

Intraspecific Variation in Growth of Marsh Macrophytes in Response to Salinity and Soil Type: Implications for Wetland Restoration

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Abstract Genetic diversity within plant populations can influence plant community structure along environmental gradients. In wetland habitats, salinity and soil type are factors that can vary along gradients and therefore affect plant growth. To test for intraspecific growth variation in response to these factors, a greenhouse study was conducted using common plants that occur in northern Gulf of Mexico brackish and salt marshes. Individual plants of *Distichlis spicata*, *Phragmites australis*, *Schoenoplectus californicus*, and *Schoenoplectus robustus* were collected from several locations along the coast in Louisiana, USA. Plant identity, based on collection location, was used as a measure of intraspecific variability. Prepared soil mixtures were organic, silt, or clay, and salinity treatments were 0 or 18 psu. Significant intraspecific variation in stem number, total stem height, or biomass was found in all species. Within species, response to soil type varied, but increased salinity significantly decreased growth in all individuals. Findings indicate that inclusion of multiple genets within species is an important consideration for marsh restoration projects that include vegetation plantings. This strategy will facilitate establishment of plant communities that have the flexibility to adapt to changing environmental conditions and, therefore, are capable of persisting over time.

Keywords Brackish marsh · Genetic diversity · Marsh restoration · Salt marsh · Salinity tolerance · Soil type

Introduction

The adaptive significance of genetic differentiation within plant populations and its role in the persistence of these populations across environmental gradients have been well documented (Silander 1979; Antlfinger 1981; Antonovics and Via 1988; Linhart and Grant 1996; Koppitz and Kühl 2000; Hangelbroek et al. 2003). Genetic diversity contributes to the resiliency of populations by affecting their ability to adapt to abiotic and biotic stressors and to disturbance. The importance of maintaining genetic variability in plant communities at restored wetland habitats has been emphasized in several studies (Seliskar 1995; Smith and Proffitt 1999; Seliskar and Gallagher 2000; Williams 2001; Proffitt et al. 2003; Travis and Sheridan 2006). Zedler (2006) noted that restoring wetland functions at a landscape scale involves site-based practices that may include manipulation of hydrologic conditions, salinity, soils, topography, and microbial communities in addition to establishing plant communities. Although intraspecific variation in flooding and salinity tolerance of wetland plant species has been the focus of many studies (e.g., Blits and Gallagher 1991; Enberg and Wu 1995; Hester et al. 1996, 1998; Lessmann et al. 1997; Krauss et al. 1999; Howard and Rafferty 2006), little information exists on the importance of such variation in response to other environmental factors, including soil characteristics.

Site hydrology and soil oxidation-reduction potential strongly affect nutrient availability and organic matter decomposition in soils, processes which, in turn, influence plant growth and distribution in wetlands (Mitsch and Gosselink 2007). Coastal wetlands in the northern Gulf of Mexico region exhibit a wide range of soil organic and mineral contents (Gosselink and Hatton 1984; Nyman et al.

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1990; Sasser et al. 1995). Wetlands in this region restored with dredged soil materials have high silt or clay content and bulk density compared to natural wetlands (Shafer and Streever 2000; Edwards and Proffitt 2003; La Peyre et al. 2009). Several studies have demonstrated that growth of wetland plant species is influenced by soil characteristics (Lenssen et al. 1999; Huckle et al. 2000; Handley and Davy 2002; Sorrell et al. 2002; Hangelbroek et al. 2003; Willis and Hester 2004). The effects of soil particle size, bulk density, and organic matter on plant growth or the distribution of plant associations has been described for freshwater systems (Barko and Smart 1978; White 1993; Poach and Faulkner 1998; Werner and Zedler 2002; Willis and Hester 2004; Martin and Shaffer 2005) and salt marshes (Eleuterius 1984; Pennings and Richards 1998; Craft et al. 1999; Lindig-Cisneros et al. 2003). Richards et al. (2005) found that soil organic matter was exceeded in importance only by salinity as a predictor of phenotypic variation in 12 salt marsh plants; they noted, however, that the relative contribution of plasticity and genetic differentiation in determining this variation was not known. The Richards et al. (2005) correlative study emphasized the need for experimental studies to elucidate the role of genetic diversity in plant adaptations to environmental conditions.

Few studies have examined intraspecific differences in plant growth related to substrate characteristics. Population variation in the hybrid grass *Spartina anglica* in response to substrate type was attributed to age-related decline in clonal vigor rather than genetic variation (Thompson et al. 1991). Hangelbroek et al. (2003), however, found adaptation of the submersed aquatic species *Potamogeton pectinatus* to substrate type was mediated by genetically determined differences in propagule provisioning. Otherwise, evidence of the relative importance of intraspecific variation in plant response to edaphic factors is lacking in the literature. If genetic differentiation within a plant species affects plant response to soil characteristics, such differentiation can have important implications regarding the resiliency of restored wetland habitats. In moderate to high-salinity restored wetlands, intraspecific variation in response to the interactive effects of salinity and soil type may also be an important consideration.

In this study, intraspecific variation of plants in response to soil type and salinity and their interaction was investigated. The study included four common macrophyte species found in brackish and salt marshes of the northern Gulf of Mexico coastal zone in Louisiana, USA. The effects of soil type and salinity on growth of several individuals of each species were determined in a greenhouse study. The hypotheses addressed in the study were (1) intraspecific growth variation in response to salinity and soil type exists in the plant species studied, and (2) intraspecific difference

in growth response to either salinity or soil type varies depending on the level of the other stressing factor.

