Red mangrove (*Rhizophora mangle*) advancing through a saltwater-freshwater ecotone demonstrates a coordinated leaf trait response to environmental resource and stress

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Abstract

The mangrove *Rhizophora mangle* L. has encroached into subtropical freshwater wetlands in the last few decades while negotiating variable landscapes. We characterized variation in *R. mangle* leaf traits related to leaf economics, water use, and plant defense across a gradient of encroachment in the Florida Everglades to assess whether *R. mangle* demonstrates a coordinated response to multiple resource and stress factors as it expands its range. Through this approach we determined the strongest environmental drivers of *R. mangle* trait variation among phosphorus availability, water salinity, flooding depth, and hydroperiod. We then interpreted what this variation means for plant productivity and ecosystem services provided. Analysis of leaf area, specific leaf area (SLA), stomatal density, stomatal size, leaf total nitrogen (TN), leaf total phosphorus (TP), leaf carbon isotope ratio ($\delta^{13}C$), and leaf total phenolic concentration showed that *R. mangle* trait response was aligned with differences in water depth, hydroperiod, and soil TP, but not with pore water salinity, a classic stress agent. *R. mangle* showed increased SLA and TP, typical of more productive plants, with increased soil phosphorus – the limiting nutrient in Everglades ecosystems. A limited coordination between water use and leaf economics traits was suggested by the negative correlation of $\delta^{13}C$, which can serve as a metric of stomatal control, with both SLA and leaf TP. Our findings suggest that local variation in soil phosphorus, water depth, and hydroperiod will drive the characteristics of and ecosystem services provided by *R. mangle* as it encroaches into freshwater wetlands.

Introduction

Subtropical coastal wetlands are experiencing an ongoing transgressive vegetation replacement of herbaceous marsh by mangrove-dominated shrublands (Osland et al., 2022; Saintilan & Rogers, 2015; Smith et al., 2013) due to sea level rise (Wanless et al., 1994) that is expected to continue (Fox-Kemper et al., 2023; Park et al., 2017). The facultative halophyte *Rhizophora mangle* L. is one of the mangrove species that has long encroached into freshwater wetlands, accelerating within the last few decades (Bernardino et al., 2022; Egler, 1952; Park et al., 2019; Ross et al., 2000). This wetland-adapted species can range widely in stature and biomass depending on water salinity, nutrient availability, and local tidal and drainage conditions (Lin & Sternberg, 1992b; Lugo & Snedaker, 1974). Such intraspecific trait variation has been shown to play a role in the ability of species to successfully occupy contrasting habitat (Silva et al., 2019) and to have effects on the ecosystem services that the species provides in plant communities of low species richness (Lammerant et al., 2025; Lugo & Snedaker, 1974; Riascos & Blanco-Libreros, 2019). Interspecific resource economics trade-offs, which have shown to be robust to intraspecific variability (Albert, Thuiller, Yoccoz, Douzet, et al., 2010), can serve as a framework to interpret such within-species trait responses. Interpretation of the intraspecific trait shifts of this dominant species may provide insight into what drives this species and other encroaching wetland- and salt-adapted species that face tradeoffs between resource acquisition, water use, and defense that differ from those faced by terrestrial species (Y. Pan et al., 2022). Plant traits can reflect tradeoffs in resource acquisitions and plant function and effectively serve as predictors of stress response or shifting environmental conditions across different species (Díaz et al., 2004; Grime et al., 1997). The leaf economics spectrum (LES; Wright et al., 2004) is one trait-based resource economics theory that proposes a model for plant investment in carbon and nutrients for leaf construction. Wright et al. (2004) describe six plant traits, - leaf mass per area (LMA, whose reciprocal is specific leaf area, or SLA), photosynthetic capacity, leaf N, leaf P, dark respiration, and leaf lifespan – that represent an axis of quick to slow return on carbon and nutrient “investments” in leaf carbon structures by plants globally. Species with a quick return on investment (also referred to as acquisitive species) typically have greater total nitrogen (TN), total phosphorus (TP), photosynthetic capacity, and dark respiration, as well as shorter leaf longevity and lower LMA.

The traits of a plant with a quick return on investments are typically found in high nutrient or high resource areas (I. J. Wright et al., 2001; Grime et al., 1997). If intraspecific trait response occurs as predicted by the LES along environmental gradients of resources, then LES traits can be used to assess species level response to local environmental change in other scenarios.

Findings on whether the global LES trait correlations hold or decouple at lower scales, such as the species level, are mixed (Fajardo & Siefert, 2018; Gorne et al., 2022; Hayes et al., 2019; Messier et al., 2017; J.P. Wright & Sutton Grier, 2012).There may also be tradeoffs in resource uptake for stress adapted plants (Y. Pan et al., 2020) that would result in deviation from the global LES trends followed by terrestrial and other wetland plant species (Wright et al., 2004; Diaz et al., 2004). For example, plant adaptive strategies of flood tolerance or salinity tolerance may compete with resource uptake (Y. Pan et al., 2022). Some argue that species' resource use strategies are similar for nutrients and water, where a quick-return species on the LES axis would also exhibit rapid water flux or stomatal conductance (Prieto et al., 2018; Reich, 2014). Other work suggests that flooding adaptation traits in wetland plants are on a trait dimension separate from leaf economic traits with no trade-off between plant functions (Y. Pan et al., 2022). In this work we address the question of whether the LES holds intraspecifically in a halophytic wetland species which has a competing cost of adapting to brackish wetland conditions (Y. Pan et al., 2020), and whether the resource use traits of the LES are coupled with traits that function in water use and plant defense (Y. Pan et al., 2022; Prieto et al., 2018; Reich, 2014). Determining whether patterns of intraspecific variation in our target species, *R. mangle*, follow a single plant economic axis as the species shifts in range with sea level rise will inform the ability to predict shifts in community traits and ecosystem services in response to resources and stressors along similar environmental gradients through the use of known trait relationships (Fajardo & Siefert, 2018; J. P. Wright & Sutton-Grier, 2012).

