Quantifying Intraspecific Variation of Red Mangrove Leaf Traits in the Southeast Saline Everglades

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Introduction

- Intraspecific variability is known to capture niche and trait overlap in species assemblages (Violle, et al., 2012) and is especially useful along gradients (Albert, 2011), as it facilitates prediction of species fate response to environmental changes (Albert, 2011; Violle et al. 2014).
- In the Southeast Saline Everglades (SESE) increased rates of sea level rise have resulted in significant landward encroachment of marine water, retreat of freshwater marsh communities (Wanless et al., 1994) and encroachment of mangroves (Ross et al. 2000).
- *R.* mangle remains ubiquitous along this gradient of nutrients and salinity. The study of intraspecific variation in leaf traits of *R. mangle* populations along these gradients may contribute to a broader understanding of how phenotypic plasticity allows plants to navigate multiple stressors, and improve prediction of the ecological repercussions of changing environments.
- Prior works have been found to focus on only one environmental variable, or to experiment mainly with seedlings or young mangroves. Others works do compare adult fringe and scrub mangroves, but not tree island mangroves, which are also taller growth forms, but not subject to tidal action as fringe mangroves are.

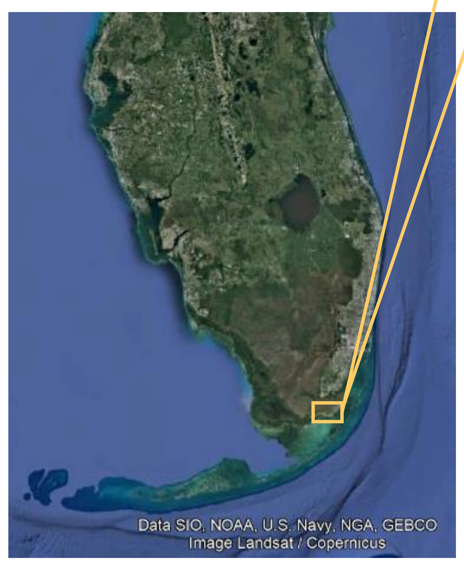

Figure 1. Satellite imagery (Google Earth) of the southern portion of Florida. The yellow square indicates the study area.

Research Questions

- Does salinity concentration have a relation to intraspecific variation in leaf traits?
- Does phosphorus limitation have a relation to intraspecific variation in leaf traits?
- Does growth form have a relation to intraspecific variation in leaf traits?

