stresses, thus promoting growth (Callaway et al. 2002).

It was hypothesized that microclimate would be modified in plots with intact grass canopy and would cause enhanced growth of shrub seedlings. This hypothesis was partially supported as grasses modified microclimate through winter insulation from minimum temperatures; however, shrub seedlings exhibited higher growth, especially in the summer, where grasses were clipped, revealing a net competitive interaction at this life stage. The hypothesis that

Table 2. Summary of linear mixed-effects models for F_v/F_m among freezing treatments (treatment) and sampling period (time).

Models	df	AIC	X^2 (df)	P
$y \sim \text{time} + (1 \text{id})$	6	-73.964		
$y \sim \mathbf{\text{treatment}} + \text{time} + (1 \text{plant id})$	11	-126.879	62.916 (5)	<0.00001
$y \sim \text{treatment} + \text{time} + (1 \text{plant id})$	11	-126.879		
$y \sim \mathbf{\text{treatment} * \text{time}} + (1 \text{plant id})$	26	-252.887	156.01 (15)	<0.00001

Notes: Sampling periods included before freezing and immediately, one day, and one week after freezing. The variability among individual plants was incorporated in the model by the random effect component (1|plant id). For each pair of models compared, chi-square statistics (X^2) and P -values were calculated using a likelihood ratio test. The specific component tested in each pairwise comparison is shown in bold text. Degrees of freedom (df) and Akaike information criterion (AIC) values are presented for each model. An α of 0.05 was assumed when testing significance of each model component.

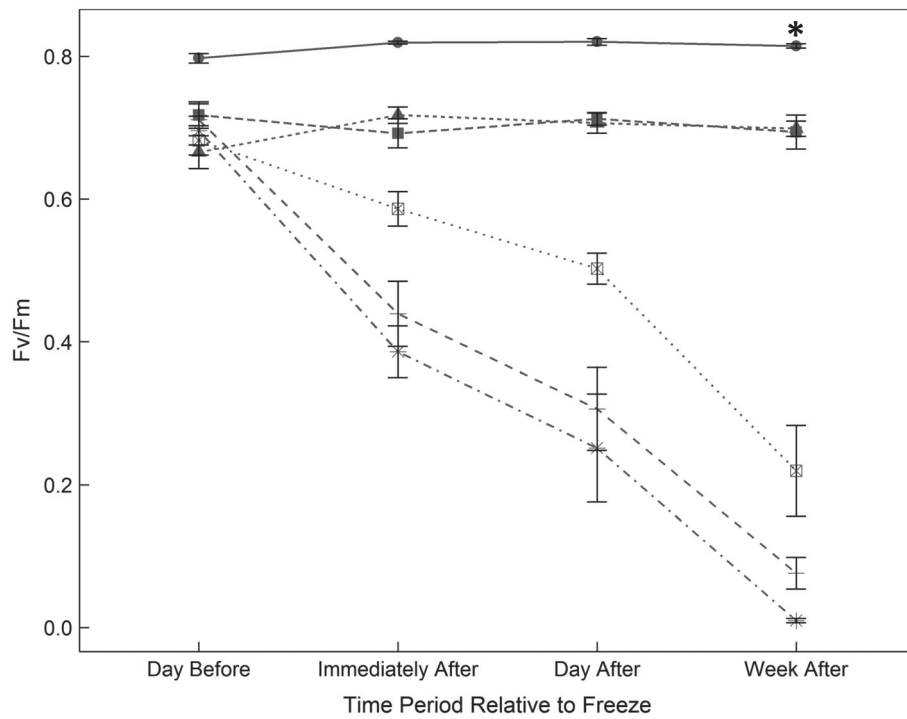


Fig. 5. Mean (\pm standard error) F_v/F_m for each freeze treatment at each sampling time. Asterisk denotes significant reduction in freeze treatments below threshold ($\leq -11^\circ\text{C}$) after one week. Treatments are denoted as circles 25°C, triangles 4°C, filled squares -6°C , crosses -11°C , hollow squares -15°C , and asterisks -20°C .

seedlings have a warmer freezing threshold than adults ($> -15^\circ\text{C}$) was supported as seedlings had a threshold between -6°C and -11°C , below which seedling physiology and electrolyte leakage measurements showed unrecoverable damage to aboveground tissues. Seed germination

rates were only mildly affected by freeze intensity and not by duration, suggesting that seedlings are the most vulnerable life stage to cold temperatures. Minimum temperatures control the range of many shrub species (Pockman and Sperry 1996, Franklin 1998, D’Odonico et al.

