PHRAGMITES AUSTRALIS DIEBACK IN THE MISSISSIPPI RIVER DELTA: CHEMICAL PROFILES OF SOIL TYPES AND RESTORATION POTENTIAL

A Thesis

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ABSTRACT

Since 2016, there has been widespread dieback of *P. australis* in the Lower Mississippi River Delta (hereafter referred to as Lower MRD, which is defined as the Birds Foot Delta) with relatively little to no signs of recovery. The cause of the current dieback is not fully understood. This thesis explores *P. australis* with emphasis on chemical profile characterization of different soil types and its effects on plant growth and the potential for restoration in the Lower MRD.

In chapter 2, I characterized the chemical profiles of soils collected from healthy and dieback stands of *Phragmites*, and from newly created dredge sites. To assess restoration potential in dieback and dredge sites, we experimentally tested whether these different soil types impact *P. australis* aboveground biomass. Dieback soils were characterized by higher % organic matter, % carbon, % nitrogen, and higher S and Fe concentrations, whereas healthy soils had higher Cu, Al, P and Zn. Whereas, dredge sites were low in nutrients and organic matter compared to healthy soils. Biomass was 16-23% and 44% lower in dieback and dredge soils, respectively, than in healthy soils. Dieback soils most negatively affected Delta followed by EU and Gulf lineages. Plants grown in dredge soils had the lowest mean biomass compared to healthy and dieback soils.

Subsequently, I provide preliminary findings from a restoration field trial to assess the potential of three lineages of *P. australis* (Delta, EU and Gulf) and common wetland plant species including *S. californicus, S. alterniflora, S. patens* and *Z. miliacia* to revegetate marsh habitats in the Lower MRD. After 6-months, plant survival of Delta and Gulf had high survival of 72.4% and 76.9%, respectively, whereas EU lineage had approximately 43% lower survival. Delta had high survival regardless of having the greatest scale abundance after one year. After 2.5 years, only 4 of 9 sites had surviving plantings which consisted of only Delta, EU, Gulf and

S. californicus. Area cover was the greatest for Delta among the lineages, thus I conclude that Delta lineage would be the most optimal for restoration success in the Lower MRD.

CHAPTER 1.
INTRODUCTION

1.1.BACKGROUND

Coastal wetlands are among the most productive ecosystems on earth and at the same time are the most threatened by global changes, including climate change, sea level rise, extreme weather and anthropogenic disturbances (Turner, 1990; White and Kaplan, 2017). Due to the accelerated economic and population growth during the 20th century, annual global losses of coastal wetlands were 0.7 – 1.2% resulting in approximately 65% of the cumulative coastal wetland losses due to the rapid economic growth (Davidson, 2014). In the contiguous United States, the Mississippi River Delta (MRD) complex encompasses approximately 40% of the remining wetlands (Turner and Rao, 1990) and comprising 25,000 km^2 of wetlands, shallow water bodies and low-lying uplands (Day et al., 2005). Over the last century, the deltaic complex has lost about 5000 km^2 of coastal wetlands (Day et al., 2000; Couvillion et al., 2017). A number of factors have led to the widespread loss of wetlands and are expected to increase in magnitude with relative sea level rise (RSLR) coupled with accelerated land subsidence, ongoing human impacts (dams, levees, canal construction and dredging) and biological mechanisms, including vegetation dieback (Day et al., 2008).

Phragmites australis (Cav.) Trin. Ex Steud, also commonly known as Roseau Cane, is a tall cosmopolitan perennial grass inhabiting a wide range of wetland ecosystems including fresh, brackish water marshes (Kiviat, 2013; Eller et al., 2017). The recent spread of European *Phragmites* from its native range in marsh upland elevations into the low interior marsh zones has led to its successful establishment in most of North America, often outcompeting native species by forming dense monospecifc stands (Silliman and Bertness, 2004; Meyerson et al., 2009).

Dieback of vegetation has been documented in various wetland habitats across the globe which has been implicated as a precursor to land loss. In the Lower MRD, *P. australis* is the dominant emergent vegetation in the freshwater and brackish marshes of the deltaic plain and has exhibited widespread dieback dating back to approximately 2015 (Ramsey III and Rangoonwala, 2017; Knight et al., 2018; Suir et al., 2018). Dieback syndrome of *P. australis* is characterized by the retreat of plants from relative deep waters, increased stand clumping habits, stunted stem growth, premature senescence of plant tissues and high occurrences of dead belowground structures (Armstrong et al., 1996a; van der Putten, 1997; Brix, 1999). Vegetation dieback is a serious threat to the stability of coastal wetlands as the aftermath involves the conversion of dense healthy stands of vegetation to bare mudflats and eventually open waters.

Figure 1.1. Stand of *Phragmites australis* (Delta lineage) in the Pass-a-Loutre Wildlife Management Area (WMA), Lower Mississippi River Delta, Plaquemines Parish, Louisiana, USA on June 16, 2020.

Given the unprecedented and substantial loss of *P. australis* in the Lower MRD, restoration of these valuable ecosystems is critical. The cause of the current dieback is not fully understood. Numerous abiotic and biotic stressors have been connected to the dieback of *P. australis* in Europe (van der Putten, 1997), China (Li et al., 2013) and the USA (McDonald, 1955; Knight et al., 2018; Knight et al., 2020; Cronin et al., 2020). Commonly attributed stressors include prolonged flooding and waterlogged conditions, elevated salinity levels, eutrophication, the accumulation of phytotoxins, heavy metals and organic matter in soils and herbivory (van der Putten, 1997; Brix, 1999). Upon the discovery of dieback in the MRD, severe infestations of the non-native Roseau Cane Scale (hereafter RCS), *Nipponaclerda biwakoensis* (Kuwana) (Hemiptera: Aclerdidae) was discovered (Knight et al., 2018).

A complicating factor of the dieback of *P. australis* is due to the lower MRD being a genetic hotspot for *P. australis* diversity comprised of both native and nonnative lineages. In the Lower MRD, the dominant lineage is Delta (haplotype M1), which accounts for 95% of the *P. australis* in the MRD (Hauber et al., 2011; Lambertini et al., 2012a; Knight et al., 2018). Next, a second common lineage is known as the invasive Eurasian lineage and is known for its invasive and superior competitive abilities in North America (Saltonstall, 2002). Lastly, the Gulf Coast lineage, also known as the Land-type (hereafter Gulf) is found throughout the Gulf coast and has recently spread to the West Coast and inhabits relatively higher elevations in the MRD (Saltonstall, 2002; Meyerson et al., 2010; Hauber et al., 2011).

1.2. STUDY OUTLINE

In Chapter 2, I aimed to investigate the soil chemical profiles from sites of dieback *P. australis*, healthy *P. australis* and recently excavated dredge soils in the MRD and to assess whether these soil types influenced the growth of three lineages of *P. australis* (Delta, EU, Gulf). In the Winter and Spring of 2019, we collected soils from replicate healthy, dieback and dredge sites in the MRD and analyzed their chemical profiles. Subsequently, we conducted greenhouse and common garden experiments to assess whether soil type, *P. australis* lineage (Delta, Gulf, and EU) and the interaction influenced aboveground biomass of *P. australis*. To my knowledge, this study is the first characterization of dieback and healthy *P. australis* soils in the MRD as well as its impacts on the three lineages of *P. australis.*

In Chapter 3, I provide the preliminary findings from a restoration field trial (LA-39 Coastwide Vegetative Planting Year 7A Mississippi River Delta Vegetative Field Trial built under the Coastal Wetlands Planning, Protection, and Restoration Act by the Natural Resources Conservation Service (NRCS) of the United States Department of Agriculture) initiated in 2018 by the Coastal Protection and Restoration Authority with the assistance of LSU researchers from the LSU Agcenter Roseau Cane Die-off research group (Figure 1.2.). The objective of this study was to evaluate the restoration potential of different lineages of *P. australis* (Delta, EU, Gulf) and various wetland vegetation species in the MRD. This is the first restoration field trial of this large scale in the MRD.

Figure 1.2. Nine site locations from the LA-39 Coastwide Vegetative Planting Year 7A Mississippi River Delta Vegetative Field Trial in the Lower Mississippi River Delta. *Source: Google Earth*

At nine sites across the Southwest Pass of the Lower MRD, the three *P. australis* lineages and various wetland plants, including *Spartina alterniflora, Spartina patens, Schoenoplectus californicus* and *Zizaniopsis miliacea*, were planted at previous dieback sites of different environmental conditions and a recent dredge site. Over the course of 2.5 years, we used different methods to measure plant health and growth. In this chapter, I provide the preliminary findings from 6-months, 11-months, and 22-months post planting. At 6-months, we visually assessed plant survival. At 11-months, we collected and analyzed the data for stem density, stem height and scale abundance. Lastly, I used an unmanned aerial vehicle (UAV) to estimate plant area cover at 22-months.

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CHAPTER 2.
PHRAGMITES AUSTRALIS DIEBACK IN THE MISSISSIPPI RIVER DELTA: CHEMICAL PROFILES OF HEALTHY, DIEBACK AND DREDGE SOILS AND IMPACTS ON PLANT BIOMASS

2.1. INTRODUCTION

Coastal wetlands are among the most productive and valuable ecosystems on the planet (Turner, 1990; Costanza et al., 2014). These essential ecosystems provide important services such as sediment stabilization and storm surge protection by tall-statured grasses and mangroves (Windham and Lathrop, 1999), removal of excess nutrients from the water column (Ouyang and Guo, 2016), pollutant trapping (Gambrell, 1994), CO_2 and CH_4 sequestration (McLeod et al., 2011), and support of nursery habitats and feeding grounds for wildlife (Aburto-Oropeza et al., 2008). However, coastal wetlands are severely threatened by changing climatic, oceanographic, ecological and anthropogenic conditions (White and Kaplan, 2017).

The perennial marsh grass, *Phragmites australis* (Cav) Tren. ex Steud (hereafter referred to as *P. australis*), which occurs in freshwater and brackish marshes worldwide, has exhibited widespread dieback in the Lower Mississippi River Delta (MRD) dating back to about 2016 (Ramsey III and Rangoonwala, 2017; Knight et al., 2018; Suir et al., 2018). Dieback syndrome of *P. australis* has been characterized as the retreat of plants from deep water, increased stem clumping within a stand, stunted stem growth, premature senescence of leaf tissue, and high occurrences of dead or decaying rhizomes (Armstrong et al., 1996a; van der Putten, 1997; Brix, 1999). *P. australis* provides valuable ecological services including providing nursery habitats and foraging grounds for various avian species, carbon sequestration, nutrient removal and water

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This chapter has been submitted for publication to *Aquatic Botany* and is currently in review as of May 13, 2021 purification. Additionally, *P. australis* plays a key role in coastal wetlands affected by high rates of relative sea-level rise and land loss, including storm surge protection, sediment trapping and soils stabilization which are essential processes for maintaining marsh elevation in the Lower MRD (Rooth et al., 2003; Howard and Turluck, 2013). The ongoing dieback of *P. australis* is of serious ecological and economic concern in the rapidly deteriorating coastal wetlands of the Lower MRD because it may perpetuate land conversion by reducing sediment trapping and consequentially allowing the infilling of essential navigation channels (Temmerman et al., 2012; Knight et al., 2018). Moreover, vegetation dieback events are often precursors to land loss because they result in the conversion of marshes into bare mudflats or open water (Turner, 1990). Land loss has been a well-established problem in the lower MRD and considerable effort and expense have been invested in creating new wetlands from dredge materials (Wingate, 2014).

Multiple abiotic and biotic stressors have been implicated in the dieback of *P. australis* in Europe (van der Putten, 1997), China (Li et al., 2013) and the USA (McDonald, 1955; Knight et al., 2018; Knight et al., 2020; Cronin et al., 2020). The European Research Project on Reed Die-back and Progression (EUREED) concluded that eutrophication coupled with prolonged waterlogging, elevated sulfide concentrations and organic matter accumulation is primarily responsible for the extensive dieback in Europe (van der Putten, 1997; Brix, 1999). Accumulation of phytotoxins such as hydrogen sulfide can negatively impact the reed's ability to efficiently uptake and assimilate nutrients for energy and growth (Armstrong et al., 1996a). Additionally, Fürtig et al. (1999) concluded that accumulation of heavy metals (e.g., copper, iron) in belowground organs might have induced reed die-back in European lakes. In Italy, Gigante et al. (2014) observed reduced growth of *P. australis* at elevated concentrations of copper, zinc, nickel, total chromium, cobalt and iron. As a result of dieback, decay and decomposition of large quantities of damaged *P. australis* and organic matter may produce and release organic acids, sulfides and heavy metals (Armstrong and Armstrong, 2001). In many cases, it is unclear whether soil conditions reported following a dieback event are a direct cause or consequence of dieback.

A complicating factor in the dieback of *P. australis* in the MRD is that this region is comprised of several phenotypically and phylogenetically distinct lineages of *P. australis,* including those of native and nonnative origin. In the Lower MRD, the dominant lineage is Delta (haplotype M1) and is of North African and Mediterranean origin (Hauber et al., 2011; Lambertini et al., 2012a; Knight et al., 2018). A second common lineage is of Eurasian origin (hereafter, the EU lineage) and is regarded as invasive across much of North America (Saltonstall, 2002). In the MRD, EU occurs in scattered monodominant stands that border larger Delta stands (Hauber et al., 2011; Knight et al., 2018). Lastly, the Gulf Coast lineage (haplotype I or subspecies *P. australis berlandieri*), also known as Land-type, is widespread throughout Central and South America (Saltonstall, 2002; Hauber et al., 2011). In North America, it is found throughout the Gulf Coast to the West Coast and inhabits more elevated areas in the MRD (Saltonstall, 2002; Meyerson et al., 2010; Hauber et al., 2011). According to Knight et al. (2018); (2020) and Cronin et al. (2020), the EU lineage appears to be less prone to exhibiting dieback syndrome than the Delta lineage in the lower MRD.

The purpose of our study was 1) to investigate the differences in soil chemistry between stands of healthy *P. australis*, stands where *P. australis* is currently experiencing dieback and sites newly constructed from dredge soils; and 2) assess whether these different soil types affect the growth of different *P. australis* lineages. Our intent was not to disentangle the chemical changes leading to dieback from those resulting from dieback. Instead, we focused on whether those dieback soils would limit *P. australis* growth and recovery following dieback. In the Winter and Spring of 2019, soils were collected from replicate healthy, dieback and dredge sites in the MRD and analyzed for their chemical composition. Subsequently, we conducted greenhouse and common garden experiments to assess whether soil type, *P. australis* lineage (Delta, Gulf, EU) and their interaction influenced *P. australis* aboveground growth (biomass, stem height and stem density). We tested the following set of predictions: (1) soils types can be discriminated by their soil chemical profiles; (2) dieback soils have the highest concentrations of organic matter, sulfur, and heavy metals, whereas dredge soils have the lowest nutrient and organic content levels; (3) plants potted in dieback soils produce less biomass than plants potted in healthy soils; (4) nutrient-poor dredge soils yield plants with the lowest biomass, and (5) as a successful invader of a wide array of environmental conditions, the EU lineage of *P. australis* outperforms the other lineages in dieback soils.

2.2. MATERIALS AND METHODS

Study System

The Mississippi River is the primary source of freshwater, nutrients, and sediments to the Gulf of Mexico, as well as the world's 7th largest in river discharge (Milliman and Meade, 1983; Allison et al., 2012). Since the 20th century, the lower MRD has been subjected to increased inputs of nutrients, contaminants and metals (e.g., Fe, Mn, Cu) from runoff containing agricultural pesticides, fertilizers and industrial byproducts (e.g., PCBs, dioxins/furans, petroleum) (Shiller, 1997) leading to further eutrophication (Elsey-Quirk et al., 2019). Additionally, saltwater intrusion and acidication, driven by natural (e.g., eustatic sea-level rise, storm surges, hurricanes) and anthropogenic (e.g., land drainage, man-made water diversions) factors, and a warming climate, have also contributed to changes in this wetland landscape (White and Kaplan, 2017). In the Mississippi River diversions, the sediment load is primarily comprised [>80%; (Elsey-Quirk et al., 2019)] of fine-grained sediment particles with a small fraction of sand [<20%;(Allison et al., 2012)].

Since 1976, the United States Army Corps of Engineers (USACE), New Orleans District (MVN) has created approximately 63 km^2 of coastal land through dredge sediment diversions in the lower MRD (Wingate, 2014). The Coastal Protection and Restoration Authority (CPRA) of Louisiana has plans for 79 additional restoration projects involving a combination of wetland creation, sediment diversions, and barrier island restoration (CPRA, 2017) in the MRD. The primary use of river sediment diversions is to create new and sustainable wetlands. As of now, we have observed natural colonization of *P. australis* in the newly constructed wetlands along the lower MRD; however, there are no known reports of the extent of colonization.

Phragmites australis is a cosmopolitan perennial grass, common in a wide range of wetland ecosystems, including fresh and brackish wetlands, lake and pond margins, disturbed wetlands, wet meadows and ditches (Kiviat, 2013). *P. australis* forms dense monospecific stands growing up to 3-5 m tall (Clevering and Lissner, 1999). The tallest among the lineages is Delta with stem heights of 4 – 5 m tall, which accounts for > 95% of the *P. australis* in the MRD, appears to be the most susceptible to dieback (Knight et al., 2018; Cronin et al., 2020; Knight et al., 2020). The introduced EU lineage is less abundant than the Delta lineage in the Lower MRD, but has high biomass productivity, prolific seed production and increased rhizome production which enables it to outcompete against other plant species, including other *P. australis* lineages (Meyerson et al., 2009). Salinity-induced stress may contribute to the dispersion of *P. australis* lineages with the Gulf lineage being the least salinity tolerant which may explain its restricted distribution in the MRD given its prevalence in upland habitats (Achenbach and Brix, 2014). Since 2016, there has been little to no evidence of recovery from the dieback events and previously dense stands of *P. australis* have been converted to bare mudflats, open waters or have been replaced by opportunistic plants such as *Colocasia esculenta* L. (elephant ear), *Alternanthera philoxeroides* (Mart.) (alligatorweed), or *Pontederia crassipes* (Mart.) (water hyacinth) (Knight et al., 2018; Cronin et al., 2020).

Healthy, dieback and dredge soil chemistry

To determine whether soils from dieback, healthy and dredge sites have different chemical profiles, soils were collected in the Winter and Spring of 2019 from within the Pass-a-Loutre Wildlife Management Area in the Lower Mississippi River Delta. The first collection of soils was made in January 2019 from three separate healthy and dieback sites (Table 2.1). The second collection was made in May 2019 from two healthy, two dieback and two dredge-disposal sites (Table 2.1). Site selection protocols and soil collection methods are described in Appendix A**.** A total of 57 l of soil was collected at each site and used in the plant-growth experiments (see below). An additional 250 g of soil was collected from three locations per site and stored at 4° C until soil chemistry tests were performed.

The characterization of the soil chemical properties was performed by the LSU AgCenter Soil Testing and Plant Analysis Lab (https://www.lsuagcenter.com/portals/our_offices/departments/spess/servicelabs/soil_testing_lab). The macronutrients analyzed included calcium (Ca), magnesium (Mg), phosphorous (P), potassium (K), sulfur (S), % carbon (% C), and % nitrogen (% N) and the micronutrients were copper (Cu), iron (Fe), manganese (Mn), and zinc (Zn). Additionally, aluminum (Al), sodium (Na), % organic material (% OM) and pH were measured. For details regarding the chemical analyses, see Appendix A, Table A1)

Table 2.1. Origin and soil type designations for Winter-Spring and Spring-Summer Experiment.

Experiment	Date	Soil Type	Latitude	Longitude
Winter-Spring	Jan-19	Healthy	29.09262	-89.21034
Winter-Spring	Jan-19	Healthy	29.08813	-89.22776
Winter-Spring	Jan-19	Healthy	29.12298	-89.23856
Winter-Spring	Jan-19	Dieback	29.08304	-89.21557
Winter-Spring	Jan-19	Dieback	29.12079	-89.25319
Winter-Spring	Jan-19	Dieback	29.11735	-89.20336
Spring-Summer	May-19	Healthy	29.103837	-89.220137
Spring-Summer	May-19	Healthy	29.083732	-89.247022
Spring-Summer	May-19	Dieback	29.082775	-89.214178
Spring-Summer	May-19	Dieback	29.117257	-89.203218
Spring-Summer	May-19	Dredge	29.137427	-89.217168
Spring-Summer	May-19	Dredge	29.137412	-89.217142

Data analysis

Our first objective was to assess whether soil types differed in their chemical profiles. To accomplish this, we first reduced the dimensionality in the data with principal component analysis (PCA). Separate PCAs were conducted for each survey period using R (R Core Team, 2019) and package *prcomp*. PCA was used to reduce the 15 chemical elements to a new set of independent (canonical) eigenvectors or principal components (PC). Separate PCAs were performed for the Winter and Spring survey datasets because dredge soils were only collected in the latter time period. Soil chemical PCs were retained for analysis if their eigenvalues were > 1 (Kaiser, 1960).

To determine if the different soil types can be differentiated based on their soil chemical profiles, we next performed linear discriminant analysis (LDA) using the retained PCs as independent variables. LDA computes directions, called linear discriminants (LDs), that represent the axes that maximize the separation between multiple classes (e.g., soil types). Separate LDAs were conducted for each survey period using R (R Core Team, 2019) and package *lda*. To evaluate the prediction performance of the model, datasets were split into a training set (75%) and a test set (25%). *A posteriori* analysis of correct classification was done using the *predict* function available in the *MASS* package. Lastly, after documenting that chemical profiles differed among soil types, we conducted separate *t*-tests for the Winter dataset (healthy vs. dieback) and one-way ANOVA for the Spring dataset (healthy, dieback, dredge) to determine whether specific chemical elements or compounds differed significantly among soil types. To minimize the risk of type I errors associated with multiple non-independent tests, we used Bonferroni-corrected levels of α for determining statistical significance. Pairwise comparisons tests for the Spring dataset were determined using Tukey's HSD test.

To evaluate whether there were seasonal changes in soil chemistry (Winter vs. Spring), we merged the two datasets and omitted the dredge sites (Combined dataset). All combinations of soil type (healthy, dieback) and survey period (Winter, Spring) were treated as classes in the LDA. Subsequent univariate tests on specific soil characteristics were conducted using a GLM with soil type and survey period as fixed factors in the model. In these analyses, our main focus was on whether soil chemistry differed between time periods.

Soil type and lineage effects on P. australis growth

To test whether soil type influenced *P. australis* growth parameters, we conducted two separate experiments in Baton Rouge, Louisiana, USA using the soils from the Winter and Spring soil surveys. We grew replicate potted plants from different *P. australis* lineages in each soil type and quantified their effects on aboveground biomass, stem density, and maximum stem height. Because aboveground biomass is most closely linked to NDVI (Suir et al., 2018), we focused primarily on this metric; although, we report how changes in stem density and stem height contributed to the change in biomass.

Winter-Spring Experiment

On January 10, 2019, a greenhouse experiment was initiated with soils collected the previous day from Pass-a-Loutre WMA (Winter soil survey). The rhizome cuttings from each of 6 source populations of *P. australis* (3 Delta, 1 EU, and 2 Gulf; Table 2.2.) were planted in each of two soil types (dieback and healthy) in 2.6 L pots. We originally intended to have two representative populations for each lineage, however, a Delta population (PLM) was initially misclassified as EU. For every *P. australis* source population × soil type combination, we had 7-8 replicates for a total of 265 pots. Additional methods for potting and growing plants can be found in Appendix A Supplemental Material and Methods.

Five months post-planting, on June 11, 2019, the experiment was terminated before the plants became pot bound. We measured the height of the tallest stem per pot (stem base to the tip of the uppermost green leaf, in cm) and number of stems per pot. Afterward, all stems per pot were cut at the base and transferred to a paper bag to dry in the greenhouse. Starting at 30 d post-harvesting, we measured the mass of a subset of 20 bags of plant material every 3-5 d. When plant mass no longer changed, we recorded the final dry mass.

Table 2.2. Lineage, location of origin and coordinates for *P. australis* populations used in (A) Winter and (B) Spring Soil Surveys.

Code	Lineage	Location of Origin	Latitude	Longitude
(A) Winter Survey				
PLM	Delta	Lower Mississippi River Delta	29.13	-89.23
Earl3	Delta	Lower Mississippi River Delta	29.17649	-89.28638
Ear4	Delta	Lower Mississippi River Delta	29.05301	-89.3327
EU3	EU	Plaquemines Parish, LA, USA	29.14991	-89.19962
HI	Gulf	High Island, TX	29.55194	-94.389531
SAU	Gulf	Bayou Sauvage NWR	30.06676	-89.8273
(B) Spring Survey				
Earl3	Delta	Lower Mississippi River Delta	29.17649	-89.28638
Ear4	Delta	Lower Mississippi River Delta	29.05301	-89.3327
TELM	EU	Missouri	38.42155	-90.34016
EU3	EU	Plaquemines Parish, LA, USA	29.14991	-89.19962

Spring-Summer Experiment

On May 30, 2019, we repeated the above experiment using the dieback, healthy, and dredge soils from the Spring soil survey. Sources of *P. australis* were limited to two Delta and two EU populations (Table 2.2.). The Gulf lineage was excluded from this experiment because there was insufficient material available from the common garden. There were four replicates of each plant population × lineage × soil type for a total of 288 potted plants. In this experiment, plants potted with the same soil source were placed in 1.2 m diameter plastic pools filled to a water depth of ≈ 15 cm. Six months later (December 5, 2019), the experiment was terminated, and we repeated the harvesting procedure outlined previously.

Data analysis

Due to differences in soil types and *P. australis* lineages used and rearing methods (plants grown in individual trays in the Winter-Spring experiment versus grouped by soil type in pools in the Spring-Summer experiment), separate statistical analyses were performed for each time period. We also performed separate analyses for each growth measure: aboveground biomass, maximum stem height and stem number. To improve the assumptions of homogeneity of variances and normality, biomass and stem number in the Winter-Spring experiment were *ln* transformed prior to analysis. Within each experiment, aboveground biomass was positively correlated with maximum stem height (Winter-Spring: $R = 0.64$, $n = 241$, $P < 0.001$; Spring-Summer: $R = 0.59$, $n = 132$, $P < 0.001$) but was mostly uncorrelated with stem number (Winter-Spring: $R = 0.05$, $n = 241$, $P = 0.44$; Spring-Summer: $R = 0.004$, $n = 132$, $P = 0.95$).

We conducted generalized linear mixed model (GLMM) analyses to test whether growth metrics were influenced by soil type, *P. australis* lineage and their interaction. Soil collection site, *P. australis* population and pool number (Spring-Summer experiment only) were treated as random effects to account for location effects within the MRD, within-lineage variation, and position effects within the garden plot, respectively.

We performed all statistical analyses in R (R Core Team, 2019) with package *lme4*. Model assumptions were assessed by visually inspecting residual plots and quantile-quantile plots. Goodness-of-fit was calculated using package *MuMIn* based on Nakagawa and Schielzeth (2013). This method reports the marginal $R^{2,}$ which provides the variance explained by all fixed effects and the conditional R^2 which provides the variance explained by the entire model with fixed and random effects combined. Marginal means ± SE for each treatment or treatment combination were computed using *ggemmeans*. Contrasts between pairs of means were assessed using package *eemeans*, where *P* values were adjusted using Tukey's method.

2.3. RESULTS

Soil chemistry analysis for Winter Survey

Based on our multivariate analysis of the chemical profiles of healthy and dieback soils, there were three principal components with eigenvalues > 1 and together, they accounted for 84.7% of the variation in soil chemistry among our soil samples. The first principal component accounted for 61.4% of the total variation (Figure 2.1) and had negative loadings for most of the chemical variables except for Al, Cu, P, and pH, which had positive loadings (i.e., the weights for each variable) (Figure A.4).

The second PC accounted for 15.9% of the total variation (Figure 2.1) and had high negative loadings for Cu (r = -0.58; contribution = 33.3%) and Al (r = 0.54; 29.2%) and thus appeared to be a factor for metals (Fig. S1B). The third PC accounted for 8.7% of the total variation and was moderately correlated with Ca (r = 0.45; 20%) and K (r = 0.45; 20%) and negatively correlated with Al (r = -0.40; contribution = 16%) (Fig. S1C). We interpret PC3 as a factor associated with soil mineral/nutrient content.

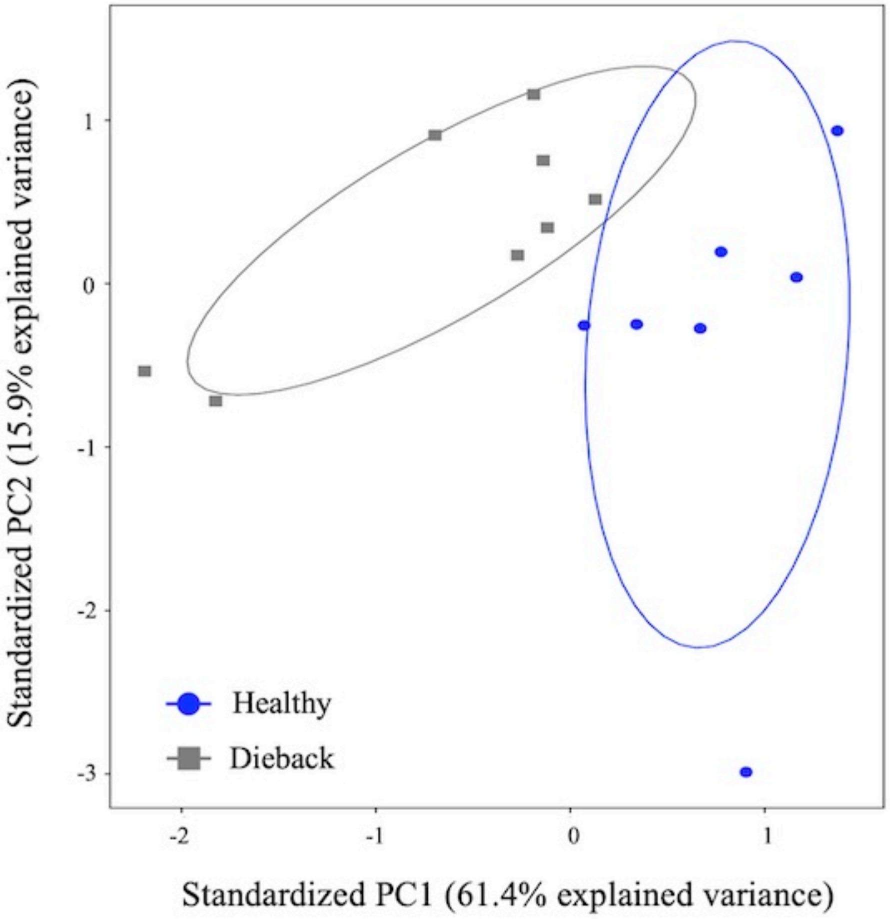

Figure 2.1. Variability explained by the first two axes (correlation biplot) of the distribution of 15 chemical variables measured in the Winter Soil Survey principal component 1 (PC1) explained 61.4% of the variability and principal component 2 explained 15.9%.