Study Sites

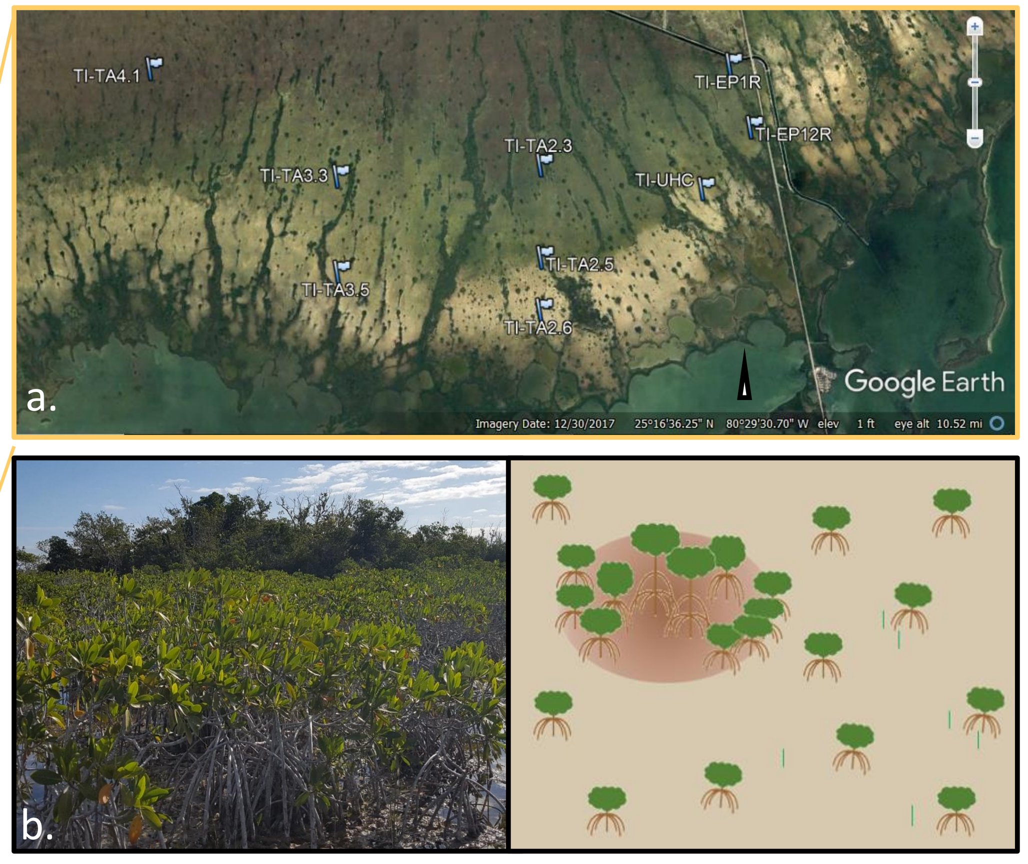

Figure 2a,b. (a) Map of study area and site locations within the Southeast Saline Everglades. The relative location of this study area can be seen on the map in Figure 1. (b)Photo of the growth variation of *Rhizophora mangle* on tree islands (background of left image) and adjacent scrub marsh (foreground). Tree islands toward the coast include species such as *Laguncularia racemosa*, and *Conocarpus erectus*, while tree islands further toward the interior contain *Metopium toxiferum*, and *Swietenia mahagoni,* among others. The illustration to the right depicts the usual placement of peat tree islands scattered among areas of scrub red mangrove in marl soils, and the growth form difference, with taller red mangrove trees on island interior, and dwarf mangroves in the scrub.

Site Specifications

						Soil						
No.	Site	Distance to Coast (km)	Type	Site	Pore Water Salinity (PPT)	Organic Matter g g-1 dw	TN mg m-1 dw	TC mg m-1 dw	TP ug g-1 dw	C:N	C:P	N:P
1	EP1R	3.11	S	EP1R	1.1	0.10	3.95	149.33	65.06	44.1	5918	134
			TI	EP1R	1.1	0.47	12.48	300.93	387.16	28.12	2004.4	71.27
2	TA2.3	4.16	S	TA2.3	0.8	0.11	4.86	155.72	86.61	37.4	4636	124
			TI	TA2.3	0.4	0.34	5.64	173.68	431.35	35.9	1038.3	28.9
3	TA4.1	8.12	S	TA4.1	2.0	0.13	6.87	157.82	71.75	26.8	5673	212
			TI	TA4.1	2.8	0.91	12.02	490.62	296.55	47.61	4266.3	89.61
4	EP12R	2.03	S	EP12R	6.0	0.17	6.84	177.01	79.58	30.2	5736	190
			TI	EP12R	3.2	0.55	15.97	328.79	599.12	24.02	1415	58.91
5	TA2.5	2.61	S	TA2.5	12.7	0.07	4.03	134.84	52.73	39.0	6595	169
			TI	TA2.5	10.5	0.75	19.47	380.77	560.24	22.81	1752.6	76.83
6	TA3.3	5.01	S	TA3.3	10.8	0.13	4.01	132.44	32.67	38.5	10454	271
			TI	TA3.3	8.9	0.83	21.32	439.27	481.19	24.03	2354.0	97.95
7	UHC	2.47	S	UHC	23.1	0.07	2.96	139.14	75.65	54.8	4743	86.5
			TI	UHC	17.9	0.56	13.78	298.37	764.88	25.27	1005.9	39.81
8	TA2.6	1.72	S	TA2.6	20.8	0.10	3.46	141.95	65.33	47.9	5603	117
			TI	TA2.6	17.7	0.59	11.62	311.95	442.65	31.31	1817.3	58.04
9	TA3.5	3.21	S	TA3.5	17.9	0.15	4.67	145.65	53.31	36.4	7046	193
			TI	TA3.5	12.0	0.79	12.31	206.72	657.55	19.59	810.70	41.38

Table 1. Table of environmental variables for each study site. Community type "S" and "TI" signifies a scrub site and tree island site respectively. Soil total nitrogen, carbon and phosphorus was determined from 30 cm cores taken at each site. Carbon, nitrogen and phosphorus ratios are molar, and all sites reflect phosphorus limitation (N:P >16). Pore water salinity was obtained during the dry season of 2018.

