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Functional traits of expanding, thicket-forming shrubs: contrasting strategies between exotic and native species

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Abstract. Woody expansion has been documented for decades in many different systems globally, often yielding vast changes in ecosystem functioning. While causes and consequences of woody expansion have been well documented, few studies have addressed plant functional traits that promote dramatic and rapid expansion in range. Our objectives were to investigate plant functional traits that contribute to the colonization, rapid expansion, and thicket formation of an invasive, N-fixing shrub, Elaeagnus umbellata Thunb. (Elaeagnaceae), and a native, N-fixing shrub Morella cerifera (L.) Small (Myricaceae) and compare to native, sympatric, non-expanding shrub species. Quantified functional traits included morphological (e.g., specific leaf area, leaf area) and physiological characteristics (e.g., electron transport rate, hydraulic conductivity) and were linked to two primary resources: light and water, which directly influence plant growth. Elaeagnus umbellata and M. cerifera rely on different strategies to maximize carbon gain, yet resulting physiological efficiency is similar. Elaeagnus umbellata invests a substantial amount of energy into growth during a short amount of time (i.e., deciduous growing season), using an acquisitive trait strategy to outcompete co-occurring woody species, while M. cerifera is productive year-round and uses a combination of conservative and acquisitive traits to outcompete co-occurring woody species. The majority of quantified functional traits of E. umbellata and several of M. cerifera are indicative of efficient light capture, utilization, and internal water movement. These factors contribute to rapid range expansion and thicket formation by promoting enhanced productivity while simultaneously inhibiting colonization and expansion of co-occurring species. Suites of functional traits are important for expansive success and thicket formation, yet differences in functional traits represent alternative strategies for colonization, rapid expansion, and thicketization.

Key words: electron transport rate; functional traits; hydraulic conductivity; nitrogen fixation; thicket formation; woody expansion.

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INTRODUCTION

2013, Thompson et al. 2017). Furthermore, many shrub species that encroach into grasslands form dense monospecific thickets that limit colonization opportunities by other species (Brantley and Young 2010). A large body of research exists documenting the causes of woody expansion into grasslands (e.g., fire suppression, reduced grazing pressures, rising atmospheric CO2 concentrations, see McCarron et al. 2003, Huxman et al. 2004, Sankaran et al. 2005, Prévosto et al. 2006, Archer 2010). In contrast, few studies have focused on mechanisms, especially plant functional traits related to morphology and physiology, leading to woody expansion and, in some cases, monospecific thicket formation (i.e., thicketization).

Functional traits linked to assimilation of water, light, and nutrients, directly influence growth and productivity (Ackerly et al. 2000, de Bello et al. 2010) and may explain success in rapidly expanding woody species (Baruch and Goldstein 1999, Zinnert et al. 2013, Shiflett et al. 2014). Traits related to light capture (e.g., specific leaf area [SLA], leaf size, leaf angle) can influence photosynthetic rates. Similarly, traits associated with water movement (e.g., sapwood area-to-leaf area ratio, vulnerability to embolism) affect hydraulic conductivity and/or hydraulic supply to leaves. Increases in hydraulic capacity tend to lead to enhanced photosynthetic capacity (Brodribb and Field 2000, Brodribb et al. 2002, Jones et al. 2010), stomatal conductance, and subsequent CO2 fixation and plant growth rates (Meinzer and Grantz 1990, Sperry 2000). Study of morphological and physiological traits is essential because these factors may represent mechanisms responsible for rapid rates of shrub expansion that often lead to thicket formation.

The shrubs, Elaeagnus umbellata Thunb. (Elaeagnaceae) and Morella cerifera (L.) Small (Myricaceae), form extensive, dense thickets reaching maximum heights of 5-6 m, minimizing subcanopy light penetration (Brantley and Young 2010). Elaeagnus umbellata (autumn olive) is deciduous, drought resistant, moderately shade tolerant and was introduced as an ornamental shrub in 1830 to North America from Asia. It has spread via cultivation in the mid- and eastern United States, from Maine to Florida and west from Wisconsin to Louisiana and occurs across a range of habitat types including edge and interior habitats, but is most prolific on disturbed or ruderal sites such as roadsides and old fields (Ebinger and Lehnen 1981, Edgin and Ebinger 2001). Elaeagnus umbellata is invasive and rapidly expanding into non-native habitat (Yates et al. 2004). It is considered “highly invasive” or ranked as a “severe threat” in many states of the southeast and is prohibited on National Forest System Lands (USDA 1987; Lund et al., public communication, Invasive Plant Species of South Carolina; https://www.se-eppc.org/southcarolina/Publications/InvasivePlantsBooklet.pdf).

Morella cerifera (southern bayberry) is a mesic evergreen, native to the Atlantic coast, and the dominant woody species on Mid-Atlantic barrier islands (Ehrenfeld 1990). It occurs from Florida to southern New Jersey and west to Texas. Cover of M. cerifera on Virginia barrier islands has increased in the past 50 yr, up to 400% on some islands (Young et al. 2007, Zinnert et al. 2011). Like E. umbellata, M. cerifera is drought resistant and moderately shade tolerant and occurs across a range of habitat types including edges and interiors. Both species, which are often among the first woody plants to colonize an area, thrive in early successional conditions, produce prolific, ornithochorous (i.e., bird-dispersed) seeds, and form an association with the actinomycete, Frankia, an N-fixing root nodule-forming endosymbiont. N-fixing symbiosis may provide an advantage over co-occurring non-N-fixing species owing to favorable alterations in nutrient acquisition, energy resources, and stress tolerance (Yates et al. 2004).