There was a strong separation of healthy and dieback soils as demonstrated in the biplot of PC1 and PC2 (Figure 2.1) and this was confirmed by the LDA. The LDA model was 100% correct in assigning soil samples to healthy or dieback sites (Table 2.3). In fact, linear discriminant 1 (LD1) alone significantly differentiated dieback soil from healthy soil ($t = 3.9$ $P < 0.05$). Many of the chemical characters considered in our study were strongly correlated with one another (Table A.5, Appendix A). Organic Matter content, % C, and % N were all strongly positively correlated with each other ($R \geq 0.97$, $P \leq 0.001$; Table A.3). Additionally, % OM, % C, and % N were positively correlated with Ca, Fe, K, Mg, Mn, Na, S and Zn, whereas pH and P content were strongly negatively correlated with the same properties (Table A2.3). It was, therefore, not surprising that suites of chemical characters exhibited similar relationships with soil type. In relation to healthy soils, dieback soils had significantly higher % OM (47%), %C (60%), and %N (62%), Fe (56%), Mg (47%) and Na (46%) (Table A.5). Dieback soils had higher concentrations of K (11%), Mn (76%), Zn (20%) and S (75%) than healthy soils, but the differences were not statistically significant (Table A.6). Dieback soil pH ranged from 4.27 to 6.93 and averaged 5.8 ± 0.27 (n = 8) and healthy soil pH ranged from 7.18 to 7.67 and averaged 7.40 ± 0.06 (n = 7) (t = -6.55, $P < 0.05$). In contrast, healthy soils had higher concentrations of Cu (39%), Al (78%), Ca (63%) and P (19%) but the differences were only significant for Cu and Al (Table A.6).

Table 2.3. Prediction accuracy results for classifying soil types using Fisher's Linear Discriminant Analysis (LDA) for Winter (dieback, healthy) and Spring (dieback, dredge, healthy) soil surveys. The first three principal components (PC) scores derived from the Principal Component Analysis (PCA) were used as the explanatory variables.

LDA-classified Winter soil type				LDA-classified Spring soil type				
Predicted	Dieback	Healthy	Sum	Predicted	Die-back	Dredge	Healthy	Sum
Dieback	8	0	8	Die-back	6	0	0	6
Healthy	0	7	7	Dredge	0	6	0	6
				Healthy	0	0	5	5
Overall accuracy			100%					100%

Soil chemistry analysis for Spring Survey

The first three principal components had eigenvalues > 1 and combined to explain 82.7% of the total variance in the soil chemical properties tested. PC1 accounted for 55.0% of the total variance and was weakly negatively loaded with pH and positively loaded with all other variables (Figures 2.2. Table A.3). PC2 explained 19% of the total variation and had moderately negative loadings by Fe (r = -0.44; contribution = 19.7%) and Al (r = -0.39; 15.5%) and moderately positive loadings P (r = 0.42; 18.0%) and Ca (r = 0.38; 14.7) (Figure A.5B). Finally, PC3 explained 8.7% of the total variation and was moderately and negatively loaded with Mn (*r* = 0.57; 32.0%) and positively loaded with S (*r* = 0.58; 34.1%) (Figure A.2). The LDA model using these PCs successfully discriminated 100% of all soil samples to their respective soil type (Table 2.3), confirming our prediction 1. The first linear discriminant (LD1) explained 82% of the variance, and the second linear discriminant (LD2) explained 18% of the remaining variance.

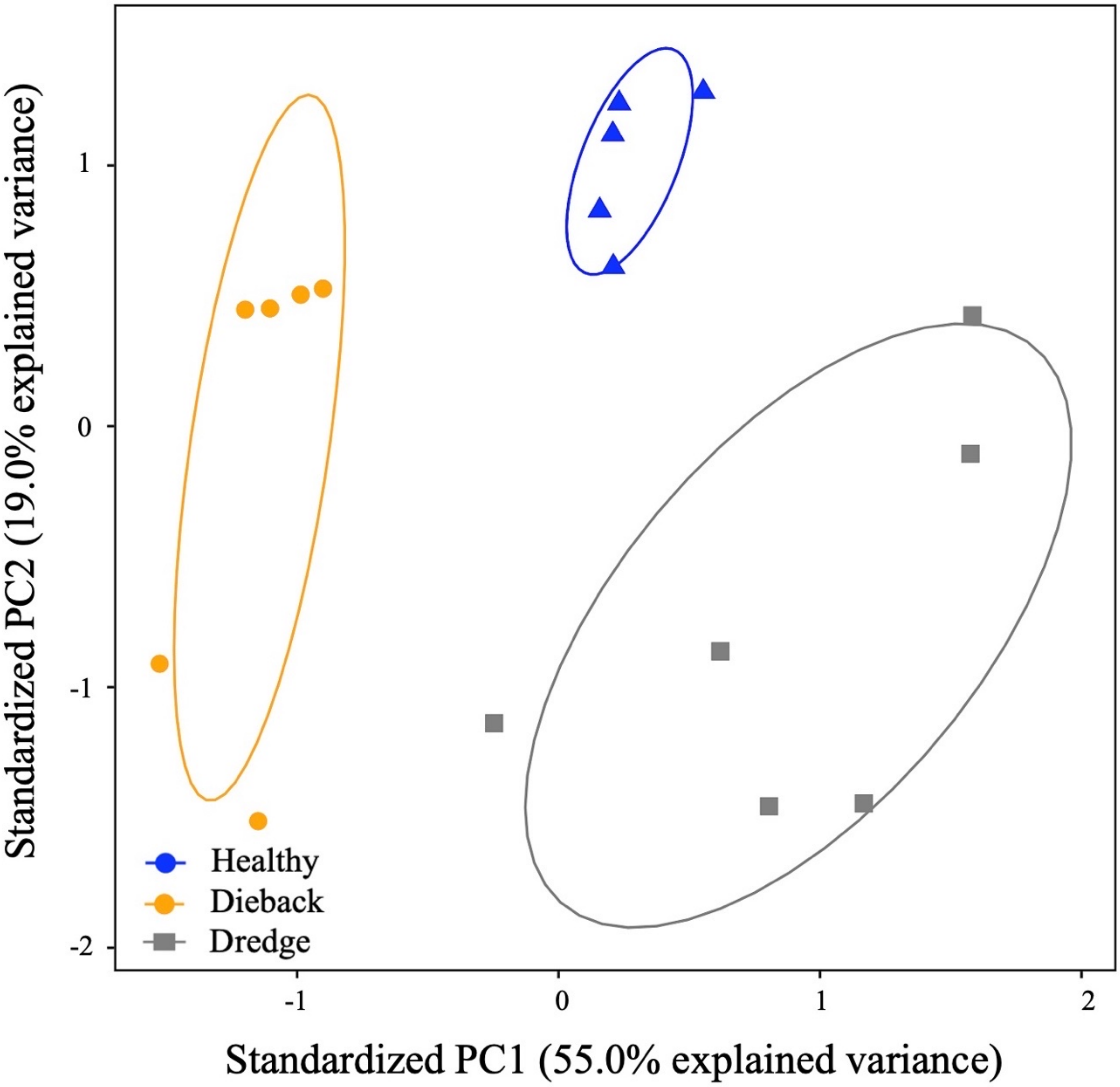

Figure 2.2. Variability explained by the first two axes (correlation biplot) of the distribution of 15 chemical variables measured in the Spring Soil Survey PC1 explained 55.0% of the variability and PC2 explained 19.0%.

As in the previous survey, there were very strong positive correlations between OM, % C, and % N ($R \geq 0.97$, $P \leq 0.001$; Table A.3B) and each of these variables were significantly positively correlated with Mg, Zn, and negatively with pH (Table A.3.B). Other positive and negative correlations corresponded with those found in the previous survey with the exceptions of Al, Ca, Cu, Fe, K, Na, P, and S. Similar to our findings with the Winter soils, dieback soils had higher %OM (45%), %C (70%), and %N (83%) than healthy soils (Table A.5), supporting our prediction 2. Dieback soils also had higher concentrations of Al (31%), Fe (44%), Mg (25%), Mn (11%), Na (25%), S (14%); however, only for S was the difference between soil types significant (Table S7). In contrast, healthy soils had higher concentrations of Ca (32%), Cu (31%), K (5%), P (26%), and Zn (3%) than dieback soils (only the former two were statistically significant). Finally, the pH of dieback soils was more acidic and averaged 6.41 ± 0.09 pH compared to healthy soils with an average of 7.43 ± 0.06 (Table A.5).

Among the three soil types, dredge soils exhibited comparably lower concentrations of Ca, Cu, Fe, K, Mg, Mn, Na, %OM, S, Zn, and % C (Table A.5). Notably, dredge soils had 7.2 and 4.0 times lower % OM than dieback and healthy soils, respectively. Finally, pH was 7.71 in dredge soils, 20.2% and 3.9% higher than in dieback or healthy soils, respectively.

Seasonal soil chemical profiles

For the combined winter and spring soil samples (healthy and dieback sites only), a PCA yielded four PCs with eigenvalues > 1. These four PC’s explained 84% of the total variance in the soil type chemical properties and the loadings for each PC are reported in Figure A.4. Linear discriminant analysis showed a clear partitioning of healthy soils between seasons and partial separation of dieback soils (Figure A.5). The posteriori analysis indicated 96% correct classification to its respective seasonal soil type. The discrimination of healthy soils between seasons appears to be driven by PC1 and PC3, which is represented by soil organic content and macronutrients (Figure A.5). At the same time, the discrimination between dieback Winter and Spring soils appears to be primarily driven by PC2 and PC4, which could be attributed to primarily micronutrients, Al and Cu, and macronutrient, Ca (Figure A.5).

Overall, the healthy Winter soils had higher concentrations of S (82%), Al (80%), % C (44%), Cu (38%), % N (19%) % OM (12%), Zn (11%), Fe (10%), Ca (5%) and K (2%), and lower concentrations of Mn (78%), P (49%), Na (30%) and Mg (11%), and a lower pH 7.29 ± 0.13 versus 7.44 ± 0.15) compared to healthy Spring soils (Table A.6). However, only for P was the difference statistically significant. As for seasonal differences of dieback soils, six out of the 15 chemical properties were significantly higher in the Winter including: S (92%), % C (48%), % N (43%), Ca (42%) Zn (21%), and Mg (32%). In contrast, dieback Spring soils contained higher concentrations of Al (35%), P (24%), Na (23%) and Mn (11%), however, only P was statistically significant (Table A.6).

Soil type and lineage effects on P. australis growth for Winter-Spring Experiment

We predicted that biomass for all three *P. australis* lineages would be lower when grown in dieback as compared to healthy soils but that the Delta lineage would be most negatively affected by dieback soils (prediction 3). Plants grown in dieback soils, irrespective of their lineage, had an average of 16.3% lower biomass (based on back-transformed estimates of the marginal means of *ln* biomass) than those grown in healthy soils (Table 2.3; Figure 2.3). The loss of biomass in dieback soils appears to be related to changes in stem production and not stem growth. We found a 20% reduction in stem counts when plants were grown in dieback relative to healthy soils (healthy: 8.45 ± 1.31, dieback: 7.07 ± 1.10; Table A.7 and Figure A.6) but no significant change in maximum stem heights between the two soil types (Table A7 and Figure A.6). Contrary to our second prediction, we found no evidence that the Delta lineage performed differently than the other two lineages when grown in the two soil types (i.e., no soil type × lineage interaction; Figure 2.3). Moreover, prediction 5, that the invasive EU lineage would outperform the other two lineages in dieback soils, was also unsupported (Figure 2.3). Surprisingly, the EU had the lowest biomass regardless of soil type, although there was no significant difference in biomass among lineages (Table 2.3A, Figure 2.3). Overall, the fixed effects in the GLMM (soil type, lineage and soil type × lineage) explained 22.3% of the variation in the model (based on the marginal R^2), whereas the combined fixed and random effects (associated with different soil sources and rhizome sources) explained 56.7% of the model variation (based on the conditional R^2).

Table 1.3. Separate generalized linear mixed model results for the effects of soil type (dieback, dredge, healthy), *P. australis* lineage (Delta, EU, Gulf), and all possible interactions on A) ln biomass for Winter-Spring Experiment and B) biomass for Spring-Summer Experiment. Highlighted sources of variation indicate significance ($P \leq 0.05$).

	Sums of Squares	*df* (num)	*df* (denom)	*F*	*P*-value
A) Winter-Spring Experiment					
Lineage	0.43	2	2.99	1.52	0.351
Soil Type	1.56	1	228.2	10.95	**0.001***
Lineage x Soil Type	0.18	2	228.23	0.62	0.541
B) Spring-Summer Experiment					
Lineage	1.87	1	2.05	0.288	0.644
Soil Type	71.83	2	17.46	5.54	**0.014***
Lineage x Soil Type	46.58	2	113.71	3.59	**0.031***

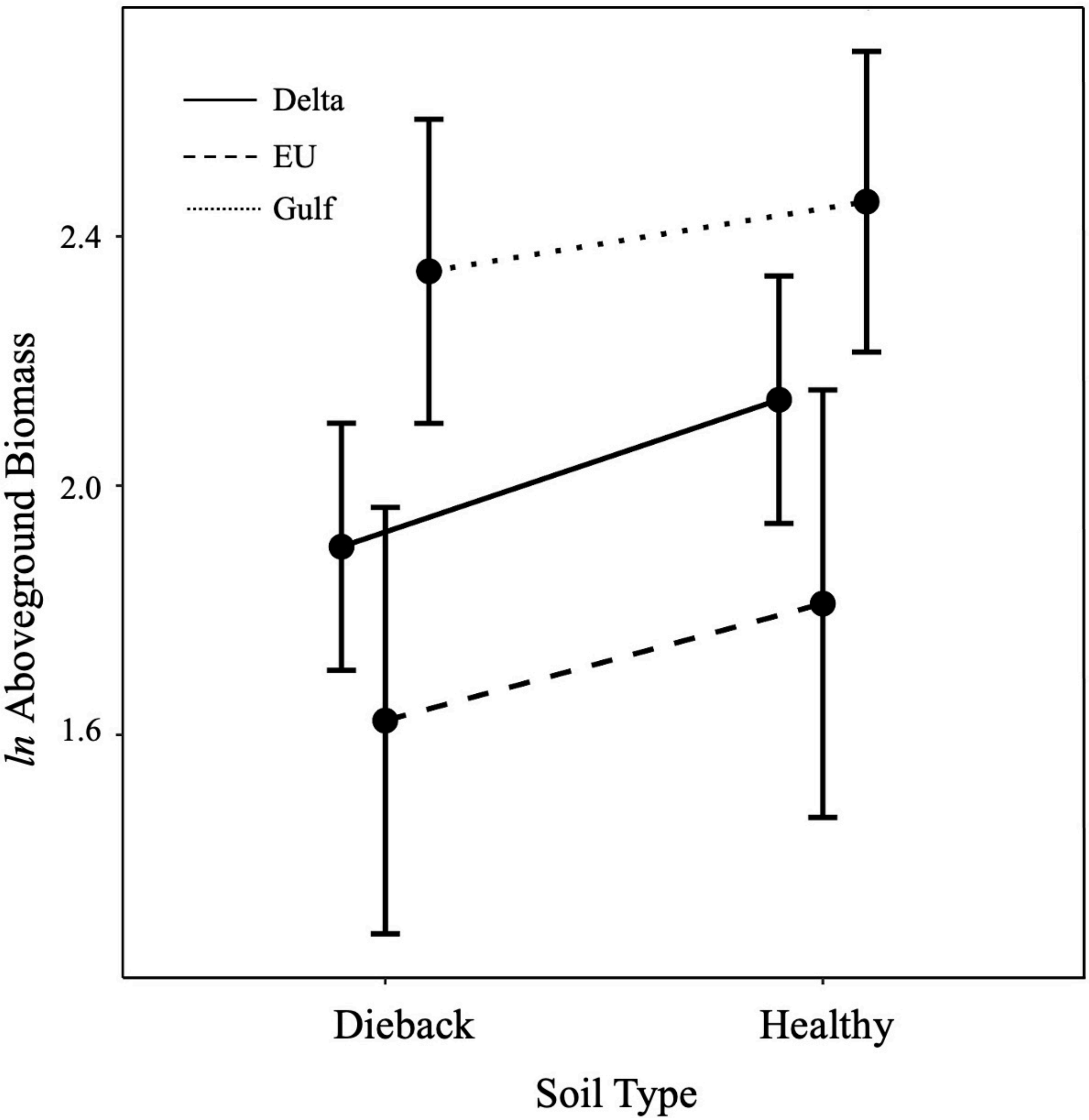

Figure 2.3. The effects of soil type (dieback, healthy, and dredge) and *P. australis* lineage on aboveground biomass for the Winter-Spring Experiment. Reported values are marginal means ± SE.

Soil type and lineage effects on P. australis growth for Spring-Summer Experiment

For the Spring-Summer experiment, 51% of the potted rhizome cuttings failed to survive to the end of the study period (148 of 288 pots). Based on a logistic regression analysis (where the response variable was live or dead), there was a significant difference in likelihood of survival between lineages (*z ratio* = -7.40, $P < 0.001$). Delta and EU plants had 23% and 73% survival, respectively. However, there was no significant difference in survival between plants grown in healthy and dieback soils (*z ratio* = - 0.36, $P = 0.932$) nor a lineage × soil type interaction (*z ratio* ≥ -0.34, $P \geq 0.32$).

Using only plants that were alive at the end of the study, we found similar results to the early-season experiment. Biomass in the Spring-Summer experiment was significantly influenced by soil type but also the soil type × lineage interaction (Table 2.3B, Figure 2.4). We had predicted that biomass would be lower for plants grown in dieback and dredge soils as compared to healthy soils (prediction 3 and 4). Consistent with these predictions, plants grown in dieback and dredge soils had 23% and 44% lower biomass, respectively than plants grown in healthy soils (Figure 2.4). However, this difference was only significant for Delta plants grown in dredge versus healthy soils (47%). Also consistent with our predictions, Delta and EU plants grown on dredge soils had biomasses 47% and 40% lower, respectively, than on dieback soils (Figure 2.4); although, differences were not statistically significant. Relative to healthy soils, biomass on dieback soils was reduced by 6% for the EU lineage and 36% for the Delta lineage. Lastly, for the model involving biomass, the fixed effects explained 21% and the full model explained 60% of the variation in biomass.

Stem counts for plants grown in healthy soils were 23% and 35% higher than for plants grown in dieback and dredge soils, irrespective of *P. australis* lineage (Figure A.6). EU plants were particularly affected by soil type (significant soil type × lineage interaction) - stem counts were reduced by 28% and 38% in dieback and dredge relative to healthy soils (Table A.7 and Figure A.6C). For Delta, the difference in stem counts among the soil types was not statistically significant. Finally, stem heights were unaffected by lineage or soil type (Figure A.6D).

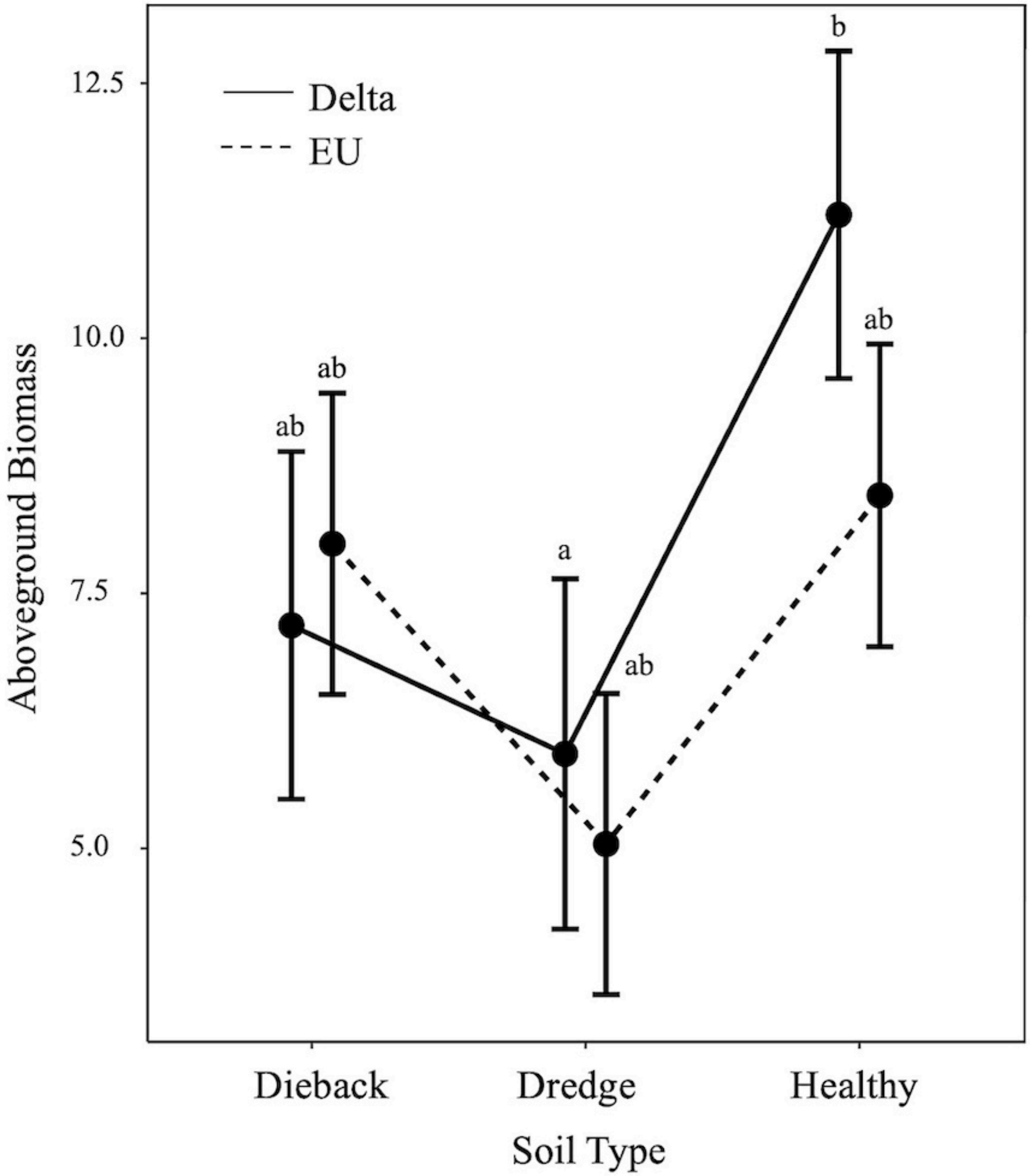

Figure 2.4. The effects of soil type (dieback, healthy, and dredge) and *P. australis* lineage on aboveground biomass for the Spring-Summer Experiment. Reported values are marginal means ± SE. Different letters between groups indicate significant differences at $P \leq 0.05$.

2.4. DISCUSSION

Since 2016, dieback of *P. australis* in the MRD has been widespread and extensive (Suir et al., 2018; Knight et al., 2018; Cronin et al., 2020; Knight et al., 2020). In the subsequent four years, there has been little evidence of recovery and, for a number of sites, the once dense stands of *P. australis* have been replaced by other invasive species or are now open water habitats (Knight et al. 2020). Our study suggests that the soil chemistry of dieback sites 1-2 years following drastic reductions in standing biomass is markedly different from healthy sites and that these soil conditions may significantly hinder *P. australis* recovery. Between our two greenhouse/common-garden experiments, dieback soils reduced aboveground biomass of *P. australis* by 13% and 23% relative to plants grown in healthy soils. Marsh habitats constructed from dredge material require vegetation like *P. australis* to stabilize sediments, however, they can be nutritionally poor and negatively affect plant productivity. In our experiment, we found *P. australis* grown in dredge soils had an average 44% lower biomass relative to those in healthy soils. Recovery of *P. australis* in the MRD may occur slowly on its own but active restoration plans with more stress-tolerant and fast-growing lineages of *P. australis*, fertilizer addition to nutrient deprived dredged sediments, and/or control of herbivory by *Myocastor coypus* (Kerr) (Nutria) may be needed to expedite reestablishment of ecological and societal services provided by this foundational plant species. Because these services are likely proportional to the biomass and productivity of *P. australis,* extensive dieback of reed stands will severely impair the efficacy of these ecosystem services (Kiviat, 2013).

Putative cause of P. australis dieback

From our study, it is uncertain whether the chemical differences reported between dieback and healthy soils are the cause for, or the consequence of, *P. australis* dieback. However, our healthy sites occurred in the same watershed as dieback sites, often along the same channel and at similar water depths. The high input of water in the MRD makes it unlikely that pollutants would differentially accumulate to the degree reported herein. As such, it is most plausible that the differences in water quality and soil chemistry between healthy and dieback sites are a consequence of dieback and indirectly by whatever caused that dieback.

One possible biotic causal agent of dieback of *P. australis* in the MRD is the Roseau Cane Scale (RCS), *Nipponaclerda biwakoensis* (Kuwana) (Hemiptera: Aclerdidae). Scale insect abundance is quite variable in the MRD; however, abundance is negatively correlated with the normalized difference vegetation index (NDVI) (I. A. Knight, G. Suir and R. Diaz, unpublished data). Corroborating these observational data, experimental studies have shown that even low to moderate scale abundances can cause significant reductions in plant height, stem abundance, stem diameter and aboveground biomass (Knight et al., 2020; Cronin et al., 2020).

Additionally, potential biotic stressors, such as the infection of viruses, fungi and soil microbes (Ratnayake et al., 1978; Armstrong et al., 1996b; Elmer et al., 2013), may potentially interact with multiple abiotic stressors, including flood exposure and the indirect effects of eutrophication and organic matter accumulation resulting in the buildup of volatile organic acids and phytotoxins, such as sulfides (Armstrong et al., 1996a; Armstrong et al., 1996c; Armstrong and Armstrong, 2001). When belowground structures of *P. australis* are exposed to phytotoxins, susceptibility to fungal infections may increase for laterals, adventitious root apices and buds ultimately leading to morbidity (Armstrong et al., 1996b). Fungal infections may be saprophytic but can also grow parasitically and augment the damaging effects of phytotoxins (Armstrong et al., 1996b). Further studies are required to assess the relative importance of *P. australis* and its relationship to pathogens and soil microbial communities across lineages.

Toxicity of dieback soils

Our study revealed that dieback and healthy soils were chemically different. We observed higher concentrations of different groups of compounds in dieback areas compared to healthy areas, including macronutrients (K, Mg and S), trace metals (Fe, Mn, and Zn), and organic matter content (% OM, % C and % N). Among the myriad of factors associated with dieback, phytotoxicity of metals and sulfides are commonly reported (van der Putten, 1997; Brix, 1999). The accumulation of toxins may impair a broad variety of essential plant processes, including internal aeration, vascular transport, photosynthesis, respiration, ion absorption, and cell membrane integrity (Armstrong and Armstrong, 2001). The reason why areas are not recolonized following dieback in the Lower MRD remains to be answered. Although dieback is not necessarily permanent, the loss of vegetation can temporarily reduce ecosystem productivity, but legacy effects of dieback may be mirrored in soils predisposing some areas to recurring dieback events over time (Crawford and Stone, 2014). Unfortunately, measurements of soil conditions are often performed weeks to years after dieback, thus the observed changes may be the resulting effects rather than causes (Alber et al., 2008) which may fluctuate over time (McKee et al., 2006).

Since trace metals cannot be degraded, their potential fates include accumulating in the water column, soil and living organisms (Ait Ali et al., 2004). Moreover, soil acidification can lead to the release of potentially toxic metals, such as Fe, Al and Mn, and the solubility of metals increases several folds with decreases in pH leading to toxic concentrations (Gambrell, 1994;

McKee et al., 2006). Although *P. australis* is commonly used for phytoremediation due to its high tolerance to metals, (Ait Ali et al., 2004), Fürtig et al. (1999) suggested that toxic concentrations of Cu and Fe ($Cu^{2+} \leq 40$ µM, $Fe^{2+} \geq 1$ mM) can potentially contribute to *P. australis* dieback by impairing root functions (e.g., nutrient uptake, root development and growth). Our results showed dieback soils had lower pH and had above the Fe threshold during both seasons but were highest in the Winter. Seasonal differences in Fe concentrations might have occurred from the accumulation of minerals in senescent leaves of *P. australis* (Hietz, 1992). Metals, such as Cu, Mn, Fe, and Zn are essential for plant growth and development in trace amounts; however, the presence of bioavailable forms in excessive quantities can potentially be toxic to vegetation (Tangahu et al., 2011).

In flooded and waterlogged soils, sulfur reduction leads to the production and accumulation of phytotoxin, hydrogen sulfide, which is often associated with dieback symptoms in various wetland plants, including *S. alterniflora* and *P. australis* (Koch and Mendelssohn, 1989; Armstrong et al., 1996a). Sulfide toxicity may hinder nutrient uptake and reduce or restrict internal aeration through blockages by callus formations within vascular systems of *P. australis* leading to subsequent reductions in root growth and death of roots and buds (Armstrong et al., 1996a). Although we did not measure soil sulfide concentrations in our study, Winter and Spring dieback soils had 75 % and 56% greater concentrations of total sulfur than healthy soils, which most is organic sulfur (Whitcomb et al., 1989). However, in these waterlogged soils are typically devoid of O_2 and reduced phytotoxins (e.g. sufides, ferrous iron, methane) readily form under reduced soil conditions (Weisner and Granéli, 1989). Studies are needed that focus on sulfide levels in the MRD, particularly how it might interact to affect plant fitness and functional traits with other factors such as water depth and redox potential.

In our survey sites, soil mineral nutrient content (%C, %N) was strongly positively correlated with organic matter content. In both seasons, the higher % OM (also, % C and % N) in dieback soils than healthy soils may be attributed to the loss of vegetation leading to increased root mortality and decomposition of root tissues and rapid peat collapse (Delaune et al., 1994). In the Winter Soil Survey, dieback soils had % C and % N levels that were more than 60% greater than healthy soils. In the Spring Soil Survey, the contrast was much greater with 70% and 85% greater % C and % N, respectively, in dieback than in healthy soils. According to Li et al. (2016), who studied *P. australis* dieback that began in 2008 in the Yangtze River Estuary in China, the initial C and N contents in leaf, sheath and stem of dieback *P. australis* were significantly greater than those of healthy *P. australis* and that stems of dieback *P. australis* decomposed faster than their healthy counterparts. Anoxic conditions that arise from flood exposure and eutrophication can also be induced by organic matter accumulation; however, we could not address this aspect of eutrophication in our experiments because we conducted our experiments in pots in shallow trays or pools where oxygen levels were likely to be consistently high.