Materials and Methods

Plant Material

The plants used in the study were *Distichlis spicata* (L.) Greene, *Phragmites australis* (Cav.) Trin. ex Steud., *Schoenoplectus californicus* (C.A. Mey.) Palla, and *Schoenoplectus robustus* (Pursh) M.T. Strong (nomenclature follows Integrated Taxonomic Information System 2008). These species are perennial and propagate primarily through clonal growth. Individual plants of each species were collected in the spring and summer of 1998. *D. spicata* and *S. robustus* are associated with salt marsh habitats (salinity >15 psu), while *P. australis* and *S. californicus* generally grow in fresh to brackish marshes (salinity <15 psu). Five to ten individuals of each species were collected on public lands (Louisiana Wildlife Management Areas and US Fish and Wildlife Service National Wildlife Refuges) across the coastal zone of Louisiana, which is approximately 340 km in length, to sample existing intraspecific variation. To ensure each collection represented a single genetic individual, several stems of healthy-appearing plants with intact rhizome connections were dug from the marsh sediment. The plants were assigned a number (plant identification number) based on collection location and were used in a series of consecutive experiments; further details on field collection procedures are provided in Howard and Rafferty (2006).

The plants were transported to a greenhouse facility in Lafayette, Louisiana (30°10'N, 92°00'W), where they were separated into ramets that included several stems and rhizome nodes supporting live roots. The ramets were planted in small pots (2.1 l) with drainage holes in a mixture of commercial potting soil and sand. Plants were vegetatively propagated over several ramet generations and were held up to 22 months in large fiberglass tanks (700 l) under common garden conditions (fresh water, flooding to 5 cm below the sediment surface). A commercial water-soluble fertilizer, 20-20-20 N:P:K, was added to the tank water at the rate of 0.26 g l⁻¹ at 3-month intervals. The ramets were standardized 10 weeks before the experiment began by separating them into individuals containing four to six stems that were clipped to a standard height within a species. These clipped ramets were planted in trays (53 cm long × 38 cm wide × 22 cm deep) of sand with drainage holes; the planted trays were placed into the large fiberglass tanks containing fresh water to allow recovery from handling stress. During recovery, water was maintained 2 cm below the sand surface, and the fertilizer described

above was added. One week prior to treatment application, the plants were transplanted a final time to large pots (20 cm diameter × 22 cm height) with bottom drainage holes containing the experimental soils; each pot contained a single species. Total acclimation time from collection to initiation of the experiment was therefore about 25 months.

Experimental Design

The study was conducted in a glass-covered greenhouse. A layer of 30% shade cloth was suspended beneath the greenhouse ceiling to provide protection from excessive heat; the experiment was subjected to the ambient photoperiod. The design was a completely randomized full factorial, with two levels of salinity (0 or 18 psu), three soil types (silt, organic, and clay), and five or six individual plants, depending on species. Silt soil was collected from a commercial soil pit along the Mississippi River in Baton Rouge, Louisiana. Because collection of a sufficient volume of organic marsh soils was considered too problematic (i.e., high cost of transportation and difficult collection conditions), an artificial soil mixture to simulate high organic soils was used. Organic soil was prepared as a mix of 90% commercial sphagnum moss and 10% river silt by volume. Clay soil was prepared by mixing 75% clay from a commercial source in Kentwood, Louisiana (Kentwood Brick Co.) with 25% river silt by volume. The organic and clay soils were combined by using a mechanical cement mixer. Mean sand, silt, and clay contents, determined from three replicate samples, were 58.5%, 33.7%, and 7.8% for the river silt mixture and 0.2%, 29.5%, and 70.3% for the clay mixture.

Five individual plants of *D. spicata*, *S. californicus*, and *S. robustus* and six plants of *P. australis* were included in the experiment. Ramets of each species were randomly assigned to one of six treatment combinations. These combinations of salinity and soil are designated as follows, with the letters F and S indicating fresh and saline conditions, respectively: FC (0 psu, clay soil), FO (0 psu, organic soil), FS (0 psu, silt soil), SC (18 psu, clay soil), SO (18 psu, organic soil), and SS (18 psu, silt soil). The experiment was designed to include five independently maintained replicates of each treatment. Because of aboveground tissue death or low vigor, however, some treatments were reduced to four or three replicates. The number of pots per species were *D. spicata*, 148; *P. australis*, 178; *S. californicus*, 110; and *S. robustus*, 112; the total number of pots in the experiment was 548. The large fiberglass tanks described above were used for treatment application. Each tank was randomly assigned a salinity level and soil type; 30 tanks were used. The potted ramets were placed in the tanks, and fresh water was added to bring the flood depth to 10 cm above the soil surface. On 18 August 2000 (experiment day 1), the first of eight equal-

weight doses of commercial aquarium salt (Forty Fathoms Marine Mix, Marine Enterprise International, Baltimore, MD, USA) was added to the water in the tanks assigned as 18 psu. The salt additions were made once per day over 8 days, and thereafter, the salinity was adjusted as required to the final level of 18 psu. A small submersible pump was used to circulate the water constantly to prevent salt precipitation. Water depth, salinity, conductivity, and temperature were monitored in all 30 tanks at 2–3-day intervals. Water depth and salinity (to within 0.5 psu) were adjusted as needed, and the experiment was not fertilized. Plants were harvested after 81 days.

Data Collection

The number and height (to nearest cm) of all live stems in each pot were measured before salt additions began. Thereafter, stem number and heights were recorded at 4-week intervals and at the conclusion of the experiment, for a total of four measurement times. Live aboveground and total belowground tissues were harvested at the conclusion of the experiment and dried at 60°C to a constant weight for biomass determination.

To document treatment effects on physicochemical conditions, interstitial water samples were collected by using a plastic syringe and tubing (described in McKee et al. 1988) from three replicates of each treatment combination at 15 cm below the soil surface twice during the experiment; several days were required to complete collections. Interstitial water salinity and pH in all pots were measured starting on days 26 and 64. Measurements were made with an Orion Model 130 Conductivity meter and Hanna Model HI9025 pH meter. Water could not be collected from the clay soils using the syringe method, so salinity and pH in those soils were measured by inserting probes directly in the soil to a depth of 5–8 cm. Other interstitial water analyses were completed for organic and silt soils only. Samples for analyses of ammonium and orthophosphate were collected twice for all plant species, starting on days 36 and 71. The samples were passed through a 0.45-micron filter and frozen until analyses. Ammonium was measured with the method described by Parsons et al. (1984), and phosphate was measured according to the method described in Alpkem Corporation (1992). Unfiltered samples for sulfide concentration were collected once during the experiment, starting on day 30 for *P. australis* and *S. californicus* and day 59 for *D. spicata* and *S. robustus*. Sulfide concentration was measured with a Corning model 103 pH/mV meter equipped with an Orion model 9616BN Sure-Flow Combination Silver/Sulfide electrode. Prior to sample collection for sulfide analysis, brightened platinum electrodes were used to measure redox potential (Eh) 15 cm below the soil surface (Faulkner et al.