Studies which compare functional trait combinations of expanding species from different habitats may provide evidence of globally shared traits that are contributing to observed shifts in growth forms (Brym et al. 2011, Funk and Cornwell 2013). Yet, research investigating functional traits and ecophysiology of expansive woody species is limited, especially in areas where they are expanding (Gong et al. 2006, Zhao et al. 2007, Brym et al. 2011). Our objectives were to investigate functional traits pertaining to light capture, processing, and water use that contribute to successful colonization, expansion, and dense thicket formation of invasive (E. umbellata) and native (M. cerifera) N-fixing shrubs and compare traits to native, sympatric, non-expanding shrubs. Specific goals were to quantify differences in light and water use physiology by examining (1) stem and leaf hydraulic...
conductivity, (2) leaf morphology, (3) leaf angle, (4) leaf biochemical traits, and (5) leaf chlorophyll fluorescence. We hypothesized that *E. umbellata* and *M. cerifera* exhibit functional traits indicative of efficient light capture, utilization, and hydraulics and that these traits promote colonization, rapid expansion, and formation of closed-canopy, monotypic thickets relative to native, sympatric shrubs.

**METHODS**

*Inland site description*

An inland portion of our study was conducted at Fort A.P. Hill, Virginia, USA (38°05’ N, 77°20’ W), during the summers of 2011 and 2012. The climate is temperate with maximum summer temperatures spanning 28–31°C and an average yearly precipitation of 1131 mm. Expansion of *Elaagnus umbellata* along roadsides, into old fields, and along forest edges has been documented here since it was planted in the 1970s (J. Applegate et al., *public communication*; EDDMapS; http://www.eddmaps.org/distribution/point.cfm?id=632886). Two native shrub species co-occur with *E. umbellata*: *Clethra alnifolia* L. (Clethraceae) and *Vaccinium corymbosum* L. (Ericaceae). *Vaccinium corymbosum* is a deciduous, multi-stemmed shrub that reaches 4 m in height and is often found in freshwater wetlands and salty or brackish marsh fringes. It is often found in thickets and also disturbed habitats. *Iva frutescens* is a deciduous, upright, multi-stemmed shrub, which can reach 3.5 m in height that preferentially grows in coastal areas and is very common in salt marshes. *Iva frutescens* is salt tolerant (i.e., halophytic), but is intolerant to flooding.

*Coastal site description*

A coastal portion of our study was conducted at the northern end of Hog Island, a barrier island located ~10 km east of the Eastern Shore of Virginia, USA (37°27’ N, 75°40’ W), during the summers of 2011 and 2012. The climate is temperate with maximum summer temperatures from 28° to 34°C and mean annual precipitation ranging from 1065 to 1167 mm/yr (Brantley and Young 2010). Hog Island is ~1000 ha, 10 km long, and 2.5 km across at its widest point. It is part of the Virginia Coast Reserve, an NSF-funded Long-Term Ecological Research (LTER) site, managed by The Nature Conservancy. A series of progressively older, dense thickets, dominated by *Morella cerifera*, has developed in mesic swales (i.e., interdunal depressions) across the northern end of the island. Individuals, even growing in close proximity, are likely to be genetically distinct (Erickson et al. 2004). Shrub thickets, consisting of predominately *M. cerifera* and which have expanded considerably over the past 50 yr, now cover more than 30% of the island (Zinnert et al. 2011). Two native shrub species co-occur with *M. cerifera*: *Baccharis halimifolia* L. (Asteraceae) and *Iva frutescens* L. (Asteraceae). *Baccharis halimifolia* is a deciduous, multi-stemmed shrub that reaches 4 m in height and is often found in freshwater wetlands and salty or brackish marsh fringes. It is often found in thickets and also disturbed habitats. *Iva frutescens* is a deciduous, upright, multi-stemmed shrub, which can reach 3.5 m in height that preferentially grows in coastal areas and is very common in salt marshes. *Iva frutescens* is salt tolerant (i.e., halophytic), but is intolerant to flooding.

*Water transport*

A suite of physiological traits linked to water transport were measured. Four branches from separate individuals, containing leaves exposed to full sun, were clipped in the field at midday, bagged, and taken back to the laboratory for hydraulic conductivity analysis using methods outlined by Sperry et al. (1988). Each branch contained unbranched stem segments at least 10 cm long and 10–13 mm diameter. In the laboratory, stem segments were excised under water to prevent xylem embolism and then initial (i.e., midday) conductivity was measured by attaching stems to a hydraulic conductivity apparatus. The shrub growth form typically exhibits vessel lengths of 10 cm or less; thus, a length of 10 cm was chosen to minimize open vessels following excision in the laboratory (Jacobsen et al. 2012, Shiflett et al. 2014a, b), and a diameter of 10–13 mm was ideal for connection to a tubing apparatus. The hydraulic conductivity apparatus consisted of an intravenous bag supplying a filtered (0.2 μm) 20 mmol/L KCl solution under low (~5 kPa) gravitational pressure to the stem. A low hydraulic head inhibited removal of in situ embolisms. Flow rate was determined using an analytical balance (Model PA64; Ohaus, Parsippany, New Jersey, USA) connected to a computer, and hydraulic conductivity was calculated as the mass flow rate of
the solution through the stem segment divided by the pressure gradient along the segment path length ($k_p \text{ kg m}^{-1} \text{s}^{-1} \text{MPa}^{-1}$). Maximum hydraulic conductivity ($k_{\text{max}}$) was measured after vacuum infiltration of stems for 2 h, which dissolved any embolisms present (Hietz et al. 2008).