Based on our combined soil chemistry survey, chemical profiles of healthy soils between Winter and Spring were different, but dieback soil chemical profiles overlapped to a greater degree between seasons. In the Winter, both healthy and dieback soil chemical profiles had overall higher concentrations of different compound groups, including nutrients (Ca, K, and S), trace metals (Cu, Fe and Zn), and organic matter content (% OM, C and N) with lower pH levels in relation to their respective Spring counterparts. In comparison, both healthy and dieback Spring soils had higher concentrations of other nutrients such as P, Na and Mn in relation to the Winter soils. The separation of healthy and dieback soils between seasons may primarily be driven by differences in oxygen inputs to the root-soil interface (Vretare Strand and Weisner, 2002). Possible drivers may also include environmental conditions influencing decomposition rates and nutrient availability, including temperature, soil pH, hydrologic fluctuations, redox potential, and microbial activity. During warmer temperatures, particularly during the summer months, nutrient levels, such as N and P, may increase as organic matter decomposition rates increase, owing to more optimal environmental conditions for microbial activity (Hietz, 1992). Although we did not find increases of % N in Spring soils, we did observe twice as much P concentrations in healthy and dieback Spring soils compared to Winter soils. In the future, it would be beneficial to monitor how long, if ever, it takes the soil chemistry of dieback sites to return to levels comparable to sites that have exhibited no dieback.

Phragmites australis lineage and response to soil condition

Our findings suggest that some aspect of the chemistry of dieback soils is harmful to *P. australis* growth. As predicted (prediction 3), across all lineages in the Winter-Spring and Spring-Summer experiments, we found on average a 16 and 23% reduction in biomass, respectively, when planted in dieback versus healthy soil. Evidence from field studies suggests that the Delta lineage is more prone to exhibiting dieback syndrome and less tolerant of environmental stressors (e.g., flooding, fertilizer, salinity, scale insects) (Knight et al., 2018; Cronin et al., 2020; Knight et al., 2020). In contrast, the successful invasion of the EU lineage into North America has been attributed to its unequaled competitive abilities, greater nutrient assimilation efficiency, high plasticity to variation in nutrient levels, and greater resistance to herbivores than the North American native lineage (Saltonstall and Court Stevenson, 2007; Holdredge et al., 2010; Cronin et al., 2015). As such, we predicted that the Delta lineage would perform proportionately worse than the EU lineage in dieback soils (prediction 5). However, we did not find support for this prediction. In our Winter-Spring experiment, both Delta and EU exhibited similar biomass reductions when grown in dieback as compared to healthy soils (21% and 17%, respectively). Additionally, the Gulf lineage was the least affected by dieback soils among the three lineages in the Winter-Spring experiment (11%). Similar results were found for the Spring-Summer experiment; although, the proportional reduction in biomass between healthy and dieback soils was greater for Delta (36%) than for EU (6%), although not significant. However, in the Spring-Summer experiment, potted rhizomes from the Delta lineage had relatively higher mortality than those from the EU lineage across all the three soil types; 68%, 59%, and 65% more in dieback, healthy, and dredge soils, respectively. The cause of the high mortality in the Delta lineage in the Spring-Summer experiment remains unknown.

The above findings, that dieback soils are harmful to *P. australis* aboveground growth, poses a serious concern for the sustainability of the lower MRD where Delta encompasses >95% of the area covered by *P. australis* (Hauber et al., 2011; Lambertini et al., 2012a; Knight et al., 2020). Although belowground biomass responses to dieback soils are unknown, several studies suggest that soil toxins and other abiotic factors can affect *P. australis* root growth, morphology and physiology (Holdredge et al., 2010). Thus, our focus solely on aboveground plant parts may underestimate the impacts of dieback soils on the whole plant. Also, a longer duration study and/or the inclusion of other plant fitness metrics (e.g., seed production, rhizome growth) may have revealed even greater effects of dieback soils on *P. australis* performance.

As predicted (prediction 4), we confirmed that nutrient-poor dredge soils yielded the lowest plant biomass for both Delta (5.93 ± 1.72) and EU (5.04 ± 1.48) lineages in the Spring-Summer experiment (Fig. 2B). The significant soil type × lineage interaction found in the

Spring-Summer experiment was solely driven by the greater biomass reduction of the Delta lineage in the dredge versus healthy soils (47%). Similarly, dredge soils reduced biomass of EU lineage by 40%. The considerably lower biomass implies that characteristics of fine-grained dredged sediments, such as accelerated decomposition rates, lower water holding capacity, and poor nutrient retention may be problematic for plant growth and productivity (Elsey-Quirk et al., 2019). However, under resource limited conditions, including low-nutrient soils, plants can compensate for biomass loss by allocating biomass production to belowground parts (Chapin et al., 1987). Further investigation of belowground biomass response to dieback and dredge soils will help illuminate the overall health of *P. australis*.

Constructed wetlands and restoration of the MRD

To offset further wetland loss of the MRD, numerous diversion plans have been implemented by the Coastal Protection and Restoration Authority (CPRA) of Louisiana for maintaining channel navigation in the MRD, including dredging and reintroducing sediments to coastal marshes to create and restore degraded marsh habitats (CPRA, 2017). The introduction of dredge sediments, composed primarily of silts and clays, increases marsh surface elevation and reduces flood stress for colonizing plants (Nyman et al., 1990; Slocum et al., 2005). However, dredge soils can pose significant problems for revegetation, either through natural colonization or restoration (Elsey-Quirk et al., 2019). Our study shows that at least in two dredge sites, many nutrients and metals, including Ca, Cu, Fe, K, Mg, Mn, Na, P, Zn, % OM, % C, and % N occur at low levels relative to healthy *P. australis* marsh sites. In soils from these dredge sites, *P. australis* produced 47% less biomass than when grown in soils from healthy sites. Despite reduced growth on these soils, colonization and establishment of *P. australis* is taking place at these dredge sites in the MRD. In a brackish marsh in the Barataria Basin of Southeast Louisiana, Howard et al. (2019) demonstrated that transplanted *P. australis* to dredge sites could rapidly spread within two-years. Additionally, our field observations at dredge sites confirm the natural colonization of *P. australis*, however, further studies are needed to estimate the extent of expansion and the time it would take for *P. australis* to achieve substantial vegetative cover in these newly constructed sites.

Dieback syndrome is a recent and widespread problem in the MRD (Ramsey III and Rangoonwala, 2017; Knight et al., 2018; Knight et al., 2020). Recovery of these dieback sites is urgently needed. The upside of our study's findings is that soils in areas of dieback are only modestly toxic (16-23% reduced biomass relative to healthy soils) and all three lineages of *P. australis* are capable of regrowing on these soils. What we do not know are the long-term effects of these soils on plant growth, reproduction and ability to compete against other aquatic plant species that are less capable of stabilizing marsh soils. However, it is possible that the factors that induced dieback in the field may be active for a limited period of time and that the removal of such factors could potentially restore suitable conditions for recovery (Lindstedt and Swenson, 2006; Marsh, 2007), but if elevation is lost to erosion and subsidence prior to vegetation establishment it may impede recovery in some areas (Nyman et al., 1993). This has been shown to be the case for field transplants of *S. alterniflora* that survived in previous *Spartina* dieback sites in Louisiana (Edwards et al., 2005). Unfortunately, our observations are that for many areas where *P. australis* has died off, they are now occupied by floating aquatics (e.g., water hyacinth, salvinia) or other shallow rooting plants (e.g., alligatorweed) (Knight et al., 2020) which is consistent with elevation loss. Whether this change in plant community composition is permanent remains to be seen. We suggest that long-term monitoring of dieback, dredge, and healthy sites will provide comprehensive data to understand patterns of dieback in the Lower MRD.

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CHAPTER 3.
RESTORATION OF *PHRAGMITES AUSTRALIS* DIEBACK IN THE LOWER MISSISSIPPI RIVER DELTA: A PRELIMINARY REPORT

3.1. INTRODUCTION

Globally, the Mississippi River has the 7th largest discharge of freshwater, nutrients and sediments in the world (Milliman and Meade, 1983; Allison et al., 2012). Currently, about two-thirds is drained via the Holocene-age deltaic plain known as the Mississippi River Delta (MRD) (Saucier, 1994) and one-third is drained via the Atchafalaya River (Day et al., 2007). The Mississippi River Delta is composed of a 2,108 km^2 heterogenous landscape of water, low-lying upland ridges and coastal marshes (fresh, intermediate, brackish) (Day et al., 2007). These coastal wetlands are among the most productive ecosystems providing invaluable ecological and economic services including flood control, wildlife habitat, and water quality (Day et al., 2000; Day et al., 2007). Economically, the lower MRD supports a myriad of natural resource-based industries as well as recreational and commercial fisheries, fur mammals and alligator (Day et al., 1997; Day et al., 2004). Despite its economic value, the MRD has experienced among the highest rates of coastal wetland loss on the planet (Day et al., 2000; Allison and Meselhe, 2010), with rates of 77 km^2/year between 1978 and 2000 (Barras et al., 2004)**.** The loss of Louisiana's coastal wetlands have been attributed to a combination of multiple interacting stressors including reduced sediment supply, land erosion, land-surface subsidence, sea level rise (SLR), storm surges and vegetation dieback (Boesch et al., 1994; Turner, 1997; Day et al., 2000; Allison and Meselhe, 2010). Current climate change predictions include increased storm frequency and intensity, which has been observed in this past century (Emanuel, 2005) with Hurricanes Katrina and Rita resulting in combined loss of over 520 km^2 of coastal marsh habitats (Barras, 2006; Howes et al., 2010).

An important contributor of accelerated wetland loss is vegetation dieback in coastal Louisiana (Webb and Mendelssohn, 1996). In 2000, over 10,000 ha of *Spartina alterniflora*-dominated salt marshes along the Mississippi River deltaic plain experienced a sudden and acute dieback event (termed "brown marsh") likely caused by a combination of prolonged flooding resulting in reduced soil conditions and toxic sulfide concentrations (Lindstedt and Swenson, 2006) In recent years, the dominant emergent vegetation in the Lower MRD, a perennial marsh grass, *Phragmites australis* (Cav.) Trin. ex Steud. (hereafter referred to as *P. australis*) has experienced ongoing dieback (Ramsey III and Rangoonwala, 2017; Knight et al., 2018; Suir et al., 2018). *P. australis* (3-5 m tall) inhabits fresh, brackish, and salt wetland systems grows in fresh, brackish and salt marsh systems in dense monospecific stands. Symptoms of *P. australis* dieback include retreat from deep waters, clumped distribution of stems, stunted stem and root growth, premature senescence and increased occurrences of dead or decaying rhizomes (Armstrong et al., 1996c; van der Putten, 1997; Brix, 1999). Dieback events often catalyze land loss because of the conversion of densely vegetated marshes into bare intertidal mudflats and replaced by open waters if erosion removes enough soil to flood the region permanently (Nyman, 2014). Complex interplays of multiple abiotic (e.g. water depth, salinity soil chemistry) and biotic (e.g. invasive scale insects) stressors have been reported to negatively affect plant health and growth of *P. australis* (Cronin et al., 2020; Knight et al., 2020; Chapter 1). Often described as ecosystem engineers (Amsberry et al., 2000), *P. australis* helps stabilize soils with its extensive root structures which maintains and improves soil surface elevation (Rooth et al., 2003). Coincidentally, the reported dieback in the MRD led to the discovery of severe infestations of the non-native Roseau Cane Scale (hereafter RCS), *Nipponaclerda biwakoensis* (Kuwana) (Hemiptera: Aclerdidae) (Knight et al., 2018).

The MRD is hotspot for several phenotypically and phylogenetically distinct lineages of both native and nonnative *P. australis*, however, little is known about the ecological and physiological differences that determine their individual susceptibility to causes of dieback (Meyerson et al., 2010; Hauber et al., 2011; Knight et al., 2018). Three distinct lineages of *P. australis* are commonly present along the MRD: (i) the dominant lineage in the MRD, Delta (haplotype M1) is of North African and Mediterranean origin (Hauber et al., 2011; Lambertini et al., 2012b; Knight et al., 2018), (ii) one of Eurasian origin (hereafter, the EU lineage), known for its invasive nature throughout North America, is found in scattered monodominant stands sympatric with Delta stands (Saltonstall, 2002) and lastly, (iii) the Gulf Coast lineage (haplotype I or subspecies *Phragmites berlandieri),* also known as Land-type, is widely distributed along the southern United States (Saltonstall, 2002; Hauber et al., 2011) and has recently spread to California (Meyerson et al., 2010) inhabiting more upland areas and less saline conditions (Achenbach and Brix, 2014; Knight et al., 2018). In the MRD, the EU lineage has been observed to be 1.5 and 2.5 m shorter than both the Gulf and Delta lineage (Hauber et al., 2011) and considered to be less prone to dieback syndrome than the Delta lineage (Knight et al., 2018; Cronin et al., 2020; Knight et al., 2020). The EU lineage of *P. australis* is a successful invader due to its superior competitive ability, greater nutrient assimilation, high phenotypic plasticity to environmental drivers (e.g., excess nutrients, salinity, temperature) and greater tolerance to scale insects than the North American native lineage (Saltonstall and Court Stevenson, 2007; Holdredge et al., 2010; Cronin et al., 2015; Croy et al., 2020).

Restoration has become a key conservation strategy to combat coastal wetland degradation and enhance biological communities and biogeochemical functions (Zhou et al., 2003; Nyman et al., 2006; Ogburn and Alber, 2006; Silliman et al., 2015). With the acceleration of coastal habitat loss due to climate change and anthropogenic activity (e.g., sediment diversions, levees, canals, dams, dredging), planting habitat-forming species in areas of stressful environmental conditions would facilitate conservation efforts, which has been widely adopted for numerous wetland plant species (Silliman et al., 2015), including *Spartina alterniflora* (Wilsey et al., 1992; Edwards et al., 2005; Silliman et al., 2015) and *P. australis* (Rolletschek et al., 1999; Howard et al., 2008; Howard and Turluck, 2013; Brix et al., 2014; Howard et al., 2019). However, natural revegetation following dieback events may be hindered by the presence of adverse edaphic or hydrologic stressors that either induced dieback or was the result of dieback (Chapter 2). Regardless of the case, transplanting marsh plants in dieback areas may aid in initializing establishment and recovery. Although dieback soils are not optimal for *P. australis* growth, I found that aboveground biomass of three lineages of Delta, EU and Gulf lineages of *P. australis* (as a measure of plant health and growth) was only modestly reduced when grown in dieback soils (collected approximately 1 year after dieback) relative to healthy soils in a garden and greenhouse experiment (16-23% lower; Chapter 1).

Given the unprecedented and substantial loss of *P. australis* in the Lower MRD, the Coastal Protection and Restoration Authority (CPRA) of Louisiana, in collaboration with our team of scientists at Louisiana State University, initiated a restoration field trial in the Lower MRD to assess the potential of three lineages of *P. australis* (Delta, EU and Gulf) and common wetland plant species including *Schoenoplectus californicus* (C.A. Meyer), Soják (California bulrush), *Spartina alterniflora* L. (tall cordgrass)*, Spartina patens* (Aiton) Muhl.(saltmeadow cordgrass) *and Zizaniopsis miliacea* (Michx.) Doell. & Asch. (giant cutgrass) to revegetate marsh habitats that experienced dieback. A total of nine widely dispersed sites at different water depths were selected to provide adequate representation of dieback habitats in the Lower MRD.

The purpose of the field trials was (1) to investigate which lineage of *P. australis* or wetland plant species would be most successful in establishing and expanding in areas subjected to extensive dieback within the past 1-2 years and (2) to assess whether different lineages of *P. australis* and other plant species have greater success in different water depths commonly found along the MRD. After 2.5 years, we provide our preliminary findings using data collected on plant survival after 6 months, scale abundance (on *P. australis* only) at 11 months, and plant area coverage after 22- and 32-months post-planting using drone-based imagery. We tested the following set of predictions: (1) among the plant species, *P. australis* (irrespective of lineage), will have the highest survival and greatest area of coverage; (2) as a successful invader in North America and presumed superior competitive ability (Saltonstall and Court Stevenson, 2007; Holdredge et al., 2010), the EU lineage of *P. australis* will have the greatest survival and area of coverage in comparison to the Delta and Gulf lineages; (3) commonly found along relatively higher elevations and drier habitats, the Gulf lineage of *P. australis* will have the lowest survival and area cover among the three lineages; and (4) because of its known greater resistance to scale insects (Cronin et al., 2020; Knight et al., 2020), plantings of the EU lineage will have lower scale abundance than the other two lineages. We conclude with updated recommendations for restoration strategies based on our preliminary findings.

3.2. MATERIALS AND METHODS

Study area

In 2018, CPRA initiated the restoration field trial known as the LA-39 Coastwide Vegetative Planting Year 7A Mississippi River Delta Vegetative Field Trial built under the Coastal Wetlands Planning, Protection, and Restoration Act by the Natural Resources

Conservation Service (NRCS) of the United States Department of Agriculture. The study was conducted in the Lower Mississippi River Delta, Plaquemines Parish, Louisiana, USA along two of three main channels stemming from the Head of Passes: Pass-a-Loutre Wildlife Management Area (WMA) (Pass-a-Loutre) and South Pass Campground (South Pass), where widespread dieback has occurred since 2016 (Suir et al., 2018). Pass-a-Loutre WMA and South Pass are composed of coastal freshwater marshes (average salinity < 1 ppt) and river channels and their accompanying banks, natural bayous and manmade canals with surrounding beach and barrier island habitats (CPRA).

We chose sites along Pass-a-Loutre and South Pass that differed in water depth and were widely dispersed so that they provided a comprehensive representation of dieback habitats in the MRD. Prior to establishing the sites, several trips were conducted to determine the most suitable sites to establish the plots. A total of nine sites were selected: Boundary Line Pond Site 1 (BLPS1), Boundary Line Pond Site 2 (BLPS2), Cheniere Crevasse (CC), Chenier Pass Splay (CPS), Rattlesnake Bayou (RB), Rattlesnake Flat (RF), Sawdust Bend (SAW), South Pass Campground (SPC), and Willow Tree Site (WTS) **(** Table 2.1, Figure 1.1**).** In Pass-a-Loutre, a total of 7 sites were selected (BLPS1, BLPS2, CC, CPS, RB, RF, SAW), and two sites were selected along South Pass (SPC, WTS) (Table 3.1). All sites were coastal freshwater marsh habitats with mean salinity < 1 ppt for each site (range: 0.15 – 3.0 ppt). Eight of nine sites had a previous history of *P. australis* dieback (within the past 1-2 years since 2018). The one site (SAW) was a recently excavated dredge disposal area created by the U.S. Army Corps of Engineers (Park, 2018). All but one of those sites were, at the time of the restoration trial, devoid of any emergent *P. australis*. The one site with *P. australis* (Rattlesnake Bayou) was sparsely populated with *P. australis* (Delta lineage) and inhabited by other marsh plant species (*Colocasia* *esculenta* (L.) (elephant ear), *Phaeiris hexagona* (Iris hexagona), *Typha latifolia (*L.), *Alternanthera philoxeroides* (Mart.) (alligatorweed), *Sagittaria lancifolia* (L.) or *Pontederia crassipes* (Mart.) (water hyacinth) (Table 3.2).

Table 3.1 Coordinate locations and site conditions of nine field site locations in Pass-a-Loutre and South Pass of Mississippi River Delta in Louisiana, USA.

Site	Location	Access Route	Latitude	Longitude
Boundary Line Pond Site 1	Pass-a-Loutre	Raphael Pass	29.178488	-89.152067
Boundary Line Pond Site 2	Pass-a-Loutre	Raphael Pass	29.176232	-89.156190
Chenier Crevasse	Pass-a-Loutre	Cheniere Pass	29.132328	-89.187155
Chenier Pass Splay	Pass-a-Loutre	Cheniere Pass	29.132082	-89.183941
Rattlesnake Bayou	Pass-a-Loutre	Pass-a-Loutre	29.142408	-89.187035
Rattlesnake Flat	Pass-a-Loutre	Pass-a-Loutre	29.144569	-89.182811
Sawdust MC South	Pass-a-Loutre	Sawdust Bend	29.112906	-89.247135
South Pass Campground	South Pass	Southpass	29.083893	-89.247135
Willow Tree Site	South Pass	Southpass	29.121811	-89.228622

Table 3.2. Site conditions for habitat type, mean salinity, water depth range from 6-months to 22-months, and indication of adjacent channel presence (Y = yes, N = No)

Site	Habitat Type	Mean Salinity (ppm)	Water depth range (cm)	Adjacent Channel
Boundary Line Pond Site 1	Unvegetated DB	0.2	11.0 - 85.0	N
Boundary Line Pond Site 2	Unvegetated DB	0.2	15.0 - 71.67	N
Chenier Crevasse	Unvegetated DB	0.3	19.0 - 64.17	N
Chenier Pass Splay	Unvegetated DB	0.3	0.00 - 25.8	Y
Rattlesnake Bayou	Vegetated DB	0.2	0.00	N/A
Rattlesnake Flat	Unvegetated DB	0.3	0.00 - 64.0	Y
Sawdust MC South	Dredge	0.2	9.0 - 54.0	N/A
South Pass Campground	Unvegetated DB	0.2	0.00 - 45.0	Y
Willow Tree Site	Unvegetated DB	0.2	18.0 - 78.0	N

Geomorphologic characteristics included whether sites had adjacent channels, which provides fresh flow of coarse sediments (actively accreting) or whether sites were isolated from an adjacent channel, which hinders inflow of fresh sediments (Holle, 2000). The edaphic conditions in isolated sites are often characterized with high water table levels and high concentrations of organic material. Sites installed along an adjacent channel included: Cheniere Pass Splay and Rattle Snake Flat (Table 3.2, Table 3.3). At 3 of 9 sites, there was no adjacent channel supplying coarse sediments: Boundary Line Pond Site 1 and 2, Cheniere Pass Splay, South Pass Campground, and Willow Tree Site.

Table 3.3. Additional descriptive information for restoration of sites

Site	Suitability
Boundary Line Pond Site 1	Isolated from fresh sediment flow, high water levels and organic matter buildup
Boundary Line Pond Site 2	Isolated from fresh sediment flow, high water levels and organic matter buildup
Chenier Crevasse	Isolated from fresh sediment flow, high water levels and organic matter buildup, protected from chenier ridges
Chenier Pass Splay	Actively accreting fresh sediment supplied from adjacent channel, shallow mudflat with firm soil, protected from storm surges by
Rattlesnake Bayou	Naturally recovered on its own with revegetation of *P. australis*
Rattlesnake Flat	Actively accreting fresh sediment supplied from adjacent channel
Sawdust MC South	Loss of site due to disposal of dredge sediments
South Pass Campground	Shallow mudflat
Willow Tree Site	High waters, high occurrence of water hyacinth

In the Lower MRD, the mean annual temperature is approximately 20 °C. The daily average maximum and minima range from 7 °C – 19 °C in January to 22 °C – 32 °C in July (Hiatt et al., 2019). Freeze events are rare but over time, severe freeze events are expected to become less frequent while severe heat (defined as a day with maximum temperature ≥ 35 °C) events are expected to increase frequency by as much as 20 days yr.$^{-1}$ by 2050 (Osland et al., 2017; Vose et al., 2017). The hydrograph of the MRD fluctuates with snowmelt and rainfall and typically the water height of the Mississippi River peaks and falls by June in most years (Gasparini and Yuill, 2020). The state of Louisiana experiences heavy precipitation with extreme storm events throughout the year, and the typical annual precipitation average range of 140 cm – 160 cm in coastal Louisiana (Keim et al., 1995). From 1986 to 2015, the upper Mississippi River watershed has experienced 5 – 15% increases in precipitation as compared to the ranges from 1901 – 1960 (Easterling et al., 2017). In 2019, the longest recorded flood occurred on the lower Mississippi River due to excessive rainfall upriver spanning over 200 days (NOAA, 2016).

Current climate change predictions include increased storm frequency and intensity, which has been observed in this past century (Emanuel, 2005) with Hurricanes Katrina and Rita, which resulted in a combined loss of over 520 km^2 of coastal marsh habitats (Barras, 2006; Howes et al., 2010). From the initial planting in 2018 till now, a total of 5 hurricanes and 1 tropical storm have made landfall in Louisiana although did not make landfall near the Lower MRD.

Sources of plant species and P. australis lineages and propagation of plants

The study species used in this restoration field trial included three *P. australis* lineages (Delta, EU, and Gulf), *Schoenoplectus californicus* (C.A. Meyer), Soják (California bulrush), *Spartina alterniflora* L. (tall cordgrass)*, Spartina Patens* (Aiton) Muhl (saltmeadow cordgrass) *and Zizaniopsis miliacia* (Michx.) Doell and Asch (giant cutgrass). Approximately four months prior to transplanting (June 2018), plant material was obtained, propagated, grown and transplanted by an external source, Stream Wetland Services, L.L.C. (SWS) (Table 3.4). For the EU and Delta lineages of *P. australis*, rhizome material was collected on May 30, 2018 along the north bank of Pass-A-Loutre Wildlife Management Area where EU was collected downstream from Delta (Table 3.4). Additional Delta was collected on July 9, 2018 at Rockefeller Refuge. Gulf lineage was collected on June 5, 2018 and on July 9, 2018 at Rockefeller Refuge (Table 3.4). For *S. californicus,* (Louisiana ecotype) and *Zizaniopsis miliaea* (Louisiana ecotype), SWS sourced the plant materials through their nursery (Stream Wetland Services Nursery) (Table 3.4). Lastly, *S. patens* (Gulf Coast ecotype) and *Spartina alterniflora* were obtained from the USDA

Natural Resources Conservation Services, Golden Meadow Plant Materials Center (PMC) (Table 3.4).

Table 3.4 Plant source locations and planting specifications required for each plant species prior to transplanting

Plant type	Source	Container type/size	Minimum stem requirements (live and actively growing)	Minimum height/length (cm) (Stem-root interface to stem tip)	Minimum Stem Height prior to planting
***Phragmites* (Delta)**	North bank of Pass-a-Loutre (29.145422°, 89.21558°) Rockefeller Refuge (Rock Road 2: 29.704100°, -92.831300°)	2.8 L (Trade gallon)	4	2 x 122 2 x 76	< 122 cm
***Phragmites* (Gulf)**	Rockefeller Refuge (29.71786389°, 92.7672667)	2.8 L (Trade gallon)	4	2 x 122 2 x 76	< 122 cm
***Phragmites* (EU)**	Upstream of north bank of Pass-a-Loutre, Louisiana (~29.145422°, 89.21558°)	2.8 L (Trade gallon)	4	2 x 122 2 x 122	< 122 cm
Schoenplectus californicus	Stream Wetland Services Nursery	2.8 L (Trade gallon)	5	2 x 101.6 3 x 76.2	< 122 cm
Zizaniopsis miliacia	Stream Wetland Services Nursery	2.8 L (Trade gallon)	3 with 4 leaves	3 x 101.6	
Spartina alterniflora	'Vermilion' released by the USDA Natural Resources Conservation Service, Golden Meadow Plant Materials Center (PMC)	Vegetative plugs	Multi-stemmed, bare root material 3 stems attached to stem-root interface	2 x 30.5 1 x 7.62	3 x 60.96
Spartina patens	'Gulf Coast; released by the USDA Natural Resources	2.8 L (Trade gallon), 0.25 L pot, and small	**2.8 L:** 8 primary stems **2.6 L**: 8 primary stems	**2.8L:** 8 x 30.5 **2.6L**: 8 x 30.5	**2.8L:** 8 x 60.96 **2.6 L:** 8 x 60.96

Conservation services, Golden Meadow Plant Materials Center (PMC)	pot containers	**0.25 L:** 3 stems attached at stem-root interface	**0.25 L pots:** 2 x 30.5 1 x 7.62	**0.25 L:** 3 x 60.96

Prior to transplantation, plant growing specifications were designated for *P. australlis* lineages and plant species regarding pot size, minimum number of stems/rhizome material and minimum stem/rhizome length (Table 3.4). All three lineages of *P. australis* were grown in 2.8 L nursery pots (trade gallon pots). Each pot contained a minimum of four live and actively growing stems with 2 of 4 stems required a minimum stem length of 122 cm from the stem-root interface to the stem tip (not leaf). The remaining two stems had a minimum stem length of 76.2 cm (Table 3.4). For *S. californicus* and *Z. miliacea,* plants were grown in 2.8 L nursery pots. For *S. californicus,* each pot contained a minimum of five live and actively growing stems per with 2 of the 5 stems had a minimum length of 101.6 cm and the remaining had a minimum of 76.2 cm. For *Z. miliacea*, each pot contained a minimum of 3 stems with each stem having a minimum of 4 leaves and a minimum height of 101.6 cm. To assess which growing conditions (pot size, size of root/rhizome material, number of stems and height) would maximize transplant survival and establishment, source material for *S. patens* were grown in three pot sizes: 2.8 L, 2.6 L, and 0.25 L nursery pots with varying requirements for each pot site (requirement specifications reported in Table 3.4). Lastly, *S. alterniflora* were raised as vegetative plugs that contained multi-stemmed, bare-root material that had a minimum of 3 live and actively growing stems attached to the stem-root interface. Two of three stems had a minimum stem height/length of 30.5 cm and 7.62 cm for the last stem. Within 10 days prior to planting at the nine sites, stems were pruned to a height of no less than 122 cm to facilitate transportation and planting. Growing specifications for all plant species and *P. australis* lineages can be found in Table 3.4.

After 4 months growing at SWS, the transplants were installed between October 18 and 19, 2018 (Table 3.5, Figure B1-5). Transplants were installed into the field in separate gridded plots specific for one plant species/lineage (Figure B1-5). A team of assistants walked each grid and sunk the potted plants (sans plastic pot) flush with the soil surface. All sites had plots of the three *P. australis* lineages but the presence/absence of plots of the other species varied from site to site. At all sites with the exception of Rattlesnake Bayou, the gridded plots were 13 rows x 4 columns (only two plants for the 13th row) for a total of 50 replicate plantings for each species/lineage. At those eight sites, individual plants within a plot were placed 4.6 m apart and the overall area of each plot was 83.6 m^2 (18.29 m x 4.57 m). The first row was oriented closest to the interior marsh side and the 13th row was closest to the channel, bayou, or open water side. Using PVC stakes and flagging, we marked the location of the first planting from the left near the marsh side

Since Rattlesnake Bayou was the only dieback site with emergent vegetation present, different grid designs were used to navigate around the existing vegetation. At Rattlesnake Bayou, plants were installed 0.9 m apart, in either a 9 x 5 grid (26.8 m^2) or 6 x 5 grid (16.7 m^2; only three plants for the last row), and plots were separated by approximately 1.5 m. In either case, the last row contained only 3 planting such that there were 43 or 28 replicate cells in each plot, respectively (Table 3.5, Figure B5). However, since Rattlesnake Bayou was a vegetated site, all *S. patens* plants were marked with pin flags and for all other species, only the perimeter was marked with pin flags to indicate the location of the plots. Appendix B shows a map of the layout of plantings for each site. Overall, a total of 443 plants per *P. australis* lineage, 428 of *S. californicus*, 143 of *S. patens*, 128 of *Z. miliacea,* and 43 *S. alterniflora* were transplanted for the restoration field trials for a grand total of 2,071 plants (Table 3.5).

