THE ROLE OF POPULATION-BASED DIVERSITY ON PRODUCTIVITY: CONSIDERATIONS FOR RESTORED SPARTINA ALTERNIFLORA SALT MARSHES

A Thesis

by

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ABSTRACT

Plant genetic diversity can augment ecosystem functions in habitats with low plant species diversity. Salt marshes are typically species-depauperate, a condition that is exacerbated when marshes are restored with a single species such as *Spartina alterniflora* (Poaceae, smooth cordgrass). Often, these transplants are from a single cultivar or donor bed, which can decrease genetic diversity and cause proliferation of maladapted genes and inbreeding depression. Increasing genetic diversity could enhance the ecological and economical potential of restored marshes. Distinct *S. alterniflora* genotypes and ecotypes can exhibit unique canopy features but the effects of increasing plant genetic diversity have not been tested. The study objective was to determine if increasing *S. alterniflora* population diversity could augment plant performance in restored salt marshes. I quantified growth and reproduction among transplants from three Texan populations in field and mesocosm experiments. I also compared plant performance in low and high population diversity assemblages in mesocosms across a range of salinities. Overall transplant growth and reproduction patterns among populations or between diversity assemblages did not differ significantly. This lack of differences might indicate that phenotypic plasticity allowed the plants to adjust to the field and mesocosm conditions. However, populations and diversity treatments might perform differently under atypical, natural stresses where the plants do not have the potential for plastic responses. Collecting different *S. alterniflora* populations has no foreseeable short term benefits towards augmenting productivity. Instead, restoration

protocols should ensure collection of native, neighboring plants or multiple, cultivated plants to mimic genetic diversity of local marshes.

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1. INTRODUCTION

Genetic diversity provides species, community, and ecosystem level benefits by yielding a wide range of phenotypic expression within a species (Hughes et al., 2008). Therefore, genetic variation, defined as the quantity of alleles or genotypes in a population, often supports viable populations, particularly within monospecific plant communities (Hughes et al., 2008; Banks et al., 2013). For example, higher genetic diversity can augment shoot density and aboveground net primary productivity (Williams, 2001; Crutsinger et al., 2006). Shoot density in genetically diverse *Zostera marina* (Zosteraceae, eelgrass) beds recovers more quickly after grazing and heat disturbances (Hughes and Stachowicz, 2004; Reusch et al., 2005). The effects of genetic variation in a single species can cascade up to higher trophic levels or ecosystem processes; this discipline is referred to as community genetics (Whitham et al., 2003; Whitham et al., 2006; Hersch-Green et al., 2011). For example, seagrass fitness is often positively associated with increased fauna richness and abundance (Hughes and Stachowicz, 2004; Reusch et al., 2005; Crutsinger et al., 2006; Reynolds et al., 2012).

The benefits of increased plant genetic diversity have been primarily studied in seagrass and terrestrial ecosystems with low species diversity (Reusch and Hughes, 2006). Less is known with regards to salt marshes, particularly in reestablished or created marshes (hereto referred to as restored). These marshes are typically species-depauperate, a condition that is exacerbated when restoration projects focus on a single species, such as *S. alterniflora* Loisel (Poaceae, smooth cordgrass). This species often

dominates salt marshes at the low intertidal zone along the East and northern Gulf coasts of the United States, and is found in a wide range of fluctuating salinities, water levels, soil pH, and soil grain sizes (Utomo et al., 2010). Distinct *S. alterniflora* genotypes from a single location and populations from different regions can display unique growth, morphology, and reproduction patterns (Seliskar et al., 2002; Travis et al., 2002; Proffitt et al., 2003). These previous studies may have application in the improvement of habitat restoration practices, but the potential benefits of increasing *S. alterniflora* genetic diversity within marsh restoration sites has not been tested.

In many regions on the East and Gulf coasts of the U.S., *S. alterniflora* is transplanted to offset marsh degradation and regain ecosystem functions and services (Travis and Grace, 2010). However, genetic diversity is given little consideration in restoration project designs, which could hinder the marsh's ecological and economical potential (Craft, 1999; Williams, 2001; Travis and Grace, 2010). Sprigs of *S. alterniflora* representing a single clone (i.e., genotype) are often cultivated in nurseries for transplant (Ryan et al., 2007; Utomo et al., 2009). In other cases, sprigs are extracted from a single donor population. These approaches may reduce genetic diversity of the plant source material, subsequently lowering marsh productivity and persistence because of increased chances of inbreeding depression caused by limited genetic diversity (Travis et al., 2002; Utomo et al., 2009).

Multiple cultivars of *S. alterniflora* are available for restoration in Louisiana (Knott et al., 2012, 2013), but in regions where these are not available, genetic variation can be manipulated by planting multiple, locally-adapted populations (Travis and Grace,

2010). Several populations collected within 300 km of the restoration site are likely to capture distinct populations and maximize transplant genetic variation (Novy et al., 2010; Travis and Grace, 2010). Given the ecosystem benefits in natural habitats, incorporating genetic diversity into restoration practices may be an efficient way to improve restored salt marsh health.

My study objective was to determine if increasing population diversity of *S. alterniflora*, as a means to increase genetic diversity, could confer an advantage in restored salt marshes. First, I sought to quantify the functional differences among three Texan *S. alterniflora* populations by comparing survivorship, reproduction, and growth patterns in a created marsh and in a controlled mesocosm experiment. I hypothesized that populations would differ in post-transplant performance. Second, I investigated whether manipulation of donor diversity increased assemblage fitness by comparing growth and reproductive patterns between low and high donor mixtures across a range of natural salinities. I hypothesized that growth and reproduction would be highest when multiple donors were grown together, relative to assemblages comprised of a single source.