1989); the electrodes were allowed to equilibrate for at least 30 min before measurement.

Statistical Analyses

The data were analyzed with the general linear model procedure (Proc GLM) of SAS Version 9 software (SAS Institute, Inc., Cary, NC, USA). Three-way analysis of variance (ANOVA) and multiple analysis of variance (MANOVA) techniques were used. Because the target high-salinity level was not achieved in interstitial water from pots containing clay soils (see “Results”), the 18 psu/clay soil data were considered missing in these models, and type-IV sums of squares were used. Plant species were analyzed separately. The models examined differences in the response variables with plant identity (five to six levels, depending on species), salinity (two levels), and soil (three levels) as fixed independent variables. Separate two-way (i.e., plant identity and salinity) ANOVA and MANOVA analyses were conducted with data from clay soil treatments. The overall significance level ($P = 0.05$) was adjusted to account for the number of comparisons of interest in each analysis; least square means tests were used for interaction comparisons with a Bonferroni adjustment, and Tukey’s test was used for main effect comparisons. Plant response variables were stem number, total stem height, and total live aboveground and belowground biomass. Aboveground and belowground biomass was analyzed with MANOVA; all other analyses were univariate. Physicochemical measurements (i.e., interstitial water salinity, pH, ammonium, phosphate and sulfide concentrations, and soil redox potential) were compared at the treatment level. Variables that did not meet the criteria for normality and homogeneity of variance were transformed. For those response variables measured multiple times (i.e., stem measurements; and interstitial water salinity, pH, and nutrients), analyses were performed separately for each time in order to avoid four-way interactions and thereby simplify interpretation of results.

Results

Plant Mortality and Growth

Five pots (one with *D. spicata*, four with *S. robustus*) in the experiment lacked any green aboveground tissue at harvest and were considered dead. When results from the clay/18 psu treatment were excluded (hereafter referred to as the complete analyses), significant treatment effects on stem characteristic were apparent for all species, and individual plant effects were persistent over time (Table 1). Biomass data for all species required rank transformations to meet

Table 1 Summary of an analysis of variance of treatment effects (clay/18 psu treatment excluded) on stem characteristics of *Distichlis spicata*, *Phragmites australis*, *Schoenoplectus californicus*, and *Schoenoplectus robustus* at three measurement times

	Day 19	Day 51	Day 81
<i>Distichlis spicata</i>			
Number of stems	Pl	Pl, So	Pl, So
Total stem height	Sa, Pl*So	Pl, Sa*So	Pl, Sa*So
<i>Phragmites australis</i>			
Number of stems	Pl*So	Pl	Pl*So
Total stem height	Pl*So	Sa	Sa, Pl*So
<i>Schoenoplectus californicus</i>			
Number of stems	Pl, Sa	Sa, Pl	Pl, Sa
Total stem height	Pl, Sa	Sa, Pl*So	Sa, Pl*So
<i>Schoenoplectus robustus</i>			
Number of stems	Pl*So	Pl, Sa	Pl*So
Total stem height	Pl	Pl, Sa	Pl, Sa

Only significant effects and interactions (indicated by *) are listed
Pl plant identity, Sa salinity, So soil type

model assumptions. Final biomass of *D. spicata* varied by plant identity regardless of soil or salinity; in other species, there were significant interactions between plant identity and the other factors (Table 2).

Because of the large number of analyses involved, only results from the final stem data collection event are presented in detail. Individual *D. spicata* plants displayed variation in stem response and biomass regardless of salinity and soil type (complete analyses, Fig. 1). Stem number in all individuals of this species was reduced in silt soil compared to organic soil (Fig. 1). In organic soil only, salinity of 18 psu significantly reduced total stem height and aboveground biomass compared to that at 0 psu in all *D. spicata* plants. These two measures were also reduced in *D. spicata* in silt compared to organic soil, at 0 psu only (Fig. 1). When response in clay soil at the two salinity levels was examined (hereafter referred to as the clay soil-only analyses), *D. spicata* stem number and stem height did not vary by plant identity or salinity (overall model, $P = 0.1956$ and 0.2511 , respectively). Intraspecific variation in biomass was identified ($P = 0.0115$); belowground biomass was greater in plant 2 (8.11 ± 0.93 g; mean ± 1 SE) compared to plant 6 (4.90 ± 0.74 g).

Total stem height, aboveground biomass, and belowground biomass in all *P. australis* plants were significantly reduced at 18 compared to 0 psu (complete analyses, Fig. 2). Stem number, height, and biomass responses to soil type were complicated and differed by plant identity, as indicated by the significant interaction between these factors (Tables 1 and 2). A few differences identified

Table 2 Results of a multiple analysis of variance of treatment effects (plant, salinity, and soil type, clay/18 psu treatment excluded) on the aboveground and belowground biomass of *Distichlis spicata*, *Phragmites australis*, *Schoenoplectus californicus*, and *Schoenoplectus robustus*