Methods

Site Selection

- Nine sites consisting of a tree island and adjacent mangrove scrub plot were selected among prior study sites to capture a spatial and environmental gradient ranging (**Fig. 1a, b**) .
  - Phosphorus limited
  - Range:
    - Distance from coast: 1.72-8.12 kilometers
    - Dry Season Salinity: 1 to 23 parts per thousand

Field Sampling

- Three trees were sampled within each tree island and scrub plot
  - Three leaves were analyzed for fresh and dry weight
- Dry season pore water salinity (Table 1).
  - YSI 30 Salinity Conductivity Meter

Lab Methods

- Leaf Stomate Imprint by nail varnish method (Hodgson et al, 1993)
  - Visualization and image capture:
    - Leitz Dialux 20 Fluorescence Light Microscope
    - Nikon Coolpix camera and MDC lens adaptor.
    - ImageJ Image Processing and Analysis in Java
      - Stomata counted within 0.48 mm^2
- Leaf Measurements
  - n=3 for each site
  - LI-COR Portable Area Meter Model LI-3000A
  - Weight, petiole removed
    - Fresh Weight
    - Dry weight, oven dried at 70°C until constant weight
- Stomatal Density (abaxial)
  - Average of stomatal count in four fields of view per leaf.
    - n=3 for each site
    - Density per mm^2
    - Total stomata per leaf

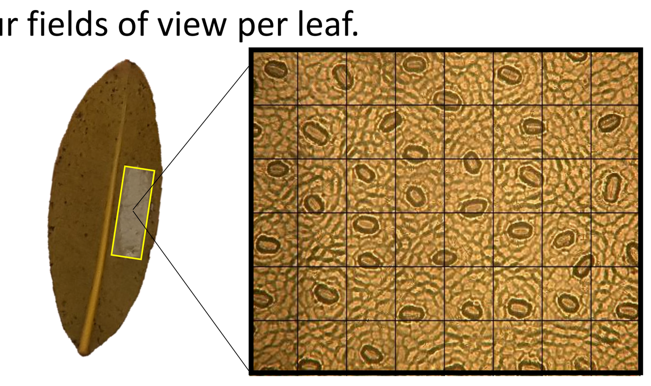

Figure 3. The abaxial side of an *R. mangle* leaf collected from tree island TA4.1, and the field of view from the magnified leaf. The overlaid grid consists of 0.1 mm wide cells.

General Terms and Traits

- Specific Leaf Area (SLA): ratio of leaf area to dry mass
  - Low SLA values are associated with denser leaf tissue, resource-poor environments, and slow-return species
- Carbon isotope ratio (δC): Ratio of δ13C : δ12C
  - Higher δC = more $\delta^{13}C$ uptake, or less plant discrimination
    - "More stress"
    - Higher long term water use efficiency
- Nutrient limitation
  - N : P molar ratios (Aerts & Chapin, 2000)
    - Phosphorus limitation at N : P > 16
    - Nitrogen limitation at N : P < 14