2. METHODS

In summer 2011 and summer 2012, *S. alterniflora* sprigs (Fig. 1) were collected from the marsh edge within three established salt marshes along the northern Texas coast in the Gulf of Mexico: Port O'Connor, Bolivar Peninsula, and Texas Point (Fig. 2). All populations were collected less than 300 km of the restoration site, which is likely to capture distinct populations while minimizing transplant stress (Table 1) (Travis and Grace, 2010). The plants were transplanted to a dredge (97-100% sand) marsh restoration project at McAllis Point (29°10'37.5"N 95°1'2.2"W) in Galveston Bay, Texas for a common garden experiment (Fig. 3), and into mesocosms (Fig. 4) located in Galveston, Texas for a controlled experiment.

Fig. 1. *Spartina alterniflora* sprig (stem, roots, and rhizome) collected for transplant

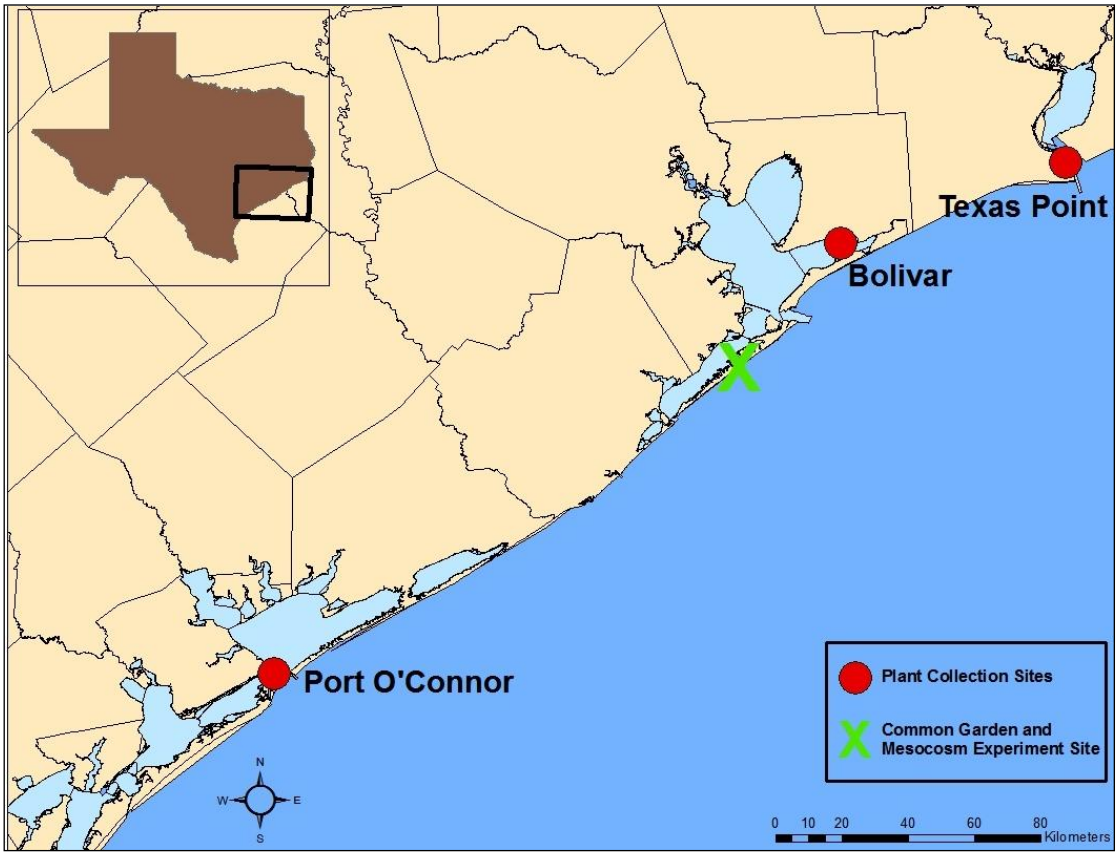

Fig. 2. Location of *Spartina alterniflora* collection sites, common garden, and mesocosm experiment site.

TABLE 1. Distance of donor sites from common garden.

	Distance (km)
Port O'Connor, TX	160
Bolivar, TX	70
Texas Point, TX	125

Fig. 3. Aerial photograph of common garden at McAllis Point in Galveston Bay, Texas. Photo Credit: Galveston Bay Foundation with aerial support provided by Lighthawk. Photo taken March 6, 2011.