	Source	ndf	ddf	F	P
<i>Distichlis spicata</i>	Plant	8	194	4.39	<0.0001
	Salinity	2	97	2.29	0.1063
	Soil	4	194	3.94	0.0042
	Pl*Sa	8	194	0.58	0.7957
	Sa*So	2	97	7.97	0.0006
	Pl*So	16	194	1.11	0.3490
	Pl*Sa*So	8	194	0.65	0.7379
<i>Phragmites australis</i>	Plant	10	236	7.54	<0.0001
	Salinity	2	118	5.61	0.0047
	Soil	4	236	2.68	0.0324
	Pl*Sa	10	236	0.92	0.5196
	Sa*So	2	118	0.62	0.5420
	Pl*So	20	236	1.64	0.0442
	Pl*Sa*So	10	236	0.67	0.7483
<i>Schoenoplectus californicus</i>	Plant	8	132	4.56	<0.0001
	Salinity	2	66	14.61	<0.0001
	Soil	4	132	0.32	0.8646
	Pl*Sa	8	132	1.38	0.2117
	Sa*So	2	66	1.26	0.2914
	Pl*So	16	132	2.16	0.0094
	Pl*Sa*So	8	132	1.37	0.2153
<i>Schoenoplectus robustus</i>	Plant	8	134	9.74	<0.0001
	Salinity	2	67	17.32	<0.0001
	Soil	4	134	3.00	0.0207
	Pl*Sa	8	134	2.14	0.0360
	Sa*So	2	67	2.83	0.0660
	Pl*So	16	134	0.51	0.9358
	Pl*Sa*So	8	134	0.99	0.4464

When interactions were significant, the results of the highest order interactions were examined (indicated in italics)

Pl plant identity, Sa salinity, So soil type

include: (1) stem number in plants 1, 2, and 9 was greater than that in plants 3 and 6 in organic soil, while in silt soil, stem number in plant 2 only exceeded that of plants 3, 4, and 6; (2) total stem height in plant 1 was greater than that in plants 3 and 6, in organic soil only; and (3) plant 4 produced lower aboveground biomass in clay (0 psu only) compared to silt and organic soils, while other plants had similar production across all three soils. In the clay soil-only analyses, salinity reduced *P. australis* total stem height ($P = 0.0014$) in the raised salinity treatment (6.37 ± 0.52 m) compared to 0 psu (9.92 ± 0.90 m). Aboveground biomass varied by population ($P = 0.0005$) and was greater in plant 3 (40.79 ± 6.60 g) compared to plants 2 (13.33 ± 3.64 g) and 4 (15.16 ± 4.17 g).

S. californicus displayed intraspecific variation in stem number across all soil types and salinity levels, while stem number, total stem height, and aboveground and belowground biomass were significantly decreased at 18 compared to 0 psu regardless of plant identity (complete analyses, Fig. 3). Significant intraspecific variation in stem

height, aboveground biomass, and belowground biomass in response to soil type was identified (Fig. 3); for example, higher belowground biomass was produced in clay compared to organic soil by plant 1, in organic compared to clay soil in plant 5, and in silt compared to clay soil by plant 9. The clay soil-only analyses for *S. californicus* indicated no plant identity effects on stem characteristics or biomass, but that salinity affected both stem number ($P < 0.0001$) and total height ($P < 0.0001$). Stem number at 0 psu (6.39 ± 0.53) was greater compared to that at the higher salinity (3.17 ± 0.23); a similar pattern was found for total stem height (8.95 ± 0.62 m compared to 3.95 ± 0.36 m). Significant salinity effects were also found for biomass ($P = 0.0003$); both aboveground and belowground biomass at 0 psu (28.00 ± 2.18 g and 35.28 ± 4.44 g, respectively) exceeded values at the higher salinity (13.69 ± 1.54 g and 18.88 ± 2.10 g, respectively).

S. robustus exhibited intraspecific variation in stem number with soil type (complete analyses, Fig. 4); plant 2 had fewer stems than one other species in clay soil (plant 5),