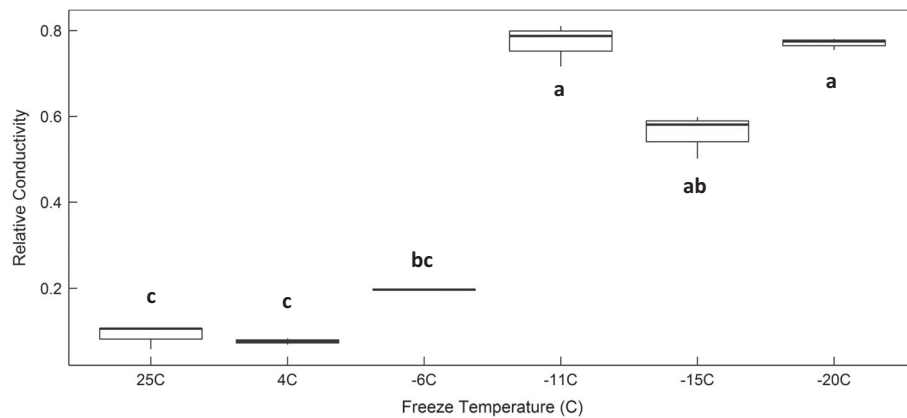


Fig. 6. Median relative conductivity (bold line) with quartiles (box and whiskers) across freeze treatments. Letters denote significant differences among treatments.

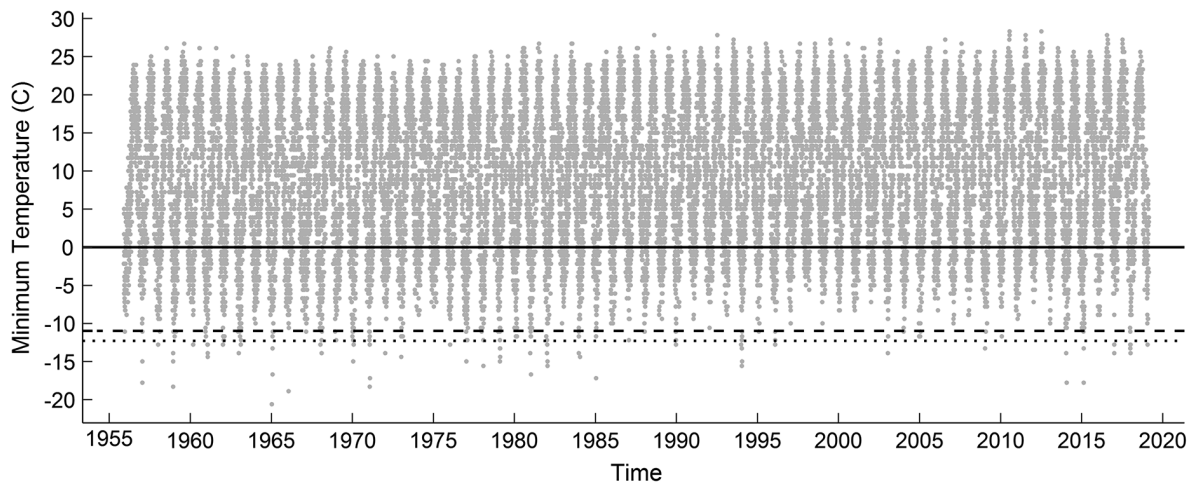


Fig. 7. Daily minimum temperatures from 1955 to 2019. Dashed line shows -11°C threshold found in freezing experiment; temperatures below this occurred 44 times since 1985. Dotted line shows -12.3°C (accounting for the 1.3°C insulation provided by grasses); temperatures below this have occurred 20 times since 1985.

2012, He et al. 2015), and freezing tolerance at the seedling stage may play a larger role than previously thought.

Although grasses ameliorate winter minimum temperatures and protect *M. cerifera* seedlings from freezing events, grasses did not moderate extreme summer temperatures. Plots with shrub seedlings had lower maximum temperatures than those without, demonstrating that *M. cerifera* moderates microclimate at earlier life stages than previously found (Thompson et al. 2017). This demonstrates that *M. cerifera* is an ecosystem engineer at the seedling stage by creating environmental conditions more favorable for growth, possibly leading to faster expansion. This is significant as, to our knowledge, shrub seedlings as an ecosystem engineer have not been observed previously and may occur in other systems. Conversely, SC seedlings grew more than those with intact grass canopy (they had wider canopies than S seedlings likely due to increased branching), suggesting that shrub seedlings compete with grasses for light and other resources, as has been seen in other systems (Berkowitz et al. 1995, Davis et al. 1998). As shrubs are rarely found in bare soils, the presence of grasses is beneficial to shrub seedlings at some earlier point in growth (Woods et al. 2019).