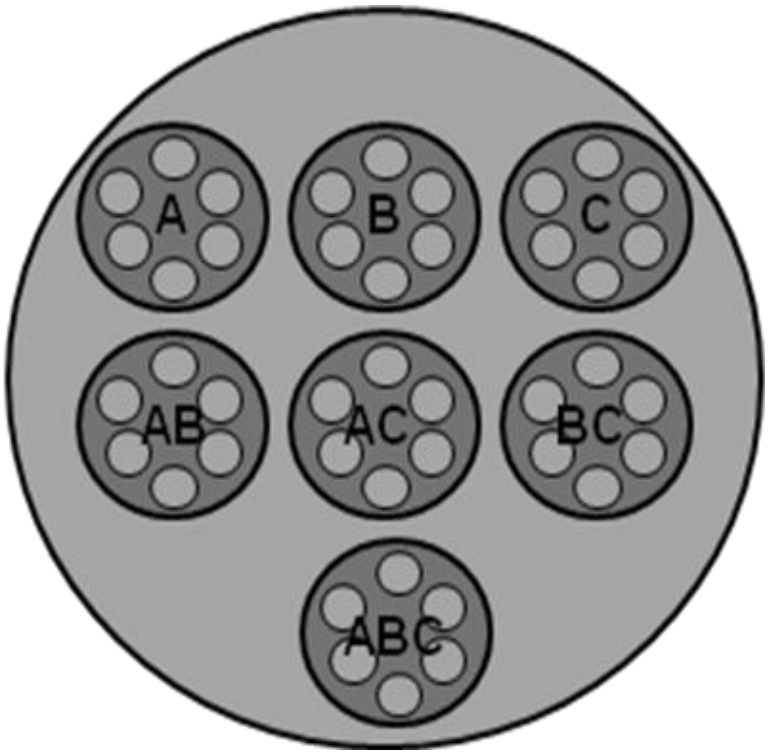

Fig. 4. Monoculture and polyculture pot layout in a single mesocosm. Note: The pots were randomized in each mesocosm.

2.1 Abiotic Features of Donor Locations and Common Garden

To compare the environmental conditions among the three donor sites and the common garden, I measured surface water salinity using an YSI Model 30 probe (YSI Inc) at six marsh edge locations haphazardly chosen within each location. A soil core (7.62 cm diameter) was taken at these locations to a depth of 20 cm, which is the characteristic rooting depth for this species (Bradley and Morris, 1991; Edwards and Mills, 2005).

After drying completely at 60°C, the soil was homogenized using a mortar and pestle and sieved (250µm). Grain size was determined following the hydrometer method of Bouyoucos (1962). Total phosphorus (%P) was determined using dry-oxidation acid hydrolysis extraction followed by colorimetric analysis of the extract using a spectrophotometer (Shimadzu Scientific Instruments) at a wavelength of 885 nm (Fourqurean and Zieman, 1992). Total carbon (%C) and nitrogen (%N) were determined using a CHNS/O analyzer (Costech Analytical Technologies).

2.2 Population Performance

To determine if *S. alterniflora* populations perform differently after transplant, post-transplant performance of three Texan *S. alterniflora* populations were determined in a common garden field experiment. In 2011, I planted 12 plants from a single population in 1 m2 plots (n = 13 for each population) haphazardly along the restored area's shoreline (Fig. 3). Because of high mortality, I replanted in new plots (n = 6 for each population) in 2012 along the same shoreline and on a neighboring restored mound. To compensate for low ambient sediment nutrient content (less than 0.01%N, 0.005%P), two grams of Osmocote, a slow-release fertilizer containing nitrogen and phosphorus, were placed directly into each sprig's planting hole during planting events. Loading rates were 17.5 g P m-2 yr-1 and 55 g N m-2 yr-1, which exceeds the anthropogenic nutrient loading rate of Galveston Bay (Santschi, 1995).

Post-transplant reproduction

For each of the two plantings, I determined percent transplant survival (future potential to reproduce) and new shoot production (asexual reproduction) and inflorescence (sexual reproduction) abundance from surviving transplants per plot (Table 2). For the 2012 transplants, inflorescences (no more than 10 per plot) were collected in October and seeds were counted.

TABLE 2. Days after transplant for collecting data in common garden.

	2011	2012
Sprig survival	27	47
Shoot abundance	111	155
Inflorescence abundance	111	177
Inflorescence collection		200

Post-transplant growth

Growth patterns of haphazardly selected stems of each population (2-10 per plot based on survival) were marked and monitored from the 2012 transplants. Stem growth (% height change, cm) and new leaf production (%) were determined over a 96 day period. The initial value was subtracted from the final value and then divided by the initial value. Leaf production rate was determined by marking the second newest leaf, 40 days later leaves above that marked leaf were quantified and divided by 40. Leaf chlorophyll (chl) *a* content was determined 155 days after transplant using a SPAD-502

portable leaf meter (Konica Minolta Corporation, USA) on the second newest leaf, generating a relative chl *a* content in units unique to the instrument.

Nutrient acquisition (% change) of each population was determined by conducting a fertilization experiment on the 2012 transplants. Leaf material was collected (from the 3rd or younger leaf) 155 days after planting, and then 24 grams of Osmocote were massaged into the sediment of each plot. After a 22 day period, leaves were collected again. Leaf material was dried at 60°C, ground, and analyzed for carbon, nitrogen, and phosphorous contents as described above for soil. Elemental quantities from the pre-fertilization collection were subtracted from the post-fertilization quantities to quantify nutrient acquisition.

Mesocosm growth

Growth patterns of haphazardly selected stems of each population (5 per pot) were monitored; see below for experimental set up. Stem growth (% height change, cm) and new leaf production (%) were determined over a 40 day period. Initial value was subtracted from final value and then divided by the initial value. Leaf production rate (20 day period) was determined, as above. Leaf chl *a* content was determined, as above, 40 days after the experiment started. In addition, belowground cores (10 cm depth, 7.5 cm wide) were collected 130 days after the experiment, rinsed, sieved (2 mm), dried (60°C), and weighed (g) to quantify belowground biomass. Stem and inflorescence abundance for each pot was quantified and inflorescences (no more than 10) were collected to count seeds130 days after the salinity experiment started.