Distichlis spicata

Stem Characteristics

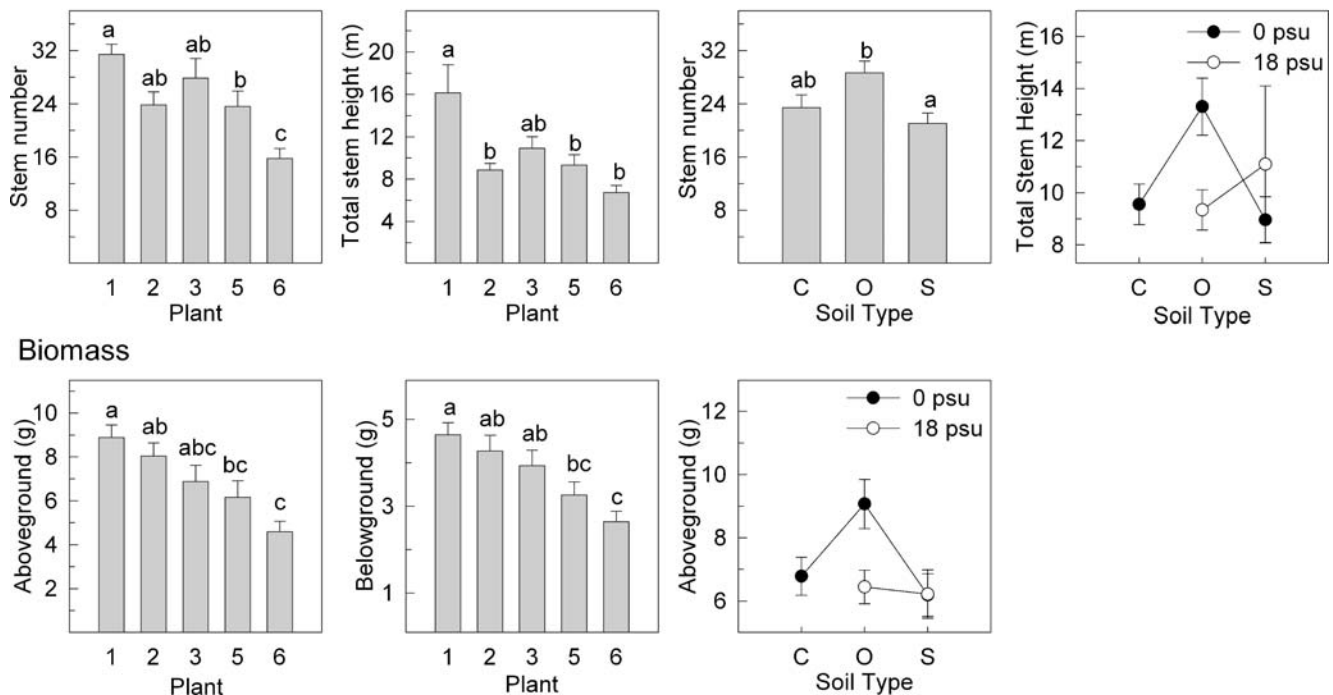


Fig. 1 Mean of significant response measures for final stem characteristics and biomass in *Distichlis spicata*. Data from the clay soil/18 psu treatment were treated as missing for the analyses. On bar

graphs, different letters represent a significant difference between means. Standard error bars are indicated for means

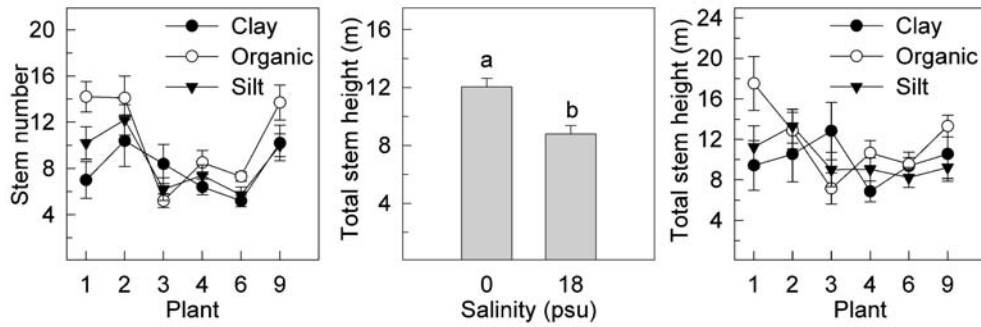
three other plants in organic soil (plants 1, 5, and 10), and all other plants in silt soil. Plant 2 also had reduced stem height compared to the others, regardless of salinity or soil. Total stem height of all plants was reduced at 18 compared to 0 psu (Fig. 4). Although soil had an overall significant effect on biomass in the model (Table 2), when adjusted for Tukey's test, this factor lost significance. Intraspecific variation in response to salinity indicated that biomass in plant 2 was lower than other plants at both salinities (e.g., aboveground biomass was lower than that in plant 10 at 0 psu and plants 8 and 10 at 18 psu), and that plant 5 biomass was reduced at 18 compared to 0 psu; biomass of other plants did not vary with salinity (Fig. 4). For the clay soil-only analyses, *S. robustus* displayed significant intraspecific variation in stem number ($P = 0.0002$) and, depending on salinity, in stem height ($P = 0.0115$). Plant 2 again performed differently than most other plants and had reduced stem number (3.00 ± 0.38) compared to plants 1 (7.50 ± 1.57) and 5 (10.10 ± 1.25). There were also salinity affects on aboveground biomass ($P < 0.0001$), with higher values at 0 psu (11.16 ± 2.05 g) than at the higher salinity (5.32 ± 0.55 g). In clay soils, intraspecific variation ($P < 0.0001$) was found in biomass, with both aboveground and belowground in plants 5 and 10 exceeding that in other plants (Fig. 5).

Interstitial Soil Water Characteristics

Physicochemical variables were pooled by salinity and soil type treatment combinations for analyses. Despite the fact that tank water salinity in the 18 psu treatments was maintained within ± 0.50 psu throughout the experiment, interstitial soil salinity levels were variable among soil types. Mean values for two measurement times (days 26 and 64) were at the target level in organic soils, about 3 psu low in silt soils, and more than 11 psu low in clay soils (Table 3). Interstitial water salinity reached the target level of 18 psu in the organic soil by experiment day 26 and in the silt soil by day 64, but remained low in the clay soil (Fig. 6). Interstitial water pH varied among treatment combination at both measurement times ($P < 0.0001$, time 1 and time 2). Significant pH differences identified by treatment were $SO < FO < SS < FS < FC$, SC . Sulfide concentration also differed by treatment ($P < 0.0001$); sulfide was lower in treatments FO and FS (0 psu) than in treatments SO and SS (18 psu), and was lower in treatment SS than in treatment SO. No significant treatment differences were found for Eh. Ammonium and phosphate concentrations were lower ($P < 0.0001$) in silt compared to organic soil treatments (Table 3).

