Investigating how tidal inundation and plant composition (species and maternal lineage) influence black mangrove (*Avicennia germinans*) growth and survival to inform coastal restoration

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Restoration often focuses on restoring foundation species as they drive ecological functions of entire ecosystems by creating the physical structure of habitats, reducing abiotic stressors, enhancing food availability and influencing species interactions[1]. Yet, changes in climate are resulting in shifts in the abundance and distribution of species[2,3], resulting in transition zones where multiple foundation species can co-occur and/or the identity of the dominant foundation species is altered[4,5]. These shifts in foundation species identity and composition have a variety of often unpredictable, cascading effects on ecosystem structure and function[6,7]. Thus, complicating whether planted restoration efforts should focus on historically dominant foundation species, predicted future ones or both.

In the northern Gulf of Mexico, coastal wetlands have been historically dominated by the clonal foundation plant, *Spartina alterniflora* (hereafter *Spartina*), in the low and mid intertidal[8]. However, due to infrequent extreme cold events and warming temperature, the identity and composition of foundation species in these coastal wetlands is changing. Nowhere is this change more acute than in southern Louisiana where black mangroves (*Avicennia germinans*; hereafter *Avicennia*) are expanding into marshes that have historically been dominated by *Spartina*[9]. Mangroves and marsh grasses differ in many physical and ecological traits, including physical structure, palatability, detrital inputs, and abiotic tolerance[10,11]. These differences in traits can have cascading effects on population, community and ecosystem-level processes[8,9,12]. As a goal of restoration is to restore ecosystem function, it is critical to understand the trade-offs among foundation species and consequences of altered composition/identity.

Prior work has illustrated that *Spartina* can rapidly grow and expand in restored sites compared to *Avicennia*[13]. But most of these studies have focused on either single tidal elevation and/or sparse plantings to reduce plant-plant interactions. However, plant-plant interactions occur across a continuum. Sparse plants were historically used to reduce competition among plants, but as restoration inherently starts as a stressful environment as typically dredge sediment is pumped into containment dikes to form new bare land, and facilitation tends to be more important under stressful environments[14]. Recent work has highlighted that clumped planting efforts have led to better restoration success and outcomes[15] – although typically with individuals of the same plant species. Clumping may enable facilitation as there is greater community oxygenation of the rhizosphere and it reduces erosion. Thus, the facilitative benefit of clumping may also tend to vary across tidal elevation[15] as tidal elevation and the associated variation in inundation alters environmental factors including sediment oxygen availability, salinity, and soil drainage and thus stress experienced by individual plants.

At the same time, individuals within species can also vary in a host of morphological, physiological, and phenological traits – including predator defenses, abiotic tolerance, resource use, and/or competitive ability[16]. Within coastal marshes, *Spartina* exhibits natural variation in small-scale genotypic diversity across the landscape[17,18]. Furthermore, *Spartina* genotypic identity can influence plant traits and have cascading ecological effects[19,20].Within Louisiana, restoration involving marsh planting has historically utilized a few *Spartina* genotypes have been propagated and planted based on specific plant traits (e.g., rapid growth, high seed set;[21,22]). Less is known about genetic and phenotypic variation within *Avicennia*. While populations at a species' range edge (such as in Louisiana) are predicted to exhibit lower genetic diversity than center populations as a consequence of smaller population size and reduced gene flow[23,24], there is limited support for this within *Avicennia* populations along the Texas-Louisiana coastline as genetic variation was similar throughout[24]. Further, field surveys of *Avicennia* along the range edge in Florida found that all 40 trees sampled were genetically unique and that this genetic variation had cascading impacts on the associated endophytic fungal community[25].

Yet, the influence of genetic identity on traits, growth and reproduction can vary both across biotic and abiotic environmental factors (i.e., environment x genotype interactions). For instance, *Spartina* genetic identity can alter and shift the direction and magnitude of plant-plant interactions[19,26]. Likewise, across several wetland plant species, plant genotypes can vary in their response to environmental stressors including inundation/ flood frequency[27-29], salinity[30], and nutrient availability[31]. Thus, given the expected increase in *Avicennia* dominance here in the future, it will be crucial to examine both how much genetic and phenotypic variation exists within *Avicennia* in Louisiana, and test how this within species variation alters ecosystem function (e.g., plant production) under potential abiotic and biotic environments of various restoration strategies to best incorporate mangroves into coastal restoration practices.