To address these questions, it is necessary to examine leaf resource use, water use, and defense traits to determine how competing environmental drivers may affect intra-specific plant response. Some commonly used leaf traits and their interpretations are as follows: Leaf trait measurements, including leaf area, specific leaf area (SLA), stomatal configuration, nutrient or phenolic concentration, and carbon isotope ratio ($\delta^{13}C$), are often used as proxies for plant function or response to the environment. Leaf area may demonstrate a response to variable environmental factors such as salinity (Watzka & Medina, 2018), nutrient availability (Lin & Sternberg, 1992b), and light availability (Khan et al., 2020). Leaf area can also be limited by tree size due to water transport requirements (Ma et al., 2022), and may itself influence stomatal density (Peel et al., 2017). SLA, the ratio of fresh leaf area to leaf dry mass ($mm^2\ mg^{-1}$), is often positively correlated with plant resource use, relative growth rate, and negatively correlated with leaf longevity (I. J. Wright et al., 2004). Foliar nitrogen (N) and phosphorus (P) concentrations can reflect patterns of plant nutrient uptake and help gauge variation in N and P limitation (Güsewell, 2004). A reduction in nutrient uptake can also be a sign of salt stress (Fageria et al., 2011). When total stomatal closure or reduced stomatal conductance is induced by environmental conditions such as low soil moisture or elevated salinity (Farquhar et al., 1989), CO_2 diffusion into the leaf becomes limited, and the Rubisco enzyme is less able to discriminate against ^{13}C (Farquhar et al., 1982); thus an increased ratio of ^{13}C to ^{12}C ($\delta^{13}C$) is commonly interpreted as increased stress or increased water use efficiency. Beyond stomatal closure, the actual configuration of stomatal size and stomatal density can physically constrain plant stomatal conductance and affect water use efficiency in stressful conditions (Bertolino et al., 2019; Sternberg & Manganiello, 2014). Plant total phenolic concentration, which can provide oxidative stress tolerance in plants (Close & McArthur, 2002), has been reported to increase with soil salinity (Kandil et al., 2004), low nutrient conditions, and drought conditions.

Variation in mangroves along coastal gradients can be influenced by regulators (e.g. salinity), resources (e.g. soil nutrients, and water), and hydroperiod (Twilley & Rivera-Monroy, 2005). We consider that these three regulators can range from having a neutral or resource-type effect to having a stress effect on mangroves depending on the increase or decrease of these factors along a gradient. For instance, an environment of low salinity, high nutrient availability, and intermediate flooding generally provides optimal conditions for mangroves, while high salinity, low nutrient availability, and drought or extensive flooding are stressful (Twilley and Rivera-Monroy 2005). The interactions among these conflicting drivers are important in understanding *R. mangle*'s trait response in light of the species' aggressive encroachment into adjacent marshes in multiple locations in recent decades.

To determine if a single plant economics axis applies to a species with considerable resilience to variable levels of nutrients, water, and salinity concentrations, we determined the multi-trait intraspecific response of adult *R. mangle* within an environmental mosaic that includes variable conditions of soil phosphorus, water depth, hydroperiod, and salinity along a coast-to-interior gradient in which the species has been extending its influence (Ross et al., 2024). We expect that plants with greater productivity will demonstrate more acquisitive traits and that plants with lower productivity will demonstrate more conservative traits. We expect that a lack of resources could lead to a stress response in the form of more conservative traits, and that stress and resource response in a wetland-adapted halophyte could differ to that of a terrestrial species or of the global LES literature (Fig. 1). We expect that increased nutrient availability would be an increased resource, that water depth and hydroperiod extremes (drought, waterlogging) would be stressors, and that moderate water depths and hydroperiods would elicit a resource-type response. We also considered that water salinity concentrations greater than that of sea water would be a stress, that decreasing water salinity would relieve water uptake stress (Lin & Sternberg, 1992b; Tomlinson, 1986), and that complete freshwater conditions might be suboptimal (Ball, 1988). In this study we did not address the extremes of drought or hypersalinity. Based on the expected resource-stress gradient we asked:

1) Does trait response in *R. mangle* hold to the assumptions of the LES with regard to both resource and stress related to soil nutrient and water conditions?

2) Are the responses of water use traits and defense traits coupled with the LES trait response?

3) Which environmental factors most strongly drive *R. mangle* trait response as it encroaches into zones of fresher water, shallower water depths, and lower phosphorus availability?

We expected that: 1) Traits would follow LES trends and shift from conservative to acquisitive along a stress-resource gradient (Fig. 1). 2) Water use and plant defense traits would covary (Y. Pan et al., 2022) with LES traits and follow the same conservative to acquisitive axis as LES traits (Reich, 2014). 3) For the wetland and salt adapted *R. mangle* a stress response would be driven by decreased soil nutrient availability, increased water depth, and increased hydroperiod. A resource-type response would be driven by decreased water salinity, and increased soil nutrient availability.

The results of this trait analysis will determine which *R. mangle* leaf traits are most responsive to variation in the environment, and which environmental factors are driving the traits of *R. mangle* individuals along the encroachment gradient. The resultant patterns of intraspecific trait variation in *R. mangle* will not only test the fit of the LES model for a halophyte advancing across a coastal terrain, but also supply clues about variation in the ecosystem services that intraspecific variation can result in across these landscapes as climate change, sea level

rise, and restoration efforts proceed (Albert, Thuiller, Yoccoz, Soudant, et al., 2010; de Bello et al., 2010; Miedema Brown & Anand, 2022; Silva et al., 2019).

Methods

Study Area

This study was conducted in the southeastern Everglades, FL, U.S.A. on a carbonate coastal setting experiencing replacement of freshwater marsh by *R. mangle* dominated shrublands (Fig. 2). Mangrove encroachment is currently reflected in a compositional gradient from a mixture of herbaceous plants and scattered *R. mangle* at the interior to open dwarf mangrove shrubland closer to the coast. Herein referred to collectively as coastal scrub, the herbaceous component is of low productivity, and the mangrove shrubs measure no more than 2 meters in height. Besides *R. mangle*, common species in interior locations include *Cladium jamaicense* (sawgrass) or *Eleocharis cellulosa* (spikerush). Toward the coast, halophytes such as *Distichlis spicata* (saltgrass) and *Sesuvium portulacastrum* (sea purslane) are also present (Ross et al., 2002). Embedded within the coastal scrub matrix are tree islands, in which *R. mangle* is also found growing in mixture with mesophytic tropical hardwood species (Ross et al., 2021). According to the functional classification proposed by Lugo and Snedaker (1974), *R. mangle*-dominated communities in the coastal scrub are of the dwarf type and those in the tree islands are of the hammock type. Both forest types in the study area face a gradient of nearly fresh to brackish pore water salinity conditions. However, the tree islands have higher phosphorus, organic matter, and elevation than the surrounding scrub, which results in local heterogeneity in environmental conditions. The mangroves in tree islands more than a few kilometers from the coast are elevated an average of 30 cm above the adjacent scrub, and experience less persistent flooding (Steinmuller et al., 2021). In comparison to the peat soils (Histosols) found in tree islands, the soils of the surrounding coastal scrub are carbonate muds or marls that have lower organic matter, total carbon (TC), TN, and TP, with higher pH and bulk density (Steinmuller et al., 2021).