Table 3.5. Total number of plantings for each plant species and lineages planted at the nine CPRA Restoration Field Trial sites (Species not planted at site is indicated by ×).

Plant species	BLPS1	BLPS2	CC	CPS	RB	RF	SAW	SPC	WTS	Total
Delta	50	50	50	50	43	50	50	50	50	443
EU	50	50	50	50	43	50	50	50	50	443
Gulf	50	50	50	50	43	50	50	50	50	443
S. alterniflora	×	×	×	×	43	×	×	×	×	43
S. patens	×	×	×	×	43	50	×	50	×	143
S. californicus	50	50	100	×	28	50	×	50	100	428
Z. miliacea	50	50	×	×	28	×	×	×	×	128
									Overall Total:	2071

Field Survey

We conducted field surveys of the plants within each restoration plot and within the nearest-naturally occurring *P. australis* patch (hereafter within-plot and nearest-patch). The first survey was conducted approximately two months post-planting on 17 December 2018 (Aerial imagery can be found in Appendix B). Subsequent surveys to monitor plant survival and health were repeated on 9 April 2019 (6-months), 29-30 July 2019 (9-months), 23 September 2019 (11 months), 15-16 June 2020 (20-months), 10-11 August 2020 (22 months) and 2 June 2021 (32 months).

Two-month survey: Plant Survival

For within-plot surveys, we visually determined whether each planting was alive or dead. Plants were marked as “alive” if green plant tissue was visible, whereas, plants were marked “dead” if no visible green tissue was present or if submerged. Plant survival was then converted to proportion alive (total # alive/total planted). At the edge of each *P. australis* lineage plot per site, we measured water depth and water quality conditions including temperature (°C), dissolved oxygen, conductivity (spc), salinity (ppt), and pH using a multiparameter instrument (YSI Professional Plus (Pro plus): YSI Inc., Yellow Springs, OH, USA). Additionally, we collected

250 g of soil at the plot edge per *P. australis* lineage for all nine sites for a total of 21 samples. Samples were stored at 4° C until soil chemistry tests were performed by the LSU AgCenter Soil Testing and Plant Analysis Lab. The soil nutrients and properties analyzed included aluminum (Al), calcium (Ca), magnesium (Mg), phosphorous (P), potassium (K), sodium (Na), sulfur (S), % carbon (% C), and % nitrogen (% N) % organic matter (% OM), copper (Cu), iron (Fe), manganese (M), zinc (Zn) and soil pH. Additionally, aluminum (Al), sodium (Na), % organic material (% OM) and pH. However, we did not analyze soil chemistry dataset for this report.

In the nearest naturally occurring *P. australis* stand from the plots, we repeated measurements for the hydrologic measurements mentioned above. To assess stand health, we measured stem heights, (nearest centimeter from soil surface) for 10 haphazardly chosen stems. Additionally, using the same stems, percent green (% green) measurements were recorded as an index of degree of plant senescence which was estimated visually as the percentage of a stem above the surface water that had green leaf sheath tissue (measured from the lowest node with green leaf to the highest node) (Knight et al., 2020). Stem density was assessed as a measure of stand healthy from five randomly chosen locations within the stand (number of live and total stems per 0.25 m^2 quadrat). Round quadrats were constructed with a 1.77 m length plastic tubing connected at the ends with a barbed coupler (Knight et al., 2020). Scale incidence (presence/absence) per stem was assessed from surveying 30 stems. Among the examined stems, we collected 10 infested stems, sealed in plastic bags and returned to the laboratory for scale counts to determine scale density.

Six-month survey: Plant survival

On 19 April 2019, six-months post-planting, we repeated the measurements for plant survival, water depth, and water quality as mentioned above. We repeated the measurements at the same nearest-patch surveyed in December 2018.

Nine-month survey: Plant survival

On 23 September 201, nine-months post-planting, we repeated our assessment of plant survival for each plant species or *P. australis* lineage as mentioned above. For the within-patch measurements, we collected water depth and water quality. Nearest-patch measurements were also collected

Eleven-month survey: Scale abundance

One of our goals was to investigate the importance of scale insects in limiting growth and health of *P. australis* and to determine if different lineages were impacted differently from another. Thus, we collected direct ground measurements of plant growth and health within the area plots including stem heights, stem density, and % green following the same protocol used in the nearest-patch. For each species, five plantings along the gridded plots were randomly chosen to measure stem density, stem height and % green for three stems. The sampled plants were chosen based on two criteria: 1) the sample areas must occur within the plot and 2) the sample areas were located ≥ 3 m apart. We did not measure growth outside of the plot due to the uncertainty of the source of the plants, whether it was from expansion and spread of transplanted material or from a naturally growing stand.

At each sampled planting, we haphazardly selected 3 green stems and inspected them for the presence of scale insects. If scales were present, we clipped the stem at the soil surface and transported it to the laboratory. In the laboratory, we measured stem height (nearest cm from base to tip of the uppermost green leaf) and basal diameter of each stem (mm). We also counted scales per stem using methods reported in Knight et al. (2020) and Cronin et al. (2020). For this study, we focused our analysis on the mean number of RCS per m of stem, which was computed as the mean number of scales per stem (adult females + juveniles) divided by mean stem length (based on 15 stems). At the same nearest-patch for each site, we repeated the measurements mentioned above.

Nine-, Twenty-, twenty-two-, and thirty-two- month surveys: Plant Area Coverage

After assessing high plant survival at 6 months, we anticipated expansion and merging of plantings which would require more comprehensive measurements of plant health and growth to assess restoration success of plantings. Since sites were only accessible by boat and in order to reduce disturbance of edaphic conditions of plantings and destructive sampling, we employed an unmanned aerial vehicle (UAV) for non-destructive measurements.

On survey dates from June 2020 – August 2020, we acquired aerial imagery using an unmanned aerial vehicle (UAV), DJI Phantom 3 Professional with a built-in 4K-color camera (DJI Corporation, Shenzhen, Guangdong, China). For each of the nine plots, autonomous aerial flights were performed using the Drone Deploy mission planning software (Drone Deploy, San Francisco, CA, USA) to create linear transects from 15 m AGL (above ground level) altitude with 80% frontal and 75% lateral overlap to ensure optimal speed, direction, and alignment of the images and to produce orthomosaic images. We used low altitudes at 15 m to cover our study sites because increasing altitude would reduce image details. However, on 2 June 2021 (32-months), I acquired aerial imagery at 20 m AGL altitude to capture the overall landscape of the surrounding area. Drone Deploy is an application for UAV image processing and analysis that includes an automated flight module. Images were automatically stored in the SD card synchronized with the UAV. The camera angle was at 90°, facing the ground throughout the flight plans. The aerial images were processed by Pix4D Cloud (Pix4D, Lausanne, Switzerland) to generate 2D orthomosiac and 3D maps. The acquired images tagged with GPS coordinates (Global Positioning System) were uploaded for image stitching and calculating three-dimensional point clouds.

Plant area cover was measured using ImageJ (Rasband, 1997-2018) with the orthomosaics obtained from Pix4D. I traced individual ellipses which was used to compute area from each ellipse and summed for each species or planting. To set our scale for the images, I utilized the length of the airboat (4.27 m) captured at the sites for images taken in August 2020 (22-months). To set our scale for the images at 20 m, I utilized the front width of the barge, a flat-bottomed boat, captured at the sites (2.37 m)

Data analysis: Plant Survival

We assessed plant survival with three separate analyses with two subsets of data. One subset included all plants species and lineages and the second subset contained proportion alive for only the three *P. australis* lineages. Separate analyses were conducted because, whereas all three lineages of *P. australis* were planted at all nine original sites, the other plant species were planted at a subset of sites (Table 3.6). Plantings of *S. californicus* were transplanted at 7 of 9 sites, *S. patens* and *Z. miliacea* were at 3 of 9 sites and *S. alterniflora* was at 1 of 9 sites (Table 3.6).

The first analysis was for *P. australis* lineages survival. To examine the relationship between water depth at each site and planting survival of *P. australis* lineages*,* we conducted a generalized linear model with water depth, lineage and the interaction term water depth x lineage as fixed effects. For *P. australis* survival, we performed a logit transformation for proportion alive (plant survival) to improve the assumptions of normality. An Analysis of Variance (ANOVA) with water depth as a covariate was performed followed by post hoc Tukey's HSD tests were applied to examine the effects of plant species and *P. australis* lineages on survival at 6 months.

Next, to determine whether plant survival at 6 months was influenced by the individual plant species and *P. australis* lineages, we conducted a linear mixed-effects model that included proportional survival of all species and lineages together ("All plant survival") with plant type/lineage as the predictor variable. We performed a logit transformation for proportion alive (plant survival) to improve the assumptions of normality for the model including all species and lineages. Since the plantings of *P. australis* lineages and species within the same site share the same environment and are not independent, we included site as a random effect to account for the shared site effects on all plantings within a site. Next, ANOVA tables for fixed effects were computed using Satterthwaite method to compute the degree of freedom for the F-test followed by a hoc Tukey's HSD test. Lastly, we compared overall survival differences between *P. australis* lineages together (regardless of lineage) and overall survival of all non-*P. australis* species (*S. patens, S. californicus* and *Z. miliacea*). Student's *t*-test was used to determine significance of plant survival between the two groups ("*P. australis* versus other").

Data analysis: Scale Abundance

We predicted that plantings of EU lineage will have lower scale abundance than the other two *P. australis* lineages due to its greater resistance to scale insects (Cronin et al., 2020; Knight et al., 2020). To examine the relationship of scale abundance of plantings and stem density and *P. australis* lineage, we performed a linear mixed model with stem density (co-variate), lineage and the interaction term stem density x lineage as the predictor variables and site was included as a random effect to account for the shared environmental conditions at the site. Scale abundances were *ln*-transformed to improve the assumptions of normality. For interpretation purposes, untransformed values were used for figures.

Data analysis: Plant area coverage

Plant area coverage was analyzed with two separate linear mixed-effects models using a subset of all lineages and plant species ("All Plant Cover") and for a subset containing *P. australis* only ("*P. australis* lineage Cover"). In the former model (All Plant Cover), we included area coverage as the predictor variable, plant species/lineage as the fixed effect and site was included as a random effect. We did not include water depth as a fixed effect in the first analysis since water depth was only measured within the plots of *P. australis* lineages.

To examine the relationship between water depth at each site and area coverage for *P. australis* only, we conducted a linear mixed-effect model with water depth (co-variate), lineage, and the interaction between water depth and lineage (lineage × water depth) as fixed effects and site was included as a random effect. Area coverage was *ln*-transformed to improve the assumptions of normality for both models.

We performed all statistical analyses using R (R Institute for Statistical Computing, Vienna, Austria) within RStudio (RStudio Inc, Boston, MA, USA). Linear mixed-effects models were performed using package *lmer.* We assessed for outliers and visually analyzed residual plots and quantile-quantile plots for homogeneity of variance and normality. Marginal means ± SE for fixed effects were computed using package *ggmmeans* and contrasts between pairs of means were performed using *eemeans* and *P*-values were adjusted with Tukey's method. Goodness-of-fit was calculated using package *MuMin* which reported the marginal R^2, which provides the variance explained by the model's fixed effects and the conditional R^2, which provides the combined variance explained by the entire model with fixed and random effects (Nakagawa and Schielzeth, 2013). Type III sums of squares were used for all tests. Finally, for presentation of results, we used untransformed means and standard errors (SE). Statistical significance was determined at $P \leq 0.05$.

3.3. RESULTS

At 6-months post-planting, the established transplants were present at all 9 sites (Table 3.6). However, we excluded the plots in Rattlesnake Bayou (RB) from the study due to the transplants being inseparable from the existing vegetation at approximately 6-months post planting. Approximately one-month after the 6-month survey, plantings in Sawdust Bend (SAW) were excluded from the remainder of the study due to deposition of dredge sediments over the experimental plots. As of May 2021, there were no surviving plants at Cheniere Crevasse (CC) (exception of one Delta planting) and Willow Tree site (WTS). However, we did not exclude the most recently lost sites (CC and WTS) from our analyses for plant survival, scale abundance and area cover.

Table 3.6 Presence of plant species and lineages of total sites during survey dates (Fractions indicate number of site present during survey/total planted sites).

Survey Dates	Delta	EU	Gulf	*S. californicus*	*S. alterniflora*	*S. patens*	*Z. miliacea*
Dec 17, 2018	9/9	9/9	9/9	6/7	1/1	3/3	3/3
April 19, 2019	9/9	9/9	9/9	5/7	1/1	3/3	3/3
July 29-30, 2019*	6/7	5/7	6/7	6/6	0/1	0/3	1/3
Sept 23, 2019*	7/7	5/7	7/7	6/6	0/1	0/3	1/3
June 15-16, 2020*	7/7	7/7	7/7	6/6	0/1	0/3	0/3
Aug10-11, 2020*	5/7	1/7	5/7	6/6	0/1	0/3	0/3
June 2, 2021*	5/7	1/7	4/7	2/6	0/1	0/3	0/3

** At this date, total number of remaining sites were reduced from nine to seven due to the removal of Rattlesnake Bayou and Sawdust Bend*

P*lant species were not planted at all nine sites but were planted at various subset of sites. Fractions represent presence/total site plantings in respect to plant species*

Six-month survey: Plant survival

We predicted that EU would have greater survivorship than both Gulf and Delta lineage (prediction 2), but that Gulf would have the lowest survival in the restoration plots (prediction 3). Specifically, we found that plant survival of Gulf was 2.6% and 43% greater than Delta, and EU, respectively (based on back-transformed estimates of the marginal means of *logit* proportion alive); although differences were only significant for the latter (Delta: $t = -0.207$, $P = 0.977$; EU: $t = 3.33$, $P = 0.009$). In contrast to our predictions, we found Delta had 42% higher survival relative to EU lineage plantings ($t = -3.45$, $P = 0.007$; Figure 3.1). In our plant survival analysis, we found that plant survival of *P. australis* was significantly influenced by water depth (the co-variate) ($F_{1,21} = 9.71$, $P = 0.005$) and lineage ($F_{2,21} = 7.40$, $P = 0.003$), but there was no significant lineage × water depth interaction ($F_{2,21} = 0.81$, $P = 0.46$). We found a significant negative relationship between water depth and survival of *P. australis* ($R^2 = 5.23$, $P = 0.003$; Figure 3.2).

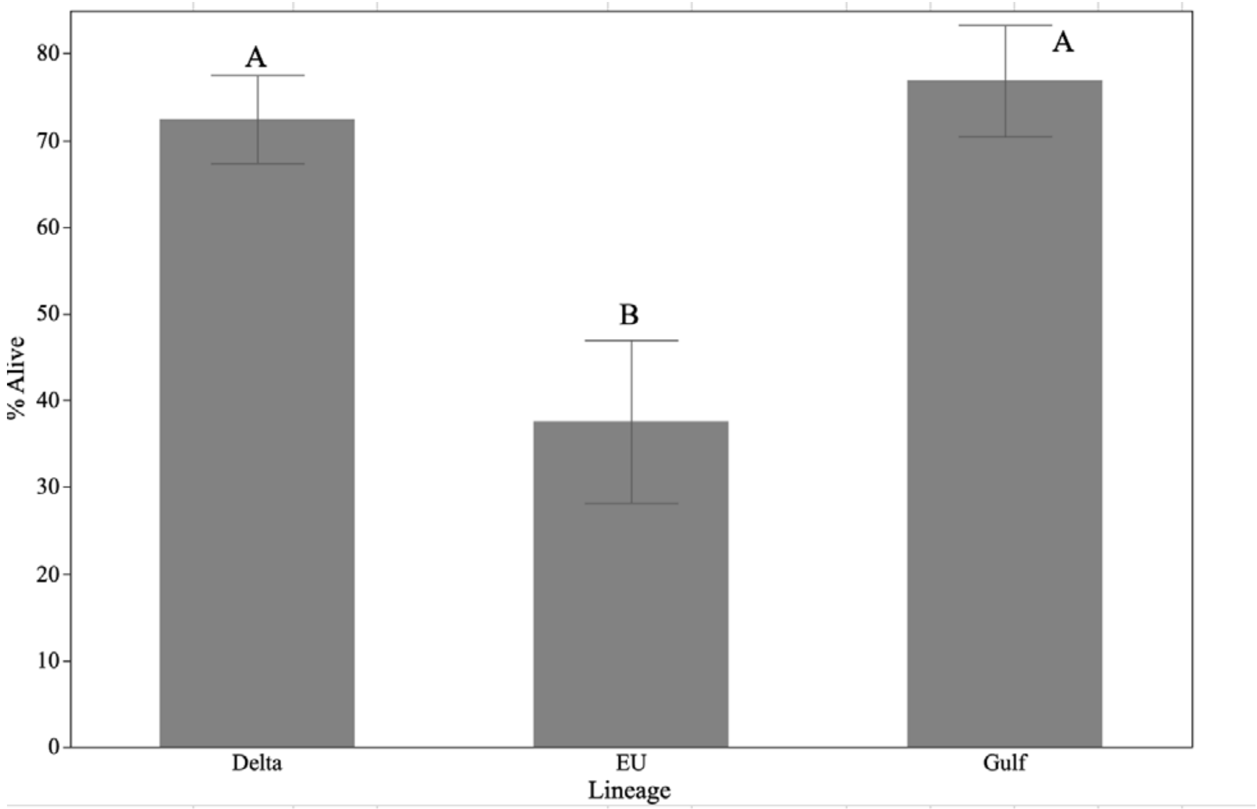

Figure 3.1. Percent (%) alive of *Phragmites* lineages at 6 months post-planting ("*Phragmites* Survival"). Means ± SE using untransformed proportion alive as a percentage value are reported for presentation reasons. Post-hoc Tukey letters were obtained using *logit*-transformed proportion alive. Different letters between groups indicate significant differences at $P \leq 0.05$.

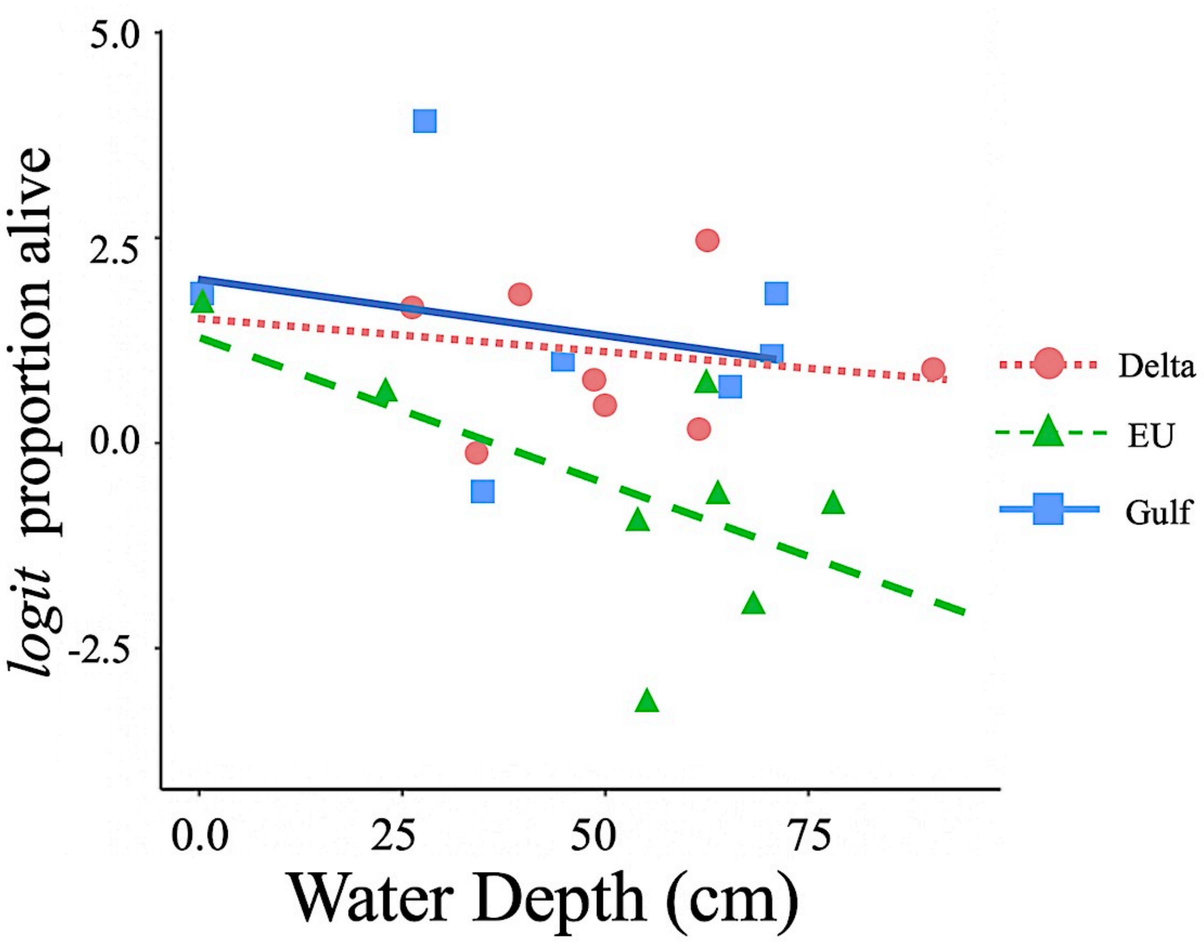

Figure 3.2. *logit* proportion alive of *Phragmites* lineage ("*Phragmites* Survival") in response to water depth. Each point represents water depth measurements at one site and the lines are the predicted relationships based on linear mixed-effects model.

Overall, results were mixed as to which plant species had the highest survivorship after 6 months post-planting. We predicted that survival of all three *P. australis* lineages would be greater as compared to the non-*P. australis* plant species. In support of prediction (1) that *P. australis* (irrespective of lineage) would have greater plant survival, we found that the mean survival of *P. australis* lineages pooled was greater by 24.0% than the survival of the other plant species pooled; although there was no significant difference in survival between *P. australis* lineages and the group of non-*P. australis* species (t = -1.19*,* P = 0.257).

Since *S. alterniflora* was planted at only 1 of 9 sites with a total of 43 replicates, we removed it from our survival analysis that included survival for all species and lineages due to insufficient replications. Among the plant species and lineages, *S. californicus* had the highest survival with comparable survival to Gulf and Delta and had greater survival by 7.7% and 9.9 in relation to the two *P. australis* lineages, respectively; although differences were not statistically significant. We found that EU and *S. patens* had the same survival of 38.8%, which was 52.5% lower than *S. californicus.* In relation to all plant species and lineages, *Z. miliacea* had between 58.5% - 80.3% lower survival (Figure 3.3). Overall, the fixed effect in the GLMM (plant species/lineages) explained 57.3% of the variation in the model and the combined fixed and random effects (associated with different sites) explained 79.6% of the model variation.

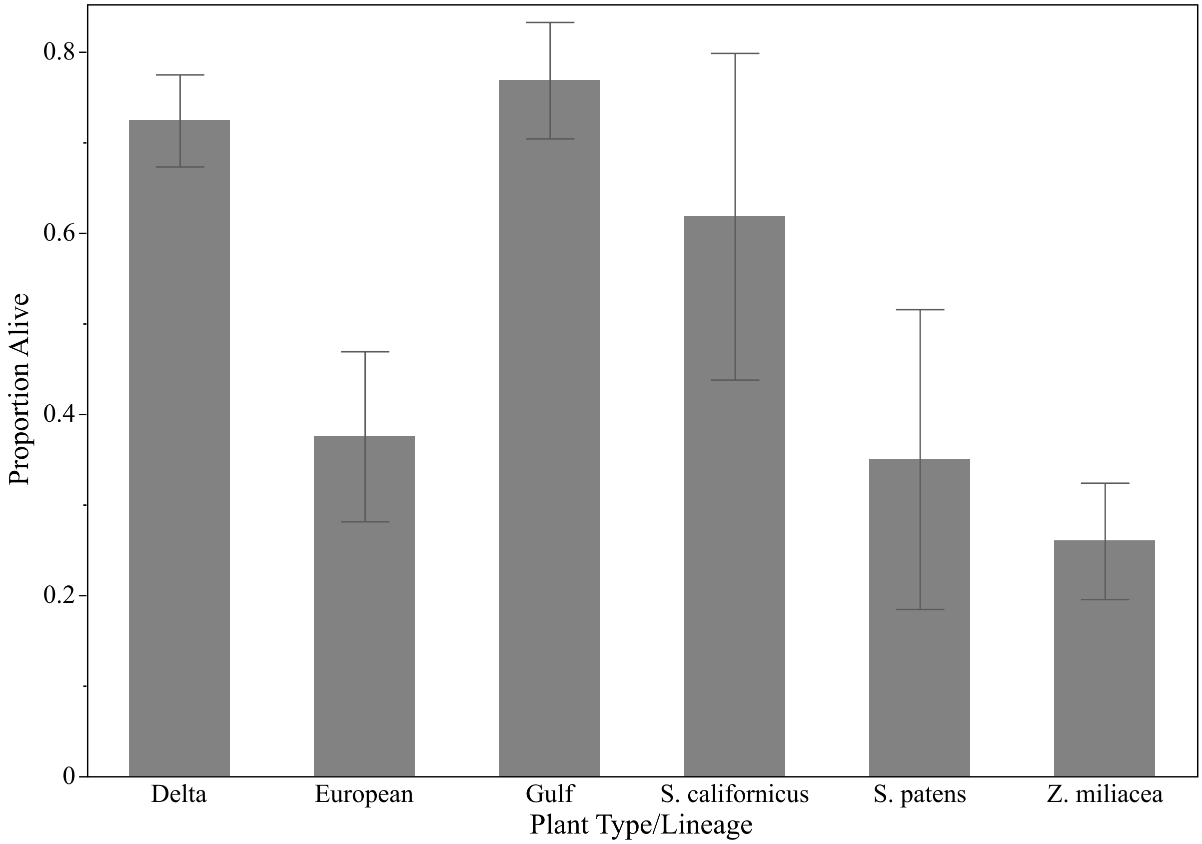

Figure 3.3. Percent (%) live of all plant species at 6 months post-planting ("All Plant Survival). Means ± SE using untransformed proportion alive as a percentage value are reported for presentation reasons. Post-hoc Tukey letters were obtained using *logit*-transformed proportion alive. No significant differences were found using marginal means.

Eleven-month survey: Scale abundance

Scales were only present at 5 of 7 sites: BLPS1, BLPS2, CC, CPS and RF. Scales were not present on plantings at SPC and WTS. Due to its greater resistance to scale insects (Cronin et al., 2020; Knight et al., 2020), we predicted (4) that plantings of the EU lineage would have lower scale abundance than Delta and Gulf. Overall, Delta lineage plantings had 92.1% and 79.1% greater scale abundance in relation to Gulf and EU lineages, respectively (Gulf: $t = 4.18$, $P < 0.001$; EU: $t = 2.78$, $P = 0.028$; based on *ln*-transformed estimates of the marginal means for scale abundance). We found that scale abundance for Gulf lineage was 62.0% greater than EU; although, differences were not statistically significant ($t = -1.69$, $P = 0.236$; Figure 3.4). Scale abundance was significantly influenced by stem density ($F_{1, 13} = 19.64$, $P = 0.0003$) but lineage and the interaction term did not significantly influence scale abundance (lineage: $F_{2,13} = 16.78$, $P = 0.647$; stem density × lineage: $F_{2,13} = 17.31$, $P = 0.180$; Figure 3.5). Lastly, for the model involving scale abundance, the fixed effects explained 70.9% and the full model explained 75.7% of the variation in scale abundance.

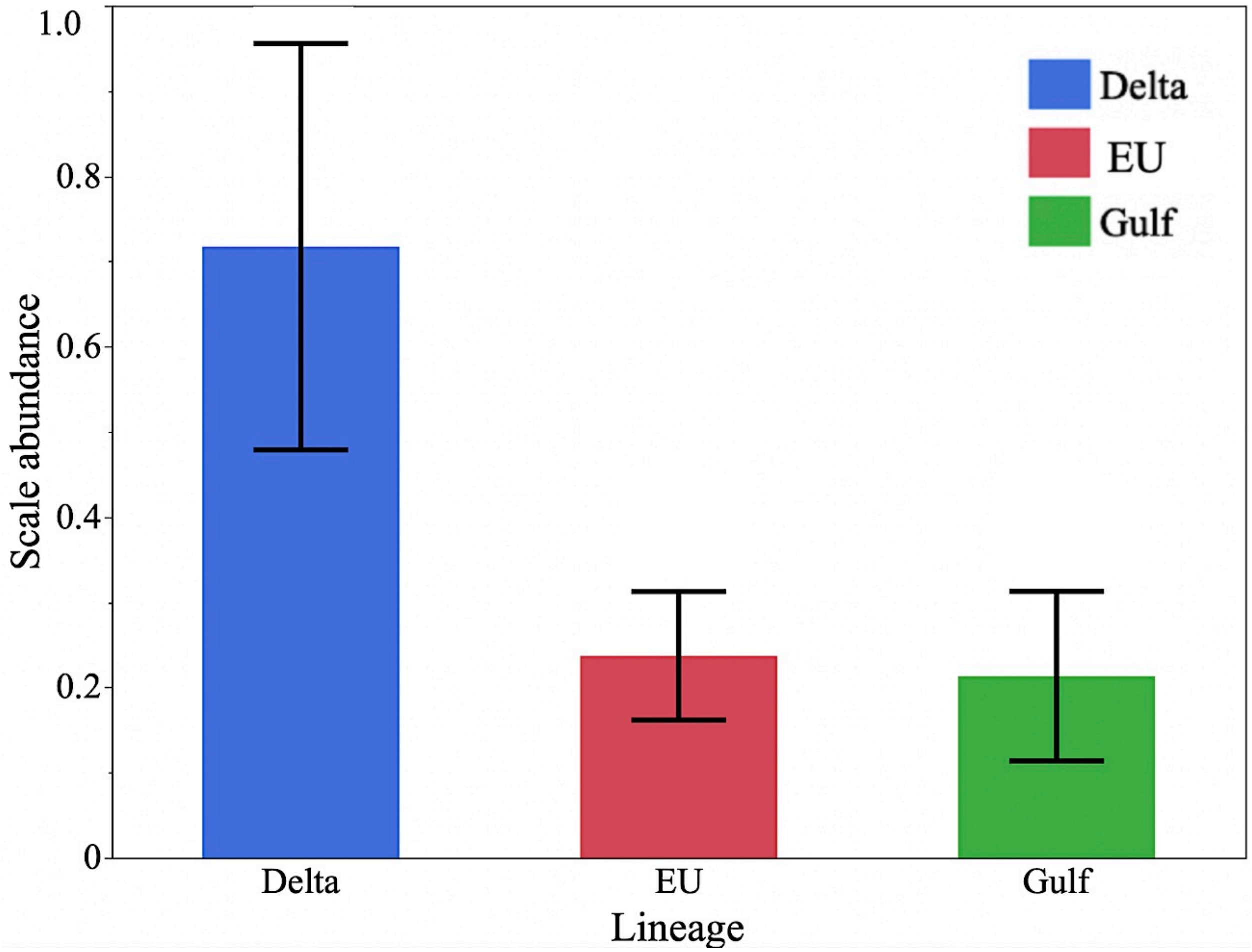

Figure 3.4. Scale abundance (total number of scales per m stem) of *Phragmites* lineages at 11 months post-planting. Means ± SE using untransformed scale abundance are reported for presentation reasons.