2.3 Diversity Assemblages Performance

My second objective was to compare productivity between low and high population diversity assemblages across a range of salinities. *Spartina alterniflora* sprigs were collected in April 2012 from the same aforementioned locations and used for a controlled experiment. Fifteen mesocosms were filled with freshwater. Each mesocosm contained seven pots, and each pot was planted with six sprigs. Pots contained a monoculture (one pot for each population) or a polyculture (all possible mixtures of two or three populations) treatment (Fig. 4). Pots (20 cm deep and 23 cm diameter) had pores at the bottom and around the upper lip to maintain sediment saturation. The sediment mix was 65% topsoil, 25% sand, and 10% manure. All potted sprigs acclimated in freshwater for two months until Instant Ocean salt was added to increase salinity to 10, 20, or 30 ppt (n=5). Throughout the duration of the experiment, June-October 2012, salinities were maintained within 2 ppt by adding water or salt as needed. Growth patterns of five haphazardly selected stems in each pot were determined as described above.

2.4 Data Analysis

To determine differences in abiotic characteristics among collection and common garden sites, and to determine differences among populations and between diversity treatments, I used a multivariate analysis called Analysis of Similarity (ANOSIM), based on a Euclidean resemblance matrix, unless otherwise noted (Primer v.6, PRIMER-E Ltd., Plymouth Marine Laboratory, United Kingdom). If the output, measured as Global

R, was greater than 0.25, then the independent factors yielded assemblages that were dissimilar from each other with some overlap. When Global R was greater than 0.50, the assemblages were strongly dissimilar. If the Global R was greater than 0.25, I used MDS (Primer's nonmetric, multidimensional scaling) ordination to represent dissimilarities among factors in two-dimensional space. As an exploratory tool, the Similarity Percentages (SIMPER) routine was used to identify the dependent variables that most strongly contributed to the MDS ordination.

Abiotic features of donor locations and common garden

I compared abiotic characteristics among donor sites and common garden using a one-way ANOSIM, where abiotic characteristics (water salinity, sediment profile: %sand, %silt, and %clay, and sediment nutrients: %C, %N, and %P) were the response variables and site was the independent factor. All data were normalized to a common scale of -1 to +1 with mean = 0.

Population performance

To determine post-transplant differences among donor populations, I compared averaged reproductive characteristics per plot (transplant survival, shoot and inflorescence abundance from surviving transplants, and seeds per inflorescence-2012 only) using a one-way ANOSIM within each year with reproductive characteristics (log transformed, normalized) as the response variables and population as the independent factor. I used a two-way ANOSIM to compare averaged reproductive characteristics per

plot (transplant survival and shoot and inflorescence abundance from surviving transplants) between years with reproductive characteristics (log transformed, normalized) as the response variables and population and year as the independent factors.

In the 2012 field experiment, additional growth metrics were measured, so a one-way ANOSIM was used to evaluate differences among populations in 2012. All growth variables were averaged per plot; variables included stem growth (%), new leaf production (%), new leaf production rate, chl *a* content, and %C, %N, and %P uptake.

In the mesocosm experiment, a two-way ANOSIM was run to evaluate differences among populations for average growth variables per pot (stem growth (%), new leaf production (%), new leaf production rate, chl *a* content, and belowground biomass); population and salinity were the independent factors and growth metrics (normalized) were the response variables. In addition, a Bray-Curtis resemblance matrix and a two-way ANOSIM was run to determine differences among population and salinity (independent factors) with average reproductive variables per pot, where stem and inflorescence abundance and seeds per inflorescence as the response variables (log transformed).

Diversity assemblages performance

To determine performance differences between low and high diversity treatments, transplant variables were averaged amongst all monocultures or all polycultures within a single mesocosm. A two-way ANOSIM was run to compare

growth metrics (stem growth (%), new leaf production (%), new leaf production rate, chl *a* content, and belowground biomass) across diversity treatments and salinity levels (independent factors); response variables were normalized. Also, a Bray-Curtis resemblance matrix and a two-way ANOSIM were used to determine differences among diversity treatments and salinity level (independent factors) for reproductive variables (stem and inflorescence abundance and seeds per inflorescence); these response variables were log transformed.

3. RESULTS

3.1 Abiotic Features of Donor Locations and Common Garden

Abiotic conditions were significantly different among source collection sites (Global R = 0.835, p = 0.001). Pair-wise comparisons indicated that all locations were significantly different from one another (all Global R values > 0.4, p < 0.01), although Texas Point and Bolivar exhibited more variability and a moderate degree of overlap (Fig. 5). The environmental characteristics at Port O'Connor and the Common Garden were statistically different from each other and all other locations; however these two locations had similarly low nutrient and high sand sediment profiles, relative to the other two source sites (Table 3).

3.2 Population Performance

Post-transplant reproduction

The analysis of population reproductive performance in the field experiment included averaged transplant survival, shoot and inflorescence abundance from surviving transplants, and seeds per inflorescence (2012 only) per plot. While some trends were apparent, populations were not significantly distinct from each other in either 2011 or 2012 field experiments (Global R = 0.000, 0.105, respectively) (Table 4). However, transplant survival, shoot and inflorescence abundance from surviving transplants differed significantly between transplant years (Global R = 0.714, p = 0.001). The MDS plot of average dissimilarities showed a clear separation between years, with low stress

(0.00) (Fig. 6A). The SIMPER analysis suggested that the largest difference between years was attributable to the production of new shoots from surviving transplants, which was nearly three times greater in 2012 (Fig. 6B).