Phragmites australis

Stem Characteristics



Biomass

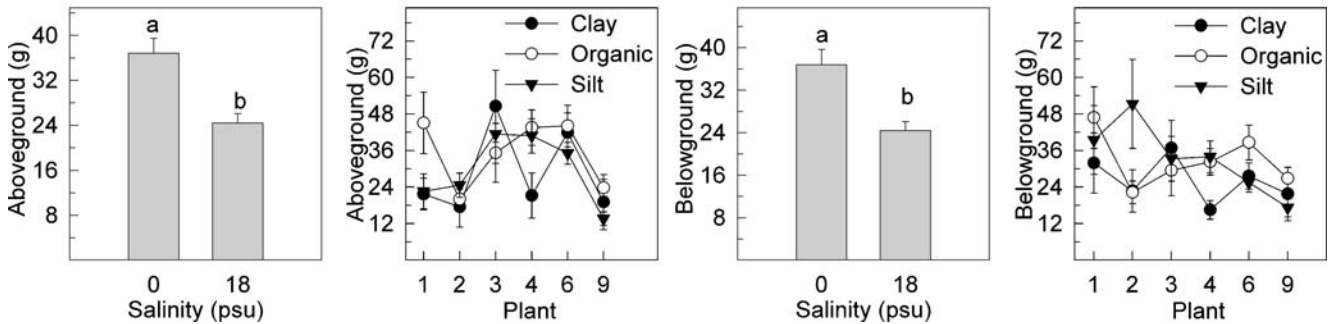
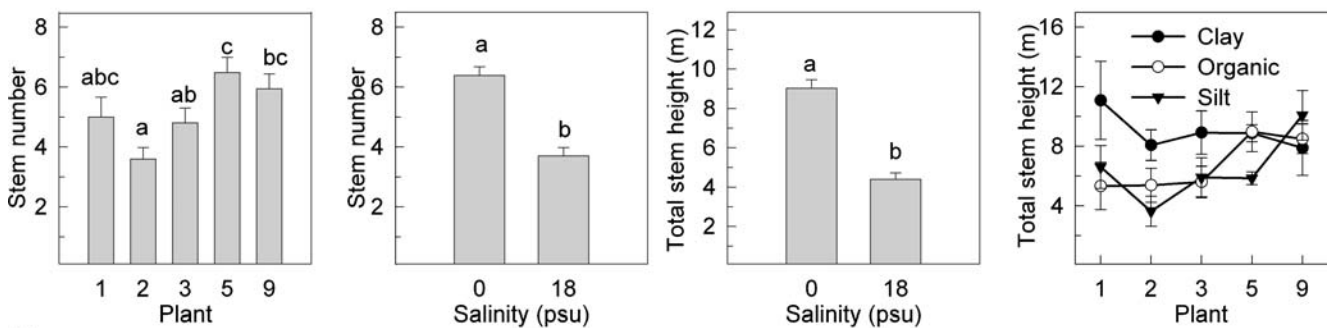


Fig. 2 Mean of significant response measures for final stem characteristics and biomass in *Phragmites australis*. Data from the clay soil/18 psu treatment were treated as missing for the analyses. On

bar graphs, different letters represent a significant difference between means. Standard error bars are indicated for means

Schoenoplectus californicus

Stem Characteristics



Biomass

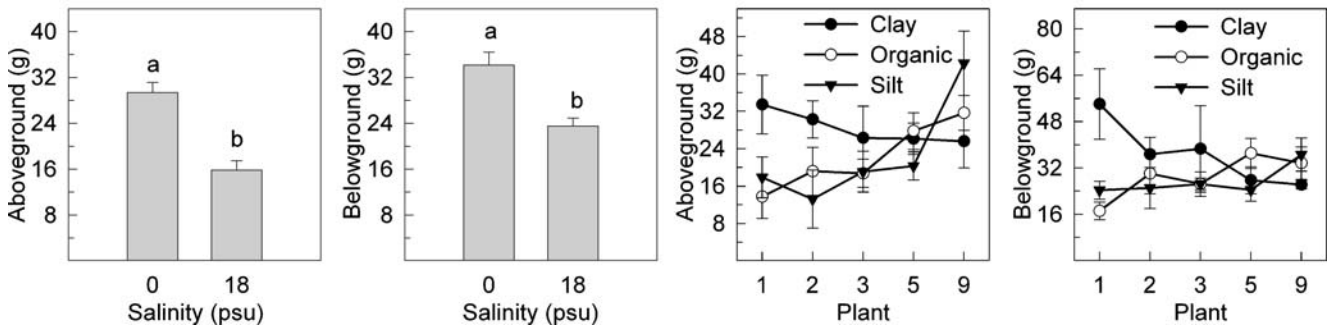
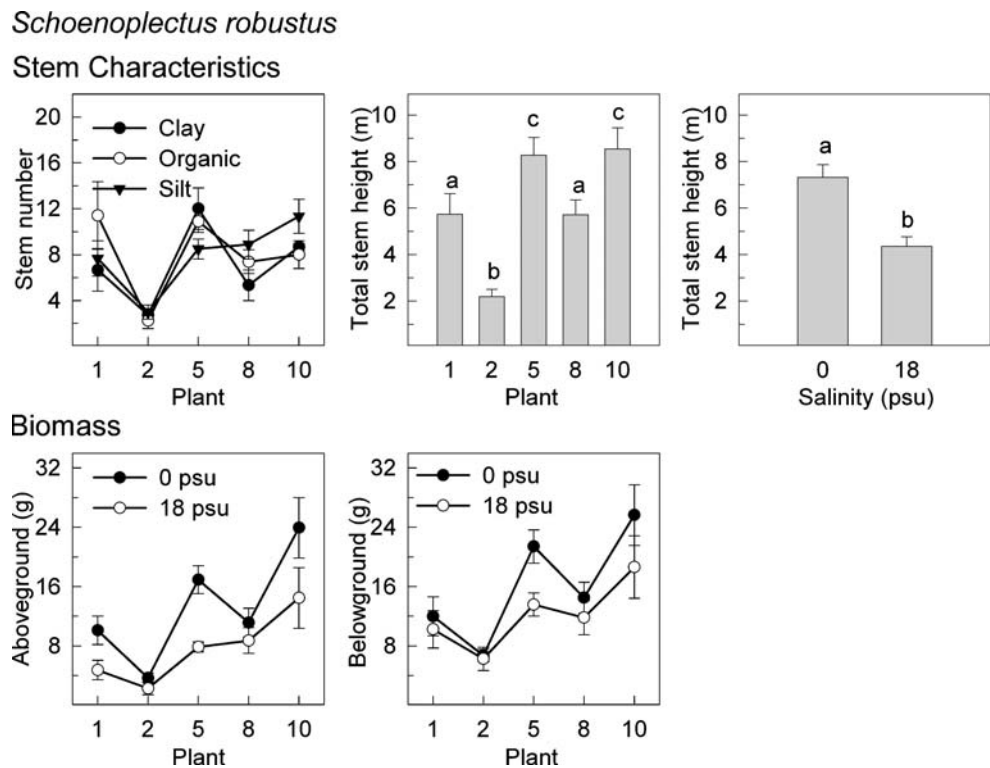


Fig. 3 Mean of significant response measures for final stem characteristics and biomass in *Schoenoplectus californicus*. Data from the clay soil/18 psu treatment were treated as missing for the analyses.