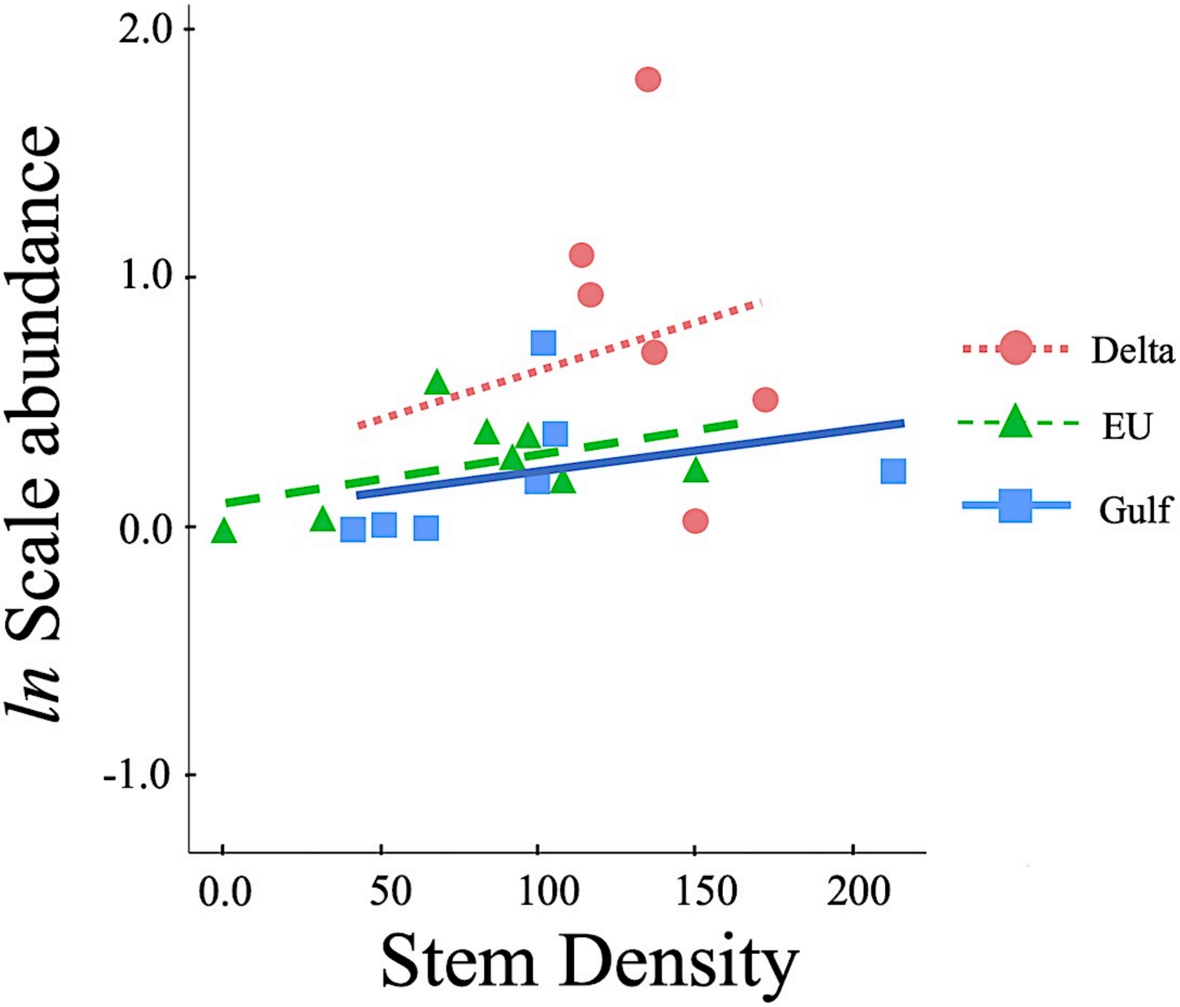

Figure 3.5. *ln-transformed* scale abundance of *P. australis* lineage response in to water depth. Each point represents water depth measurements at one site and the lines are the predicted relationships based on linear mixed-effects model

Twenty-two months survey: Aerial survey

After 22-months, plantings of Delta, EU, Gulf, *S. californicus* and *Z. miliacea* were present. However, due to lost aerial imagery for BLPS1 and CC, for our analysis, we estimated area cover for only 5 of 7 remaining sites (BLPS2, CPS, RF, SPC, and WTS). Delta and Gulf were present at 5 of 7 sites (BLPS1, BLPS2, CPS, RF and SPC), however, area cover was only estimated for 4 of 7 sites (Table 3.6). EU lineage was present at 1 of 7 sites (CPS) (Table 3.6). *S. californicus* was present in all six sites it was planted in (originally 7 sites sans Rattlesnake Bayou) and was the only surviving plant species at two sites where no *P. australis* was present (CC and WTS; Table 3.6). Area cover for *S. californicus* was estimated for only 4 of 6 sites (BLPS2, RF, SPC and WTS). Lastly, *Z. miliacea* was present at only BLPS1, however, due to missing aerial imagery for BLPS1 it was excluded from our analysis.

For prediction 1, we predicted that *P. australis* lineages would have greater area cover than the other study species and that the invasive EU lineage would be most successful at establishing and expanding in the field restoration trials in comparison to the Gulf and Delta lineages (prediction 2 & 3). Area cover was not significantly affected by any fixed effects and its interactions (water depth: $F_{1,3.03} = 3.03$, $P = 0.1795$; lineage: ($F_{2,6.02} = 0.743$, $P = 0.515$); water depth x lineage: $F_{2,6.01} = 0.026$, $P = 0.975$; sites area cover data included zeroes for sites not present) across the sites area. The fixed effects explained 43.8 % and the full model explained 57.0% of the variation in plant area cover. Gulf and Delta lineages had approximately 88% greater area cover than EU; although, differences were not statistically significant (Gulf: $t = -2.41$, $P = 0.114$; Delta: $t = 2.34$, $P = 0.127$; based on *ln*-transformed estimates of the marginal means for scale abundance). There was considerable overlap in total area cover between Delta and Gulf with the former having 2.1% greater total area cover (Figure 3.7). Among the lineages, Delta had the greatest total area cover across five sites (233.17 m^2), followed by Gulf (228.44 m^2) and lastly EU (28.162. m^2) after 22-months (using raw values) Lastly, *S. californicus* had greatest overall area cover (564.0 m^2).

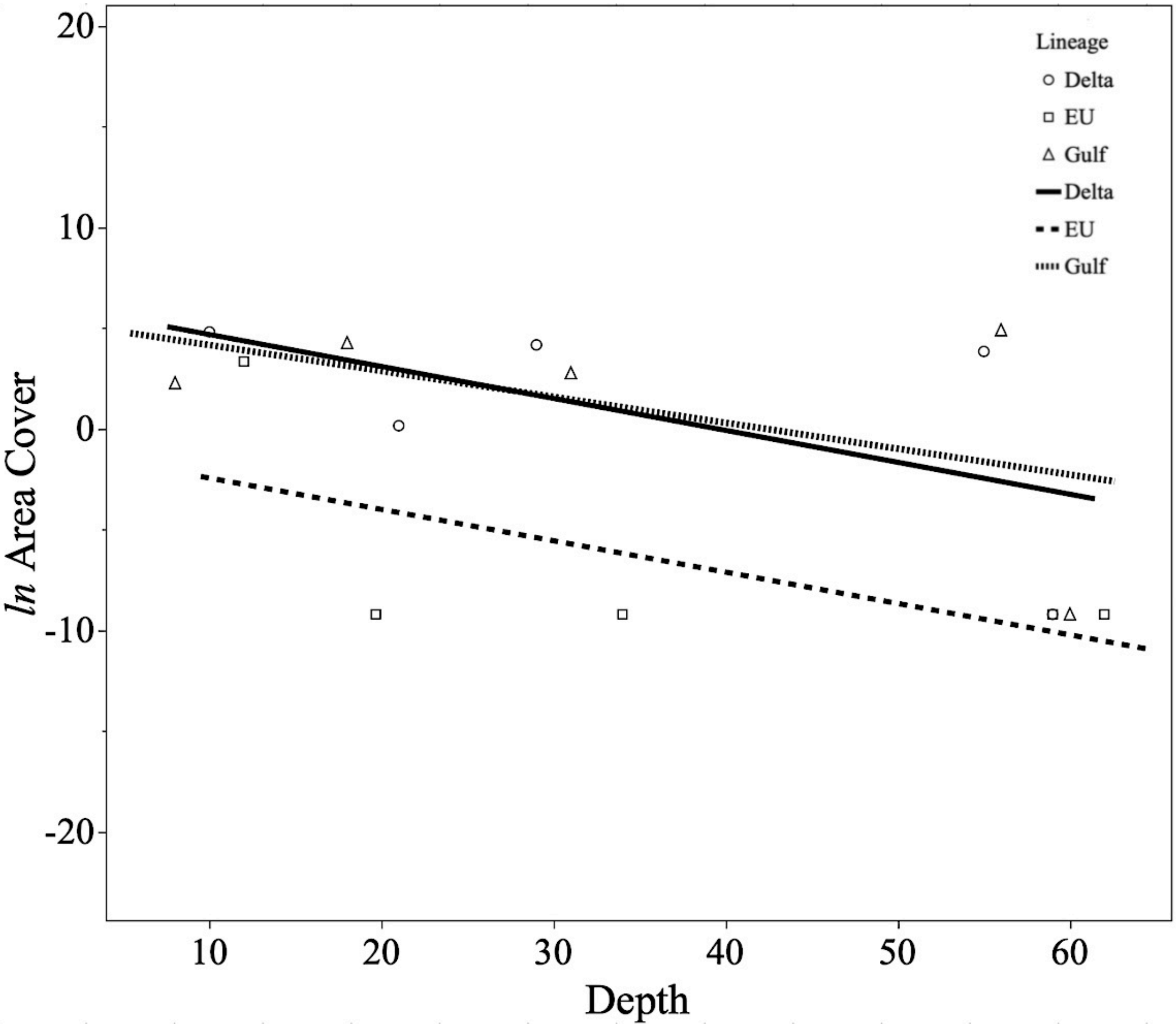

Figure 3.6. *ln* area cover of *P.australis* lineages in response to water depth. Each point represents individual water depth measurements taken within the plots of *P. australis* lineages and the lines are the predicted relationships based on linear mixed-effects model.

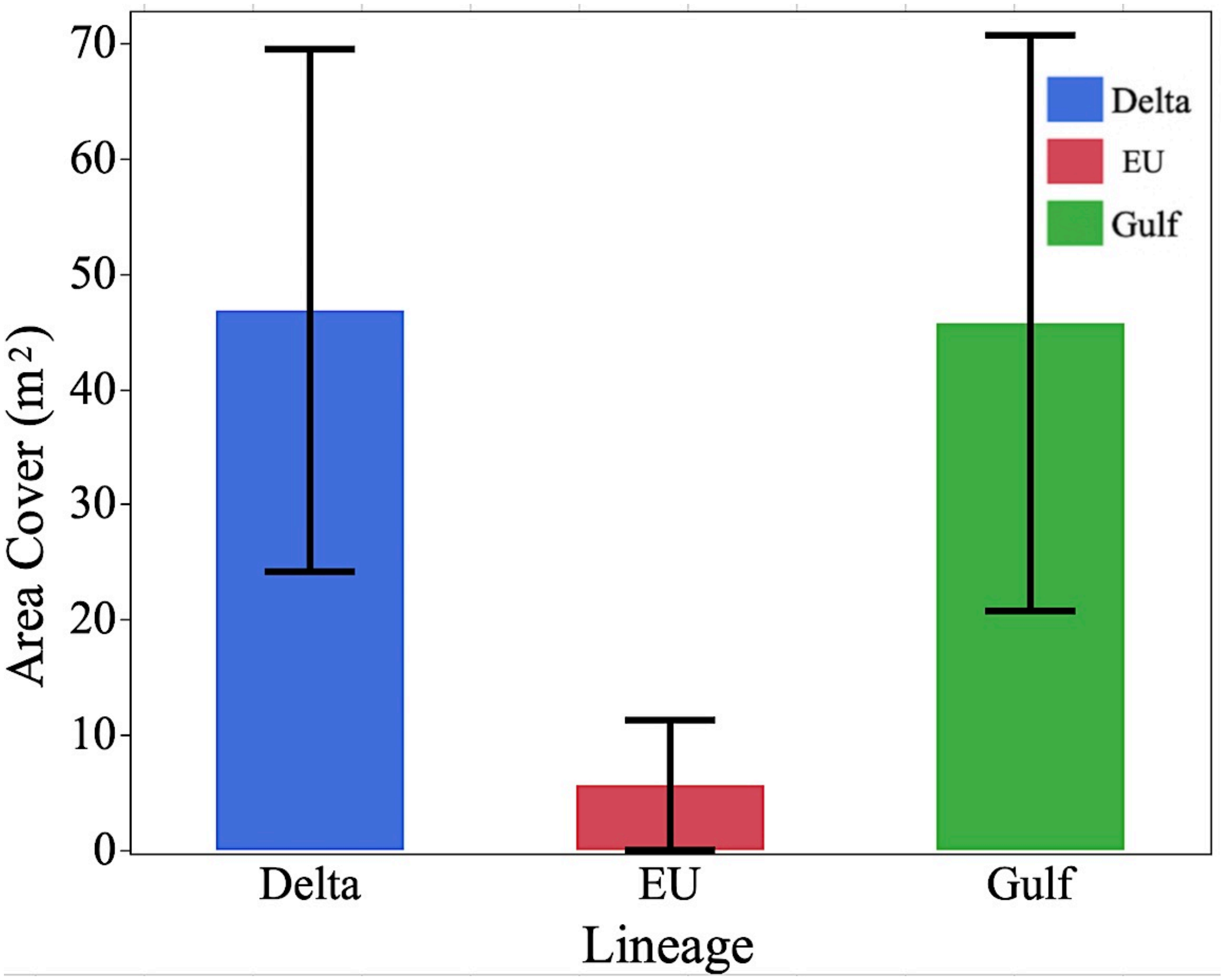

Figure 3.7. Area cover of *P.australis* lineages at 22 months post-planting. Means ± SE using untransformed area cover are reported for presentation reasons.

Thirty-two months survey: Aerial survey

At 32 mo. post-planting, we acquired aerial imagery on 2 June 2021. Two of 7 sites had no surviving plantings with the exception of one Delta planting at CC (CC, WTS); although the lone Delta planting was not visible during our survey in August 2020. Delta was found in 5 of 7 sites and in two sites (CPS and RF), plantings appeared to be growing vigorously and with more coverage than at 22 months. The Gulf lineage was found in 4 of 7 sites and in two sites (CC, SPC), plantings appeared to be growing with similar coverage to 22 months. However, at BLPS1, plantings appeared to have reduced cover in relation to coverage at 22 months. For

BLPS2, we were unable to determine whether area coverage at 32 months appeared to differ from BLPS2 due to missing imagery. Plantings of EU was found at only one site (CC) and appeared to be growing with similar coverage to area measured at 22 months. Lastly, *S. californicus* was found in 2 of 7 sites and in two sites (SPC and RF), plantings appeared to be growing of similar degree of cover at 22 months. Previously in August 2020 at CC and WTS, the only remaining plant species was *S. californicus*, however, approximately 10 months later, we found no surviving plantings of *S. californicus.* At this time, we did not estimate area coverage for this report. Aerial imagery of sites from 22-month and 32-month are found in the Appendix for Chapter 3 (Appendix B).

3.4. DISCUSSION

This study demonstrated that *P. australis* (Delta, EU and Gulf) and *S. californicus* could survive and grow when transplanted to dieback *P. australis* marsh habitats along the lower MRD with varying differences in plant survival and growth. Early plant survival of *P. australis* (regardless of lineage) was greater by 24.0% in relation to the survival of the other plant species (*P. australis* versus other). Surprisingly, we found that early plant survival for Gulf and Delta had comparably high survival whereas EU had ≥ 48 % lower survival relative to Gulf and Delta lineages. Overall *P. australis* survival was best in lower water depths, indicating potential sensitivity to deeper water levels. Among the non-*P. australis* species, *S, californicus* had the highest plant survival, which was comparable to both Gulf and Delta followed by *S. patens* and *Z. miliacea*.

Based on our findings, transplanting of vegetation can facilitate recovery in dieback areas but revegetation success may vary. The present observations suggest that none of the common wetland species other than *P. australis* were successful in revegetating dieback sites (at 32 months). Among *P. australis*, the most dominant lineage of the MRD, Delta, performed the best and surprisingly, EU had the lowest survival and area cover. Despite being more abundant on Delta, scale insects were likely not a limiting factor in the establishment and expansion of *P. australis* at about one-year post-planting. The observed scale abundances in our study were far less abundant than what has been normally seen in the MRD as observed in September by over 90 times greater (Knight et al., 2020).

Early Plant Survival

Successful revegetation may depend on habitat suitability and greatly on the choice of species or lineage. Also, short-term assessments (< 2 years) may not yield a clear picture of what plant sources will ultimately be successful in establishment. At the time of the survey, the *P. australis* lineages had survived at all of the seven sites. We found variable survival of plant species and *P. australis* lineages across the sites where *S.alterniflora,* Gulf and Delta lineage and *S. californicus* had > 60% mean survival whereas EU lineage, *S. patens*, and *Z. miliacea* had < 40% mean survival. Although the majority of the plants had relatively high survival rates at the beginning, subsequent surveys conducted from July 2019-June 2021 showed further loss of the planted vegetation for *P. australis* and other plant species. In Georgia, transplants of *S. alterniflora* and *Juncus roemerianus* in dieback areas had 100% survival after 6 months and planted clones grew and produced new tillers, suggesting that whatever caused the dieback had abated or disappeared within 1-2 years amount of time (Ogburn and Alber, 2006). However, early high mortality of plantings may be linked to environmental stressors (e.g. salinity, flooding, phytotoxins) if they are outside the normal range of intensity and duration, which is species-specific (Otte, 2001).

A number of studies have found that *S. alterniflora* and *S. patens* had substantial biomass reductions in lower surface elevations, prolonged waterlogging and elevated salinity suggesting that even subtle increases in sea level rise will be detrimental to plant health (Mendelssohn and McKee, 1988; McKee and Mendelssohn, 1989; Reed and Cahoon, 1992; Snedden et al., 2015). Following *P. australis* dieback in the MRD, both *S. californicus* and *Z. miliacea* appear unsuccessful to replace the newly open spaces (Hu et al., 2021), however, these *P. australis* habitats are often conditioned to high water levels and salinity stress which are unsuitable for these species. According to our findings of early plant survival, we suggest that non-*P. australis* plant species are not suitable for revegetating areas of dieback due to the highly dynamic environment of the MRD coupled with current climate change predictions of sea level rise and saltwater intrusion.

Plant survival was variable among the three *P. australis* lineages; however, this may be attributed to phenotypic differences between lineages influencing their survival in the early stages (Eller et al., 2017). After 6-months, Gulf had the highest total surviving plantings (346 out of 443) followed by Delta (326 out of 443) and EU (169 out of 443). This observation suggests that dieback conditions may have abated due to high survival of both Gulf and Delta. Whereas the low survival of EU lineage may be due to phenotypic differences that influence their survival in combination of environmental stressors involving growing and planting conditions (e.g., timing of planting) or site-specific environmental conditions (e.g., soil composition, flooding, salinity, temperature). Contrary to the high survival of Delta lineages, previous studies have reported high mortality of Delta lineage in the early stages of sprouting in individual nursery pots propagated from rhizome cuttings (Cronin et al., 2020). In Chapter 1, for our Spring-Summer Experiment (May 2019 – December 2019), 77% and 27% of the potted rhizome cuttings of Delta and EU populations failed to survive in 2.6 L pots containing soils sourced from either healthy or dieback soils, respectively (Gulf lineage was excluded from the Spring-Summer experiment). However, it remains unclear as to what causative agent induced the high mortality of Delta populations.

In the present study, we observed increasing water depth negatively affected early plant survival of EU lineage. Potential effects of planting season could have impeded the establishment of EU plantings since plants were installed at the end of the growing season in mid-October, in which, hydrologic conditions such as flooding and duration of inundation can influence plant growth and survival (Howard and Rafferty, 2006).

Specifically, high water levels in the following spring and summer shortens the survival window of plantings by hindering plant development due to submergence of shoots preventing oxygenation to any surviving rhizomes and new shoot growth (Pyšek et al., 2020). Weisner (1996) reported that young *P. australis* grown in organic sediments from a eutrophic lake had significant growth reductions when subjected to deep water levels (77 ± 2.0 cm) compared to shallow water levels (8.0 ± 2.0 cm) after 7 weeks. Harmful edaphic conditions induced by flooding can restrict oxygen transport to the roots from stems and rhizomes upslope which is necessary for vegetative spread (Weisner and Granéli, 1989; Weisner, 1996; Vretare Strand and Weisner, 2002; Weis and Weis, 2004). Armstrong et al. (1996b) observed that disrupted root functioning often interferes with nutrient uptake in *P. australis,* leading to stunted root and rhizome growth and high mortality of plants. High accumulation of organic materials and production of phytotoxins may cause further plant death impeding recovery of certain areas thus repeating the vicious cycle of dieback (Armstrong et al., 1996c). Adjustments to planting season could improve plant survival in which the timing would be complicated with high rates of sea level rise and recent unprecedented flooded conditions of the MRD (Day et al., 1993; NOAA, 2016).

Scale abundance differences of P. australis lineages

Our results support the growing evidence that support differences in susceptibility of *P. australis* lineages to Roseau Cane Scale infestations with the Delta lineage having higher scale abundances (Bumby, 2020; Cronin et al., 2020; Knight et al., 2020). In the MRD, Knight et al. (2018) found scale densities in Delta lineage were 2-7 times greater than in EU; however, our densities were much lower relative to previous findings. We found that scale density in Delta (1.08 ± 0.19) was 92.1% and 79.1% significantly greater relative to EU (0.52 ± 0.11) and Gulf (0.41 ± 0.11). At the same time, we found that the Delta lineage had the highest survival of 42% whereas EU lineage had only 11% survival across the sites. Together with the findings that Delta had more scales yet did better than the other lineages and high survival that scales were not a limiting factor in the establishment of *P. australis* within the first year. In addition, other biotic stressors, plant pathogens, microbial communities, or other marsh herbivores such as nutria (*Myocastor coypus)*, which can reduce plant biomass and inhibit plant recovery (Nyman et al., 1993). However, this may fluctuate over time and the buildup of scales could lead to reduced fitness of Delta lineage due to its greater susceptibility to scales (Bumby, 2020; Cronin et al., 2020; Knight et al., 2020).

Plant area cover at 22 months post-planting

While we did not find significant relationships between plant area cover and lineages at 22-months, the overall direction of our results showed promising trends that revegetation is feasible in dieback areas. Although Delta seems to be the most susceptible to exhibiting dieback syndrome, our findings suggest that within two years of monitoring that Delta lineage was able to tolerate flooding stress at the early stages of the trial, whereas EU lineage had low survival within the first 6-months of the study. However, survival was significantly lower in relatively high-water depth for the three lineages with deeper water levels most negatively affected survival of EU. These findings are not consistent with our findings in Chapter 1 and our predictions for the current chapter that EU lineage would be the most suitable plant for revegetation due to its phenotypic plasticity and greater capacity to adapt to a wide range of environmental conditions outside of its natural ranges allowing it to expand into higher salinity marshes (Chambers et al., 1999; Burdick and Konisky, 2003; Vasquez et al., 2005), efficiently assimilate nutrients in eutrophic conditions (Saltonstall and Court Stevenson, 2007; Mozdzer and Zieman, 2010; Tho et al., 2016) and increase aboveground biomass production in high water depths (Cronin et al., 2020). We suggest that harmful dieback soil conditions were no longer present within the plots but that low survival EU lineage may be less tolerant to the compounding stressors in the lower MRD. Interestingly, the Gulf lineage performed far beyond our expectations with comparable total area cover as Delta after 22-months. However, as a common inhabitant of dry, upland marsh habitats and the least salinity tolerant among the three lineages, we do not recommend planting Gulf lineage for restoration along rapidly subsiding marsh habitats in the Lower MRD (Achenbach and Brix, 2014). Further research is needed to understand the haplotypic differences and adaptability to environmental stressors among the lineages.

Of the habitat types, the most anticipated sites for successful revegetation were two shallow mudflats, Rattlesnake Flat and Chenier Pass Splay, which are actively accreting coarse sediments discharged from an adjacent channel. Here, we observed vigorous growth of the Delta lineage that had established a dense stand comparable to the nearest-neighboring *P. australis* patch (Figure B.8). The deposit of fresh sediments can provide a more favorable environment for plant establishment and growth by improving soil aeration and release from sulfides (Nyman et al., 1990; Mendelssohn and Kuhn, 2003; Slocum et al., 2005; Lane et al., 2006; Stagg and Mendelssohn, 2010; Baustian et al., 2012; Baustian and Mendelssohn, 2015), which should facilitate vertical accretion via vegetative growth (Nyman et al., 1993; Nyman et al., 2006).

In an effort to delay or reverse land loss, construction of artificial crevasses has become a powerful tool to mimic the natural deltaic processes restore hydrologic processes in the Lower MRD (Turner, 1997; Boyer et al., 1997). Natural crevasses occur as levee breaks under elevated water events resulting in fresh sediment flux to create land formations known as splays, which provides newly open habitats such as mudflats (Boyer et al., 1997). The unvegetated bare mudflats provide optimal conditions for the colonization of emergent vegetation such as fresh sediment deposits promoting rapid accretion and increased elevation. In our field trial, we had one artificially created crevasse (Chenier Crevasse) and its respective pass splay (Chenier Pass Splay). We observed high plant survival and vigorous growth of the Delta lineage in the shallow mudflat of CPS whereas survival of EU and Gulf plantings were reduced significantly after 2.5 years. In contrast, at Chenier Crevasse, no plantings (with the exception of on Delta planting) were present after 2.5 years which may be attributed to the submergence of plantings by floating vegetation, such as water hyacinths, and elevated water levels. However, these crevasse splays are not a permanent solution as the life spans of these habitats range from over 10 years to less than 150 years where the rapid vegetative growth will eventually lead to slower plant colonization, high root mortality and increasing decomposition resulting in peat collapse (Delaune et al., 1994; Boyer et al., 1997; Cahoon et al., 2011). Prior to transplantation, habitat suitability should be assessed to identify viable areas for successful establishment of plantings.

Within a year's span (22-month versus 32-month), we lost two sites that were vulnerable to high water levels and floating vegetation, particularly due to beds of water hyacinths outcompeting emergent vegetation of nutrients or through direct submergence (Villamagna and Murphy, 2010).

Implications for Restoration

The fate of the Lower MRD will depend on the stabilization of elevation by emergent vegetation, and as such, wetland elevation is at risk to the same stressors that affect plant growth such as eutrophication, salinity intrusion, elevated atmospheric CO_2 concentrations, increasing intensity and frequency of hurricanes and storms and flooding events (Cahoon et al., 2011). The Mississippi River delta is undoubtedly a profound case given its high rates of sea level rise (8.5-9.5 mm year^{-1}) and coastal wetland degradation (Day et al., 1993). As such, it is no surprise that the ongoing *P. australis* dieback in the Lower MRD is attributed to complex interactions of multiple abiotic and biotic stressors, making identification of the causal agent(s) difficult. While natural recolonization following dieback may occur, the rate of growth may be insufficient to revegetate vast regions of the MRD within an acceptable time frame with increasing anthropogenic activity and the changing climate (Barras et al., 2004; Cahoon et al., 2011). Since the 1950's, the Mississippi River's suspended sediment loading has been reduced by 50%, owing mainly to infrastructure construction of over 50,000 dams in the Mississippi basin (Maloney et al., 2018). The combination of multiple natural and anthropogenic activities has altered the geomorphology, hydrology and vegetation communities of the Lower MRD (Cahoon et al., 2011). With extensive reduction of sediment loadings, long-term survival of plantings will be contingent on the major drivers of land loss in the Lower MRD: vertical accretion deficient and high land subsidence rates that can exceed 1 cm/yr^{-1}. in some areas of Louisiana (Penland and Ramsey, 1990)

Contrary to the common assumption that vertical accretion is dependent on mineral sedimentation or in conjunction with organic matter accumulation, our preliminary findings support the growing number of practical restoration applications using vegetation plantings to promote vertical accretion for maintenance of marsh elevation. (McCaffrey and Thomson, 1980; Hatton et al., 1983; Bricker-Urso et al., 1989; Nyman et al., 1993; Callaway et al., 1997; Anisfeld et al., 1999; Rooth et al., 2003; Chmura and Hung, 2004). As a robust competitor against other wetland plants, the use of *P. australis* will be the best ecological and economical option in anticipation to predicted climate change impacts and increasing anthropogenic disturbances along the Mississippi River.