Post-transplant growth

In the field experiment, growth characteristics (stem growth (%), new leaf production (%), leaf production rate, chl *a* content, %C, %N, %P for 2012 only) did not distinguish populations from one another at the end of the 2012 growing season (Global R = -0.045). Although there were no statistically significant differences, a few trends emerged that suggest that the populations may diverge over time. Specifically, Port O'Connor and Bolivar transplants produced more leaves and had marginally higher chlorophyll *a* content in leaves (Table 5). Additionally, Texas Point transplants had lower phosphorous uptake compared to the other populations (Table 5).

Mesocosm growth

In the mesocosm experiment, neither growth characteristics (stem growth (%), new leaf production (%), leaf production rate, chl *a* content, and belowground biomass) (salinity: Global R = -0.01; population: Global R = 0.092) nor reproductive characteristics (pot stem and inflorescence abundance, seeds per inflorescence; salinity: Global R = 0.037; population: Global R = 0.088) differed significantly among populations or salinities. While growth characteristics did not differ significantly, trends suggest that the populations may differentiate over a longer time span. For example, Port

O'Connor and Texas Point populations had increased stem growth for all salinities, as much as 40% at 30 ppt (Table 6). Port O'Connor produced at least 25% more leaves in all salinities, and Bolivar had marginally higher leaf chlorophyll *a* concentration (Table 6). In general, Bolivar inflorescences produced at least 25% fewer seeds than the other source populations for all salinities (Table 6).

3.3 Diversity Assemblages Performance

Monoculture and polyculture performance (stem growth (%), new leaf production (%), leaf production rate, chl *a* content, and root biomass) was similar among all salinities (salinity: Global R = 0.010; culture: Global R = -0.039). Reproduction metrics (stem and inflorescence abundance, and seeds per inflorescence) were also similar among population and salinities (salinity: Global R = 0.081; culture: Global R = -0.127). While these multivariate analyses did not reveal significant differences between mono- and polycultures, trends emerged for a few metrics. For example, root biomass increased two-fold in polycultures at the highest salinity (Table 7). In addition, seed production in polycultures doubled compared to monocultures at the lowest salinity (Table 7).

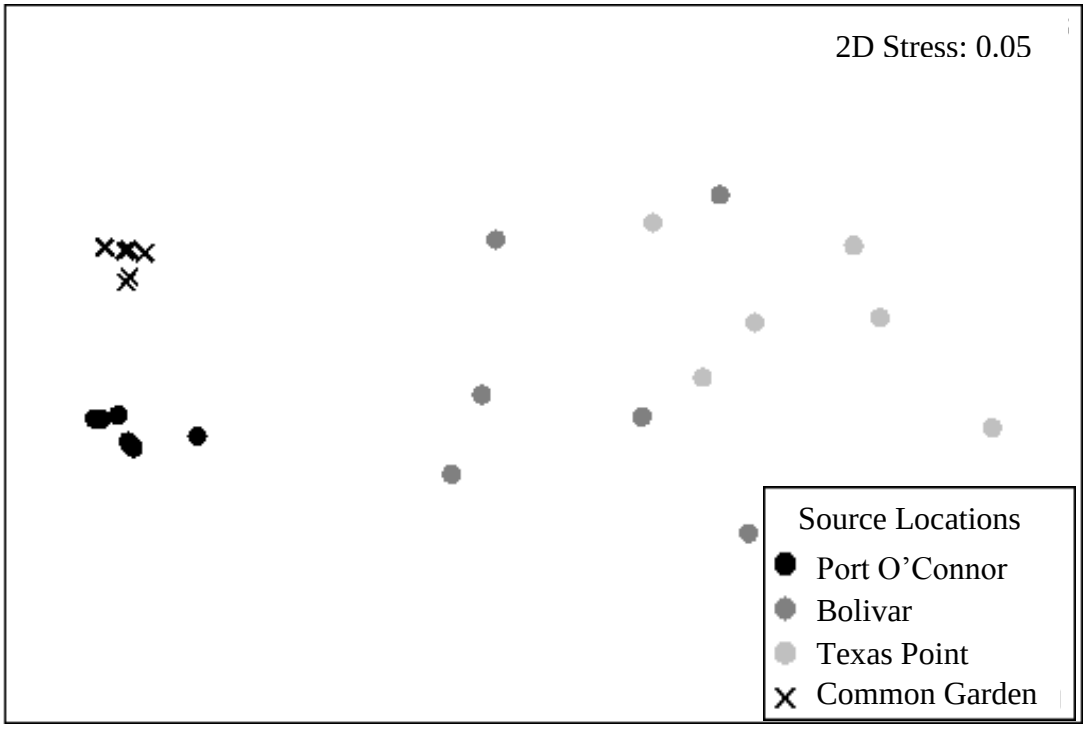

Fig. 5. MDS (non-metric multi-dimensional scaling) ordination graphically depicts that source locations are different from each other based on abiotic parameters.

TABLE 3. Average values (±SE) for aboitic characteristics of three source locations and common garden. Water salinity was recorded as parts per thousand and soil measures were recorded as percentages.

	Salinity	Sand	Silt	Clay	Carbon	Nitrogen	Phosphorous
Port O'Connor	33.1 ± 0.15	91.7 ± 0.7	3.9 ± 0.5	4.4 ± 0.7	0.36 ± 0.10	0.03 ± 0.01	0.003 ± 0.002
Bolivar	25.3 ± 0.25	49.6 ± 5.5	20.1 ± 4.4	30.2 ± 4.3	1.54 ± 0.31	0.07 ± 0.03	0.028 ± 0.005
Texas Point	21.8 ± 0.41	40.7 ± 3.6	32.7 ± 1.3	26.6 ± 3.4	2.25 ± 0.27	0.17 ± 0.03	0.039 ± 0.006
Common Garden	25.3 ± 0.21	98.9 ± 1.1	1.1 ± 0.7	0.0 ± 0.9	0.16 ± 0.04	0.00 ± 0.00	0.000 ± 0.000

TABLE 4. Post-transplant reproductive performance of populations in common garden: average values (±SE) at plot level for transplant survival, shoot and inflorescence abundance per surviving transplant, and seeds per inflorescence.