Beginning a few km from the Florida Bay coast, vegetation grades from 1) a mangrove dominated “white zone” – an unproductive environment with little to no graminoid presence and exposed marl soils – through 2) a transitional area, before reaching 3) the *C. jamaicense* (sawgrass) dominated freshwater marsh. The portion of the coastal gradient considered here is the leading edge of mangrove encroachment in the southeastern Everglades, and does not include the fringing swamp closest to the coast, where mangrove canopy cover approaches 100%. Within the target study area, pore water salinity under peak dry season conditions ranged from > 20 ppt at its coastal reach to < 1 ppt at the interior. Nine sites were selected to represent three zones within this sequence, and three sampling sites were selected in each zone. At each site, a paired tree island and coastal scrub plot were sampled, for a total of nine tree island plots and nine coastal scrub plots. Soil characteristics, average water depth, and pore water salinity conditions in each plot were referenced from datasets or models for the time period between 2016 and 2018, as reported in Steinmuller et al. (2021), and Ross et al. (2021, 2024). The environmental value range captured in our study area is listed in Table 1. Soil parameters were calculated from a soil core (5.5 cm diameter) collected to 30 cm depth. Soil TN and TP are reported per cm^2 to make values comparable among soils of different bulk densities. The soil nitrogen to phosphorus molar ratio (N:P) was calculated using the molar soil TN and TP values per cm^2. Pore water salinity values were one-time measurements obtained during the peak of the dry season between April and May 2018, when salinity was near its annual high. While water salinity is expected to vary across seasons, a general gradient of low to moderate salinity from interior to coast is expected to prevail throughout the year.

Precipitation patterns throughout south Florida result in seasonal wet and dry periods, with a rainy season from May to October. However, in the coastal Everglades, coastal scrub remains inundated for about 10 months of the year, while the surface of tree islands is flooded for about one month a year and water otherwise remains below the soil surface (Steinmuller et al., 2021). The water depth and hydroperiod for each of our eighteen plots was estimated by using daily water stage values from the MIKE Marsh Model developed by Florida International University's Applied Research Center and the National Park Service in 2011 which provides reconstructed historical water stages based on a network of long-term water level monitoring gauges (Cook, 2012). Stage values were converted to coastal scrub water depths using localized elevation estimates and tree island relative elevation to the coastal scrub as in Ross et al. (2021). Daily water stage data for the 24 month 2017–2018 period was used to account for the water depth and hydroperiod conditions approximately coincident with the leaf set of the three most recent leaf cohorts based on the leaf production calculations for *R. mangle* in Ross et al. (2001). Average water depth and hydroperiod were calculated for each plot. The average hydroperiod ranged from 37 to 152 days in the nine tree island plots and from 112 to 365 days in the coastal scrub plots (Table 1). The maximum water depth recorded per year averaged 30.6 cm in the tree island plots and 59.4 in the coastal scrub plots between 2017 and 2018. The environmental heterogeneity encompassed in the study area thus provides an ideal template for quantifying the main drivers of intraspecific variation in *R. mangle*.

Table 1
Minimum and maximum values for environmental parameters in tree island and coastal scrub plots. Soil and water depth parameters were subset from a dataset reported on by Ross et al. (2021, 2024) and Steinmuller et al. (2021). Water depth data were estimated using the stage data outputs from the Mike Marsh Model developed by Everglades National Park (M3ENP) for the calendar years of 2017 and 2018. Negative water depth values denote water levels below the soil surface.

Variable	Tree Islands		Scrub	
	Min.	Max.	Min.	Max.
Soil TN (mg cm^{-2})	18.07	60.46	15.81	56.10
Soil TP (µg cm^{-2})	472.52	2910.83	315.21	977.29
Soil N:P	30.35	96.89	77.93	321.43
Soil OM (g g^{-1} dw soil)	0.29	0.91	0.06	0.18
Pore Water Salinity (ppt)	0.40	17.90	0.80	23.10
Mean Annual Water Depth (cm)	-11.7	-2.8	-5.7	28.4
Mean Annual Water Depth Extrema (cm)	-32.1	30.6	-26.1	59.4
Mean Annual Hydroperiod (days)	37	152	112	365

Field Sampling and Lab Processing

In the month of December 2018, three *R. mangle* individuals were sampled in each of the 18 plots, for a total of 54 mangrove individuals. Six fully expanded leaves were collected from a single branch of each individual while avoiding the youngest leaf pair at the terminal node. Based on a leaf lifespan of 189 to 281 days in *R. mangle* coastal scrub and fringe trees (Ross et al., 2001) leaf emergence across the three leaf pairs collected was assumed to have occurred within the two calendar years discussed above. Fresh leaves were separated into two

subsets of three leaves each, one for stomatal analysis (size and density) and the second for analyses of leaf area, SLA, TN, TP, $\delta^{13}C$, and total phenolic concentration.

Stomatal analysis was completed on the first leaf subset using the nail varnish imprint method (Hodgson et al., 1993) and imprints were mounted on glass microscope slides. All imprints were from the middle of the leaf abaxial side, avoiding the midrib and leaf margins. Stomatal size and density were measured by photo analysis. A Leitz Dialux 20 fluorescence light microscope at 160X magnification equipped with a Nikon Coolpix camera and Microscope Digital Camera lens adapter was used to photograph three fields of view from different sections of each leaf impression. A micrometer slide was used to scale image pixels to a known unit using ImageJ version 1.52a (Schneider et al., 2012) image processing software. A 0.01 mm^2 grid was overlaid on each image, and stomata were counted within the 0.48 mm^2 field of view (6 x 8 grid cells). Stomatal dimensions were measured in the same photographs. From each field of view, four stomata in the central grid cells were measured. Accurate stomatal pore measurements require pores to be completely open, but this was not possible due to the sampling methods, so stomatal length and width were assessed on the basis of guard cell measurements (Subedi et al., 2018; Xu & Zhou, 2008). The length and width of each stomate was determined using the outer edge of the guard cells. The size (area) of each stomate was calculated using the formula for the area of an ellipse (area = πab, with “a" being the radius of the major axis of the ellipse (guard cell length), and “b” being the radius of the minor axis (guard cell width).

From the second leaf collection subset, the area of fresh leaves (cm^2) with petiole excluded was determined with a LI-COR portable area meter model LI-3000A adapted with the LI3050C Transparent Belt Conveyor Accessory. Once leaf area was determined, the leaves were placed in paper bags, dried to constant weight (72 hours) at 70°C, and the dry leaf weight was obtained. After removal of the leaf midrib (Fry et al., 2000), the dry leaf sample was placed into 2 mL centrifuge tubes along with two grinding balls and ground to a fine powder using a Retsch MM 200 Ball Mill or a QIAGEN TissueLyser II as available. Samples for analysis were at the tree level, i.e., the three leaf samples per tree were homogenized in a single sample. TN and $\delta^{13}C$ analysis were completed by mass spectrometry. Between 0.4 and 0.5 mg of dry leaf sample was encapsulated in 8 x 5 mm Elemental Microanalysis tin capsules and submitted to the Laboratory of Stable Isotope Ecology in Tropical Ecosystems at the University of Miami for analysis on a Pyrocube elemental analyzer connected to an IsoPrime isotope ratio mass spectrometer. For analysis of leaf TP content, 17–21 mg of dry leaf powder was weighed out into 20 mL glass scintillation vials. Samples were submitted to the Seagrass Ecosystems Research Lab at Florida International University where they were analyzed on a UV-2101 Shimadzu Spectrophotometer using a modified colorimetric method (Solórzano & Sharp, 1980). Total phenolic concentration was determined by the modified Folin-Ciocalteu bioassay protocol used in the FIU Lab for Plant Chemical Ecology, consisting of an extraction with 100 mg of plant tissue and 70% methanol. Sample absorbance was measured using the Thermo Scientific Genesys 30 Visible Spectrophotometer.