Vertical accretion via plant growth (McCaffrey and Thomson, 1980) involves mechanisms that promote the accumulation of organic matter originating from aboveground plant tissues (Craft et al., 1993; Rybczyk et al., 1998; Nyman et al., 2006), expansion of belowground root structures (Wolaver et al., 1988) and sediment trapping via surface litter has been attributed to increased soil elevation (Rooth et al., 2003). According to Rooth et al. (2003), *P. australis* may be 30% more effective than *S. alterniflora* in retaining storm sediments in subsiding marsh habitats due to litter accumulation and greater belowground productivity in marsh habitats in New England, USA. Additionally, the dense clonal stands of *P. australis* are connected through its extensive belowground rhizome network which facilitate nutrient acquisition and minimize potential biological and physiological stress (Lissner and Schierup, 1997). The large root system of *P. australis* allows for greater rhizosphere oxygenation under anoxic soil conditions which can outcompete other species for belowground nutrients and penetrate into deep, permeable soil layers for lower salinity water (Burdick et al., 2001). The advantages of its larger roots allow *P. australis* to occupy greater soil volume (Burdick and

Konisky, 2003) and the deep soil penetration enhances soil stabilization (Burdick et al., 2001). In brackish tidal marshes of Chesapeake Bay, Maryland McCormick et al. (2010) reported that area cover of *P. australis* patches increased by 25 times, from 7,294 m^2 to 183,369 m^2, over the 36 years from 1971 to 2007. Thus, if *P. australis* continues to expand at the restoration sites, it is anticipated to have a substantial long-term effect on the dynamics of soil surface elevation. Although our study is still ongoing and analysis of all data is incomplete, it is clear that transplanting the Delta lineage of *P. australis* would be the most efficient to facilitate recovery after dieback events the Lower MRD, indicated by the high plant area cover after two and half years.

In light of the considerable and ongoing land loss along Louisiana's coastal ecosystems, the addition of dredged sediments from adjacent water bodies has become a prominent restoration technique used to restore degraded marshes and to create new marsh habitats. The newly deposited dredge sediments provide suitable conditions for plant colonization with increased surface elevation, reduced flooding and improved edaphic conditions which enhances root aeration for plant survival (Nyman et al., 1990; Mendelssohn and Kuhn, 2003; Slocum et al., 2005; Lane et al., 2006; Stagg and Mendelssohn, 2010; Baustian et al., 2012; Baustian and Mendelssohn, 2015), which should facilitate vertical accretion via vegetative growth (Nyman et al., 1993; Nyman et al., 2006). Unfortunately, we were unable to assess the effects of dredge sediments on plant health of *P. australis* lineages in a field setting (plantings only included Delta, EU and Gulf) due to disposal of dredge sediments on the plantings after 6-months (SAW). However, in Chapter 1, our results indicated that Delta and EU plants grown in dredge soils had 36% and 6% lower biomass in relation to those grown in soils from healthy *P. australis* sites. Interestingly, relative to dieback soils, biomass on dredge soils was reduced by 47% and 40% for

Delta and EU, respectively. Facilitating vegetation establishment using plantings that rapidly increase area cover and increase soil surface elevation, particularly *P. australis* (Howard et al., 2019), may be critical for wetland restoration when natural colonization is delayed along dredge-restored marsh habitats. Future research should focus on the growth differences of *P. australis* lineages and potential limiting factors controlling plant growth in dredged sites.

Conclusions

Allocation of restoration efforts should be directed towards areas that are suitable for restoration to optimize successful revegetation; however, it will depend on the restoration strategy and the desired outcome whether it is possible to vegetate open water habitats, in areas following dieback or to facilitate vegetation establishment in areas restored with dredge sediment additions. In the Lower MRD, flooding and salinity stress can be too great for plant species to recolonize in mudflats created by subsidence of crevasse splays, but restoration potential could be optimized by providing suitable habitat conditions that increase elevation, reduced flooding and improved soil conditions. For *P. australis,* stressors including flooding, anoxic soil conditions eutrophication and reduced soil conditions have been implicated to induce blockages within the reed's gas transport pathways which impedes the aeration of the rhizome system leading to dieback syndrome (Armstrong et al., 1996a; Armstrong et al., 1996b; Armstrong et al., 2006). Thus, we recommend adding a thin layer of dredge sediment along degraded marsh habitats to alleviate flooding stress and lower buildup of soil organic matter preventing the release of phytotoxins (e.g., hydrogen sulfide). Based on our findings, geomorphological conditions may be the most critical indicator for determining the success of plantings.

Lastly, we provide recommendations for future restoration framework: (1) Consider geomorphological conditions from a wider geographic region and across multiple time scales for wetland formation. We suggest determining whether planting will be successful based on the surrounding geomorphological and hydrological conditions at sites. Additionally, data on age and condition of crevasse splay environments is needed for accurate habitat suitability. Rapid growth in areas are subjected to decline over time which could cause early loss of plantings.; (2) Consider cost effectiveness and less labor-intensive methods for plantings. Each planting was approximately $48.29 with an estimated 92% mortality of the total 2,071 plantings resulting in a total estimated loss of $92,0007 after 2.5 years. In addition, less labor-intensive methods should be considered, which will also reduce costs, such as transplanting of shoots and rhizomes of *P. austrais* has been deemed to be successful in China (Wen et al., 2012). Lastly, initial screening of site suitability such as collecting site-specific soil chemistry to determine key factors that may potentially restrict plant growth (Zhou et al., 2003). Finally, we suggest (3) considering long-term monitoring for climate change and adaptability. As the tallest lineage in the MRD, the Delta lineage may have greater adaptations to the rising sea levels and increasing intensity and frequency of hurricanes. Although our study is still ongoing and analysis of all data is incomplete, it is clear that transplanting the Delta lineage of *P. australis* would be the most beneficial to facilitate recovery in areas with slow natural revegetation.

The anthropogenic activity along the Mississippi River Delta has altered the geomorphology, hydrodynamics and vegetation communities including detrimental sediment load reductions and hydrologic fragmentation of the riverine system (Hu et al., 2021). To optimize restoration efforts and to improve wetland sustainability, further research is needed on the compounding environmental processes that govern the deltaic plain and their interaction with the plant communities. It is critical to improve our understanding of the dynamics of wetland formation and its relation to historical processes to use as a potential predictive tool for restoring degraded coastal wetlands in a rapidly changing river delta

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CHAPTER 4.
CONCLUSIONS.

Since 2015, there has been relatively little to no signs of recovery of *P. australis* dieback with concerns of whether it could grow in dieback soils. Given that previous dieback events have been associated with elevated levels of soil chemicals such as trace metals, phytotoxins, and organic matter content, I characterized chemical profiles of soils collected in the MRD from healthy and dieback stands of *P. australis*, and from recently excavated dredge disposal areas. Subsequently, I tested whether these different soil types impacted *P. australis* growth and also included three different *P. australis* lineages currently found in the Mississippi River Delta. Until now, there has been no characterization of the chemical profiles from these soil types in the MRD which provides considerable insight regarding the recovery and restoration of these coastal wetlands.

The potential for revegetation in areas of dieback is shown to be possible. *P.australis* has been able to grow in dieback soils in experimental garden and greenhouse settings as well as in the field. While dieback soils were found to have high concentrations of trace metals, sulfur and organic matter content, *P. australis* was able to grow in dieback soils within one growing season. Delta lineage, which is also the most dominant lineage, has been suspected to be the most susceptible to exhibiting dieback syndrome. The aboveground biomass of Delta, EU and Gulf responded differently to dieback soils with Gulf being the least affected and Delta being the most negatively affected in relation to plants that were grown in healthy soils; although these differences were not significant. While dredging and dieback locations are not ideal for *P. australis* development, plants may survive on these soils, and we suggest initiating restorative efforts as soon as possible to promote vertical accretion via vegetative growth.

Since the transplanting of *P. australis* lineages (Delta, EU and Gulf) and other wetland plant species including *S. alterniflora, S. patens, S. californicus* and *Z. miliacea* to assess which lineage or plant species will be the most successful for revegetation in the Lower MRD, we determined that Delta lineage had the highest survival and establishment after 2.5 years. The invasive EU lineage that has been shown to adapt to a wide range of environmental conditions had the lowest survival in the early stages of the trial and had failed to form monospecific stands. Instead, Delta and Gulf had high survival at 6 months and were able to successfully establish at the sites, proving that revegetation is possible. Additionally, both lineages had similar area cover after 2.5 years. We determined that Delta lineage had significantly higher scale densities as compared to EU and Gulf which supports previous findings suggesting Delta lineage is more susceptible to scales (Knight et al., 2018; Cronin et al., 2020; Knight et al., 2020). As a known causative agent for dieback, the buildup of Roseau Cane Scale on Delta could lead to a repeat of dieback within a few years, in which high densities of scale can be detrimental to plant health (Knight et al., 2018; Cronin et al., 2020; Knight et al., 2020).Thus, in the face of climate change, monitoring of short-term restoration projects is insufficient to understanding how the vegetation responds to the changing landscape of the lower Mississippi River Delta. Specifically, further studies should focus on long-term restoration strategies using *P. australis* to determine its ability to adapt to future sea-level rise and the changing climate

One way to prevent and manage dieback is to understand the factors that lead to it. White biotic factors, such as RCS, appear to cause dieback, abiotic factors such as environmental soil characteristics, salinity and hydrologic regime appear to determine future restoration success. Further research is needed to better understand how site and species characteristics affect recolonization following dieback events in the Lower Mississippi River. Continuing these lines of research is required to determine if revegetating degraded wetlands can be affordable.

APPENDIX A. SUPPLEMENTARY MATERIAL FOR CHAPTER 2.

Supplementary Material and Methods

Soil collection

Candidate sites were preselected from maps of the reported change in NDVI between 2008 and 2017 (Suir et al., 2018) and confirmation of stand status was confirmed by expert knowledge of Louisiana Department of Wildlife and Fisheries personnel. Sites categorized as healthy had minimal NDVI change in the previous three years and at the time of survey had dense stands of live *P. australis* stems often spanning > 1 ha in size. Sites categorized as dieback were from the highest category of dieback NDVI change (i.e., greatest decline in NDVI between 2016 and 2019). On-site inspection of those stands clearly revealed the telltale symptoms of dieback (van der Putten, 1997; Brix, 1999): stunted growth of stems, premature senescence of leaf tissue, the presence of dead meristematic tissue, high patchiness in the distribution of plants, few new live stems and extensive open spaces (Table 2.1).

The two dredged sediment locations were selected within the Pass-a-Loutre WMA of the Head of Passes Hopper Dredge Disposal Area (HDDA) created by the U.S. Army Corps of Engineers (Wingate, 2014) (Table 2.1). The first site was from South Sawdust Bend that had 115 acres of dredge sediment discharged approximately 6 months prior to collection (Wingate, 2014). The second site was from North Sawdust Bend that had 72 acres discharged approximately 1 year prior to collection (Wingate, 2014). Sediment material was dredged upriver and hydraulically discharged out at both locations. We note that at both sites, *P. australis* already had begun to establish during the collection trip on May 29, 2019.

At each site, 19 L of soil was excavated from each of three locations (separated by at least 5 m) and obtained from the surface to a depth of 0.5 m. For the dredge sites, soils were collected approximately 50 m from the water's edge in open areas free of vegetation. Soils from within a site were combined for use in the Winter-Spring or Spring-Summer plant-biomass experiments. At the same time, we also collected an additional ~ 250 g of soil from each of the three locations per site, placed each sample in a separate plastic bag and transported the bags on ice to the laboratory. Samples were stored at 4° C until soil chemistry tests were performed.

Chemical profile analysis of healthy, dieback and dredge soils

Procedures performed by the LSU AgCenter Soil Testing and Plant Analysis Lab for soil chemistry analysis were obtained from their website and are provided below (Table A.1; Stevens, 2017). For each soil sample, concentrations of Ca, Cu, K, Mg, Na, P, S, and Zn (ppm) were extracted using Mehlich 3 as the extractant in 2 g soil with 20 mL solution of 20 mL solution of 3.75 M NH_4F – 0.25 M EDTA NH_4NO_3, CH_3COOH, and HNO_3. Concentrations of Fe and Mn (ppm) were extracted from 10 g of soil with 20 mL of pH 7.3 and 0.005 M diethylenetriaminepentaacetic acid solution (DTPA). Determination of Al (ppm) was extracted from 2 g of soil with 20 mL solution of 0.1 M of $BaCl_2/NH_4Cl$. All three soil tests were analyzed using Inductively Coupled Plasma Optical Emission Spectroscopy (ICP - OES). For pH, the lab used a pH meter and electrode to measure the pH of the supernatant obtained from the mixture of 10 g of soil with 10 mL of deionized H_2O. Soil organic matter (OM), as a percentage, was determined from 1 g of soil using an acid-dichromate oxidation solution of 10 mL of 0.1 N potassium dichromate ($K_2Cr_2O_7$), 20 mL of concentrated sulfuric acid (H_2SO_4), and 90 mL of H_2O and analyzed using a Dip-Probe colorimeter. Lastly, % C and % N was measured by dry combustion using a LECO Carbon/Nitrogen Dumas Analyzer. Methods are summarized in Table A.2.

Table A.1. Overview of soil testing methods conducted by the LSU AgCenter Soil testing and Plant Analysis Lab.

Soil Test	Extractant	Conditions	Analysis	Reference
Phosphorous, Potassium, Calcium, Magnesium, Sodium, Sulfur, Copper, Zinc	Mehlich 3	2 g soil/20 mL solution, 5 min shaking (3.75 M NH4F – 0.25 M EDTA NH4NO3, CH3COOH, and HNO3)	ICP	Mehlich, 1984
pH	Water	10 g soil / 10 mL deionized H2O 2 hr. equilibration	pH meter + electrode	McLean, 1982
Organic Matter	1 g soil / 10 mL 1 N K2Cr2O7 + 20 mL conc. H2SO4 (wait 2 hours) + 90 mL H2O, 16 hr. equilibration	1 g soil / 10 mL 1 N K2Cr2O7 + 20 mL conc. H2SO4 (wait 2 hours) + 90 mL H2O, 16 hr. equilibration	Nelson and Sommer, 1982	Nelson and Sommer, 1982
Manganese, Iron, Copper, Zinc	DTPA	10 g soil / 20 mL pH 7.3, 0.005 M DTPA, 2 hr. shaking	ICP	Baker and Amacher, 1982
Aluminum	BaCl2 / NH4Cl	2 g soil / 20 mL 0.1 M BaCl2 / NH4Cl, 15 min shaking	ICP	Barnhisel and Bertsch, 1982
% C and % N		0.25 g soil	LECO Carbon/Nitrogen Dumas Analyzer.	Dumas Dry-Combustion

Winter-Spring Experiment

Rhizome cuttings from each source population were obtained from a common garden that has been maintained at Louisiana State University since 2010 (Bhattarai et al., 2017). The source populations used for this study (Table 2.2) had been growing under identical conditions in the garden for at least two years; thus, maternal environmental effects on plant growth metrics were likely to be minimal. We originally intended to have two representative populations for each lineage, however, a Delta population (PLM) was misclassified as EU. The EU population (EU3) and all three Delta populations originated from the lower MRD (Earl3, Earl4, PLM). Gulf populations originated from High Island, TX (HI) and Bayou Sauvage National Wildlife Refuge (SAU) in St. Tammany Parish, Louisiana.

Harvested rhizome material was rinsed to remove all sediment and cut into single fragments (15-20 g wet weight with at least one node). A single rhizome cutting was inserted upright into a 10.2 × 10.2 cm square plastic nursery pots and filled with either dieback or healthy soil. Each pot was placed into a separate 23 cm x 23 cm x 8 cm clear plastic tray filled with water. Separate watering trays were necessary to avoid movement of soil nutrients/contaminants between pots.

Pots were watered as often as needed by wetting the soil surface and filling the trays. On March 4, we added 5 ml fertilizer solution to each pot. The fertilizer was comprised of a mixture of 45 g of Miracle Gro (24-8-16 NPK, The Scotts Miracle-Gro Company®, Marysville, Ohio), 132 ml of Liquinox® (ironand zinc supplement; Liquinox Co., Orange, California) and 11.3 L of water. Because the purpose of this study was to evaluate plant growth in these different soils, we only provisioned the plants with an initial small inoculum of nutrients and iron at the time when plants were first sprouting new shoots (Cronin et al., 2020; Meyerson et al., 2020). Approximately five weeks later, pots that had no growth were repotted with fresh rhizome material from the same plant source population.

Spring-Summer Experiment

Rhizome sources for this experiment are provided in Table 2.3. For Delta, we used two of the three population sources used in Winter-Spring experiment (Earl3, Earl4; Table 2.3). For EU populations, we used TELM, originally sourced from Missouri and Earl2 from the Lower MRD

(Table 2.3). The Gulf lineage was excluded from this experiment because there was insufficient material available from the common garden.

Pools were watered twice a week, or as needed. After one month, we added 236 mL of the Miracle Gro – Liquinox solution to each pool (an equivalent amount to what was added to each individual pot in the previous experiment).

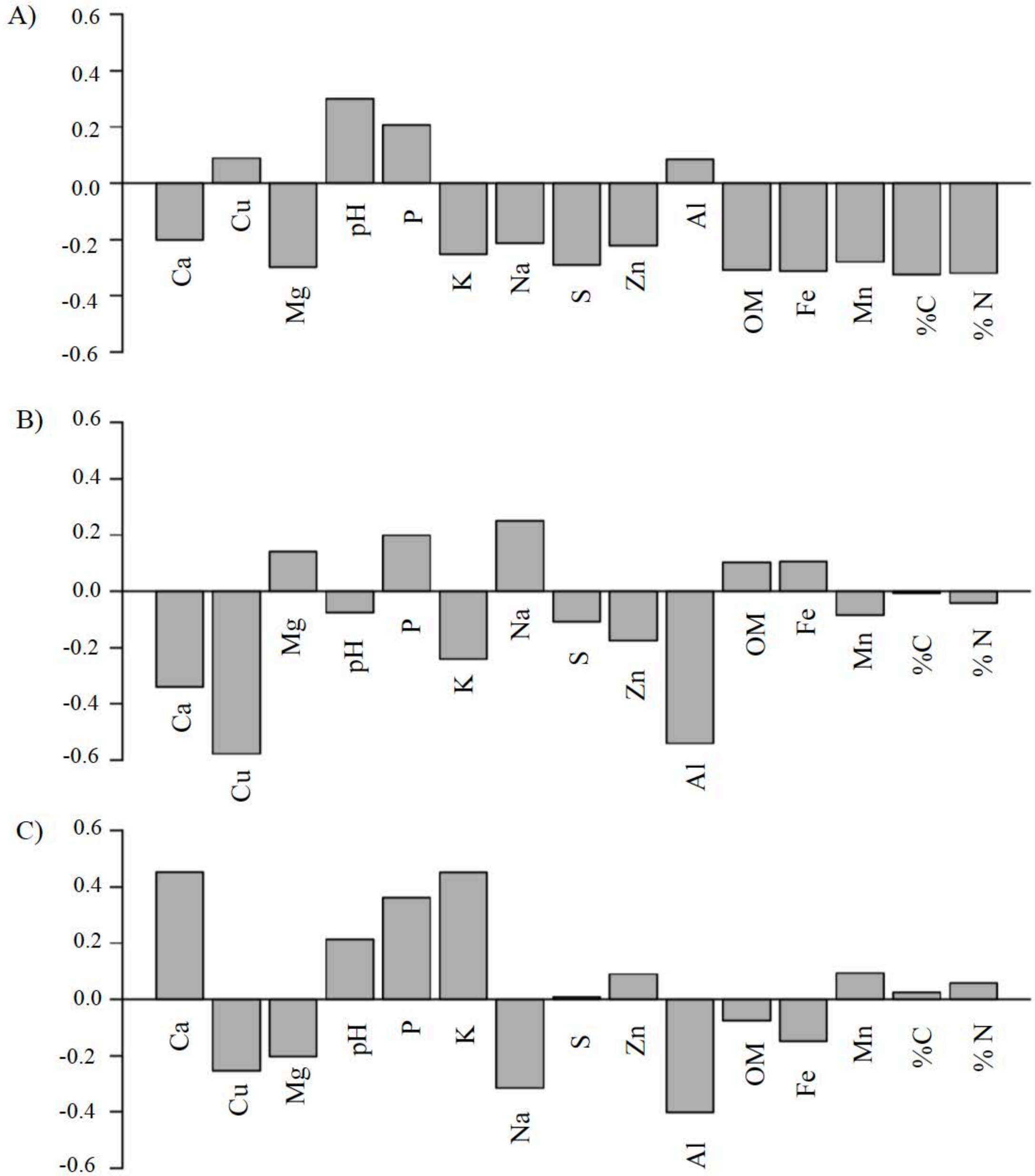


Figure A.1. The individual loadings of soil chemical properties for (A) PC1, (B) PC2 and (C) PC3 from the principal component analysis of Winter Soil Survey.

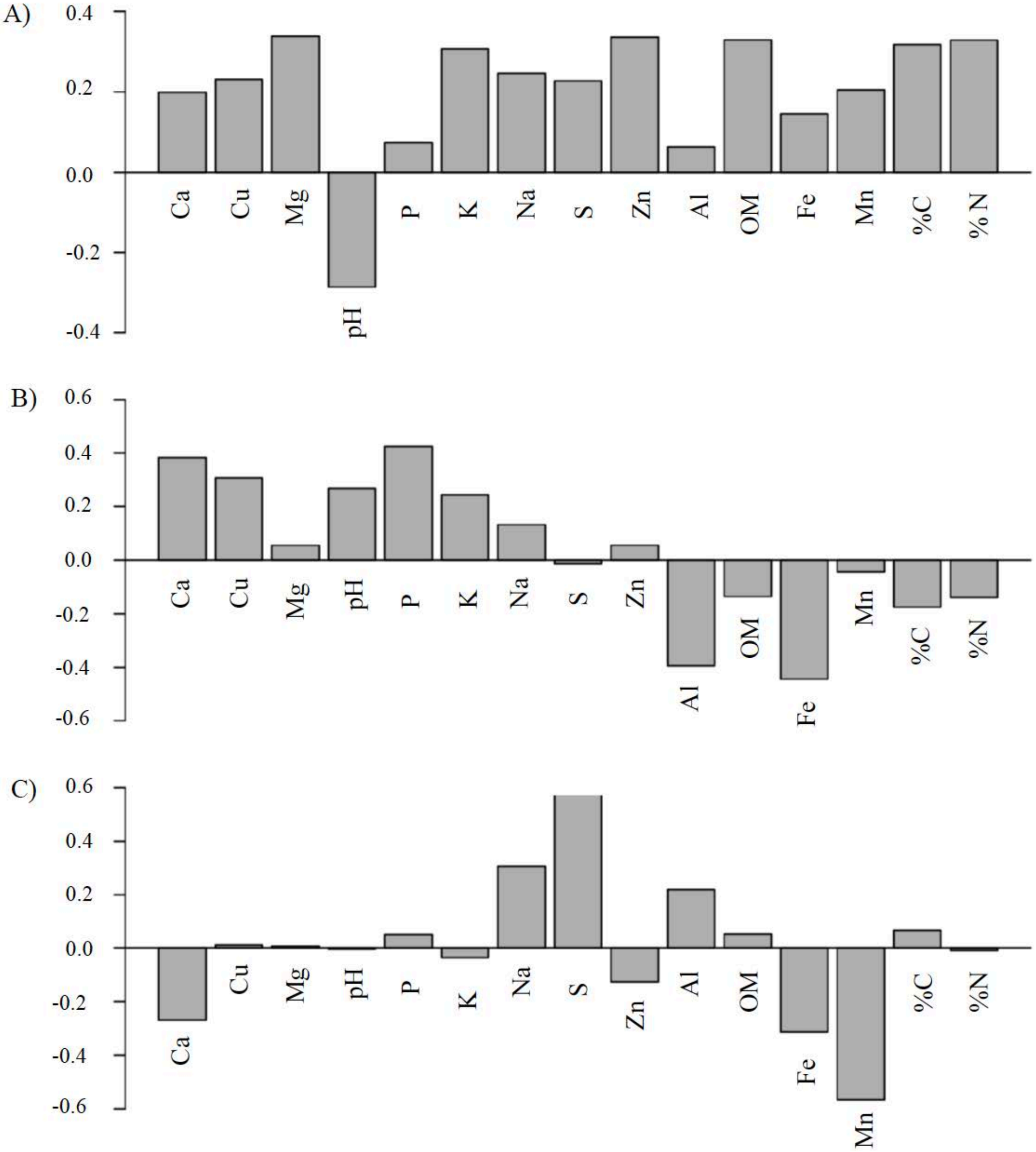


Figure A.2. The individual loadings of soil chemical properties for (A) PC1, (B) PC2 and (C) PC3 from the principal component analysis of Spring Soil Survey.

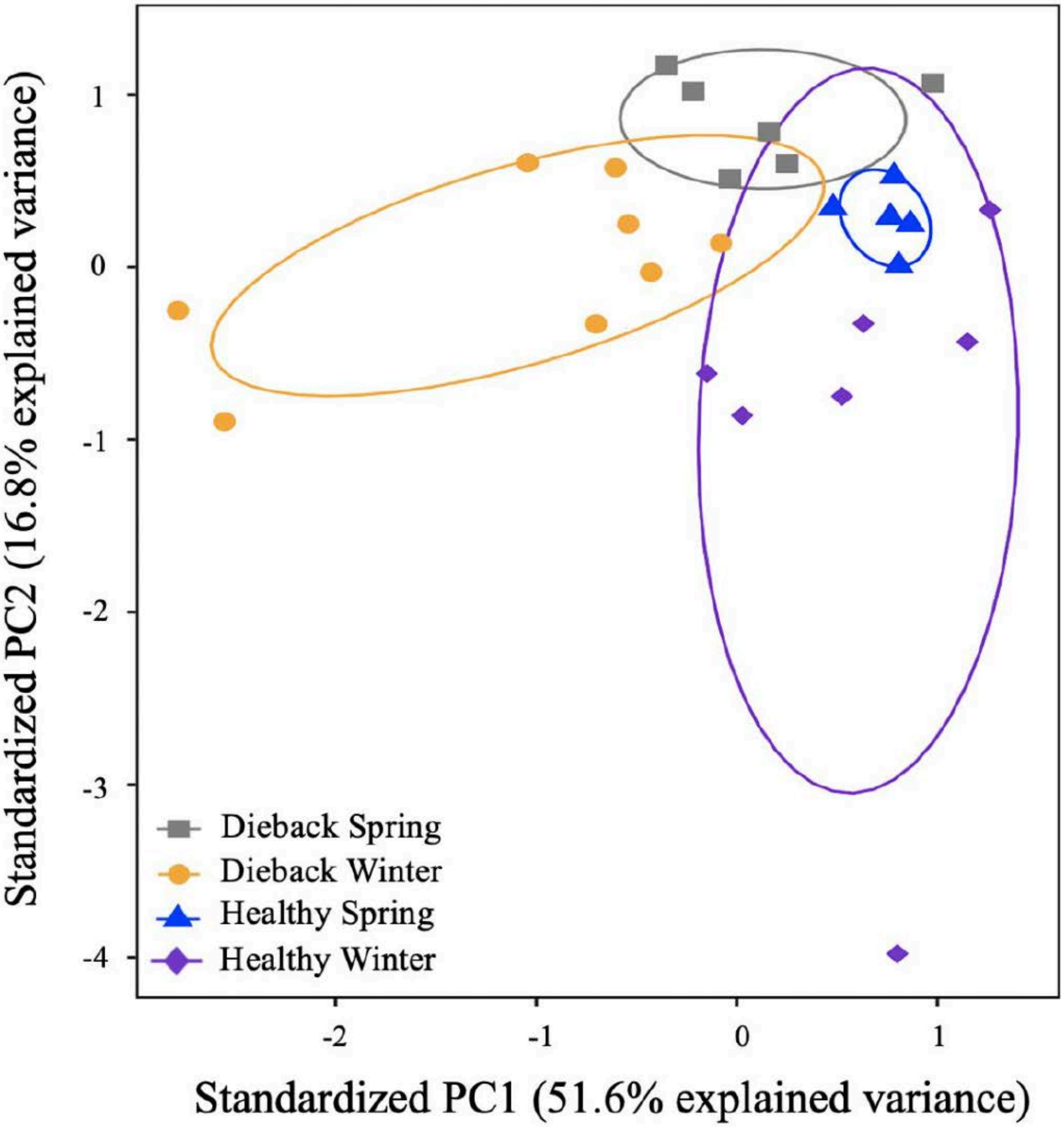


Figure A.3. Biplot of the first two principal components axes (PC1 and PC2) derived from 15 soil chemical characters in the combined Winter and Spring soil surveys. Principal component 1 (PC1) explained 51.6% of the variability and principal component 2 explained 16.8% variability.

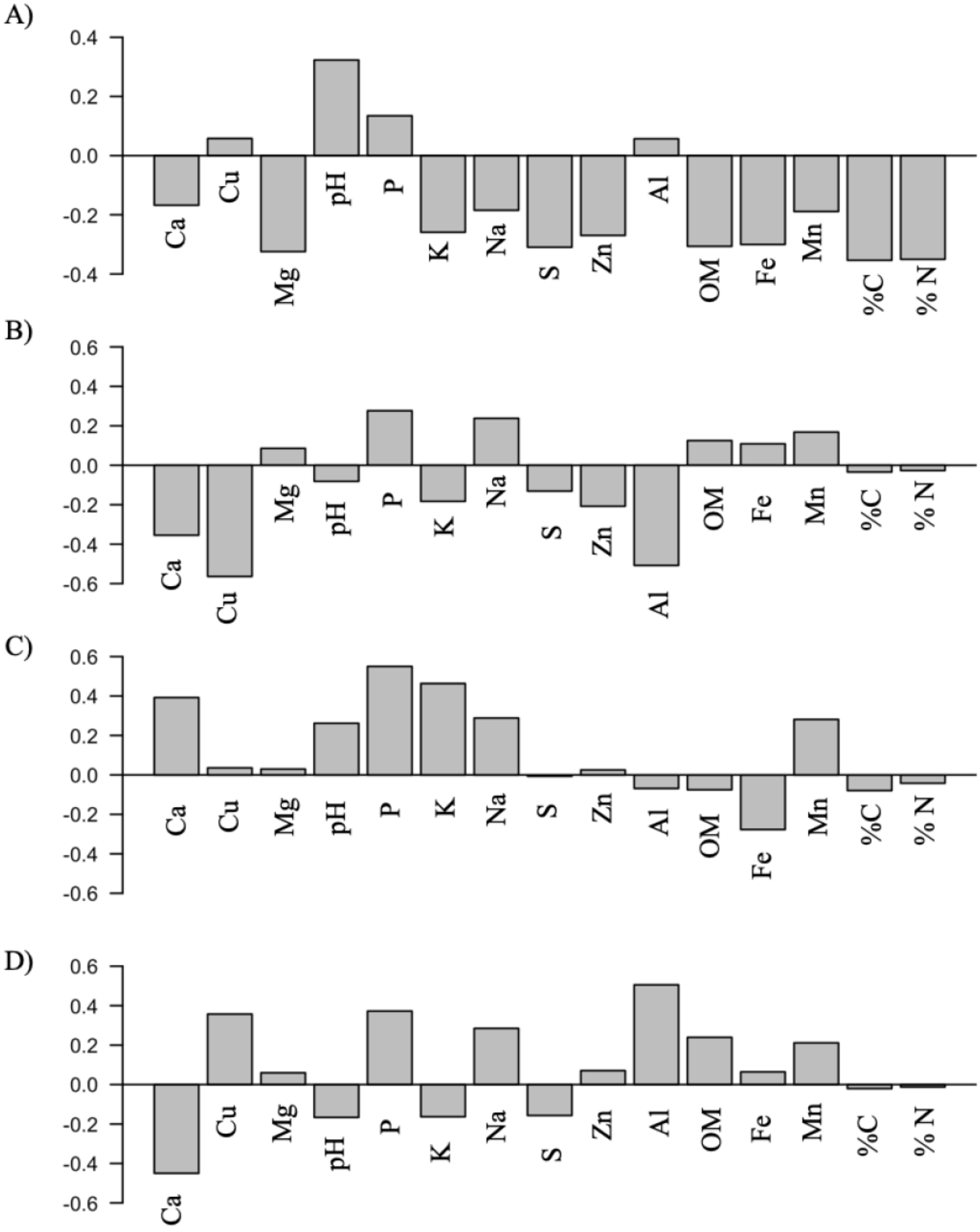


Figure A.4. The individual loadings of soil chemical properties for (A) PC1, (B) PC2, (C) PC3 and (D) PC4 from the principal component analysis of combined dataset of Winter and Spring Soil Surveys.