Specific conductivity ($k_s \text{ kg m}^{-1} \text{s}^{-1} \text{MPa}^{-1}$), which adjusts for variations in stem diameter, was calculated from $k_p$ divided by sapwood area following safranin staining of stems as outlined by Sperry et al. 1988. Percent loss of conductivity (PLC), which represented native embolism at midday, was measured that were linked to light capture and physiological traits. Total leaf area distal to excised stem segments was quantified with a leaf area meter (Model LI-3100c; LICOR, Lincoln, Nebraska, USA) in order to determine leaf specific conductivity ($k_s \text{ kg m}^{-1} \text{s}^{-1} \text{MPa}^{-1}$) and the leaf area-to-sapwood area ratio ($A_L : A_S \text{ m}^2/\text{cm}^2$), which equals the sum of leaf area fed by the stem segment divided by the sapwood area (i.e., outer layer of wood that contains water-conducting tissue; Tyree and Sperry 1988).

**Light capture and processing**

At the individual level of each species, morphological, chemical, and physiological traits were measured that were linked to light capture and processing. Leaf morphological traits include leaf area and SLA. For leaf area and SLA, leaves ($n = 10$) were sampled evenly on terminal branches of outer canopy of five individuals from each species. Leaf area was quantified using a leaf area meter (LI-3100C). Specific leaf area was calculated from leaf area and mass after oven-drying samples at 70°C for 72 h. Leaf angle was measured to link leaf morphology to leaf physiology. Leaf angle from the horizontal ($n = 100$) was quantified to the nearest 5° using a clinometer placed along the leaf mid-axis by taking evenly distributed samples from five individual shrubs of each species.

Leaf chlorophyll content was measured to further compare light capture and processing capabilities among species. Leaf samples ($n = 20$) were obtained evenly distributed from five individuals of each species. Sample preparation included obtaining uniformly sized disks from each leaf followed by sampling grinding using mortar and pestle and pigment extraction using acetone. Analysis of leaf pigments was conducted using standard spectrophotometric methods (Lichtenhaler 1987).

Electron transport rate (ETR) was quantified as a physiological trait representative of light use and photosynthetic capacity. Rapid light curves (RLCs, $n = 5$ curves) were sampled on fully expanded, light-adapted leaves on terminal branches at ~1.5 m height of five individuals of each species using a pulse amplitude-modulated leaf fluorometer and attached leaf clip (MINI-PAM, Leaf-Clip Holder 2030-B; Walz, Effeltrich, Germany). Leaves were exposed to eight increasing steps of PAR (Photosynthetically active radiation) ranging from 50 to 1500 $\mu$mol m$^{-2}$ s$^{-1}$. Above-canopy PAR was ≥1600 $\mu$mol m$^{-2}$ s$^{-1}$ at solar noon for both sites. After exposure to each light intensity, quantum yield of PSII ($\Delta F/F_m$) was measured. Electron transport rate was calculated at multiple light intensities using the fluorometer. Electron transport rate was calculated as follows: $\text{ETR} = (\Delta F/F_m) \times \text{PAR} \times \text{ETR-Factor} \times P_{\text{PS2}}/P_{\text{PS1+2}}$, where $(\Delta F/F_m)$ represents maximal fluorescence in a light-adapted leaf after a saturating pulse of light, and $(F_m)$ represents steady-state fluorescence prior to any saturating pulse (Genty et al. 1989). The ETR-Factor is equivalent to the fraction of incident photons absorbed by the sample, and a default value of 0.84 was used. $P_{\text{PS2}}/P_{\text{PS1+2}}$ is the relative distribution of absorbed PAR to photosystem II, and a default value of 0.5 was used.

**Nutrient and water use**

Biochemical traits measured for nutrient and water use included %N, %C, C:N, $\delta^{15}$N, and $\delta^{13}$C analysis. Leaf samples ($n = 5$) were dried at leaves at 70°C for 48 h and ground to a fine powder with a mortar and pestle. Further preparation (i.e., weighing and encapsulating) and nutrient and isotope analyses were conducted on an elemental analyzer–isotope ratio mass spectrometer at the University of Georgia Stable Isotope and Soil Biology Laboratory (Athens, Georgia, USA).

**Statistical analysis**

Variations in morphological, physiological, and biochemical traits at the individual level among species at each site (i.e., inland or coastal) were analyzed with one-way analysis of variance (Zar 2010). Significant differences were identified with Tukey post hoc tests ($\alpha = 0.05$). Computations were performed with SAS statistical software (version 9.2; SAS, Cary, North Carolina, USA).
To detect general trends among thicketizers vs. co-occurring native species, we organized the data into a single 19 traits \times 6 species matrix and performed a principal component analysis (PCA). Five values were assigned per species for each trait and were randomly selected, except in the case of hydraulic traits, leaf angle, and ETR. Given that only four branches were sampled per species for all hydraulic traits, the input matrix was gap-filled using mean values. Leaf angles were sorted by increasing values across all individuals sampled, and the median value within each quartile was selected to obtain five representative input trait values. Only five light curves were obtained for each species; therefore, all five were used as matrix inputs; however, we limited the input to ETR at 1500 \( \mu \text{mol-m}^{-2} \text{s}^{-1} \). Data were normalized using a scaling function. Main loading traits of the ordination were identified by performing correlation analysis between PCA axes and trait values. Principal component analysis was performed using R studio software (version 1.0.143, Boston, Massachusetts, USA).