Statistical Methods

To characterize the variation in each independent trait, the coefficient of variation (CV) was calculated as the ratio of the standard deviation to the mean plot average trait value. To estimate the degree of association among the eight leaf traits (TN, TP, $\delta^{13}C$, total phenolic concentration, leaf area, SLA, stomatal density, and stomatal size) and whether these associations matched LES expectations, a Spearman’s rank correlation was used based on the

range-scaled average trait values by plot (n = 18 plots) using the “rcorr” (Matrix of Correlations and *p* values) function of the “Hmisc” (Harrel Miscellaneous) R package version 4.4-0 in RStudio (R Core Team, 2019).

To identify the environmental variables driving variance of the multiple leaf traits, non-metric multidimensional scaling (NMDS) ordination was used. A data matrix of average leaf traits at the plot level and environmental metrics was created, and each parameter was scaled by its range to account for differences in parameter units by using the formula $(x - minimum) \div (maximum - minimum)$, where x is the original value, minimum is the minimum value for the parameter, and maximum is the maximum value for the parameter. The “metaMDS” function of the vegan package version 2.6-4 in RStudio version 4.2.2 (R Core Team, 2019) was used to complete the NMDS based on the Bray-Curtis dissimilarity distance matrix of the scaled trait matrix and the output was rotated so that the vectors representing the largest variation were along the first NMDS axis. Environmental variables were fit to the NMDS using the “envfit” function of the vegan package which used a permutation-based statistical test of fit with 999 permutations. The environment matrix consisted of pore water salinity (ppt), two-year (2017–2018) average water depth (cm), two-year average hydroperiod (2017–2018), soil TN, soil TP, soil N:P, and distance to coast as vectors, with the plot type (coastal scrub or tree island) as a factor.

Results

Among the eight traits, the coefficient of variation ranged from 4.41 to 21.51% (Table 2). Leaf TP was the most variable trait, followed by total phenolic concentration and SLA. The least variable trait was $\delta^{13}C$, followed by leaf area (Table 2). The Spearman’s rank correlation analysis (Table 3) of *R. mangle* leaf traits showed significant interactions of traits typically attributed both to the LES (e.g. SLA, and leaf TP) and water use ($\delta^{13}C$). Across the 18 plots SLA had a very strong positive correlation with leaf TP (0.91, $p < 0.001$), and a very strong negative correlation with leaf $\delta^{13}C$ (-0.88, $p < 0.001$) while leaf TP and leaf $\delta^{13}C$ had strong negative correlation (-0.68, $p = 0.002$). Stomatal density had a moderate positive correlation with leaf $\delta^{13}C$ (0.48, $p = 0.044$) while leaf area, total nitrogen, total phenolic concentration, and stomatal size were not significantly correlated with any variables ($p > 0.05$).

Table 2
Trait ranges, trait means, and the absolute value of coefficient of variation of *R. mangle* leaves expressed in percent (n = 18).

Variable	**Trait Values**			
	Minimum	**Maximum**	**Mean**	**CV (%)**
Area (cm^2)	17.68	27.47	22.90	11.47
SLA ($mm^2\ mg^{-1}$ dw)	3.72	6.21	4.71	15.25
Stomatal Size (mm^2)	8.0×10^{-4}	1.3×10^{-3}	1.0×10^{-3}	13.05
Stomatal Density (No. mm^{-2})	74	113	88	13.67
TN (mg g^{-1} dw)	8.9	12.9	10.8	13.09
TP (µg g^{-1} dw)	349.4	808.1	557.4	21.51
$\delta^{13}C$	-30.2	-26.2	-28.1	4.41
Total Phenolics (mg g^{-1} dw)	50.66	99.57	81.31	15.74

Table 3
Spearman rank correlation matrix of mean plot trait values of leaf percent total nitrogen (TN), leaf percent total phosphorus (TP), carbon isotope ratio ($\delta^{13}C$), total phenolics (mg g^{-1} dw), leaf area (cm^2), stomatal density (mm^{-2}), and stomatal size (mm^2). Correlation values are on the left side of the matrix and *p* values are on the right side of the matrix. Significant correlation values ($p < 0.05$) and corresponding *p* values are in bold.

	Traits	TN	TP	$\delta^{13}C$	TtlPhen	Area	SLA	StomD	StomS	
Correlations	TN		0.061	0.437	0.575	0.201	0.119	0.248	0.189	**p values**
	TP	0.45		**0.002**	0.160	0.662	**0.000**	0.215	0.713	
	$\delta^{13}C$	-0.2	**-0.68**		0.367	0.062	**0.000**	**0.044**	0.813	
	TtlPhen	-0.14	-0.35	0.23		0.291	0.345	0.327	0.349	
	Area	0.32	-0.11	0.45	-0.26		0.322	0.084	0.836	
	SLA	0.38	**0.91**	**-0.88**	-0.24	-0.25		0.162	0.959	
	StomD	-0.29	-0.31	**0.48**	0.25	0.42	-0.34		0.238	
	StomS	0.32	0.09	0.06	-0.23	0.05	-0.01	-0.29		

Strong environmental drivers of *R. mangle* intraspecific trait variation

The two-dimensional NMDS ordination of plot leaf trait data converged at a stress of 0.12, producing a figure in which points close to each other in the ordination space have greater trait similarity and those far from each other are less similar (Fig. 3). Across the 18 plots the environmental vectors driving a majority of the trait variance included average water depth ($R^2 = 0.670$, $p = 0.001$), average hydroperiod ($R^2 = 0.632$, $p = 0.002$), soil N:P ($R^2 = 0.443$, $p = 0.014$), and soil TP ($R^2 = 0.441$, $p = 0.012$) (Fig. 2). The community type factor was also significant ($R^2 =$

0.408, p = 0.001), with the centroid (diamond shape) for scrub plots located on the left side of the ordination, and the centroid for tree island plots on the right. The significant environmental vectors, represented as the longest vectors (highest R^2 values) in the ordination, suggest that the largest proportion of trait change in *R. mangle* was related to a shared axis along a gradient of greater hydroperiod and water depth and high soil N:P (phosphorus limitation) on one end and high soil TP on the other – an axis that can be referred to as a stress-resource gradient of nutrients and hydroperiod combined. Pore water salinity (R^2 = 0.242, p = 0.124), distance to coast (R^2 = 0.110, p = 0.410), and soil TN (R^2 = 0.085, p = 0.517) were not significantly related to trait variation and their vectors were mostly discordant from the other vectors.