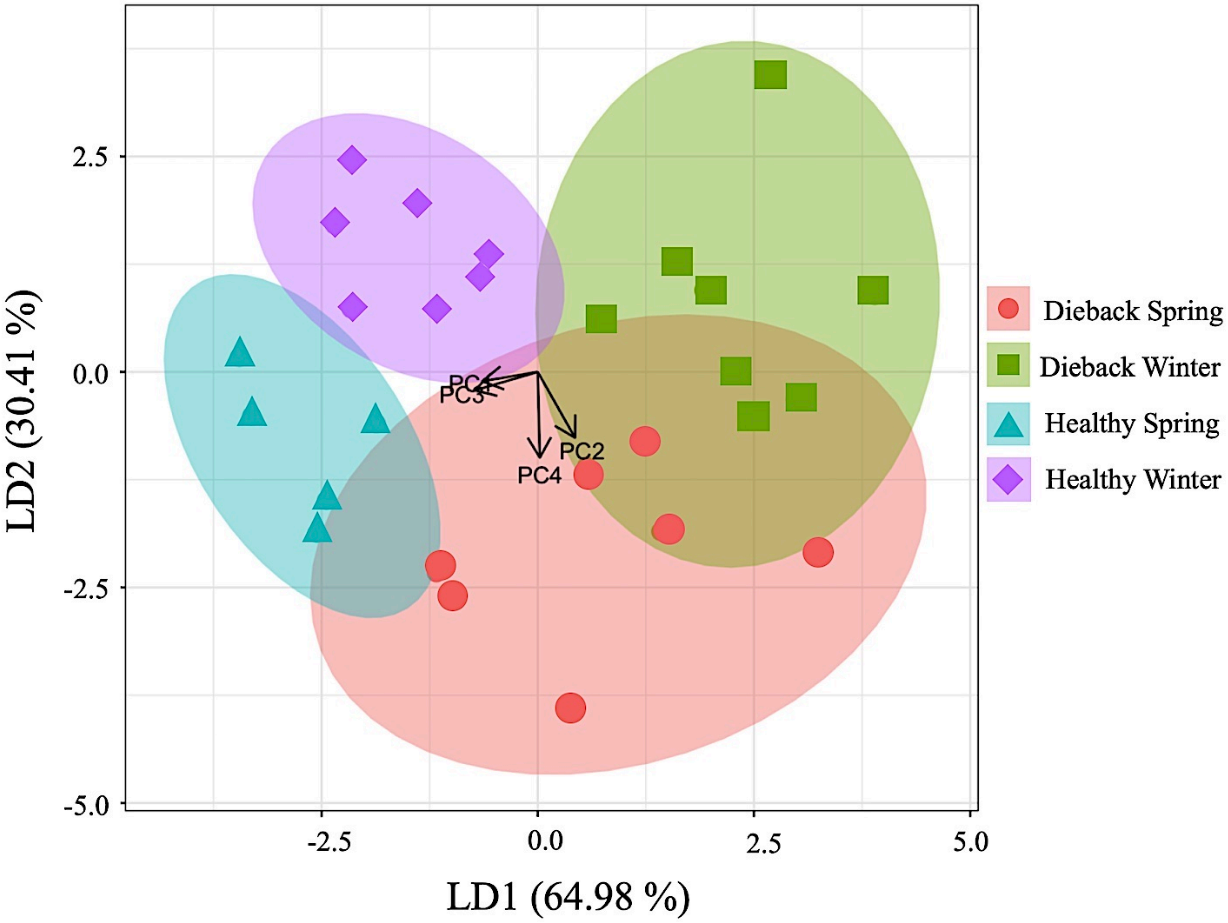


Figure A.5. Plot of linear discriminant analysis of PC1-4 obtained from the principal component analysis (PCA) using combined data set of Winter-Spring and Spring-Summer. Sample points and ellipses are color-coded based on soil type and season. 95% confidence ellipses from the linear discriminant analysis are reported.

Table A2.4. Prediction accuracy results for classifying soil types based on season using the Fisher's Linear Discriminant Analysis (LDA) for the combined dataset of Winter (dieback, healthy) and Spring (dieback, healthy) soils. The first four principal components (PC) scores derived from the Principal Component Analysis (PCA) were used as the explanatory variable.

	LDA-classified seasonal soil types			
Predicted	Dieback Spring	Dieback Winter	Healthy Spring	Healthy Winter
Dieback Spring	5	0	0	0
Dieback Winter	0	8	0	0
Healthy Spring	1	0	5	0
Healthy Winter	0	0	0	7
Overall accuracy				96%

Table 2.5. Correlation matrix (Pearson's product moment correlation, *R*) of correlations between soil chemical properties for the soils in the (**A**) Winter and (**B**) Spring soil surveys. Values in bold indicate strong correlations (critical value > 0.80) and (**C**) Combined dataset *** $P \leq 0.001$, ** $0.001 < P < 0.01$, * $0.01 < P \leq 0.05$.

A) Winter Spring Survey

	Ca	Cu	Mg	pH	P	K	Na	S	Zn	Al	%OM	Fe	Mn	%C	%N
Ca	1.00														
Cu	-0.24	1.00													
Mg	0.70**	-0.71**	1.00												
pH	-0.72**	0.65**	**-0.99*****	1.00											
P	**-0.82*****	0.31	**-0.86*****	**0.87*****	1.00										
K	**0.96*****	-0.45	**0.85*****	**-0.86*****	**-0.86*****	1.00									
Na	0.53*	-0.79***	**0.95*****	**-0.91*****	-0.75**	0.69**	1.00								
S	**0.85*****	-0.55*	**0.95*****	**-0.96*****	**-0.93*****	**0.94*****	**0.84*****	1.00							
Zn	0.79***	-0.42	**0.87*****	**-0.91*****	**-0.80*****	**0.90*****	0.69**	**0.89*****	1.00						
Al	-0.27	**0.99*****	-0.68**	0.62*	0.27	-0.47	-0.74**	-0.53*	-0.42	1.00					
%OM	0.74**	-0.71**	**1.00*****	**-0.99*****	**-0.86*****	**0.88*****	**0.94*****	**0.95*****	**0.88*****	-0.68**	1.00				
Fe	0.72**	-0.70**	**0.99*****	**-0.99*****	**-0.86*****	**0.87*****	**0.93*****	**0.96*****	**0.88*****	-0.67**	**0.99*****	1.00			
Mn	**0.85*****	-0.58*	**0.94*****	**-0.94*****	**-0.92*****	**0.94*****	**0.85*****	**0.99*****	**0.84*****	-0.57	**0.95*****	**0.96*****	1.00		
%C	**0.80*****	-0.64**	**0.98*****	**-0.99*****	**-0.89*****	**0.92*****	**0.89*****	**0.98*****	**0.91*****	-0.62*	**0.99*****	**0.99*****	**0.98*****	1.00	
%N	**0.82*****	-0.61*	**0.97*****	**-0.98*****	**-0.90*****	**0.94*****	**0.86*****	**0.99*****	**0.92*****	-0.60*	**0.98*****	**0.98*****	**0.98*****	**1.00*****	1.00

B) Spring Soil Survey

	Ca	Cu	Mg	pH	P	K	Na	S	Zn	Al	%OM	Fe	Mn	%C	%N
Ca	1.00														
Cu	0.29	1.00													
Mg	0.65**	0.08	1.00												
pH	-0.12	-0.11	-0.72	1.00											
P	0.20	0.71**	-0.12	0.17	1.00										
K	**0.81*****	0.37	**0.88*****	-0.53*	0.24	1.00									
Na	0.45	-0.07	0.79	-0.40	0.04	0.74	1.00								
S	0.17	0.14	0.65**	-0.55*	-0.14	0.55*	0.67**	1.00							
Zn	0.45	**0.80*****	0.52*	-0.58*	0.56*	0.68**	0.27	0.28	1.00						
Al	-0.22	-0.20	0.17	-0.29	-0.28	-0.12	0.13	0.26	0.00	1.00					
%OM	0.33	0.36	0.79	**-0.90*****	0.09	0.70**	0.49*	0.62**	0.78	0.28	1.00				
Fe	-0.06	-0.20	0.35	-0.60*	-0.45	0.13	0.14	0.03	0.37	0.56*	0.48*	1.00			
Mn	0.38	0.37	0.55*	-0.48*	0.08	0.49*	0.25	0.04	0.59*	0.00	0.50*	0.50*	1.00		
%C	0.24	0.27	0.76	**-0.90*****	0.10	0.64**	0.54*	0.58*	0.72	0.31	**0.97*****	0.53*	0.50*	1.00	
%N	0.33	0.35	0.78	**-0.92*****	0.11	0.70**	0.48*	0.56*	0.78	0.26	**0.99*****	0.52*	0.55*	**0.98*****	1.00

C) Combined Dataset

	Ca	Cu	Mg	pH	P	K	Na	S	Zn	Al	% OM	Fe	Mn	% C	% N
Ca	1.00														
Cu	0.18*	1.00													
Mg	0.45*	-0.27	1.00												
pH	-0.17	0.19	**-0.80*****	1.00											
P	-0.44*	0.05	-0.47**	0.34	1.00										
K	**0.80*****	0.06	0.62***	-0.45*	-0.24	1.00									
Na	0.20	-0.32	0.74***	-0.44*	-0.11	0.42*	1.00								
S	0.58***	0.00	0.66***	-0.76***	-0.41*	0.65***	0.34	1.00							
Zn	0.29	0.29	0.37*	-0.55**	0.07	0.49**	0.10	0.47**	1.00						
Al	0.13	**0.86*****	-0.16	0.12	-0.15	0.03	-0.19	-0.01	0.09	1.00					
% OM	0.17	-0.20	**0.80*****	**-0.84*****	-0.20	0.54**	0.56**	0.55**	0.57***	-0.15	1.00				
Fe	0.16	-0.29	0.70***	**-0.85*****	-0.43*	0.36	0.39*	0.67***	0.53**	-0.18	0.72***	1.00			
Mn	0.14	-0.20	0.37	-0.45*	0.19	0.40*	0.30	0.49*	0.14	-0.18	0.40*	0.52	1.00		
% C	0.45*	-0.12	**0.81*****	**-0.90*****	-0.39*	0.67***	0.46**	**0.86*****	0.63***	-0.09	**0.85*****	**0.83*****	0.48*	1.00	
% N	0.44*	-0.11	0.78***	**-0.91*****	-0.32	0.70***	0.39*	**0.87*****	0.64***	-0.10	**0.83*****	**0.80*****	0.54**	**0.98*****	1.00

Table A.1. Separate *t*-test results for the difference in soil chemicals between healthy and dieback sites from the Winter soil survey. Significance, denoted by * adjacent to the *P*-value, was determined using Bonferroni-corrected values of α.

Variable	Soil Type	*n*	Mean	SE	*t*	*P*-value
Al (ppm)	Dieback	8	0.58	0.10	-1.22	0.308
	Healthy	7	2.58	1.64		
Ca (ppm)	Dieback	8	3992.48	297.21	0.04	0.968
	Healthy	7	4017.69	284.89		
Cu (ppm)	Dieback	8	1.16	0.11	-1.40	0.261
	Healthy	7	1.89	0.53		
Fe (ppm)	Dieback	8	160.82	16.12	5.33	**<0.001***
	Healthy	7	71.06	4.93		
K (ppm)	Dieback	8	261.66	28.61	0.90	0.414
	Healthy	7	231.66	23.37		
Mg (ppm)	Dieback	8	1174.23	53.25	7.47	**<0.001***
	Healthy	7	620.78	52.18		
Mn (ppm)	Dieback	8	127.84	52.49	1.85	0.145
	Healthy	7	30.27	6.28		
Na (ppm)	Dieback	8	426.77	51.71	2.98	**0.027***
	Healthy	7	230.48	28.65		
% OM	Dieback	8	5.77	0.34	5.21	**<0.001***
	Healthy	7	3.07	0.36		
P (ppm)	Dieback	8	27.76	1.42	-2.21	0.085
	Healthy	7	34.20	2.46		
pH	Dieback	8	5.80	0.27	-6.55	**<0.001***
	Healthy	7	7.40	0.06		
S (ppm)	Dieback	8	1121.41	404.80	2.19	0.106
	Healthy	7	280.07	57.66		
Zn (ppm)	Dieback	8	9.07	0.57	1.94	0.112
	Healthy	7	7.28	0.58		
% C	Dieback	8	5.51	0.80	3.84	**0.011***
	Healthy	7	2.19	0.30		
% N	Dieback	8	0.51	0.08	3.69	**0.014***
	Healthy	7	0.19	0.02		

Table A.2. Analysis of variance results for the effect of different soil types (healthy, dieback, dredge) on separate soil chemical characteristics from the Spring survey. Post-Hoc Tukey HSD tests were used to assess differences between treatment pairs. For each variable, means with different letters are significantly different (*P* ≤ 0.05). Significance denoted by * adjacent to the *P*-value.

Variable	Soil Type	Mean	SE	*F*	*P*-value
Al (ppm)	Healthy	0.64^{a}	0.05	1.51	0.25
	Dieback	0.93^{a}	0.07		
	Dredge	0.79^{a}	0.18		
Ca (ppm)	Healthy	3546.70^{a}	366.1	12.30	**0.001***
	Dieback	2412.31^{b}	199.37		
	Dredge	1653.18^{b}	218.48		
Cu (ppm)	Healthy	1.57^{a}	0.26	5.983	**0.012***
	Dieback	1.09^{ab}	0.14		
	Dredge	0.74^{b}	0.03		
Fe (ppm)	Healthy	64.3^{ab}	7.52	4.41	**0.033***
	Dieback	114.01^{a}	20.04		
	Dredge	51.11^{b}	15.83		
K (ppm)	Healthy	219.87^{a}	21.45	14.51	**<0.001***
	Dieback	209.63^{b}	23.83		
	Dredge	82.47^{b}	13.52		
Mg (ppm)	Healthy	617.43^{a}	94.82	31.87	**<0.001***
	Dieback	823.07^{a}	76.96		
	Dredge	279.04^{b}	23.55		
Mn (ppm)	Healthy	136.84^{ab}	36.66	5.33	**0.02***
	Dieback	153.66^{a}	28.40		
	Dredge	42.25^{b}	12.68		
Na (ppm)	Healthy	282.87^{a}	66.58	2.072	0.161
	Dieback	381.88^{a}	87.75		
	Dredge	192.60^{a}	29.1		
% OM	Healthy	2.89^{a}	0.23	35.75	**<0.001***

	Dieback	5.29^{b}	0.61		
	Dredge	0.73^{c}	0.12		
P (ppm)	Healthy	72.89^{a}	8.75	1.973	0.174
	Dieback	54.10^{a}	6.39		
	Dredge	58.7^{a}	5.33		
pH	Healthy	7.43^{a}	0.06	104.20	**<0.001***
	Dieback	6.41^{b}	0.09		
	Dredge	7.71^{a}	0.04		
S (ppm)	Healthy	44.70^{ab}	10.37	4.171	**0.036***
	Dieback	100.89^{a}	29.98		
	Dredge	27.68^{b}	7.02		
Zn (ppm)	Healthy	7.58^{a}	0.89	15.70	**<0.001***
	Dieback	7.34^{a}	0.22		
	Dredge	3.01^{b}	0.89		
% C	Healthy	1.42^{a}	0.12	25.79	**<0.001***
	Dieback	3.04^{b}	0.42		
	Dredge	0.49^{c}	0.27		
% N	Healthy	0.05^{a}	0.01	31.77	**<0.001***
	Dieback	0.30^{b}	0.04		
	Dredge	0.17^{c}	0.00		

Table A.3. Analysis of variance results for the effect of seasons and different soil types on separate soil chemical characteristics from combined data sets from the Winter and Spring soil surveys. Post-Hoc Tukey groups for significant differences are denoted by letters. For each variable, means with different letters are significantly different ($P \leq 0.05$). Significance, denoted by * adjacent to the *P*-value

Variable	Soil Type	Season	Mean	SE	*F*	P - value
Al	Healthy	Winter	3.16^{a}	1.09	1.24	0.32
	Healthy	Spring	0.65^{a}	1.29		
	Dieback	Winter	0.61^{a}	1.02		
	Dieback	Spring	0.93^{a}	1.18		
Ca	Healthy	Winter	4117.17^{a}	265	8.73	**<0.001***
	Healthy	Spring	3899.00^{a}	314		
	Dieback	Winter	4135.36^{a}	248		
	Dieback	Spring	2412.31^{b}	286		
Cu	Healthy	Winter	2.14^{a}	0.36	1.73	0.189
	Healthy	Spring	1.32^{a}	0.43		
	Dieback	Winter	1.20^{a}	0.34		
	Dieback	Spring	1.09^{a}	0.39		
Fe	Healthy	Winter	71.06^{a}	13.7	10.62	**<0.001***
	Healthy	Spring	64.30^{a}	16.2		
	Dieback	Winter	160.82^{b}	12.8		
	Dieback	Spring	114.01^{ab}	14.8		
K	Healthy	Winter	239.87^{a}	25.2	1.12	0.362
	Healthy	Spring	235.50^{a}	29.8		
	Dieback	Winter	274.52^{a}	23.6		
	Dieback	Spring	209.63^{a}	27.2		
Mg	Healthy	Winter	628.16^{a}	56	21.72	**<0.001***
	Healthy	Spring	708.68^{a}	66.3		
	Dieback	Winter	1201.34^{b}	52.4		
	Dieback	Spring	823.07^{a}	60.5		
Mn	Healthy	Winter	30.27^{a}	36.7	2.20	0.117
	Healthy	Spring	136.84^{a}	43.4		
	Dieback	Winter	127.84^{a}	34.3		
	Dieback	Spring	153.66^{a}	39.6		
Na	Healthy	Winter	59.20^{a}	59.2	2.19	0.117
	Healthy	Spring	70.10^{a}	70.1		
	Dieback	Winter	55.40^{a}	55.4		
	Dieback	Spring	64.00^{a}	64		
OM	Healthy	Winter	3.15^{a}	0.41	13.77	**<0.001***
	Healthy	Spring	2.76^{a}	0.49		
	Dieback	Winter	5.96^{b}	0.38		
	Dieback	Spring	5.29^{b}	0.44		
P	Healthy	Winter	32.88^{a}	3.34	23.70	**<0.001***
	Healthy	Spring	64.67^{b}	3.95		

	Dieback	Winter	28.20 ^{a}	3.12		
	Dieback	Spring	54.10 ^{b}	3.61		
pH	Healthy	Winter	7.39 ^{a}	0.17	26.36	**<0.001***
	Healthy	Spring	7.44 ^{a}	0.2		
	Dieback	Winter	5.66 ^{b}	0.16		
	Dieback	Spring	6.41 ^{c}	0.18		
S	Healthy	Winter	288.80 ^{ab}	265	4.60	**0.012***
	Healthy	Spring	51.28 ^{a}	313		
	Dieback	Winter	1249.89 ^{b}	248		
	Dieback	Spring	100.89 ^{a}	286		
Zn	Healthy	Winter	7.49 ^{ab}	0.57	3.47	**0.034***
	Healthy	Spring	6.69 ^{a}	0.67		
	Dieback	Winter	9.26 ^{b}	0.53		
	Dieback	Spring	7.34 ^{ab}	0.61		
% C	Healthy	Winter	2.36 ^{a}	0.57	11.47	**<0.001***
	Healthy	Spring	1.33 ^{a}	0.67		
	Dieback	Winter	5.83 ^{b}	0.53		
	Dieback	Spring	3.04 ^{a}	0.61		
% N	Healthy	Winter	0.20 ^{a}	0.06	8.84	**<0.001***
	Healthy	Spring	0.16 ^{a}	0.07		
	Dieback	Winter	0.54 ^{b}	0.05		
	Dieback	Spring	0.31 ^{a}	0.06		

Table A.4. Analysis of variance results for the effects of soil type (dieback, healthy), *P. australis* lineage (Delta, EU, Gulf), and the lineage × soil type interaction on A) *ln* total stem count per pot and B) max stem height for Winter-Spring Experiment and for the effects of soil type (dieback, healthy, dredge), *P. australis* lineage (Delta, EU), and the lineage × soil type interaction on C) total stem count per pot and D) max stem height for Spring-Summer Experiment. Bolded sources of variation indicate significance ($P \leq 0.05$).

	Sums of Squares	*df* (num)	*df* (denom)	*F*	*P*-value
A) *ln* total stem count per pot					
Lineage	0.38	2	2.99	1.87	0.297
Soil Type	0.78	1	9.5	7.7	**0.021***
Lineage × Soil Type	0.12	2	225.63	0.58	0.562
B) Max stem height					
Lineage	0.15	2	3	2.16	0.263
Soil Type	0.01	1	228.26	0.19	0.66
Lineage × Soil Type	0.13	2	228.3	1.91	0.151
C) Total stem count per pot					
Lineage	2.67	1	1.77	0.16	0.733
Soil Type	405.9	2	22.86	12.1	**< 0.001***
Lineage × Soil Type	15.06	2	122.64	0.45	0.639
D) Max stem height					
Lineage	818.5	1	1.68	3.66	0.22
Soil Type	784.74	2	17.51	1.75	0.202
Lineage × Soil Type	1579.58	2	113.07	3.53	**0.033***

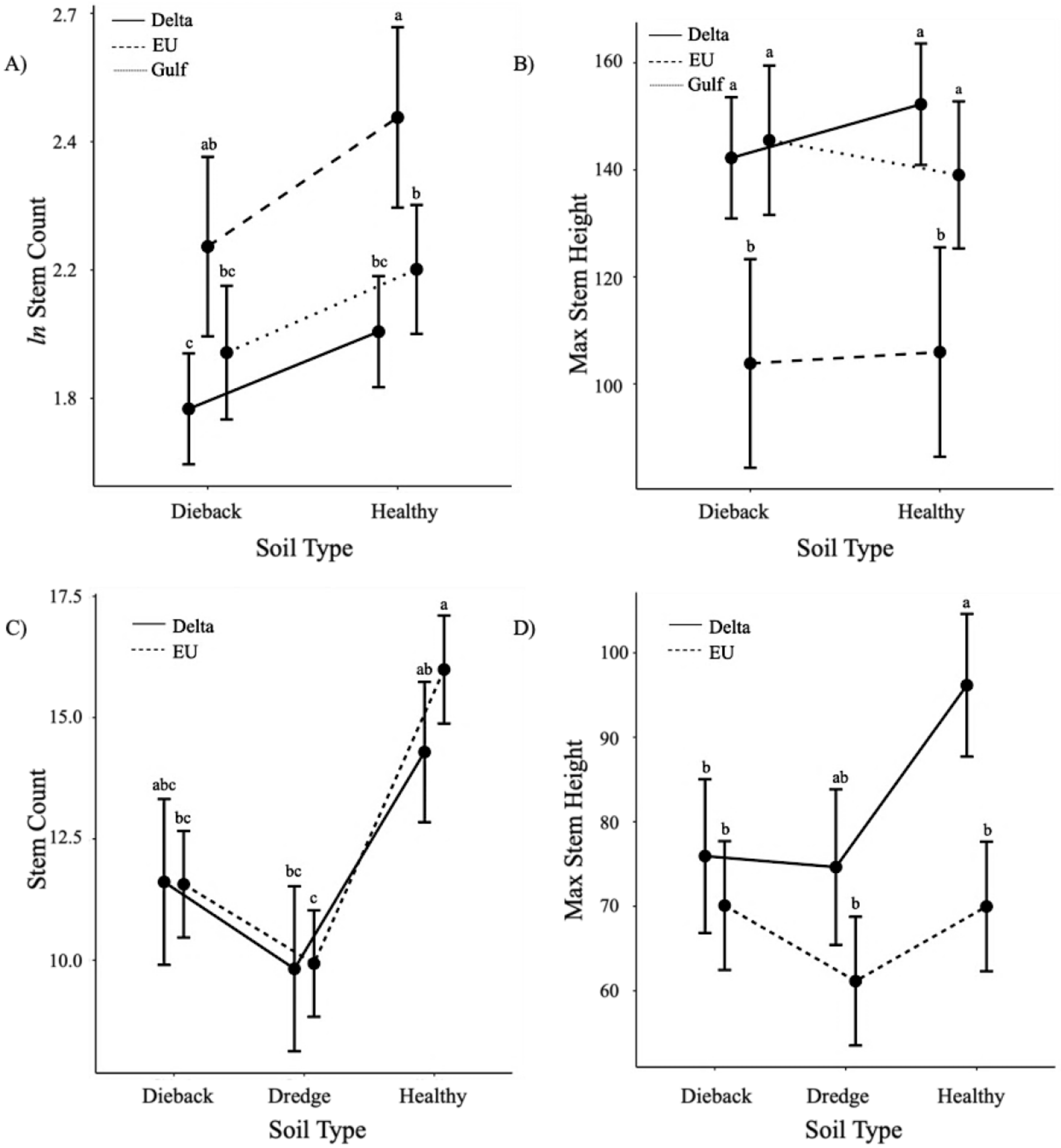


Figure A.6. The effects of soil type (dieback, healthy) and *P. australis* lineage (Delta, EU, Gulf) on A) *ln* total stem count per pot and B) max stem height (cm) for Winter-Spring Experiment and the effects of soil type (dieback, healthy, dredge) and *P. australis* lineage (Delta, EU) on C) total stem count per pot and D) max stem height (cm) for Spring-Summer Experiment. Reported values are marginal means ± SE. Different letters between groups indicate significant differences at $P \leq 0.05$.