Source	Survival (%)		Shoot Abundance		Inflorescence Abundance		Seeds per Inflorescence
Year	2011	2012	2011	2012	2011	2012	2012
Port O'Connor	0.34 ± 0.10	0.44 ± 0.10	1.2 ± 0.6	10.1 ± 1.5	0.06 ± 0.04	3.58 ± 0.53	134 ± 11
Bolivar	0.10 ± 0.06	0.39 ± 0.08	0.7 ± 0.4	7.5 ± 0.7	0.07 ± 0.06	1.11 ± 0.19	189 ± 18
Texas Point	0.19 ± 0.08	0.31 ± 0.10	1.3 ± 0.6	6.7 ± 1.5	0.16 ± 0.12	2.78 ± 1.12	159 ± 44

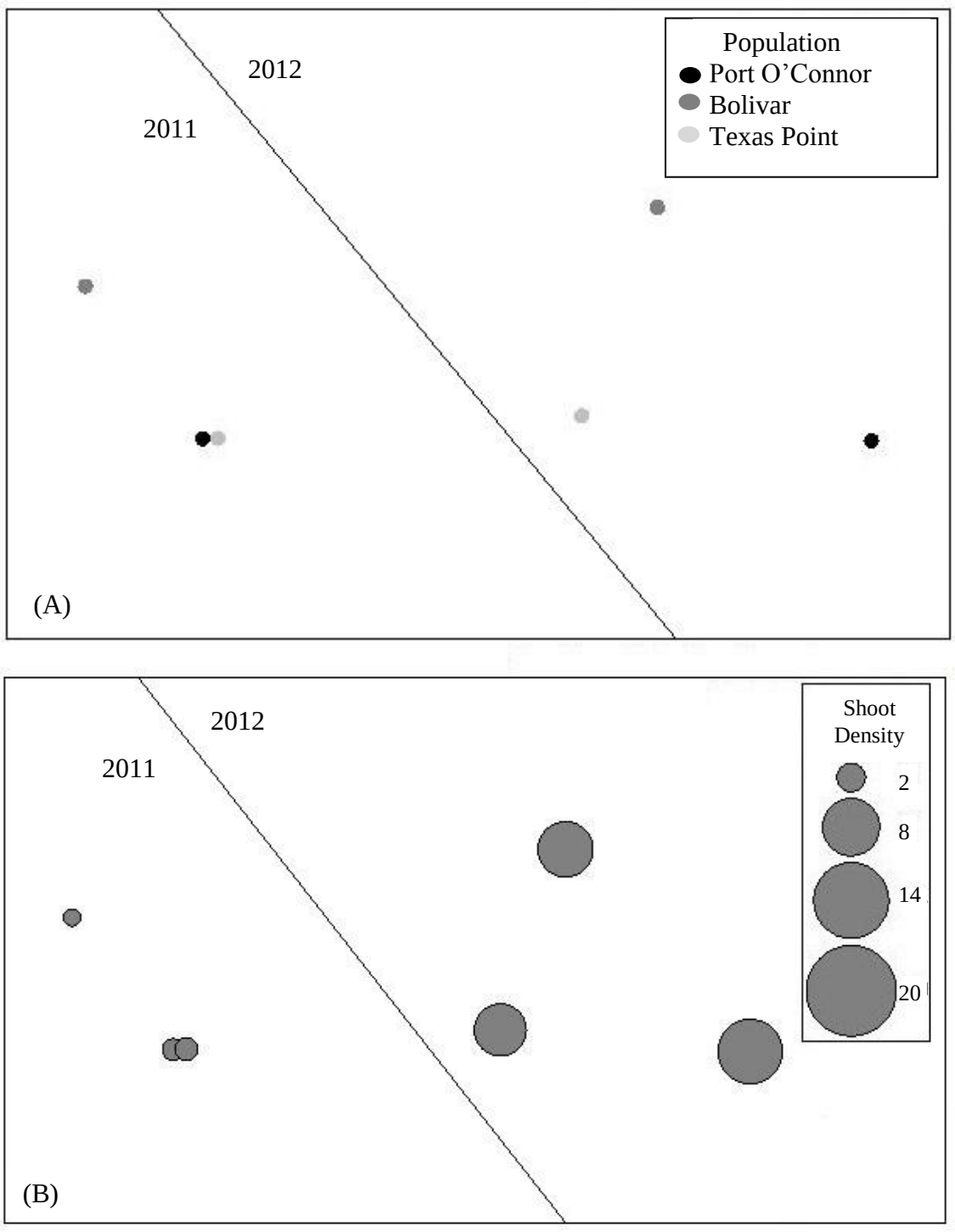

Fig. 6. MDS ordination of average dissimilarities among years and populations depicts separation between years in common garden experiment (A). Bubble plot for shoot abundance overlaid on MDS ordination (B).

TABLE 5. Mean (±SE) growth values from field experiment. Stem growth and leaf production were measured over a 96 day period; leaf production rate was over a 40 day period. Chlorophyll (chl) *a* content was estimated 155 days after transplant. Nutrients are percentage change over a 22 day period.