The leaf trait vectors most aligned with the stress-resource gradient included SLA and leaf TP, which increased toward the resource side of the gradient, and $\delta^{13}C$ which increased on the stress side of the gradient. Leaf area was more associated with soil N:P than the overall water depth, hydroperiod, and phosphorus gradient. Leaf TN and total phenolic concentration were not aligned with the stress-resource gradient and instead with the weak soil TN vector. Stomatal density was negatively associated with soil TN, even while positively associated with the $\delta^{13}C$ leaf trait. Stomatal size corresponded to the shortest trait vector with limited variation opposite to leaf area.

Discussion

R. mangle multi-trait response partially follows the global LES

The LES suggests that low SLA, low leaf TP, and low leaf TN are typical of conservative species globally. For this reason, it was expected that intraspecific variation in red mangrove would demonstrate more conservative traits with decreasing stress, due in part or in whole to decreased soil TP or TN, increased hydroperiod and water depth, or increased salinity. These expectations were supported in large part by the positive correlation between SLA and leaf TP, the positive association of these two traits with soil TP, and the negative association of these two traits with increasing annual mean water depth, hydroperiod, and soil N:P. Leaf TN, although a global LES trait, was not correlated to the other LES traits in *R. mangle* and its variation was not associated with the same environmental variables as SLA and leaf TP. This is likely attributable to the strong P limitation that prevails across most Everglades communities, including those in the coastal zone (Noe et al., 2001; Steinmuller et al., 2021). Leaf area was expected to decrease along with the occurrence of conservative LES traits under increased environmental stresses (Lin & Sternberg, 1992b; Liu et al., 2024; Watzka & Medina, 2018). Instead, leaf area *increased* with increased phosphorus limitation (N:P) in the soil. Among the traits measured in *R. mangle*, those following expectations for the LES appear limited to SLA and leaf TP in a phosphorus-limited fresh to brackish water environment of variable hydroperiod and water depth.

Water use traits had a partially coordinated intraspecific response with LES traits

When comparing water use and defense trait response to LES response, it was expected that in *R. mangle* a greater $\delta^{13}C$, decreased stomatal density or size (water use traits), and a greater total phenolic concentration (defense trait) would co-occur with conservative LES traits. This correlation of traits would signal tradeoffs among the related plant functions and would suggest a single fast-slow economic spectrum among the different plant functions (Reich, 2014). Among the water use and defense traits only $\delta^{13}C$ was negatively correlated with SLA and leaf TP. These results suggest that there may be tradeoffs between water use efficiency and nutrient uptake functions in *R. mangle*, and that foliar $\delta^{13}C$ and WUE trends can be related not only to water stress (J. Pan

et al., 2021), but also to P deficiency or P loading in mangroves (Mancera-Pineda et al., 2009; Krauss et al., 2026). Trends in leaf $\delta^{13}C$ aligned with the LES expectations of decreased photosynthetic capacity in more conservative individuals. Tradeoffs between nutrient uptake and water use efficiency have previously been observed in red mangrove seedlings in which low nutrient concentrations resulted in further decrease in carbon isotope discrimination, CO_2 assimilation, and stomatal conductance than salinity differences alone (Lin & Sternberg, 1992). This link between nutrient and water use traits could also be due to the role of P in stomatal function (Tiziani et al., 2020), or the effect of P on the relationship between photosynthetic capacity and N in a P-limited system (Reich et al., 2009). Phenolic concentration, stomatal size, and stomatal density, which were expected to respond to stress conditions of increased salinity or decreased soil nutrient availability, were not correlated with the LES traits. This result suggests that no tradeoffs occur between the LES and stomatal characteristics nor the defense trait of phenolic concentration in this wetland and salt adapted species (Prieto et al., 2018; Reich, 2014), or that these traits vary independently of the LES response within the range of environmental conditions observed.

Trait response of encroaching *R. mangle* in comparison to the existing literature

We compare the trait response of the encroaching *R. mangle* in our study sites to that of other *R. mangle* growth forms described in the literature. Leaf area values remained close to the average leaf area of 23.4 cm^2 reported for *R. mangle* in scrub forests (Lin & Sternberg, 1992a). SLA values in our study ranged from 3.72 to 6.21 mm^2 mg^{-1} which was comparable or greater than the range of SLA values of 4.18 to 5.55 mm^2mg^{-1} reported for *R. mangle* in hypersaline and low salinity sites respectively (Watzka & Medina, 2018). The $\delta^{13}C$ trait ranged from −30.2 to -26.2‰, which is a range similar to the reported difference of 1.0 to 4.0‰ between adult scrub *R. mangle* and the highly productive coastal fringe mangrove (Lin & Sternberg, 1992a). The link of *R. mangle* nutrient use (P) with water depth or hydroperiod found in our study has been similarly demonstrated by Hogan et al. (2022) who found heterogeneity in inundation regime led to differences in gas exchange and nutrient acquisition in *R. mangle*. The relation between soil phosphorus and plant carbon isotope discrimination found in our study is also supported by the decreased water use efficiency and increased water use resulting from phosphorus fertilization in mangroves by Krauss et al. (2026). The links demonstrated between phosphorus availability, hydroperiod, nutrient uptake, and water use efficiency across multiple studies support the idea that nutrient and water use in *R. mangle* are coupled.

Variation in stomatal density and size did not align with our expectations for a plant under increased stress conditions. Stomatal density was moderately positively correlated to $\delta^{13}C$ without a concurrent decrease in stomatal size or reduction in leaf size (Table 3), which was contrary to the expectation of lower stomatal surface area for decreased gas exchange, stomatal conductance, or lower rates of photosynthesis and respiration (Hogan et al., 2022; Wright et al., 2004). It is possible that the increased stomatal density we found is an initial response to moderate water stress and that density will decrease with more severe stress conditions (Xu & Zhou, 2008). Alternately, the acclimation of stomatal structure may not be a useful investment at the current rate of changing or fluctuating water salinity conditions (DeWitt et al., 1998), especially due to potential differences in *R. mangle* source water across surface water, shallow soil, and deep soil water between the wet and dry season in this region (Ewe et al., 2007). Even without stomatal acclimation in this scenario, carbon isotope discrimination has been demonstrated to occur and still demonstrates plant response to changing conditions (Rodriguez-Rodriguez et al., 2018). Further, greater stomatal density does not always equate to greater gas exchange; rather, it only demonstrates potential for this (Ellison & Farnsworth, 1997; Kiani-Pouya et al., 2020). Average stomatal density

was within the range of 72–183 stomata mm^{-2} previously reported for *R. mangle* within both oligohaline and euhaline basins (Rodriguez-Rodriguez et al., 2018). Average stomatal density was characteristic of the mangrove water conservation strategy of larger pore size and lower stomatal density in comparison to glycophytic plants (below 100 stomata mm^{-2} with an average guard cell length greater than 30 μm) (Sternberg & Manganiello, 2014).