Results

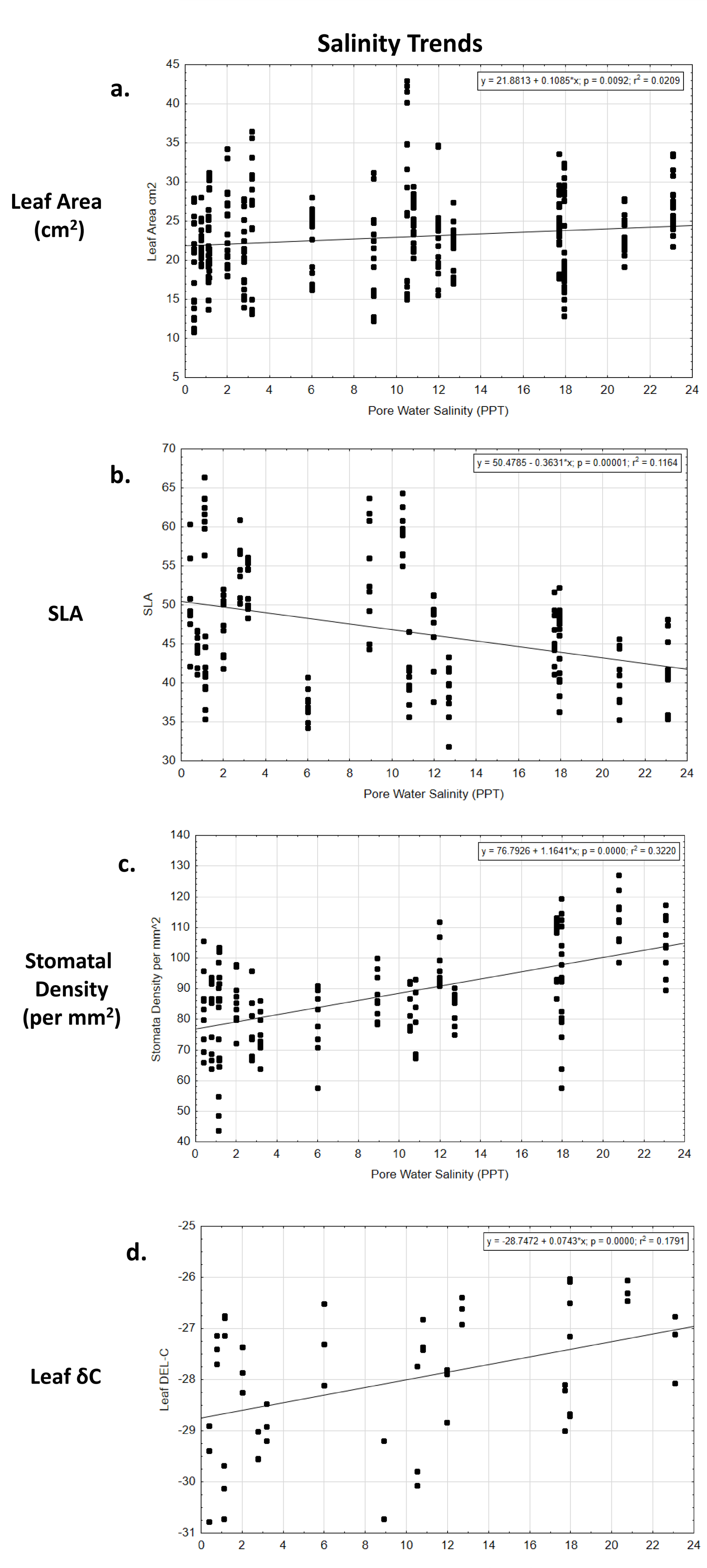

Figure 4a, b, c, d. Trends between pore water salinity and leaf traits across all sites. Pore water salinities were measured in the dry season of 2018. There was a positive correlation between pore water salinity and leaf area (a), stomatal density (c), and leaf δC ratio(d). There was a negative correlation between pore water salinity and SLA.

Nutrient Limitation Trends

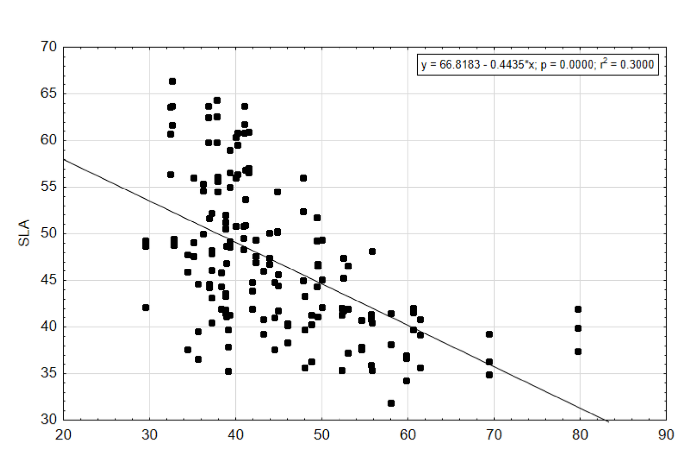

Figure 5. Trend between plant phosphorus limitation and specific leaf area across all sites. With increased phosphorus limitation (increased N:P ratio, and N:P>16) there is a decrease in SLA.

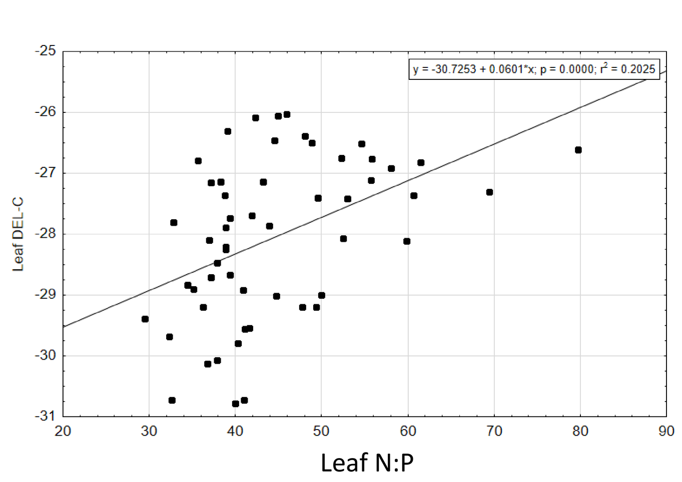

Figure 6. Trends between plant phosphorus limitation and leaf δC ratio across all sites. With increased phosphorus limitation (increased N:P ratio, and N:P>16) there is an increase in plant stress as determined by a less negative δC ratio.