**RESULTS**

**Water use**

Initial specific conductivity \( (k_s) \) was similar between *Elaeagnus umbellata* and *Vaccinium corymbosum* (1.39 ± 0.25 and 1.22 ± 0.23 \( \text{kg-s}^{-1} \text{MPa}^{-1} \text{m}^{-1} \), respectively), and higher than *Clethra alnifolia* (0.55 ± 0.06 \( \text{kg-s}^{-1} \text{MPa}^{-1} \text{m}^{-1} \); \( F = 5.02, P = 0.034 \)); however, maximum \( k_s \) was highest for *E. umbellata* relative to the other two inland species \(( F = 60.43, P < 0.001 \); Fig. 1). Maximum \( k_s \) of *E. umbellata* (3.01 ± 0.13 \( \text{kg-s}^{-1} \text{MPa}^{-1} \text{m}^{-1} \)) was four times higher than that of *C. alnifolia* (0.74 ± 0.08 \( \text{kg-s}^{-1} \text{MPa}^{-1} \text{m}^{-1} \)), and more than

![Fig. 1. (A) Initial and maximum leaf specific conductivity \((k_L)\), (B) initial and maximum specific conductivity \((k_s)\), (C) midday percent loss of conductivity (PLC), and (D) leaf area-to-sapwood area ratio \((A_L/A_S)\) comparing a thicketized shrub species to sympatric non-thicketized shrub species at an inland and a coastal site. Error bars represent ±1 SE of the mean. Significant differences \((P < 0.05)\) among species at each site for all variables except maximum \(k_L\) and \(k_s\) are represented by letters a and b. Differences in maximum \(k_L\) and \(k_s\) among species are expressed with the letters c and d.](image-url)
two times higher than *V. corymbosum* (1.33 ± 0.22 kg·s⁻¹·MPa⁻¹·m⁻¹; Fig. 1). Initial *k₁* did not differ among species, yet maximum values differed such that *E. umbellata* had the highest *k₁* (5.06 ± 0.62 kg·m⁻¹·s⁻¹·MPa⁻¹), which was significantly higher than *C. alnifolia* (2.18 ± 0.32 kg·m⁻¹·s⁻¹·MPa⁻¹) but not significantly higher than *V. corymbosum* (3.33 ± 0.69 kg·m⁻¹·s⁻¹·MPa⁻¹; *F* = 6.53, *P* = 0.018, Fig. 1). *Elaeagnus umbellata* displayed a greater native (i.e., midday) PLC (53% ± 9%), than either *C. alnifolia* (26% ± 0.6%) or *V. corymbosum* (9% ± 4%; *F* = 15.72, *P* = 0.001; Fig. 1). *Elaeagnus umbellata* also supported more leaf area per stem area (*A_L: A_W* 0.66 ± 0.07 m²·cm⁻²) than either *C. alnifolia* (0.31 ± 0.04 m²·cm⁻²), or *V. corymbosum* (0.44 ± 0.04 m²·cm⁻²; *F* = 11.93, *P* = 0.003; Fig. 1).

Initial *k∞* of *Morella cerifera* (1.59 ± 0.27 kg·s⁻¹·MPa⁻¹·m⁻¹) was higher than *Baccharis halimifolia* (0.72 ± 0.12 kg·s⁻¹·MPa⁻¹·m⁻¹) and *Iva frutescens* (0.77 ± 0.06 kg·s⁻¹·MPa⁻¹·m⁻¹; *F* = 7.72, *P* = 0.011, Fig. 1). Maximum *k∞* was similar between *M. cerifera* and *B. halimifolia* (2.69 ± 0.24 and 2.32 ± 0.26 kg·s⁻¹·MPa⁻¹·m⁻¹, respectively) and higher than *I. frutescens* (1.06 ± 0.48 kg·s⁻¹·MPa⁻¹·m⁻¹; *F* = 17.49, *P* = 0.001). No difference was observed among island species for either initial or maximum *k₁* (*F* = 1.94 and 2.83, *P* = 0.199 and 0.111, respectively), though patterns mirrored initial and maximum *k∞* from *Baccharis halimifolia* displayed the greatest (i.e., midday) PLC, while *I. frutescens* displayed the lowest, and that of *M. cerifera* was similar to both of the other island species (*F* = 7.87, *P* = 0.011; Fig. 1). There were no differences in *A_L: A_S* among island species (*F* = 2.04, *P* = 0.186, Fig. 1).

**Light capture and processing**

Leaves of *E. umbellata* had larger average leaf size than *V. corymbosum* and were similar to *C. alnifolia*, yet leaves of *E. umbellata* had a lower SLA than either co-occurring species (*F* = 9.09, *P* < 0.001; *F* = 18.14, *P* < 0.001, respectively; Table 1). Both leaf area and SLA of *M. cerifera* were similar to *I. frutescens* and lower than *B. halimifolia* (*F* = 8.58, *P* = 0.001; *F* = 68.29, *P* < 0.001, respectively; Table 1). Leaf angle varied significantly among inland species such that *E. umbellata* and *V. corymbosum* had similarly horizontal leaf angles, while *C. alnifolia* had a steeper leaf angle (*F* = 23.02, *P* < 0.001; Fig. 2).