Source	Stem Growth (% cm)	Leaf Production (%)	Leaf Production Rate	Chl *a* Content	Carbon	Nitrogen	Phosphorous
Port O'Connor	185 ± 28	55 ± 10	0.08 ± 0.01	35.2 ± 1.5	-1.1 ± 0.3	0.3 ± 0.1	0.013 ± 0.011
Bolivar	203 ± 28	68 ± 16	0.08 ± 0.01	39.3 ± 1.7	-1.3 ± 0.2	0.3 ± 0.0	0.023 ± 0.009
Texas Point	176 ± 20	10 ± 11	0.09 ± 0.01	32.8 ± 2.4	-1.6 ± 0.2	0.2 ± 0.1	-0.006 ± 0.010

TABLE 6. Mean (±SE) growth values from each population averaged across salinities (n = 5) in a controlled, mesocosm experiment. Stem growth and leaf production were determined over a 40 day period; leaf production rate was over a 20 day period. Chlorophyll (chl) *a* content was determined 40 days after the experiment and stem and inflorescence metrics were determined 130 days after the experiment.

	Source	Stem Growth (% cm)	Leaf Production (%)	Leaf Production Rate	Chl *a* Content	Root Biomass (g)	Stem Density	Inflorescence Density	Seeds per Inflorescence
10 ppt	Port O'Connor	83 ± 13	40 ± 6	0.07 ± 0.01	36.1 ± 1.4	0.45 ± 0.20	28 ± 4	6 ± 2	87 ± 28
	Bolivar	62 ± 11	26 ± 11	0.06 ± 0.02	41.8 ± 1.4	0.27 ± 0.05	35 ± 4	3 ± 2	29 ± 21
	Texas Point	83 ± 9	25 ± 13	0.08 ± 0.01	37.0 ± 1.5	0.62 ± 0.24	32 ± 4	4 ± 2	51 ± 32
20 ppt	Port O'Connor	61 ± 9	43 ± 7	0.07 ± 0.01	38.7 ± 1.6	0.32 ± 0.18	31 ± 2	11 ± 2	120 ± 12
	Bolivar	47 ± 4	12 ± 12	0.06 ± 0.01	40.1 ± 2.4	0.18 ± 0.04	39 ± 5	5 ± 2	82 ± 21
	Texas Point	52 ± 5	17 ± 9	0.08 ± 0.01	38.8 ± 1.3	0.32 ± 0.08	32 ± 3	7 ± 2	122 ± 17
30 ppt	Port O'Connor	54 ± 6	40 ± 12	0.07 ± 0.01	33.9 ± 1.5	0.22 ± 0.03	36 ± 5	10 ± 2	88 ± 14
	Bolivar	28 ± 10	30 ± 11	0.07 ± 0.02	40.0 ± 1.0	0.28 ± 0.12	37 ± 3	3 ± 2	34 ± 21
	Texas Point	46 ± 10	14 ± 6	0.09 ± 0.00	37.0 ± 0.9	0.22 ± 0.06	25 ± 2	2 ± 1	84 ± 34

TABLE 7. Mean (±SE) values from monocultures and polycultures averaged within the same mesocosm and then averaged across salinities (n = 5). Stem growth and leaf production were determined over a 40 day period; leaf production rate was over a 20 day period. Chlorophyll (chl) *a* content was determined 40 days after the experiment and root, stem, and inflorescence metrics were determined 130 days after the experiment.

	Diversity Treatment	Stem Growth (% cm)	Leaf Production (%)	Leaf Production Rate	Chl *a* Content	Root Biomass (g)	Stem Density	Inflorescence Density	Seeds per Inflorescence
10 ppt	Monoculture	76 ± 8	30 ± 9	0.07 ± 0.01	38.3 ± 1.0	0.45 ± 0.13	32 ± 2	5 ± 2	56 ± 22
	Polyculture	57 ± 7	29 ± 8	0.06 ± 0.01	37.8 ± 0.9	0.36 ± 0.04	35 ± 2	6 ± 2	113 ± 15
20 ppt	Monoculture	52 ± 5	24 ± 7	0.07 ± 0.01	39.2 ± 1.2	0.27 ± 0.05	34 ± 1	8 ± 1	108 ± 8
	Polyculture	60 ± 6	26 ± 8	0.08 ± 0.01	37.5 ± 0.8	0.33 ± 0.03	37 ± 1	7 ± 1	104 ± 13
30 ppt	Monoculture	43 ± 6	28 ± 6	0.08 ± 0.01	37.0 ± 0.6	0.24 ± 0.05	33 ± 2	5 ± 1	69 ± 12
	Polyculture	43 ± 6	20 ± 8	0.07 ± 0.01	37.3 ± 0.7	0.48 ± 0.10	33 ± 2	4 ± 1	66 ± 9

4. DISCUSSION AND CONCLUSIONS

The three Texan *S. alterniflora* populations had similar post-transplant performance. Although this finding differed from the original hypothesis, there have been similar findings in previous studies that compared populations from similar distances. For example, two *S. alterniflora* populations 500 km from each other along the U.S. east coast had similar plant heights and stem densities after five growing seasons in a common garden (Seliskar et al., 2002). Additionally, ten *S. alterniflora* populations within a 500 km range along the Gulf of Mexico had variable stem heights and stem and inflorescence abundances in a common garden after a single growing season (Travis and Grace, 2010). Similarly, fourteen transplanted populations of *Ammophila breviligulata* (Poaceae, American beachgrass) within a 40 km range had similar aboveground biomass after two growing seasons in a common garden (Crawford and Rudgers, 2012). A possible explanation for minimal differences in performance among plant populations is phenotypic variation caused by plasticity. Trait plasticity might explain why the transplants adjusted to local environmental conditions and performed similarly throughout the growing season (Seliskar et al., 2002; Richards et al., 2005; Richards et al., 2010). In the abiotic environment of the common garden and the salinity range, population identity was not strong enough to influence populations to respond to treatments differently. Phenotypic plasticity is a strong candidate to explain high variation in plant morphology of salt tolerant plants (Richards et al., 2010).