In many systems, interactions between shrub seedlings and grasses might vary seasonally.

Morella cerifera presents a trade-off between summer competition and winter facilitation. At the VCR, shrub seedlings which grow in dense grasses experience increased competition for light but are less likely to suffer mortality during a freeze event. Temperature events colder than -11°C , a conservative estimate of the threshold found in the freezing experiment, have occurred 44 times at the VCR since 1985, when shrubs began to rapidly expand across the landscape (Young et al. 2007, Zinnert et al. 2011). Seeds are only mildly affected below this threshold, but seedlings are vulnerable during these relatively common events and would likely experience irreversible damage without insulation by grasses. Accounting for the 1.3°C insulation provided by grasses, fewer $<-12.3^{\circ}\text{C}$ events occurred. Insulation by grasses considerably reduced the frequency of potentially lethal temperatures for seedlings (D'Odorico et al. 2012). The interaction between grasses and *M. cerifera* seedlings varies seasonally from facilitative to competitive.

This temporal shift between facilitation and competition has been demonstrated in other systems (Maestre et al. 2003, Kikvidze et al. 2006, Wright et al. 2014, Loranger et al. 2017). Although we expected summer to be stressful for shrub seedlings, we found that grasses did not ameliorate maximum temperatures. It appears that summer temperatures are not stressful for

M. cerifera seedlings, which is not the case for all shrub species. *Morella cerifera* has a relatively high photosynthetic temperature optimum (~30°C) and maintains some level of photosynthesis (although reduced) up to 50°C (Young 1992); no temperatures occurred above 50°C in any shrub plots. Shrub response from the freezing experiment suggests that winter may be the most stressful time of the year for *M. cerifera* seedlings. As winter minimum temperatures in the region have warmed due to climate change (Goldstein et al. 2018), *M. cerifera* seedlings benefit. Thus, interactions between shrub seedlings and grasses have likely become increasingly competitive, providing evidence that species interactions shift with climate change (Klanderud 2005, Van Der Putten et al. 2010), potentially enhancing expansion rates across the landscape.

As climate changes, shifts in interactions at the seedling level are leading to emergent properties at the landscape level seen in the rapid expansion of *M. cerifera*. *Morella cerifera* seedlings compete with grasses for resources and grow more when grasses are clipped away, as has been seen in many ecosystems for tree seedlings (Berkowitz et al. 1995, Ward and Esler 2011). Shrub seedlings had a total increase of ~600% in canopy area during the study; thus, seedlings are shading out other species earlier than previously observed (Thompson et al. 2017). At later stages, competitive effects intensify as shrub thickets exclude nearly all other species by low light availability via high leaf area and thick litter layers (Brantley and Young 2007, Zinnert et al. 2011, Thompson et al. 2017). *Morella cerifera* exerts a strong biotic control similar to hemlock forests (Martin and Goebel 2013). Thickets arrest succession under current climatic conditions, preventing the historic progression to maritime forest (Bissett et al. 2016, Zinnert et al. 2017) and likely representing an irreversible state change similar to shrub encroachment in the southwestern United States (D'Odorico et al. 2012).

This new ecosystem is a result of ecosystem engineering by *M. cerifera*. Seedlings modify microclimate, and when combined with warming winter temperatures, enable the rapid expansion of *M. cerifera* across the barrier islands. Although shrub seedlings are small, the

biotic interactions which govern their success appear to have significant implications for successional trajectories and perhaps even whole-ecosystem stability (Walker et al. 2003, Angelini et al. 2011, D'Odorico et al. 2012, Zinnert et al. 2019). Few studies investigating shrub expansion look at the role of the seedling life stage. While much is known about how adult shrubs interact with nearby species (Shumway 2000, Al-Namazi et al. 2017), modify microclimate (Devaney et al. 2017, Thompson et al. 2017), and respond to disturbances and climate change (Archer et al. 1995, Morgan et al. 2007, Huang et al. 2018), these are understudied at younger life stages. It is necessary to understand the dynamics of shrub encroachment at all life stages in order to better manage and study this global phenomenon.

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