In contrast, leaf angle distributions were similar among the three coastal species (*F* = 1.56, *P* = 0.212; Fig. 2). Chlorophyll *a*, chlorophyll *b*, total chlorophyll, and carotenoids were similar for *E. umbellata* and *V. corymbosum*, and higher than in *C. alnifolia* (*F* = 9.89, *P* = 0.003; *F* = 7.0, *P* = 0.10; *F* = 8.87, *P* = 0.004; *F* = 18.12, *P* < 0.0001, respectively; Table 1). No differences were observed in chlorophyll *a:b* ratio among inland species. Chlorophyll *a* was highest in *B. halimifolia* and lowest in *M. cerifera*, with *I. frutescens* having a similar chlorophyll *a* content as the other coastal species (*F* = 4.62, *P* = 0.033; Table 1). No differences were observed in chlorophyll *b*, total chlorophyll, or chlorophyll *a:b* ratio for coastal species; however, *M. cerifera* had the highest carotenoid content, and *I. frutescens* had the lowest (*F* = 7.41, *P* = 0.008; Table 1).

Rapid light curves showed significant increases in ETR for *E. umbellata* from the other inland species at 550 μmol·m⁻²·s⁻¹ (*F* = 28.18, *P* < 0.001; Table 1). Leaves of *E. umbellata* had larger average leaf size than *V. corymbosum* and were similar to *C. alnifolia*, yet leaves of *E. umbellata* had a lower SLA than either co-occurring species (*F* = 9.09, *P* < 0.001; *F* = 18.14, *P* < 0.001, respectively; Table 1). Both leaf area and SLA of *M. cerifera* were similar to *I. frutescens* and lower than *B. halimifolia* (*F* = 8.58, *P* = 0.001; *F* = 68.29, *P* < 0.001, respectively; Table 1). Leaf angle varied significantly among inland species such that *E. umbellata* and *V. corymbosum* had similarly horizontal leaf angles, while *C. alnifolia* had a steeper leaf angle (*F* = 23.02, *P* < 0.001; Fig. 2).

Table 1. Leaf traits and leaf chlorophyll (chl) content of monotypic, dense thicket-forming shrubs (*Elaeagnus umbellata* and *Morella cerifera*) relative to co-occurring, non-thicketed shrub species.

<table>
<thead>
<tr>
<th>Leaf characteristics</th>
<th>Clethra alnifolia</th>
<th>Vaccinium corymbosum</th>
<th><em>E. umbellata</em></th>
<th>Baccharis halimifolia</th>
<th>Iva frutescens</th>
<th><em>M. cerifera</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Leaf area (cm²)</td>
<td>11.5 ± 1.2B</td>
<td>7.1 ± 0.5A</td>
<td>11.0 ± 0.5G</td>
<td>8.4 ± 0.6F</td>
<td>7.1 ± 0.4E</td>
<td>5.8 ± 0.4D,E</td>
</tr>
<tr>
<td>SLA (cm²/g)</td>
<td>149.5 ± 9.9C</td>
<td>120.2 ± 1.9A</td>
<td>99.5 ± 1.6A</td>
<td>165.5 ± 6.3E</td>
<td>95.5 ± 2.5D</td>
<td>100.4 ± 4.6D</td>
</tr>
<tr>
<td>Chl a (mg/m²)</td>
<td>130.4 ± 32.5A</td>
<td>187.2 ± 33.7G</td>
<td>220.1 ± 30.4G</td>
<td>230.6 ± 8.6E</td>
<td>195.8 ± 31.4D</td>
<td>192.7 ± 19.4E</td>
</tr>
<tr>
<td>Chl b (mg/m²)</td>
<td>114.6 ± 31.9A</td>
<td>172.3 ± 36.0A</td>
<td>180.7 ± 26.4G</td>
<td>186.3 ± 10.2</td>
<td>165.5 ± 27.7</td>
<td>157.6 ± 13.5</td>
</tr>
<tr>
<td>Total chl (mg/m²)</td>
<td>245.3 ± 63.2A</td>
<td>358.5 ± 65.6B</td>
<td>405.8 ± 56.6B</td>
<td>416.9 ± 18.1</td>
<td>361.3 ± 57.8</td>
<td>350.3 ± 32.4</td>
</tr>
<tr>
<td>Chl a:b ratio</td>
<td>1.1 ± 0.1</td>
<td>1.1 ± 0.1</td>
<td>1.2 ± 0.0</td>
<td>1.2 ± 0.04</td>
<td>1.2 ± 0.1</td>
<td>1.2 ± 0.04</td>
</tr>
<tr>
<td>Carotenoids (mg/m²)</td>
<td>27.3 ± 3.4A</td>
<td>41.0 ± 8.6B</td>
<td>51.1 ± 5.7E</td>
<td>27.6 ± 10.8D</td>
<td>19.2 ± 6.6D</td>
<td>37.7 ± 4.1E</td>
</tr>
</tbody>
</table>

Notes: SLA, specific leaf area. Values are presented as means ± 1 SE. Superscript letters denote significant differences (*P* < 0.05) among species at each site where A, B, and C are used at the inland site and D, E, and F are used at the coastal site.
Electron transport rate remained consistently higher for *E. umbellata* at subsequent light levels. At the highest light intensity (~1500 \( \mu \text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1} \)), ETR for *E. umbellata* was 30–50% greater than in *C. alnifolia* and *V. corymbosum*. For the coastal species, ETR was highest in *I. frutescens* at 150, 550, and 750 \( \mu \text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1} \); however, there were no differences among species at the other light intensities (Fig. 3).