Total phenolic concentration was expected to present higher concentrations on the conservative side of the trait axis but was not correlated with any LES traits. The negative association of phenolic concentration with the non-significant soil TN vector rather than with soil TP may suggest N availability rather than P availability determines the production of phenolic defense compounds as has been demonstrated on other phosphorus impoverished soils (D. M. Wright et al., 2010).

Water depth, hydroperiod, and soil phosphorus availability are the main drivers of *R. mangle* strategy as it encroaches into freshwater areas

Previous studies on trait variation in *R. mangle* are limited in the number of environmental variables considered (Peel et al., 2017; Watzka & Medina, 2018). This study addresses gaps in the literature of prior studies that: 1) Do not separate the effects of increased freshwater from increased phosphorus availability (Adame et al., 2013; Ahmed et al., 2022), 2) focus on seedling response rather than the response of adult individuals (Lin & Sternberg, 1992b), and 3) do not characterize trait variation in mangroves found in hammock forests (Lin & Sternberg, 1992a, Rodriguez-Rodriguez et al., 2018; Peel et al., 2017). As *R. mangle* faces variable hydroperiod and salinity conditions due to increased coastal influence and human-managed freshwater inputs or restoration efforts, it is important to address how trait variation is driven when multiple environmental variables are considered, how widely traits range intraspecifically, and if trait response is predictable via existing global plant economics theories. Our work has found that intraspecific leaf trait variation in *R. mangle* does partly follow the global LES, and that the conservative to acquisitive response is partially coupled with water use as expressed by $\delta^{13}C$ values. The range of *R. mangle* trait values in adult trees on the stress side of the axis demonstrated a trait response to soil phosphorus limitation and extended hydroperiod comparable to that of decreased SLA and increased $\delta^{13}C$ as salinity stress or hypersaline conditions in the literature (Watzka & Medina, 2018; Lin & Sternberg 1992b). Meanwhile, decreased salinity from brackish to low salinity (0.4–23.1 ppt) did not significantly decrease the conservative response to the other stressors in our study area. In our study we were able to distinguished the effects of increased freshwater influence from increased phosphorus inputs and found that the acquisitive traits of greater height and biomass production attributed to lower salinity in mangrove ecosystems, including the Caribbean coast of Honduras and the Sundarbans (Ahmed et al., 2022; Bhomia et al., 2016), may also bear the influence of increased soil P similarly to mangroves of the karstic Mexican Caribbean (Adame et al., 2013).

Our analysis shows that local differences in water depth, hydroperiod, and soil P drove most of the variance in *R. mangle* traits rather than shifts in fresh to brackish pore water salinity. This suggests that freshwater inputs alone will not increase mangrove productivity. Instead, there is a complex influence of nutrient availability and inundation patterns. Across a landscape such as the southeastern Everglades with heterogeneity in soil types, soil nutrient concentrations, ground elevation, water depths, and hydroperiods, *R. mangle* function and expected ecosystem services can be expected to be variable across areas facing mangrove encroachment.

Intraspecific variation in encroaching *R. mangle* has implications for ecosystem services

Based on our findings, as mangroves encroach into interior marshes on phosphorus-deficient carbonate soils in the southeast Everglades, especially those where flooding is prolonged, the dominant invading *R. mangle* will take on a conservative strategy with higher $\delta^{13}C$, lower SLA, and lower leaf TP, regardless of freshwater supply. This conservative strategy is associated with traits that promote greater water use efficiency (Farquhar & Richards, 1984), a slower relative growth rate and a longer leaf lifespan (Wright et al., 2004) and in turn, low aboveground biomass production (Ross et al., 2001) unlike the highly productive coastal fringe mangrove forests.

As the pace of *R. mangle* establishment on phosphorus-deficient soils of coastal marsh communities continues, the result will be an increase nutrient uptake, storage, and belowground biomass production compared to the herbaceous plant communities that are replaced (Doughty et al 2026; Charles, 2018; Kelleway et al., 2017; Wellman et al., 2026). Still, benefits to carbon sequestration and vertical accretion by mangrove encroachment into low nutrient marshes would be limited in comparison to forested fringe sites (Meeder et al., 2017) and presumably the hammock mangrove types growing in high-phosphorus areas. As mangrove encroachment continues, scrub type mangrove in the southeastern Everglades with minimal accretion rates and patchy establishment (Charles, 2018) cannot be expected to build soils at a similar rate as mangrove peat-marl in high density or high productivity mangrove forests, nor at a rate sufficient to counteract sea level rise (Meeder, 2024; Meeder et al., 2017). More immediately, however, the open canopy structure that typifies these relatively unproductive *R. mangle* shrublands offers ideal foraging conditions to the threatened roseate spoonbill (*Platalea ajaja*) and others among the region's diverse wading bird community, as prey fish become concentrated and vulnerable during seasonal drydowns (Lorenz, 1999; Ogden et al., 2014).

R. mangle in areas with increased soil P are expected to have an increasingly acquisitive strategy but the species may face a concurrent limit to acquisitive strategy with increased water depth conditions (Fig. 2). More acquisitive individuals tend to have greater aboveground biomass, which can be inferred to mean increased carbon storage, but excess increase in soil P may lead to less root production and soil accretion in favor of shoot production which could lead to mangrove mortality (Krauss et al., 2026). At the same time, a shift to conservative traits in response to increased water depth or hydroperiod can also lead to a slower growth rate and accretion rate. Conservative traits would be an issue in transitional marshes facing subsidence (Howard et al., 2020) and in areas with sediment accumulation rates already in deficit to regional rates of sea level rise (Meeder, 2024; Parkinson & Wdowinski, 2022) which would not have sufficient productivity to maintain the necessary accretion rates for survival.

Our work shows that intraspecific variation in the halophytic wetland-adapted species *R. mangle* does demonstrate a conservative to acquisitive shift with a shift from stress to resource conditions along with the coupling of LES traits and some water use adaptations (but uncoupling of phenolic plant defense). *R. mangle* leaf traits along the coupled LES and water use axis responded to variation in water depth, soil TP, and soil N:P rather than decreased pore water salinity. These environmental drivers result in differences of strategy in the *R. mangle* individuals that establish along the current gradient of encroachment. This intraspecific variation will have a direct effect on the ecosystem services we can expect from this foundational species as its range increases or shifts.

Declarations

Ethics approval and consent to participate:

This research did not involve human participants, their data or biological material.

Consent for publication:

The publication has been approved by all coauthors.

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Authors' contributions:

Rosario Vidales: Conceptualization, Methodology, Investigation, Formal Analysis, Data Curation, Writing – original draft, Visualization, Project administration. Michael S. Ross: Conceptualization, Methodology, Investigation, Writing – Review & Editing, Supervision, Resources, Funding acquisition.

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Competing interests:

The authors have no competing interests to declare that are relevant to the content of this article.