There was no significant relation between plant phosphorus limitation and stomatal density, nor leaf area.

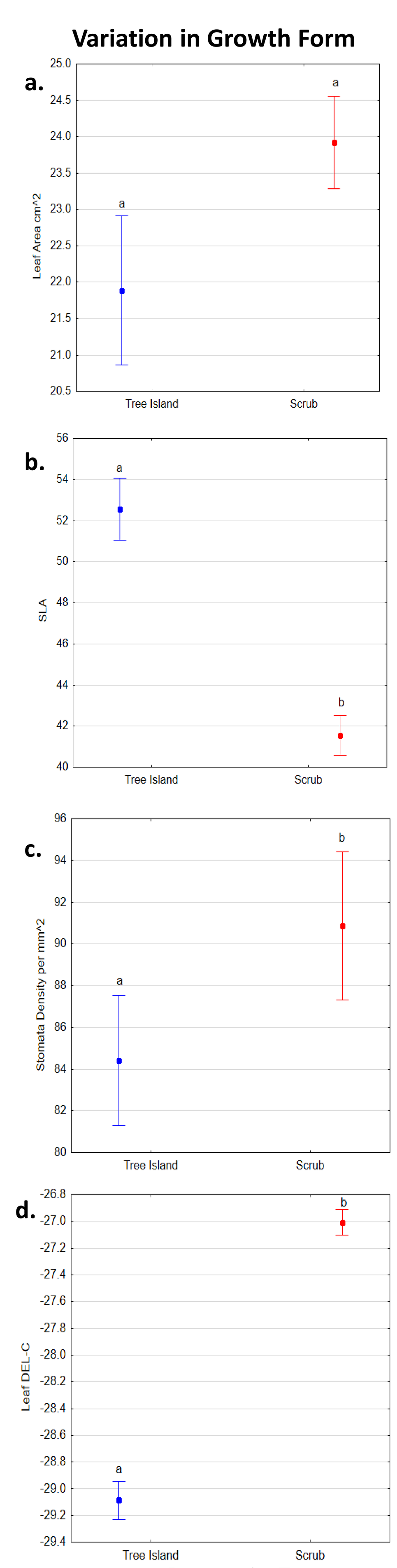

Figure 7a, b, c, d. Mean and standard error of leaf traits compared across the tree island and scrub mangrove growth forms. In all cases, means were significantly different at a significance of p<0.05 and 95% confidence interval.

Discussion and Conclusions

Variation in Leaf Traits with Salinity and Phosphorus Limitation:

With increased pore water salinity there was decreased SLA (**Fig. 4b),** increased stomatal density (**Fig. 4c**), and increased δC ratio (**Fig 4d).** With increased phosphorus limitation there was decreased SLA, and increased δC ratio. Both of these parameters had a higher R^2 in the regression for P limitation than in the model for salinity.

Intraspecific variation in *R. mangle* stomatal density appears to be salinity driven (**Fig. 4c**) rather than phosphorus driven (p>0.05) within our dataset. The model for leaf area and pore water salinity, although significant, had a low R^2 (**Fig. 4a**)

Variation in Leaf Traits of Tree Island and Scrub Mangrove Growth Forms:

Overall, mean leaf area was higher in the scrub growth form of *R. mangle* than in the tree island growth form (**Fig. 7a**). Mean values for the scrub leaves are well within Lin & Sternberg's (1992a) 23.4 $\pm$ 1 cm^2 mean area in scrub. This would mean leaf area is not solely attributed to growth form, as tall fringe mangroves in the aforementioned study had a higher area than the scrub counterpart, contrary to this case with tall, tree island red mangrove.

There was lower plant stress in tree islands. The δC ratio was lower (**Fig. 7d**) and the SLA higher in tree island *R. mangle* (**Fig. 7b**) than in scrub mangrove, which is typical of resource rich environments. This is unlikely to be the result of growth form alone, and further analysis taking into consideration the simultaneous effect of variation in site salinity, available nutrients, and community competition is an important next step. Stomatal density was lower (**Fig. 7c**) in tree island *R. mangle*, which coupled with smaller leaf area also resulted in significantly less total stomata per leaf in tree island *R. mangle*. While stomatal density does not appear to be nutrient driven, the effect of nutrient limitation on leaf area, may have some implications on plant dynamics in relation to stomatal activity as well. Future work will address stomatal dimensions, as this is an important component in implications for gas exchange, and water use efficiency along with stomatal density.

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