**Nutrient and water use**

All inland species had significantly disparate \( \delta^{15}\text{N} \) and foliar %N, with *E. umbellata* exhibiting the highest values compared to the non-fixers \( (F = 379.76, P < 0.001 \) and \( F = 84.58, P < 0.001 \), respectively; Table 2). C:N ratios also varied across species and were much lower for *E. umbellata* \( (F = 116.64, P < 0.001 \) Table 2, Fig. 4). *Vaccinium corymbosum* had the highest C:N ratio and the lowest %N values. \( \delta^{13}\text{C} \) values were highest for *E. umbellata* followed by *C. alnifolia* and then *V. corymbosum*, which had the most negative values \( (F = 28.95, P < 0.001 \) Table 2, Fig. 4). Among coastal species, the non-fixers, which were also deciduous, had higher foliar %N and lower \( \delta^{15}\text{N} \) than N-fixing, evergreen *M. cerifera*. C:N ratios were also lower in the non-fixers than in *M. cerifera* \( (F = 57.13, P < 0.001 \) Table 2, Fig. 4). Foliar %C varied among coastal species such that *I. frutescens* had the lowest leaf %C and *M. cerifera* had the highest \( (F = 122.61, P < 0.001 \) Table 2). Leaf \( \delta^{13}\text{C} \) values were highest for *M. cerifera* and
Fig. 3. Electron transport rate (ETR) resulting from increased light intensity of rapid light curves for thicket-forming shrubs (*Elaeagnus umbellata* and *Morella cerifera*) and co-occurring shrub species. Error bars represent ± 1 SE of the mean. Significant differences (*P* ≤ 0.001) among species at specified PAR are denoted by ****: lowest for *B. halimifolia*, while δ¹³C of *I. frutescens* was similar to both groups (*F* = 4.48, *P* = 0.035; Table 1, Fig. 4).

δ¹³C and C:N were used as proxies for water use efficiency and N use efficiency (WUE and NUE), respectively. There was a negative trend between WUE and NUE for inland species (*F* = 25.14, *P* < 0.001, *b* = −25.73, *r²* = 0.66; Fig. 4). *Elaeagnus umbellata* had the highest WUE but lowest NUE, whereas *V. corymbosum* had the lowest WUE and highest NUE (Fig. 4). The inland species showed distinct segregation across ranges of WUE and NUE. Comparisons among coastal species showed that *M. cerifera* had the highest WUE and NUE, but that species were clustered closer together and island species encompassed a narrower range in both WUE and NUE.

Table 2. Leaf tissue chemistry comparing thicket-forming shrubs (*Elaeagnus umbellata* and *Morella cerifera*) to sympatric, non-thicket-forming shrubs.

<table>
<thead>
<tr>
<th>Foliar trait</th>
<th>Clethra alnifolia</th>
<th>Vaccinium corymbosum</th>
<th>E. umbellata</th>
<th>Baccharis halimifolia</th>
<th>Iva frutescens</th>
<th>M. cerifera</th>
</tr>
</thead>
<tbody>
<tr>
<td>δ¹⁵N</td>
<td>−4.25 ± 0.09³³</td>
<td>−3.81 ± 0.11³³</td>
<td>−3.43 ± 0.12³³</td>
<td>−1.77 ± 0.17³³D</td>
<td>−2.32 ± 0.15³³D</td>
<td>−1.06 ± 0.15³³E</td>
</tr>
<tr>
<td>%N</td>
<td>1.99 ± 0.06³³</td>
<td>1.13 ± 0.03³³A</td>
<td>3.38 ± 0.19³³C</td>
<td>2.92 ± 0.15³³E</td>
<td>2.89 ± 0.17³³E</td>
<td>1.85 ± 0.06³³D</td>
</tr>
<tr>
<td>C:N</td>
<td>23.01 ± 0.70³³</td>
<td>44.55 ± 2.28³³C</td>
<td>14.31 ± 0.73³³A</td>
<td>15.79 ± 0.91³³D</td>
<td>13.86 ± 0.79³³D</td>
<td>26.08 ± 0.90³³E</td>
</tr>
<tr>
<td>%C</td>
<td>45.69 ± 0.25³³</td>
<td>49.59 ± 0.33³³C</td>
<td>47.86 ± 0.24³³B</td>
<td>45.52 ± 0.20³³E</td>
<td>39.56 ± 0.63³³D</td>
<td>48.63 ± 0.29³³F</td>
</tr>
<tr>
<td>δ¹³C</td>
<td>−29.03 ± 0.27³³A</td>
<td>−30.22 ± 0.32³³A</td>
<td>−26.54 ± 0.44³³B</td>
<td>−29.10 ± 0.29³³D</td>
<td>−28.08 ± 0.25³³D,E</td>
<td>−27.94 ± 0.36³³E</td>
</tr>
</tbody>
</table>

Notes: Values are presented as means ± 1 SE. Significant differences (*P* ≤ 0.05) among species within a site are represented by letters where A, B, and C are used at the inland site and D, E, and F are used at the coastal site.
and NUE compared to inland species ($F = 1.51$, $P = 0.241$; Fig. 4).

**Trait comparisons among species**

The first PCA axis was identified as an axis of resource capture (e.g., chlorophyll content, N fixation) and utilization capacity (e.g., $k_s$, ETR 1500), accounting for 30% of the total variance (Fig. 5). The second PCA axis, associated with leaf construction costs, secondary leaf pigments, NUE, and leaf angle, accounted for 19% of the total variation. Expansive thicketizers clustered together with N fixation, maximum hydraulic conductivity, and chlorophyll content as key drivers of observed clustering (Fig. 5). Inland species were separated across trait space, particularly along the second axis for *C. alnifolia* and *V. corymbosum*, and the first axis for *E. umbellata* (Fig. 5). Coastal species were closer together in trait space than inland species, with distinct similarities between *B. halimifolia* and *I. frutescens* along the second axis and low separation along the first axis (Fig. 5).