In contrast, populations may adapt to their local environments over time leading to specialized traits within distinct populations (Seliskar et al., 2002; Richards et al., 2005; Richards et al., 2010). In such cases, plant phenotypic variation is dissimilar enough that post-transplant performance can differ among populations, possibly scaling up to influence associated fauna communities. The consequences of community genetics has been observed in *S. alterniflora* populations grown in a common garden: after five growing seasons, two populations had different belowground biomass, edaphic respiration, and larval fish use (Seliskar et al., 2002). Additionally, different bacterial communities were present among Chinese *S. alterniflora* populations collected from within 500 km of each other after a growing season in a common garden (Nie et al., 2010). Similarly, fourteen *A. breviligulata* populations had different shoot densities, root hair thickness, and maximum plant height (Crawford and Rudgers, 2012). Analyzing plant morphologies influenced by local adaptation, rather than phenotypic variation, could help distinguish these Texan *S. alterniflora* populations from one another.

This study did not show evidence to support the hypothesis that assemblages with multiple *S. alterniflora* populations would outperform single population assemblages. Similarly, as *A. breviligulata* population diversity increased, aboveground biomass was not augmented in high population diversity treatments compared to single population treatments (Crawford and Rudgers, 2012). Additionally, as *Z. marina* genotypic diversity increased, shoot biomass did not before a disturbance (Hughes and Stachowicz, 2004). Plant phenotypic plasticity could have compromised the potential

benefits of the polyculture treatment because monoculture plants adjusted performance similarly to plants in polycultures.

The mesocosm experiment exposed plants to several different salinities, but none were outside the typical salinity range in Galveston Bay. Therefore, the abiotic conditions in the experiment might not have been stressful enough to observe the benefits of increased population diversity in *S. alterniflora*. Previous studies have demonstrated that increased genetic diversity can augment plant performance and recovery in response to extreme abiotic stresses. For example, *Z. marina* exposed to lethal temperatures, 25% higher than recorded sublethal levels, experienced mortality but beds with higher genotypic diversity had increased regrowth compared to monocultures (Reusch et al., 2005). In addition, high allelic diversity treatments of *Z. marina* survived longer than low diversity plots through chronic light stress (Reynolds et al., 2012). Effects of increased *S. alterniflora* genetic diversity may only be important in situations in which plastic responses are not enough to cope with environmental challenges rather adaptive responses are essential, such as atypical stresses including long-term droughts.

Belowground biomass was two times higher in polycultures than monocultures at the highest salinity, but because of high variability, this and other response variables did not differ significantly among populations or diversity treatments. In the mesocosm experiment, at the highest salinity, populations did not have different root biomasses potentially because of high variability and similar plastic responses for this trait. However, root biomass was marginally higher in polycultures compared to monocultures

at the highest salinity. While it was not explored, additive ('sampling effect') or non-additive ('complementary') factors could have facilitated this trend seen in the polycultures (Hughes et al., 2008). A population with augmented belowground biomass production at higher salinities might influence the overall belowground biomass when this population is grown with others. For example, the Bolivar population had the lowest biomass at the low and moderate salinities but had a comparable biomass to the other two populations at the highest salinity. When grown in the polyculture, this population could have influenced the increase in biomass observed in polycultures. Future experiments that manipulate genetic diversity of *S. alterniflora* should consider investigating belowground characteristics and associated processes in stressful conditions, particularly saline stresses.

In regards to current restoration practices, there are no foreseeable short term benefits to collecting different *S. alterniflora* populations to augment assemblage productivity. These populations were collected from a spatially wide spread area, so collecting outside of this range might not increase chances for different transplant performance and could increase chances of poor transplant performance (Travis and Grace, 2010). However considering the time of restoration is important. The first transplant to the common garden (2011) occurred during an exceptional drought while the second was in a recovery year (2012). Transplant reproductive performance was significantly higher during the recovery, demonstrating the importance of transplant timing. Productivity of the restored marsh could be augmented if weather conditions are explicitly considered.

While there were no clear short-term population-level benefits of increased population diversity, there may be longer-term benefits because as genetic diversity is the basis for evolution and adaptation to potential environmental changes (Hughes et al., 2008; Knott et al., 2012). Restoration practices should focus on determining if genetic variation of the plant material mimics the genetic diversity in native, local salt marshes (Travis et al., 2002; Ort et al., 2014). Native *S. alterniflora* marshes harbor substantial amounts of fine scale genetic diversity that could be captured if transplant material is collected from these areas with high genetic diversity (Hughes and Lotterhos, 2014). Additionally, using multiple *S. alterniflora* cultivars for transplant could increase genetic diversity in restored marshes. In Louisiana, six new cultivars have been registered and recommended for use along northern Gulf of Mexico coasts because of their augmented performance over Vermilion, formerly the only cultivar available for this region (Knott et al., 2012, 2013). Maintaining high levels of genetic diversity in restored marshes by using native, neighboring plants or multiple, local cultivars could prevent negative outcomes of low genetic diversity such as inbreeding depression while supporting long term growth and health (Travis et al., 2002; Williams, 2001).

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