On bar graphs, different letters represent a significant difference between means. Standard error bars are indicated for means

Fig. 4 Mean of significant response measures for final stem characteristics and biomass in *Schoenoplectus robustus*. Data from the clay soil/18 psu treatment were treated as missing for the analyses. On bar graphs, different letters represent a significant difference between means. Standard error bars are indicated for means



Discussion

Intraspecific variation in response to soil type and/or salinity was found in all four species, thereby supporting the first proposed hypothesis. For *D. spicata*, this variation was identified early (about week 3, Table 1) and disappeared during the course of the study. Results failed, however, to support the second proposed hypothesis as no three-way interactions between salinity, soil, and plant

identity were identified. The demonstrated existence of intraspecific variation in the four species studied is consistent with other studies that have identified clonal variation in populations of salt marsh perennials (Silander 1984; Brewer and Bertness 1996; Eppley et al. 1998; Travis et al. 2002; Richards et al. 2004; Utomo et al. 2009). Although genetic information defining distinct clones does not exist for three of the species studied, amplified fragment length polymorphism analyses conducted on a group of *P. australis* plants that included those used in the study confirmed that four clones of this species were represented. Plants 2 and 9 composed one genet, as did plants 4 and 6 (Howard et al. 2008). The stem responses of individuals of both these genets were similar. A difference in biomass response, however, was indicated; plant 4 aboveground and belowground biomass were lower in clay compared to silt and organic soils, while plant 6 exhibited no difference by soil type. It is important to note that when plants were compared within soil type, plants 4 and 6 did not differ. Therefore, it seems the more compelling evidence points to no difference between these plants in response to soil type.

It is interesting to note that an overall species response to the interaction of salinity and soil type was found for *D. spicata*. Total stem height in this species was greater at 0 psu in organic soil only; height was equivalent at the two salinities in silt soils. This species also accumulated more aboveground biomass in organic compared to silt at 0 psu, but this advantage was lost at 18 psu (Fig. 1). This information has little practical value, however, because *D.*

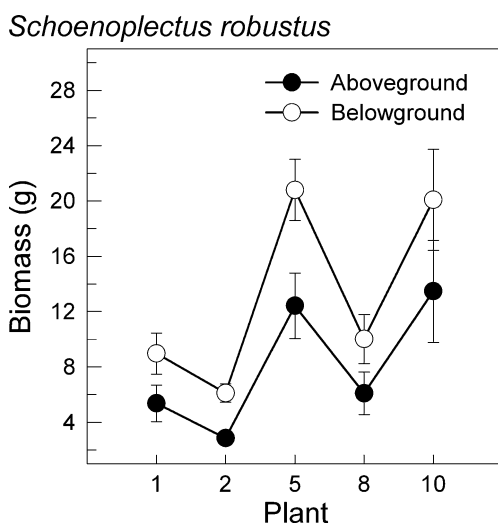


Fig. 5 Mean values for aboveground and belowground biomass in *Schoenoplectus robustus* in clay soil at two salinities. Standard error bars are indicated for means

Table 3 Mean of physicochemical measurements in pot interstitial soil water by treatment combination

Treatment	Salinity (psu)	pH	Sulfide (ppm)	Eh (mV)	Ammonium (μM)	Phosphate (μM)
FC	0.00 (0.00)	7.73 (0.03)	–	–65.48 (16.12)	–	–
FO	0.02 (0.01)	5.47 (0.04)	0.08 (0.01)	–64.40 (15.21)	15.93 (2.95)	63.20 (5.44)
FS	0.26 (0.02)	7.25 (0.01)	0.07 (0.01)	–13.85 (15.91)	2.29 (0.21)	0.28 (0.02)
SC	6.47 (0.20)	7.63 (0.05)	–	–58.03 (17.60)	–	–
SO	17.97 (0.04)	4.73 (0.06)	0.60 (0.06)	–70.21 (15.75)	16.78 (3.06)	4.90 (0.65)
SS	15.38 (0.32)	6.87 (0.01)	0.20 (0.06)	–53.53 (16.90)	4.15 (0.63)	0.30 (0.30)

Salinity and pH values averaged over two measurement times, and the ammonium and phosphate measurements are means from the second measurement time. Salinity and soil combinations are indicated by FC (0 psu, clay soil), FO (0 psu, organic soil), FS (0 psu, silt soil), SC (18 psu, clay soil), SO (18 psu, organic soil), and SS (18 psu, silt soil). Numbers in parentheses indicate 1 standard error

– missing data

spicata would not be a species selected for a restoration site with freshwater conditions, regardless of soil type, because it would soon be replaced by freshwater species. Howard and Rafferty (2006) found that salinity and water depth also interacted to influence several growth measures in *D. spicata* and *S. californicus*. Therefore, it is probable that the additional stress imposed by the constant flooding (10 cm above the sediment surface) maintained throughout this study intensified response at the higher salinity level. The design of the study, however, does not allow quantification of a water depth effect. The presence of interactive environmental effects on plant growth are consistent with a study by Spalding and Hester (2007), who identified interactions of flooding depth and salinity on productivity of three marsh macrophytes (*Panicum hemitomon*, *Sagittaria lancifolia*, and *Spartina patens*). Similar to the findings of those authors, redox potentials in this study were not reduced

to the level where reduction of sulfate to sulfide occurs, i.e., between -100 and -200 mV (Connell and Patrick 1968; Mitsch and Gosselink 2007). Therefore, the low sulfide concentrations measured in the 18 psu treatment were expected, and sulfide toxicity (Ingold and Havill 1984; Koch and Mendelssohn 1989) was not a factor affecting plant growth. The soils used in this study were from either upland (silt and clay) or commercial (organic) sources and, with probable high Fe content, would likely poise Eh at a relatively high level despite flooded conditions (Spalding and Hester 2007).