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Figures

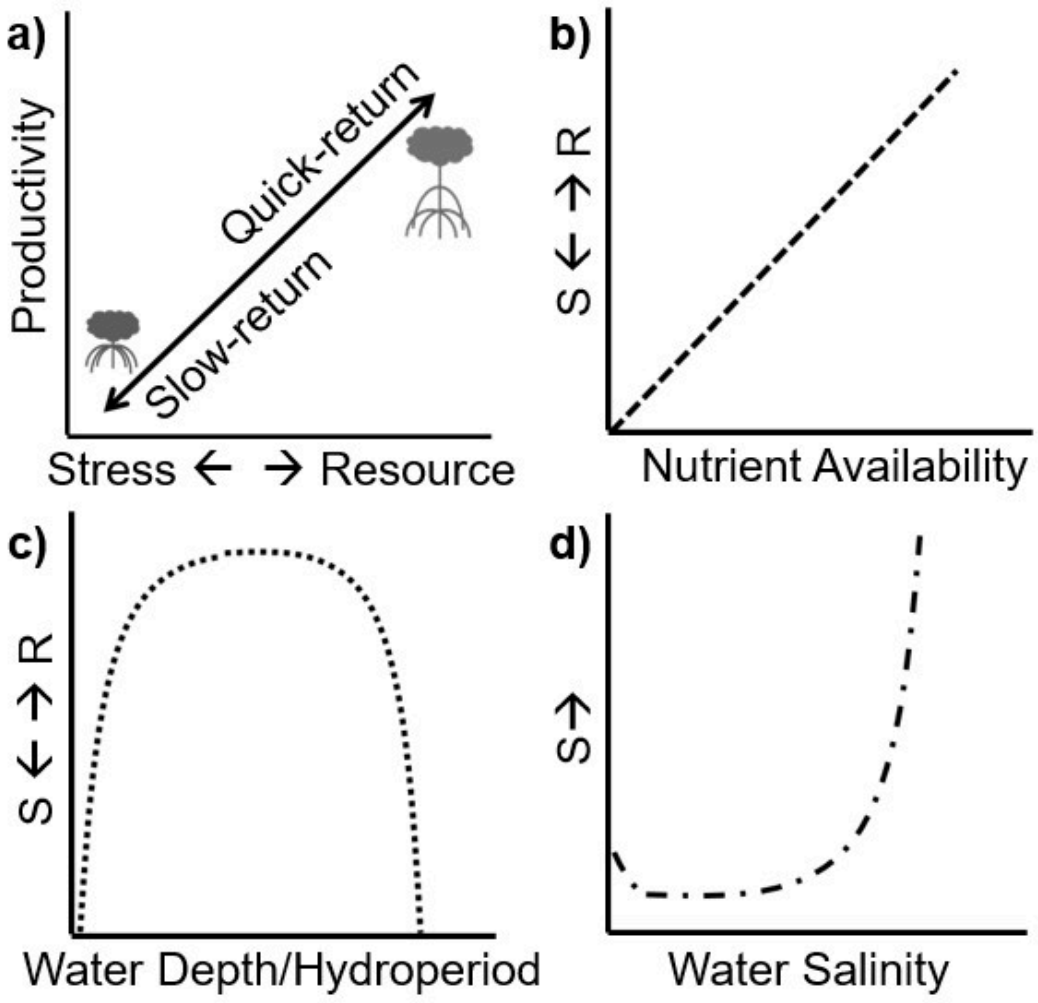

Figure 1

(a) Here we represent the expected single axis of slow-return to quick-return intraspecific response for leaf economics, water use, and stress response traits in *R. mangle* across with low productivity and slow-return traits expected with greater stress (Stress) and greater productivity and quick-return traits expected with increased resource availability (Resource) or with decreased stress. The environmental regulators of *R. mangle*, a wetland and salt adapted plant, are expected to have both resource and stress effects depending on their availability. (b) In the case of soil nutrients, higher nutrient availability is expected to be a resource (R) while nutrient limitation is expected to be a plant stressor (S). (c) Increased water availability is generally a resource, but water depth and hydroperiod extrema (drought and waterlogging) are expected to lead to stress. (d) In *R. mangle,* brackish to moderate salinity does not stress the plant and salinity can be tolerated up to a certain threshold where it would

then become a stressor. Decreased salinity may reduce stress and facilitate water uptake, but too low of a salinity (freshwater) may be suboptimal.