**DISCUSSION**

Functional traits, which characterize species by their ecological roles and highlight species–environment interactions, have received recent
attention for explaining patterns of habitat filtering and mechanisms of community assemblages (de Bello et al. 2010, 2012, Brym et al. 2011, Zinnert et al. 2013). We quantified functional traits that represent morphological and physiological characteristics and were linked to three primary resources: water, light, and nutrients, which directly influence plant growth. A combination of morphological and physiological traits contributes to the rapid growth, expansion, and thicket formation observed for *Elaeagnus umbellata* and *Morella cerifera*, compared to native, co-occurring, non-expanding species. While there was some convergence in functional traits of expanding species (e.g., $k_s$, ETR), several functional traits were different between expanding species (e.g., $k_L$, $A_L$ : $A_S$, leaf area, and leaf angle) and one trait (e.g., SLA) was unexpectedly similar given that it is typically associated with resource conservation. Physiological performance traits (e.g., $k_s$, ETR) measured were similarly high for both species despite differences in several structural and anatomical traits and low SLA observed for both expanding species. Our hypothesis that *E. umbellata* and *M. cerifera* exhibit functional traits that promote efficient light capture, utilization, and hydraulic relative to co-occurring, non-expanding shrub species was supported; yet, differences in functional traits represent alternative strategies for colonization, rapid expansion, and dense, monotypic thicket formation.

Among inland species, there was a gradient in functional trait response of low to high performance with *Clethra alnifolia* having lowest performance, *E. umbellata* having highest, and *Vaccinium corymbosum* frequently falling in the middle or having a similar trait response as *E. umbellata*. For instance, this pattern occurred with maximum $k_s$ and $k_L$, $A_L$ : $A_S$, leaf chlorophyll and carotenoid content, ETR, and the majority of leaf chemistry traits. In many environments, *V. corymbosum* forms thickets (Pritts and Hancock 1985), though they are smaller in stature than those of *E. umbellata*. Our data suggest that where *E. umbellata* co-occurs with *V. corymbosum*, its capacity for rapid growth and higher relative physiological performance will give it a competitive advantage for physical dominance on the landscape. *Elaeagnus umbellata* outperformed co-occurring inland species for almost every trait measured. It had higher maximum $k_s$ and $k_L$, more leaves supported per sapwood area, WUE, leaf %N, and ETR across light levels relative to co-occurring, non-expanding species.

Many quantified functional traits were similar among coastal species, especially for parameters related to light capture and processing. Coastal species exhibited functional traits indicative of a high-light, high-stress environment such as vertically angled leaves, which were generally small and thick, high ETR at solar noon, high total chlorophyll content, and reduced $A_L$ : $A_S$ relative to inland species. However, differences among species existed when comparing hydraulic traits and leaf traits. For instance, while hydraulic traits were similar between *M. cerifera* and *Baccharis halimifolia*, *Iva frutescens* exhibited reduced maximum $k_s$ relative to the other two coastal species. *Iva frutescens* is a halophyte, typically observed on hypersaline soils, and accumulates salt within its leaves (Young et al. 1994, Nau mann et al. 2009). To our knowledge, salinity concentration of xylem sap in *I. frutescens* or species-specific anatomical adaptations for maintaining hydraulic conductivity under saline conditions have not been documented, yet growth in highly saline soils likely influences the capacity of *I. frutescens* to transport water and may explain lower relative maximum $k_s$ (López-Portillo et al. 2005, Reef and Lovelock 2015). For instance, work in mangroves has shown that mangroves at high salinities use less water than similarly sized freshwater wetland trees and have lower rates of sap flow and higher resilience to cavitation (Krauss et al. 2007). In terms of leaf traits and biochemistry, C:N ratio was substantially higher in *M. cerifera* relative to co-occurring coastal shrubs. Leaf %N content of *M. cerifera* was lowest, but it has a constant source of N via symbiotic N fixation. Notably, this contrasts with the trend observed among inland species, whereby the expansive *E. umbellata* had the highest leaf %N and lowest C:N ratio.

Differences in spatial heterogeneity between sites act as drivers for colonization and expansion strategies, which is expressed via dichotomy in trait responses at the different sites. The inland site was characterized as a relatively homogenous area consisting of an old field and the edge of a forest understory. The coastal site, in
contrast, is an extreme environment with complex spatial heterogeneity and habitat patchiness associated with relative landscape position (e.g., distance to the ocean, elevation, depth to the water table), creating a mosaic of environmental stressors and available habitat types (e.g., dune/swale complexes, salt flats, brackish marsh, freshwater marsh). At the inland site, where there is less habitat complexity, a colonization strategy for rapid growth to quickly establish dominance and form a tall, dense canopy to shade out other species is promoted. At the coastal site, a strategy which alternates between slow and fast growth leads to successful colonization and woody expansion. Colonization and establishment of *M. cerifera* are first facilitated by grasses, which provide shade and reduce micro-habitat air temperature to optimize seedling establishment (Thompson et al. 2017). Eventually, the young shrubs grow taller than the grasses and continue to expand dense canopy coverage, effectively preventing establishment of understory species, by radiating outward (Zinnert et al. 2011).