Study results were complicated by the fact that the high-salinity treatment in clay soil was apparently not achieved. Obtaining accurate pore water salinity measurements in this soil type was problematic because the predominance of fine particles precluded water collection using the syringe and tubing method. The alternate technique applied, i.e., inserting the electrode directly into the soil at the surface, measured salinity of relatively stagnant water at the pot surface. It is probable that some inorganic solutes precipitated out on the soil surface, leading to the overall low mean salinity of 6.5 psu measured in the 18 psu treatment. Because plants continued to grow in clay soil, tank water did move through the pots despite low permeability. A possible explanation for low interstitial water salinity is adsorption of sodium ions onto the clay particles. In comparison, the organic soil, with its associated low bulk density and high permeability, tracked the tank water salinity closely. The clay soil-only analysis indicated that even the moderate salinity level reached reduced growth in *P. australis*, *S. californicus*, and *S. robustus*. This may be of interest to restoration practitioners when soils at brackish marsh sites have high clay content.

Findings of this study are in agreement with others that concluded plant growth can vary with sediment type (van den Brink et al. 1995; Lenssen et al. 1999; Sorrell et al. 2002; Willis and Hester 2004). Although sand was not included in this experiment because it is not a common

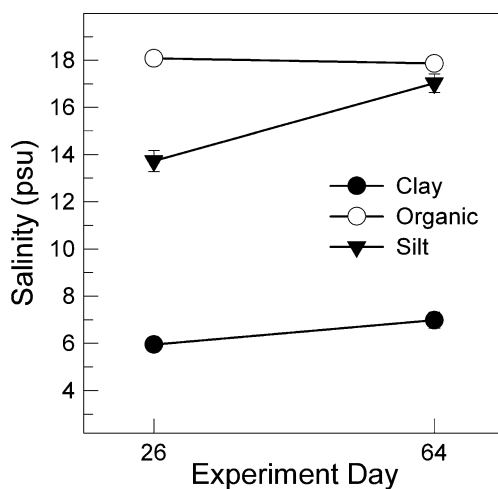


Fig. 6 Mean value of interstitial soil salinity in pots of four species of plants subjected to an 18-psu salinity treatment, in three soil types at two measurement times. Time is indicated by day of the experiment; salinity treatments were initiated on the first day of the experiment. Standard error bars are indicated for means

substrate type in Louisiana marshes, studies have demonstrated plant growth enhancement in organic soils compared to sand; this was attributed to higher nutrient availability in organic soil (Huckle et al. 2000; Sorrell et al. 2002). Increased growth with increased soil organic matter content has been documented for *Spartina alterniflora* in salt marsh habitat (Padgett and Brown 1999) and for several freshwater emergent species along lakeshores (Wilson and Keddy 1985). As Lenssen et al. (1999) noted, however, the nature of soil organic matter must be considered when attributing beneficial growth effects to increased organic matter. Increasing content of labile organic matter enhances nutrient availability, while refractory organic matter does not affect nutrient availability (Lenssen et al. 1999).

Sphagnum peat is recalcitrant and therefore is typically characterized by low nutrient supply (van Breemen 1995). Peat in this experiment was mixed with river silt (10% by volume), so inorganic nutrients were added to the soil mixture. However, the higher nutrient levels found in organic compared to silt soils (i.e., 100% silt) is puzzling. Nutrient uptake by plants was apparently not reduced in organic soils because growth was often equivalent across soils or, in some cases, was greater in organic soils compared to silt and clay. Despite the fact that the soil treatments were not fertilized, ammonium and phosphate concentrations in interstitial water (Table 3) were within range of levels found in Gulf of Mexico estuaries (Pennock et al. 1999). It is therefore unlikely that these nutrients were limiting during the experiment.

Differences in growth attributed to soil type may be related to other chemical (i.e., other than nutrient concentrations) or to physical soil characteristics. Organic soils in general have lower pH and bulk density compared to mineral soils and higher water holding capacity, porosity, and cation exchange capacity (Mitsch and Gosselink 2007). Interstitial water pH was slightly acidic in the organic soil (Table 3) but, because plants generally grew well in organic soils, the low pH did not have an adverse effect. Variation in growth may be related to the physical structure of the soils; the higher bulk density of silt and especially clay soils compared to organic may have inhibited root development in some species or genets.

Genetic diversity within plant populations has been found to influence ecosystem functions in salt marshes (Seliskar et al. 2002) and in seagrass populations (Reusch et al. 2005). Kühl et al. (1999) noted that *P. australis* populations with a large number of different clones were associated with a greater degree of morphological plasticity than those with just a few. Hangelbroek et al. (2003) described the importance of clonal diversity in *P. pectinatus* in adapting to soil characteristics; they demonstrated that within a population of this species, clones producing larger tubers had higher fitness in sandy soils, while clones with smaller tubers had

higher fitness in clay soils. Such studies have important implications for wetland restoration in highly variable environments. In regions experiencing high rates of relative sea-level rise, including the northern Gulf of Mexico, the interaction between plant growth and sediment accretion can be an important factor affecting marsh stability (Nyman et al. 1995). Because a key component of successful marsh restoration is the persistence of vigorous plant communities, it is crucial to consider the composition of those communities.

This study, as the first to indicate that intraspecific variation in response to soil type exists in common emergent macrophytes of northern Gulf of Mexico marshes, has practical applications for wetland restoration planning. Characteristics of the sediments that will be used to restore degraded wetlands should be determined so that a variety of plant species and genets within those species that grow well under both the prevailing soil and salinity conditions can be included in the suite of plants introduced through seeding or planting. Under this scenario, opportunities for outcrossing between genets and the associated reduced chance of inbreeding depression will act to increase overall plant population diversity (Montalvo et al. 1997; Travis et al. 2004). If some species or genets are eventually eliminated under stressful or changing environmental conditions, restored wetlands that have genetically diverse plant communities will be resilient and will therefore have a high probability of persisting over time.

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