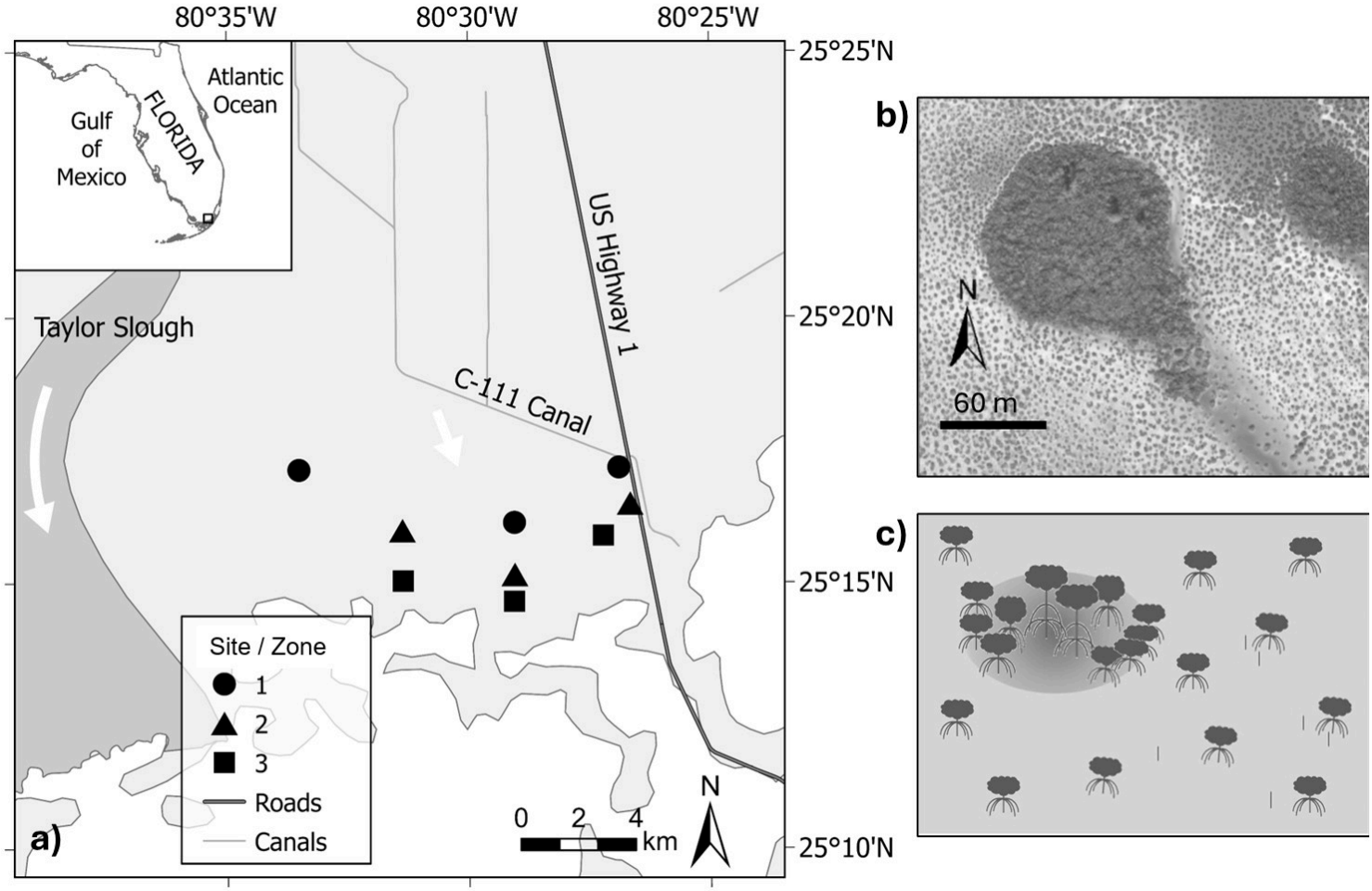

Figure 2

(a) A map of the nine sampling sites within the southeast Everglades, a few kilometers north of the coastal fringe. Sites are represented by circle, triangle, and square symbols, which distinguish the zone in which the site is located. The white arrows in the map denote the southward direction of oligotrophic freshwater flow from Taylor Slough and the C-111 spreader canal. A paired coastal scrub and tree island plot were sampled at each site. Map modified from Mike Rugge, Florida Coastal Everglades LTER, CC BY 4.0. (b) An aerial image and (c) illustrated schema of a tree island embedded within a coastal scrub at each site are shown in the panels to the right. Aerial image from Google Earth.

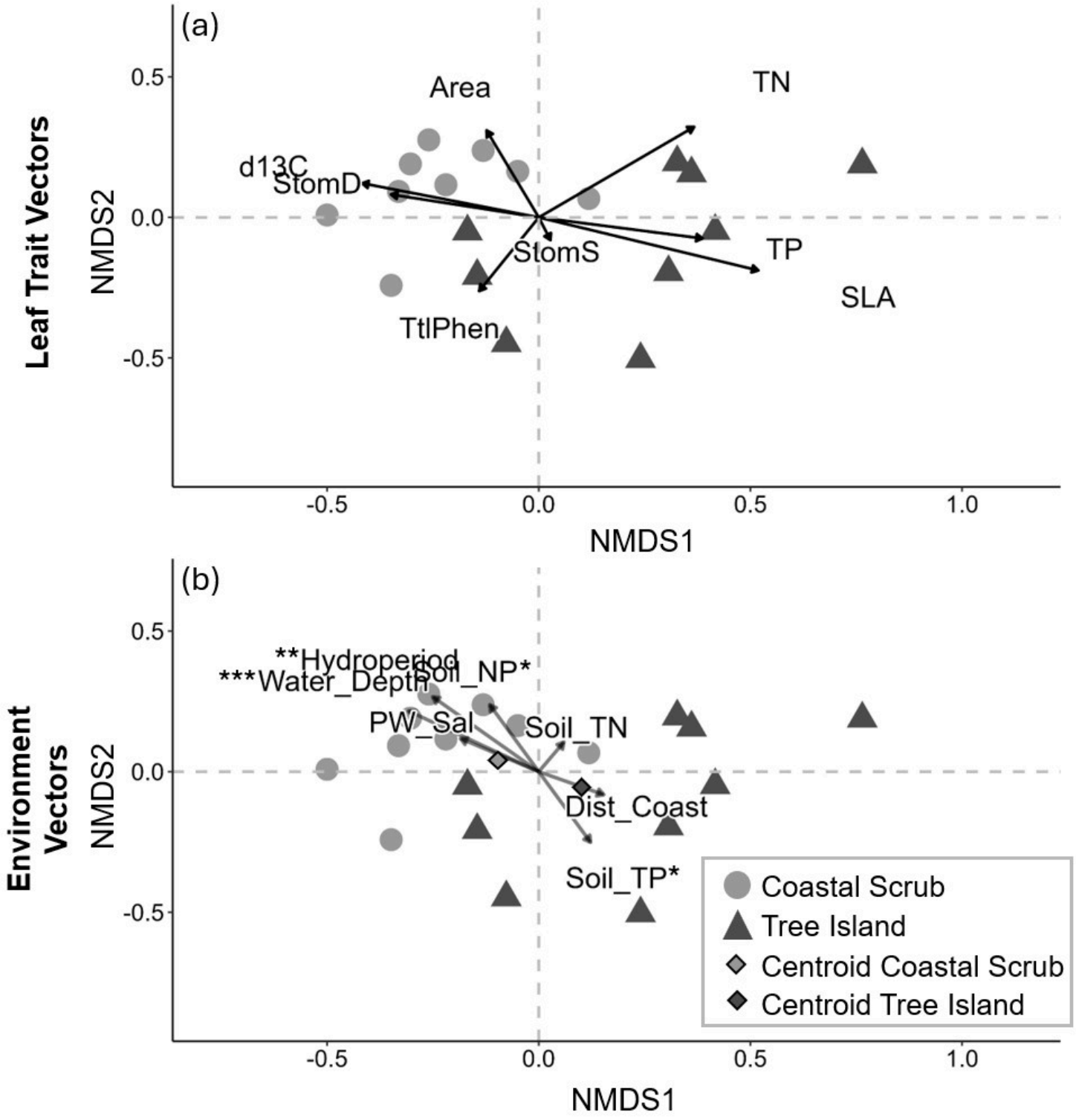

Figure 3

Nonmetric multidimensional scaling (NMDS) of *R. mangle* leaf traits by plot. The points in both graphs demonstrate the ordination of leaf traits in two-dimensional NMDS space based on the average of eight leaf trait values per plot (n = 18). The arrow length of the trait and environmental vectors is related to the strength of the association between the vector and the ordination. a) The top graph shows the leaf trait vectors. Notably, SLA and TP increase toward the right side of the ordination, while $\delta^{13}C$ increases toward the left side of the ordination. b) The bottom graph shows the environmental vectors fit to the same trait ordination. The significance of the

environmental vectors was tested with permutation. Significant environmental vectors represented the stress-resource axis and included mean annual water depth (Water_Depth), mean annual hydroperiod, soil total phosphorus (Soil_TP), soil N:P (Soil_NP), and average water depth (Avg_WD). The soil phosphorus resource increased toward the right side of the ordination, while the environmental stress vectors increased toward the left side of the ordination. Significant environmental vectors are marked with asterisks: $p<0.05$ (*), $p<0.01$ (**). Plot type (coastal scrub or tree island) was a significant factor ($p = 0.001$). Coastal scrub plots are represented by circle symbols and tree island plots are represented by triangle symbols. The diamond shape represents the centroids for each plot type.