APPENDIX B. SUPPLEMENTARY MATERIAL FOR CHAPTER 3

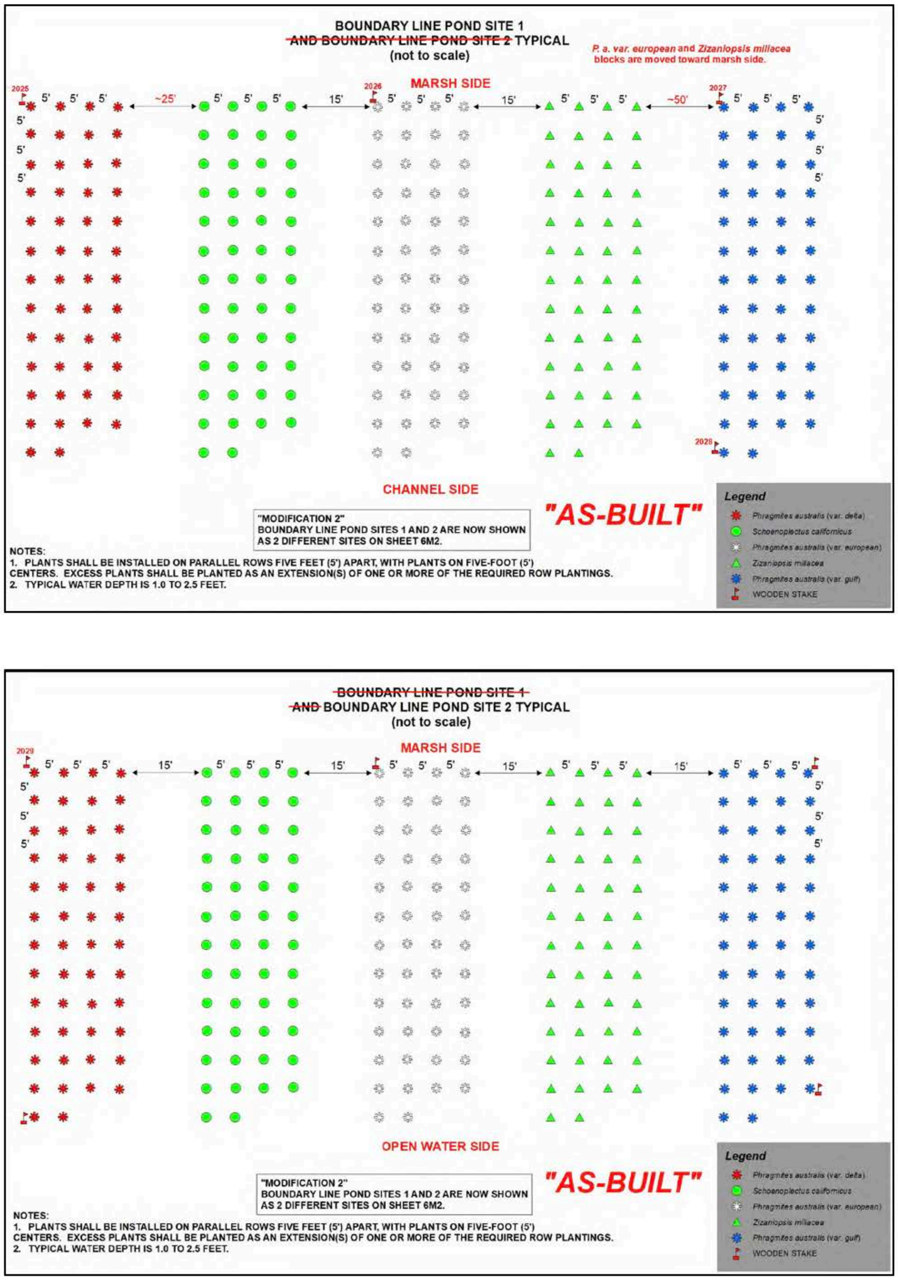
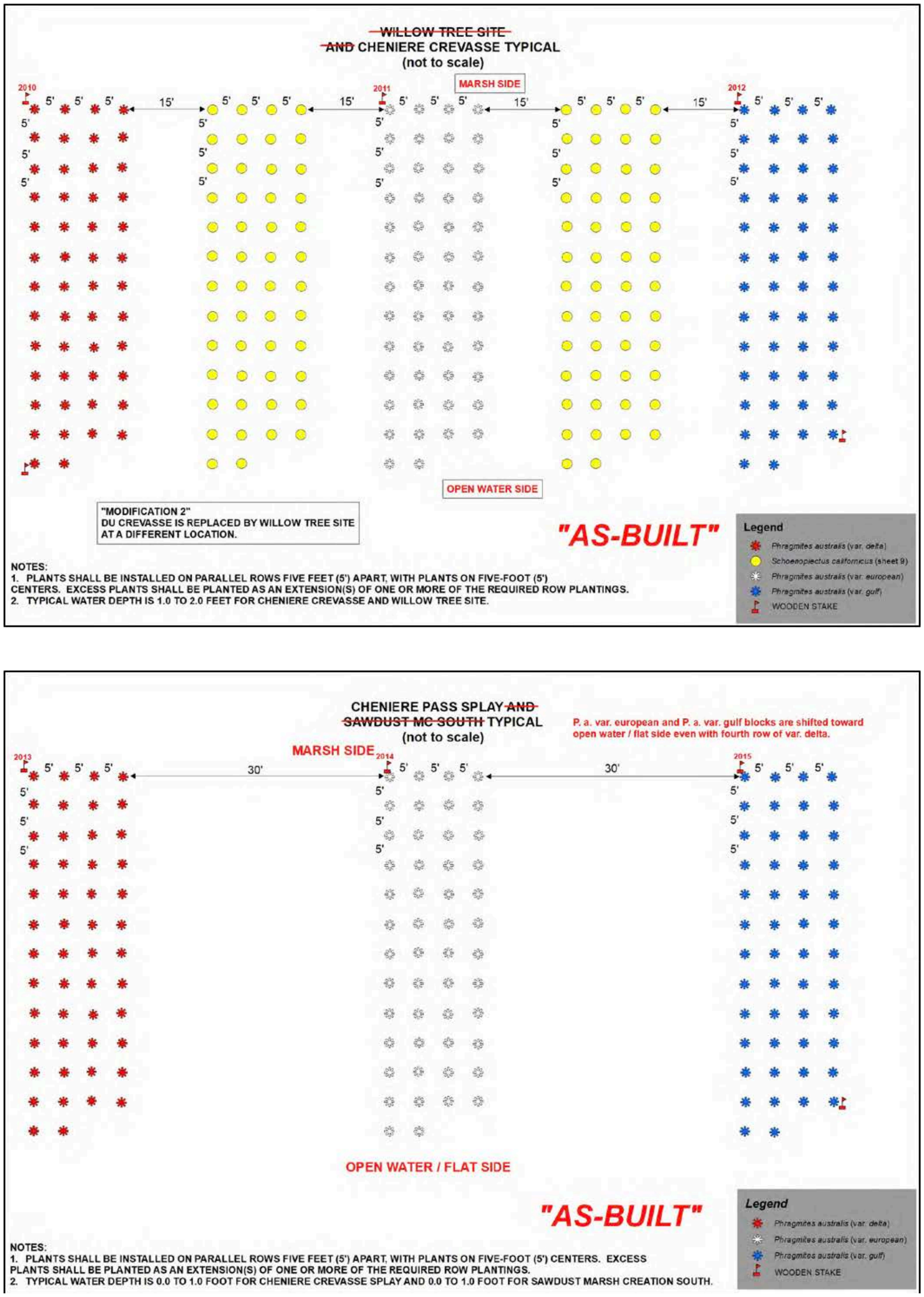


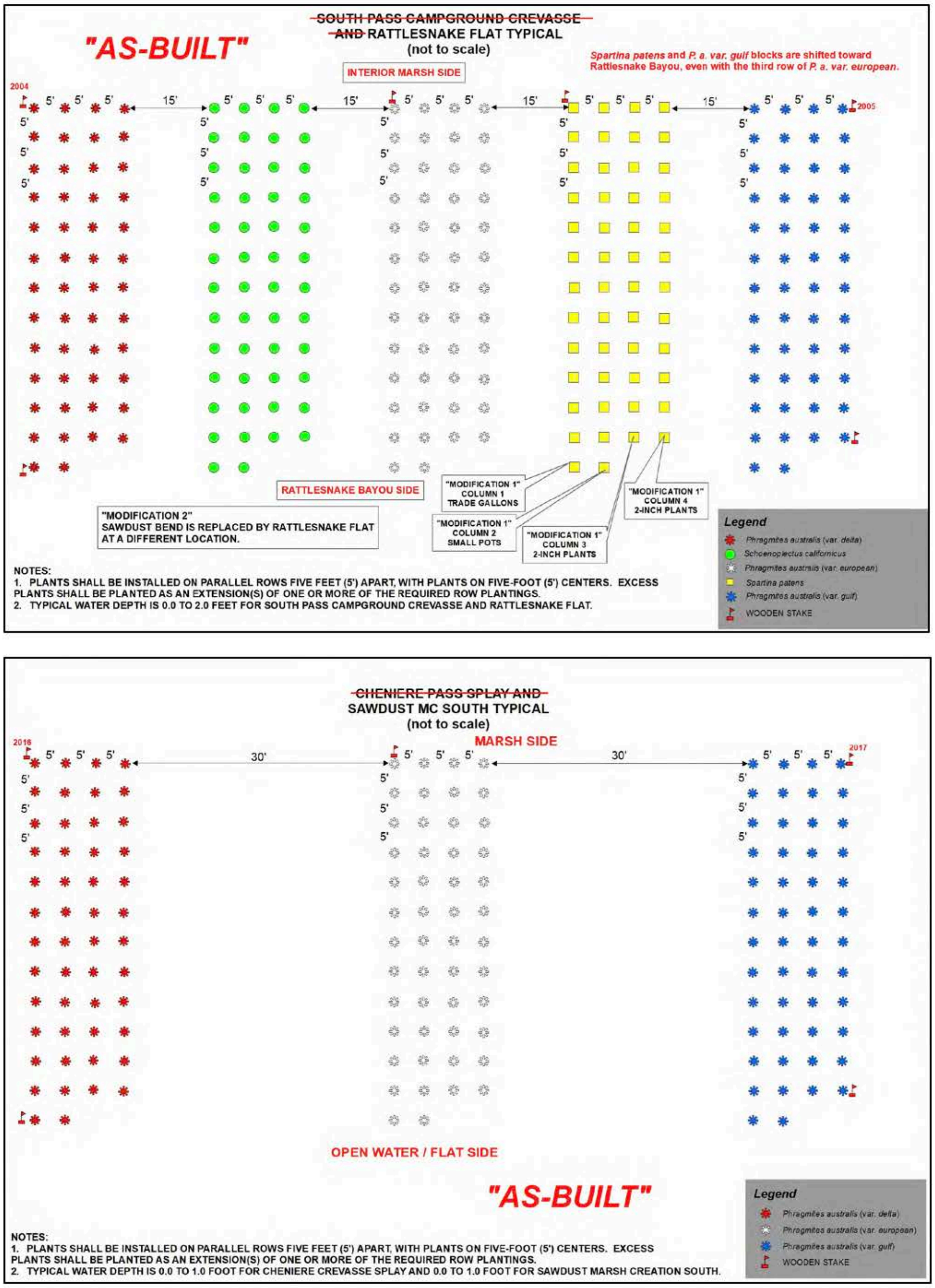
Figure B.1. LA-39 7A Mississippi River Field Trial As-Built plants for vegetation plantings in the Lower Mississippi River Delta at nine sites. Legend: Red astrick – *Phragmites* Delta, White astrik with black outline – *Phragmites* Euro, Blue astrick – *Phragmites* Gulf, Green circle – *S. californicus, Source: Quinn Kinler (NRCS, National Resources Conservation Service) and United States Department of Agriculture*



NOTES:
1. PLANTS SHALL BE INSTALLED ON PARALLEL ROWS FIVE FEET (5') APART, WITH PLANTS ON FIVE-FOOT (5') CENTERS. EXCESS PLANTS SHALL BE PLANTED AS AN EXTENSION(S) OF ONE OR MORE OF THE REQUIRED ROW PLANTINGS.
2. TYPICAL WATER DEPTH IS 1.0 TO 2.0 FEET FOR CHENIERE CREVASSE AND WILLOW TREE SITE.

NOTES:
1. PLANTS SHALL BE INSTALLED ON PARALLEL ROWS FIVE FEET (5') APART, WITH PLANTS ON FIVE-FOOT (5') CENTERS. EXCESS PLANTS SHALL BE PLANTED AS AN EXTENSION(S) OF ONE OR MORE OF THE REQUIRED ROW PLANTINGS.
2. TYPICAL WATER DEPTH IS 0.0 TO 1.0 FOOT FOR CHENIERE CREVASSE SPLAY AND 0.0 TO 1.0 FOOT FOR SAWDUST MARSH CREATION SOUTH.

Figure B.2. LA-39 7A Mississippi River Field Trial As-Built plants for vegetation plantings in the Lower Mississippi River Delta at nine sites. Legend: Red astrick – *Phragmites* Delta, White astrik with black outline – *Phragmites* Euro, Blue astrick – *Phragmites* Gulf, Yellow circle – *S. californicus, Source: Quinn Kinler (NRCS, National Resources Conservation Service) and United States Department of Agriculture*

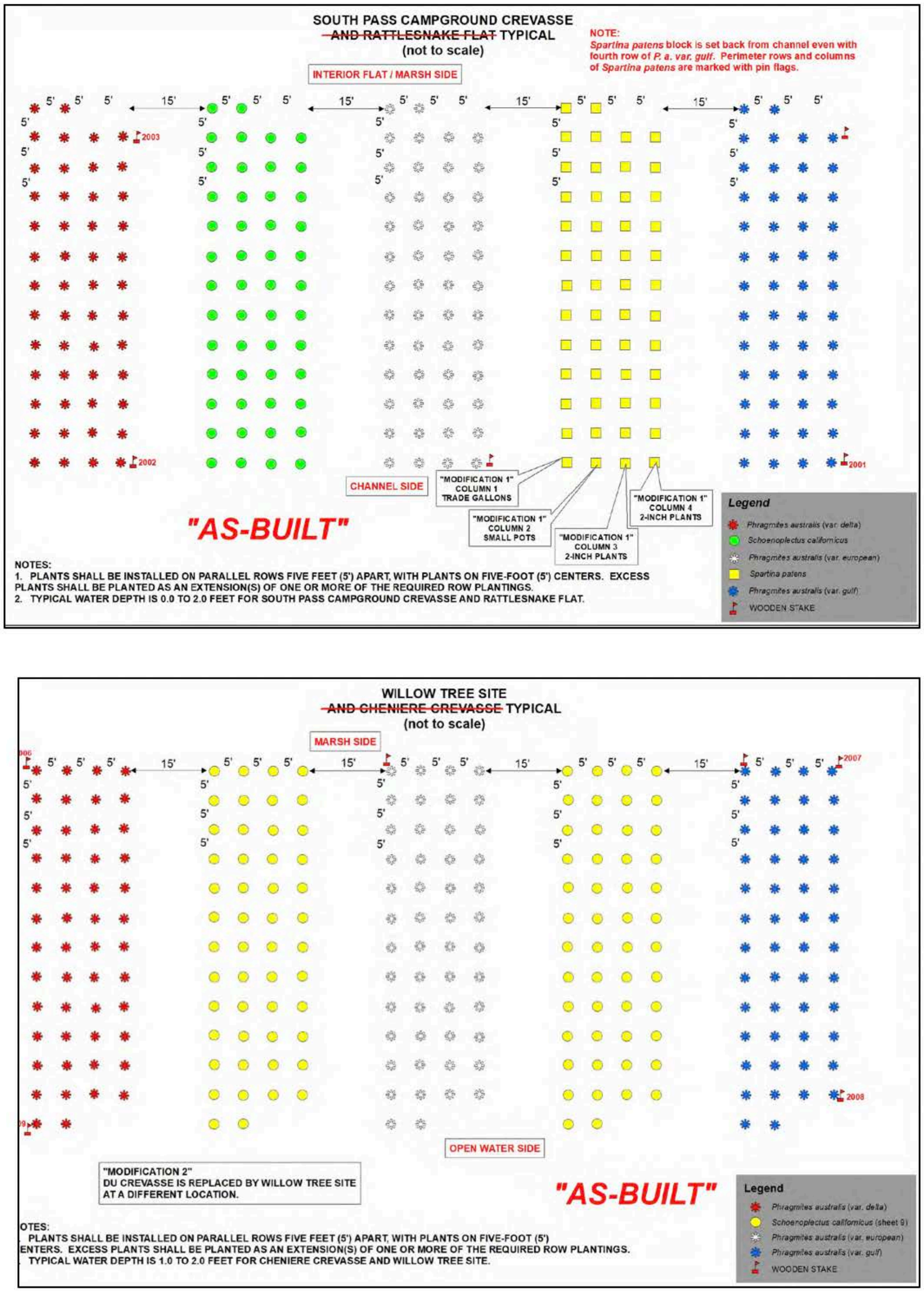

~~SOUTH PASS CAMPGROUND CREVASSE~~
~~AND~~ RATTLESNAKE FLAT TYPICAL
(not to scale)

NOTES:
1. PLANTS SHALL BE INSTALLED ON PARALLEL ROWS FIVE FEET (5') APART, WITH PLANTS ON FIVE-FOOT (5') CENTERS. EXCESS PLANTS SHALL BE PLANTED AS AN EXTENSION(S) OF ONE OR MORE OF THE REQUIRED ROW PLANTINGS.
2. TYPICAL WATER DEPTH IS 0.0 TO 2.0 FEET FOR SOUTH PASS CAMPGROUND CREVASSE AND RATTLESNAKE FLAT.

~~CHENIERE PASS SPLAY AND~~
SAWDUST MC SOUTH TYPICAL
(not to scale)

NOTES:
1. PLANTS SHALL BE INSTALLED ON PARALLEL ROWS FIVE FEET (5') APART, WITH PLANTS ON FIVE-FOOT (5') CENTERS. EXCESS PLANTS SHALL BE PLANTED AS AN EXTENSION(S) OF ONE OR MORE OF THE REQUIRED ROW PLANTINGS.
2. TYPICAL WATER DEPTH IS 0.0 TO 1.0 FOOT FOR CHENIERE CREVASSE SPLAY AND 0.0 TO 1.0 FOOT FOR SAWDUST MARSH CREATION SOUTH.

Figure B.3. LA-39 7A Mississippi River Field Trial As-Built plants for vegetation plantings in the Lower Mississippi River Delta at nine sites. Legend for RF: Red astrick – *Phragmites* Delta, White astrik with black outline – *Phragmites* Euro, Blue astrick – *Phragmites* Gulf, Green circle – *S. californicus,* Yellow Square – *S. patens .Source: Quinn Kinler (NRCS, National Resources Conservation Service) and United States Department of Agriculture*



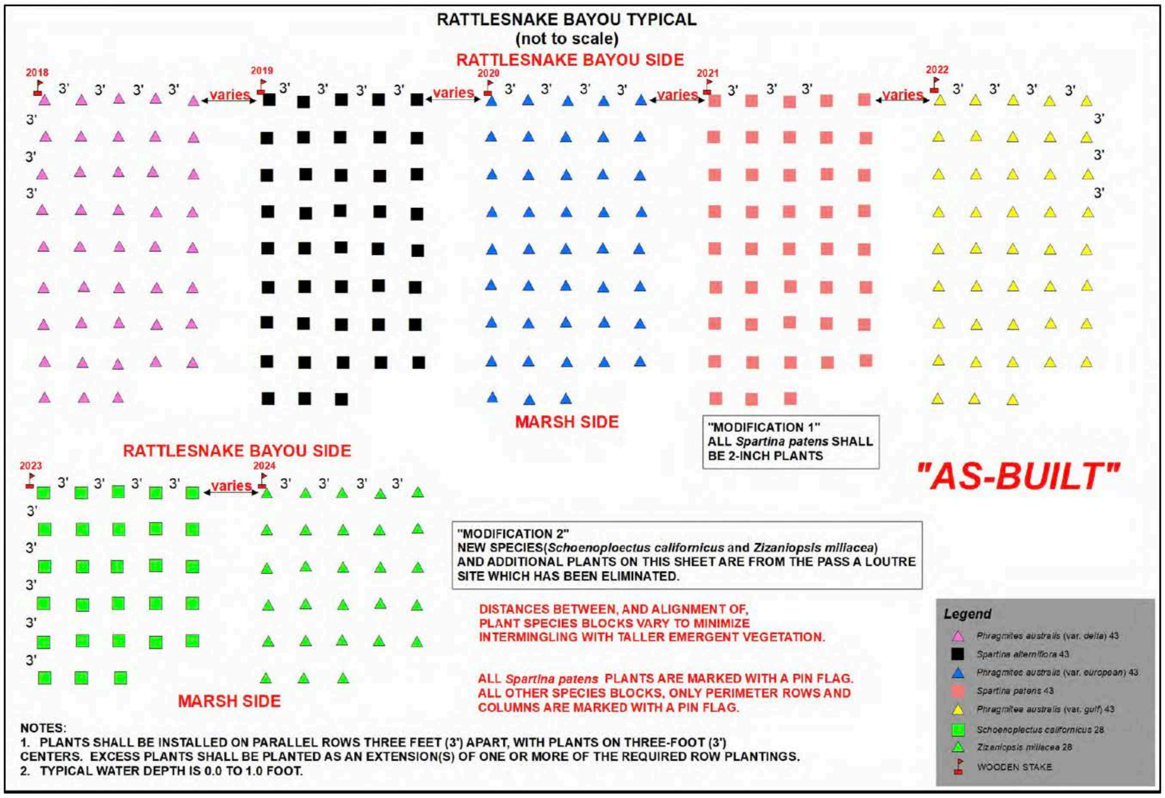
NOTE:
Spartina patens block is set back from channel even with fourth row of *P. a. var. gulf*. Perimeter rows and columns of *Spartina patens* are marked with pin flags.

NOTES:
1. PLANTS SHALL BE INSTALLED ON PARALLEL ROWS FIVE FEET (5') APART, WITH PLANTS ON FIVE-FOOT (5') CENTERS. EXCESS PLANTS SHALL BE PLANTED AS AN EXTENSION(S) OF ONE OR MORE OF THE REQUIRED ROW PLANTINGS.
2. TYPICAL WATER DEPTH IS 0.0 TO 2.0 FEET FOR SOUTH PASS CAMPGROUND CREVASSE AND RATTLESNAKE FLAT.

"MODIFICATION 2"
DU CREVASSE IS REPLACED BY WILLOW TREE SITE AT A DIFFERENT LOCATION.

OTES:
PLANTS SHALL BE INSTALLED ON PARALLEL ROWS FIVE FEET (5') APART, WITH PLANTS ON FIVE-FOOT (5') ENTERS. EXCESS PLANTS SHALL BE PLANTED AS AN EXTENSION(S) OF ONE OR MORE OF THE REQUIRED ROW PLANTINGS.
TYPICAL WATER DEPTH IS 1.0 TO 2.0 FEET FOR CHENIERE CREVASSE AND WILLOW TREE SITE.

Figure B.4. LA-39 7A Mississippi River Field Trial As-Built plants for vegetation plantings in the Lower Mississippi River Delta at nine sites. Legend for RF: Red astrick – *Phragmites* Delta, White astrik with black outline – *Phragmites* Euro, Blue astrick – *Phragmites* Gulf, Green circle/yellow circle – *S. californicus,* Yellow Square – *S. patens .Source: Quinn Kinler (NRCS, National Resources Conservation Service) and United States Department of Agriculture*



"MODIFICATION 1"
ALL *Spartina patens* SHALL BE 2-INCH PLANTS

"MODIFICATION 2"
NEW SPECIES(*Schoenoploectus californicus* and *Zizaniopsis miliacea*) AND ADDITIONAL PLANTS ON THIS SHEET ARE FROM THE PASS A LOUTRE SITE WHICH HAS BEEN ELIMINATED.

DISTANCES BETWEEN, AND ALIGNMENT OF, PLANT SPECIES BLOCKS VARY TO MINIMIZE INTERMINGLING WITH TALLER EMERGENT VEGETATION.

ALL *Spartina patens* PLANTS ARE MARKED WITH A PIN FLAG. ALL OTHER SPECIES BLOCKS, ONLY PERIMETER ROWS AND COLUMNS ARE MARKED WITH A PIN FLAG.

NOTES:
1. PLANTS SHALL BE INSTALLED ON PARALLEL ROWS THREE FEET (3') APART, WITH PLANTS ON THREE-FOOT (3') CENTERS. EXCESS PLANTS SHALL BE PLANTED AS AN EXTENSION(S) OF ONE OR MORE OF THE REQUIRED ROW PLANTINGS.
2. TYPICAL WATER DEPTH IS 0.0 TO 1.0 FOOT.

Figure B.5. Rattlesnake Bayou planting layout for LA-39 7A Mississippi River Field Trial As-Built plants for vegetation plantings in the Lower Mississippi River Delta. Legend: Pink Triangle – Delta, Black Square: *S. alterniflora,* Blue triangle - European, Pink square - *S. patens,* Yellow triangle –Gulf, Green square – *S. californicus,* Green triangle – *Z. miliacea. Source: Quinn Kinler (NRCS, National Resources Conservation Service) and United States Department of Agriculture*

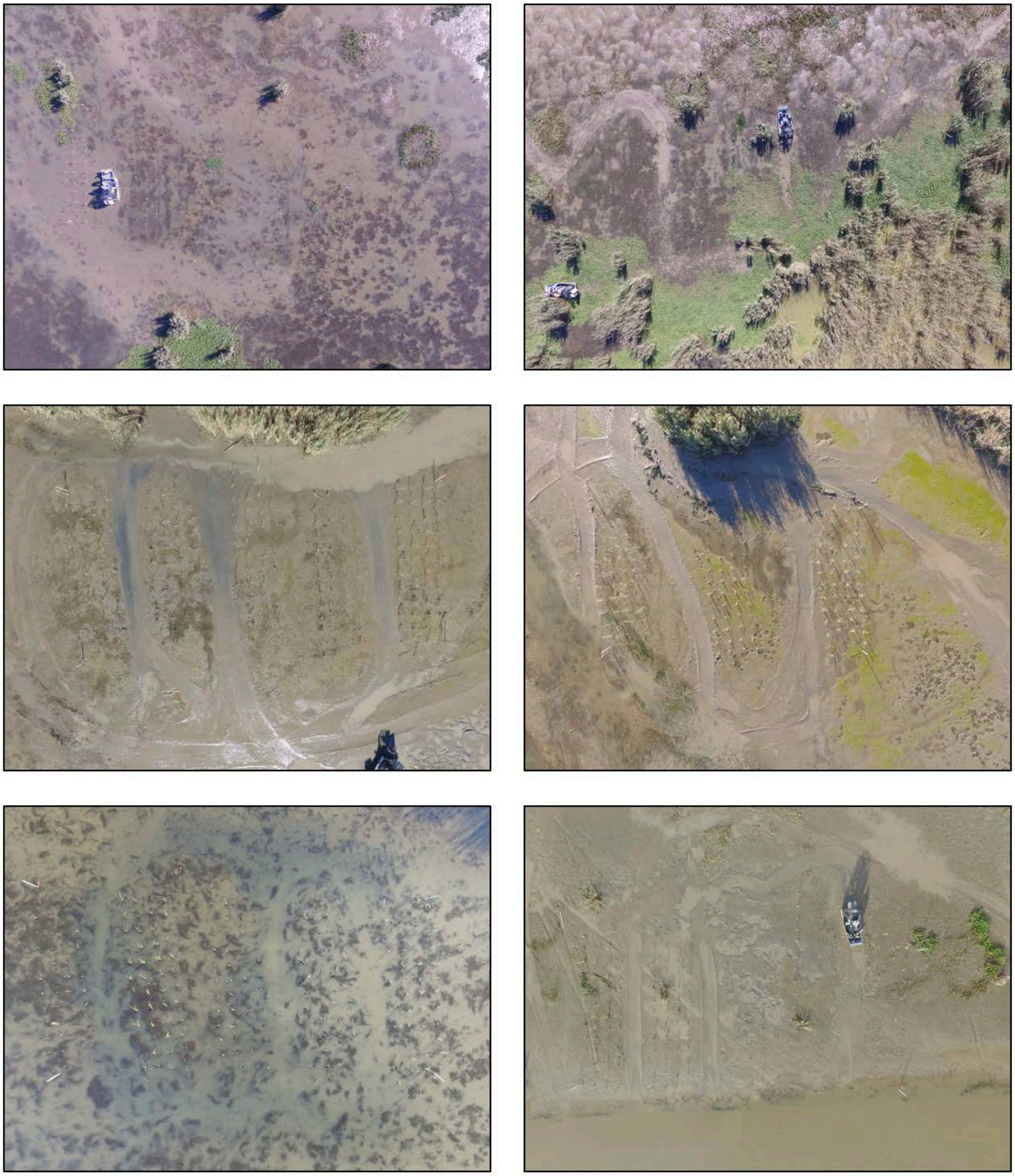


Figure B.6 Aerial imagery of Boundary Line Pond Site 1, Boundary Line Pond Site 2, Cheniere Crevasse, Cheniere Pass Splay, Willow Tree Site, and South Pass Campground captured on December 17, 2018 (From left to right). *Source: M. Gill*

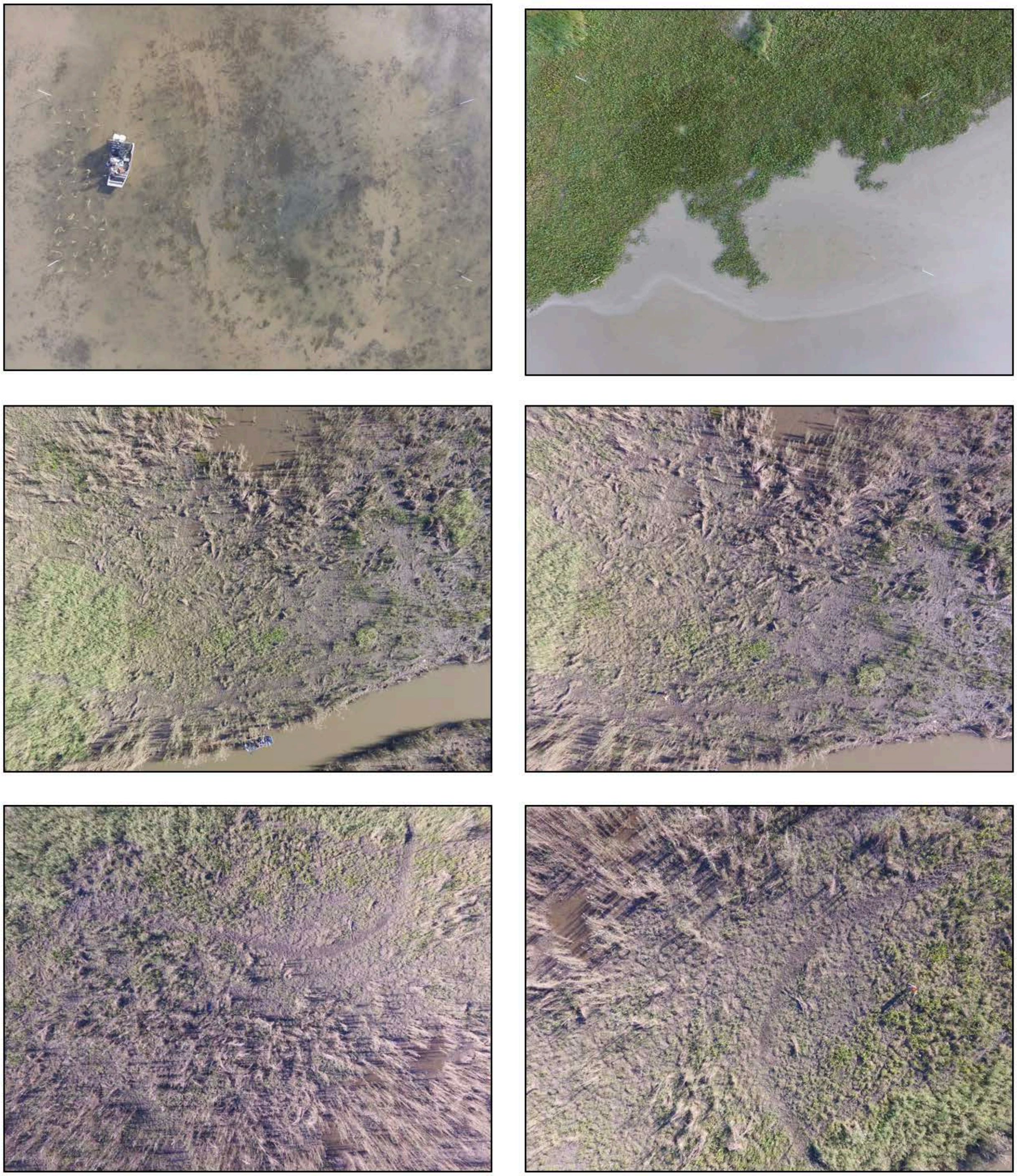


Figure B.7 Aerial imagery of Rattlesnake Flat (upper left), Sawdust (upper right) and Rattlesnake Bayou (from 2nd – 3rd row) on December 17, 2018. *Source: M. Gill*

Figure B.8 Orthomosaic imagery of Chenier Pass Splay from (A) 22-month survey (taken on August 10, 2020 (scale used was 4.37 meters) and from (B) 32-month survey taken on June 2, 2021 (Scale used was 2.37 meters)

Figure B.9 Orthomosaic imagery of Rattlesnake Flat from (A) 22-month survey (taken on August 10, 2020 (scale used was 4.37 meters) and from (B) 32-month survey taken on June 2, 2021 (Scale used was 2.37 meters)

Figure B.10. Orthomosaic imagery of South Pass Campground from (A) 22-month survey (taken on August 10, 2020 (scale used was 4.37 meters) and from (B) 32-month survey taken on June 2, 2021 (Scale used was 2.37 meters)

Figure B.11. Orthomosaic imagery of Boundary Line Pond Site 2 from (A) 22-month survey (taken on August 10, 2020 (scale used was 4.37 meters) and from (B) 32-month survey taken on June 2, 2021 (Scale used was 2.37 meters)