In open, full-sun environments, *M. cerifera* utilizes a strategy that alternates between fast and slow dynamics, whereas *E. umbellata* relies on classic invasion mechanics underpinned by fast growth and efficient use of light and nutrients. Unlike *E. umbellata*, success for *M. cerifera* does not hinge upon rapid establishment of dominance to outcompete co-occurring species. However, similar to *E. umbellata*, once established, both form dense, closed-canopy thickets, which inhibit competition and establishment of other species within their understories (Brantley and Young 2010). Not only do these species utilize two different strategies for colonization and establishing dominance on the landscape, but the different approaches between these expansive species also mirror differences in their strategies for carbon gain.

While *E. umbellata* and *M. cerifera* rely on two different strategies to maximize carbon gain, resulting efficiencies and mono-thicket formation capabilities are similar. *Elaeagnus umbellata* relies on acquisitive traits (e.g., deciduous, shorter lived, horizontal leaves, increased ETR, lower $A_1:A_0$) that enable maximal carbon gain in a short time period (Naumann et al. 2010), while *M. cerifera* relies on traits (e.g., evergreen, longer lived, vertical, and smaller leaves) that enable a “hybrid” strategy combining conservative and acquisitive traits, where it is productive throughout the year and with enhanced physiological activity during the growing season, enabling it to physiologically compete with co-occurring species (Aerts 1995, Shiflett et al. 2013, 2014b, Tecco et al. 2013). Similar strategies for altering physiological activity have been observed for species which grow in dynamic thermal environments. For instance, in the desert shrub *Larrea tridentata*, optimal photosynthetic temperature shifts from 22°C during winter to 32°C during summer (Mooney et al. 1978). During cooler temperate fall, winter, and spring months, light use-related physiological activity of *M. cerifera* slows down dramatically compared to summer months when physiological activity of *M. cerifera* is similar to co-occurring deciduous species (Young 1992, Shiflett et al. 2013, 2014b).

Despite several differences between the deciduous, non-native, invader *E. umbellata* and the evergreen, native, expander *M. cerifera*, both species maintain advantages over co-occurring woody species via shared traits such as bird-dispersed seeds, and N fixation. For instance, evergreenness and fleshy, persistent, ornithochorous seeds facilitate expansion of *M. cerifera* relative to co-occurring species by providing a seed source for overwintering birds when co-occurring shrubs are dormant. Fixation of atmospheric also N confers many advantages, especially in nutrient-poor environments (Huss-Dbnell 1990). This may be especially important because *E. umbellata* readily invades disturbed and poor-quality soils (Yates et al. 2004) and *M. cerifera* rapidly expands in nutrient-limited sandy soils (Young 1992). N fixation also contributes to observations that *M. cerifera* occupies more area on Virginia barrier islands than *B. halimiolia* (Young et al. 2007, Zinnert et al. 2013). Both coastal species occur across the island in swales, but *M. cerifera* is more successful in nutrient-poor sandy soils than *B. halimiolia*. Research has shown that when *B. halimiolia* grows adjacent to *M. cerifera*, the $\delta^{15}N$ signature of its leaves mirrors that of *M. cerifera*, whereas individuals, which grow farther away from *M. cerifera*, do not show any evidence of enhanced leaf nitrogen supply (Vick 2011). Based on trait similarities among coastal species, our data suggest that
B. halimifolia should be competitive with M. cerifera, but N fixation, coupled with evergreenness, and high physiological activity during the growing season favor dominance of M. cerifera.

Similar to M. cerifera, E. umbellata also had a lower SLA than co-occurring species, a trait which is typically associated with a stress-tolerant life-history strategy, aiding in nutrient retention and long-term photosynthetic NUE (Gibson and Rundel 2012). Low SLA is not associated with rapid resource acquisition and utilization per conventional leaf economics spectrum theory (Wright et al. 2004), and thus, it was unexpectedly low for the invasive E. umbellata, but not for the evergreen M. cerifera. In general, increased SLA is associated with lower leaf construction costs and higher N allocation to photosynthesis (Feng et al. 2008). Both expansive species are N-fixers, and both had reduced NUE relative to non-expanders, indicating that nitrogen is not likely a limiting resource. A readily available N supply enables E. umbellata to invest more in leaf construction, relative to deciduous species, which are non-N-fixers. Some authors have also shown that reduced SLA may be seen as an adaptation to drought stress, providing further advantages to species which have low SLA, but high N allocation to photosynthesis and photosynthetic capacity (Aspelmeier and Leuschner 2006).

CONCLUSIONS

Functional traits may explain why some species dominate the landscape (Baruch and Goldstein 1999), yet no universal suite of traits exist that make a species or group of species ubiquitously successful (Pysek and Richardson 2007). Elaeagnus umbellata and Morella cerifera thrive in different habitats, display differences in several functional traits, yet show convergence of some traits, and similar physiology during the temperate growing season. Both species form dense thickets and have traits that maximize light attenuation, capture, and processing while minimizing light stress. Moreover, both species have traits that contribute to drought tolerance, yet enable high hydraulic efficiency. However, each species utilizes different strategies for expansion. Elaeagnus umbellata relies on acquisitive traits and enhanced physiology relative to co-occurring, non-invasive, and non-expanding woody species (Zinnert et al. 2013), whereas M. cerifera relies on a mixture of conservative and acquisitive traits with varying degree seasonally and at several timeframes during colonization and expansion (Shiflett et al. 2013). The majority of quantified functional traits of E. umbellata and many of M. cerifera allow for efficient light capture, utilization, and internal water movement. These factors contribute to rapid range expansion and thicket formation by promoting enhanced productivity while inhibiting successful colonization of other species. Suites of functional traits may be linked to expansive success and thicket formation, yet differences across a broad range of functional traits represent alternative strategies leading to rapid growth and thicketization.

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