Objectives- We propose to investigate how plant composition (*Spartina* presence or absence), *Avicennia* maternal lineage, and tidal inundation influence *Avicennia* growth and survival to understand how mangroves may be incorporated into successful future coastal restoration projects. We predict that *Avicennia* propagules (early life stage) may particularly benefit from the presence of *Spartina* under greater inundation stress as at earlier life stages (typical of planting) they have not yet formed pneumatophore roots (that oxygenate soils). Thus, the present of *Spartina* – with its aerenchyma tissue that can aerate the soil – likely benefits *Avicennia* via root oxygenation under higher inundation stress. Furthermore, we predict that different maternal lines will vary in plant traits (e.g., plant production, nutrient content), and this variation could have cascading impacts on restored wetland ecosystem function (e.g., carbon sequestration, trophic interactions). Lastly, we expect *Avicennia* maternal lines will vary in response to the environment (inundation treatment and neighbor presence) and thus, determining which lineage may be best for restoration efforts will likely depend on the type of environment being planted into.

To address our question, we plan to conduct a fully-factorial greenhouse mesocosm experiment at the UL Ecology Center that manipulates tidal inundation (i.e., low vs high), *Spartina* (neighbor) presence or absence, and *Avicennia* maternal lineage to assess impacts on plant traits, growth and survival monthly during a year-long experiment.

Methods and Preliminary Data- In the fall, we will collect *Avicennia* propagules from at least 10 unique trees in Port Fourchon; tree will be separated by at least 50 meters. *Spartina* transplants will be collected adjacent to sampled *Avicennia* trees. Plants will be grown in the greenhouse prior to experimental set-up in the spring. Tidal inundation will be manipulated within 8 large mesocosm tanks as either low (2-3 hours) or high (8+ hours) inundation duration. Within each tank, 8-10 large pots will be placed within which *Avicennia* maternal line and neighbor presence will be manipulated (N = 4 for each treatment combination). We will plan to measure *Avicennia* stem height, number of leaves, number of branches, leaf length/width (2-3 leaves per tree), and canopy area along with *Spartina* stem density and height monthly. At the end of the experiment, we will also measure specific leaf area, above- and belowground biomass, and collect a leaf sample to analyze nutrient content (i.e., carbon and nitrogen). Statistical comparisons will compare individual response variables using a 3-way ANOVA with tidal inundation, plant neighbor and *Avicennia* maternal lineage as fixed effects. We will use principal component analysis to compare trait variation among maternal lineages.

This past summer I ran a pilot project that tested variation in traits among *Avicennia* maternal lineages across sediment treatments (i.e., dredge sand or sand/soil mixture). Preliminary data indicates that there is significant variation in the measured traits (i.e., stem height, number of leaves, leaf area, branch number and canopy area) across maternal lineages either independently or interactively with sediment type (Figure 1). This indicates that within-species variation in *Avicennia* is present within trees throughout Port Fourchon, and thus the goal of this proposed project will be to build off this by examining these and additional traits across abiotic (inundation) and biotic (plant neighbor) environments that plants would be exposed to during potential restoration projects.

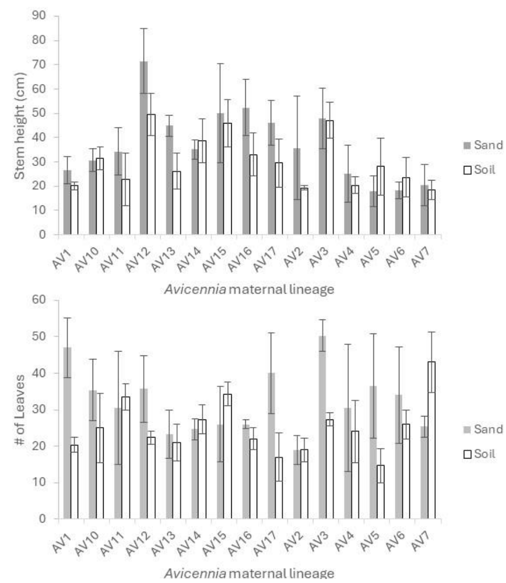

Figure 1. Variation in stem height and leaf # among *Avicennia* lineages across sediment treatments.

Relevance to CPRA and Louisiana's Master Plan – This research will provide CPRA with information on how the alteration of coastal wetlands by environmental change (i.e., species range expansion, and sea-level rise) may need to be accounted for in future restoration practices to manage and maintain coastal ecosystem function and structure. Specifically, we will address how to best incorporate mangrove planting into future coastal restoration projects. This project addresses objectives of the Master Plan in terms of quantifying how environmental context – including plant species composition and within-species variation – can influence restoration success to promote a sustainable coast, and thus, our findings can inform future revisions of the Master Plan, particularly, with respect to best practices for restoration outcomes and objectives.