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Leaf Tissue Indicators of Flooding Stress in the Above- and Belowground Biomass of *Spartina patens*

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ABSTRACT

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Many factors, such as rising sea levels and human alterations, threaten coastal wetlands in the United States and around the world. To reverse some wetland loss, dredge material, tidal flow, or river flow can be used to create new wetlands, or existing wetlands can be managed to increase plant productivity. Identifying the causes of limited production can improve management plans by suggesting possible remedies. Managing and restoring marshes depends on understanding which stress factors limit growth of key marsh-building plants. *Spartina patens* is a common marsh-building species of grass in brackish marshes along the Gulf of Mexico and Atlantic coasts of North America, and it is often the target of management and restoration plans. *Spartina patens* was grown under six flooding levels in a field experiment. *Spartina patens* plants grown at lower elevations had consistently lower biomass, in contrast to *Spartina alterniflora*, which has been shown to exhibit peak biomass at intermediate levels of flooding. Critical values of elemental concentrations in plant tissue are widely used to diagnose mineral deficiencies in agricultural crops and are just beginning to be developed to aid wetland management and restoration. For leaf tissue harvested in summer, [Mn] < 256 ppm and [Ca] $< 0.3\%$ indicated that plants grew at lower elevations and had limited biomass. The results suggest that concentrations of Mn and Ca in the leaf tissue of *S. patens* could form part of an indicator to monitor belowground productivity of marshes. Although low concentrations of these elements were associated with the smaller root and shoot biomass of plants grown at low elevations, variability in the relationship suggests that additional factors may need to be considered. Marsh managers should monitor soil elevation loss carefully if they choose to drain marshes to increase plant production to prevent excessive loss of soil elevation.

ADDITIONAL INDEX WORDS: *Wetlands, hydrology, inundation, stoichiometry, management, manganese, calcium.*

INTRODUCTION

Many factors threaten wetlands in the United States and around the world. Wetlands are susceptible to the influences of climate change, sea level rise, and urbanization, among many other threats that alter hydrology. These hydrologic alterations reduce the resiliency of marshes by intensifying factors such as high salinity, low nutrient availability, and flooding. Root productivity in marshes in coastal Louisiana is essential for maintaining stability against factors that contribute to marsh loss. Vegetative growth of roots controls vertical accretion in these marshes, making productivity an important factor in the ability of marshes to keep up with sea level rise (Fox, Valiela, and Kinney, 2012; Nyman *et al*., 2006). Live root material contributes to the strength of marsh soils (McGinnis, 1997), which increases their resistance to erosion (Nyman *et al*., 1995).

In highly affected coastal wetlands, such as those in Louisiana, loss of soil stability and diminished ability to accrete vertically results in a conversion of marsh to open water. In coastal Louisiana, estimates of land loss rates range from approximately 66 to 90 km^2/y (Barras *et al*., 2003; Britsch and Dunbar, 1993; Gagliano, Meyer-Arendt, and Wiker, 1981).The loss of coastal wetlands results in a loss of wetland functions such as storm protection, nutrient removal from runoff, and nursery habitat for marine species such as fish and crustaceans.

Development of a bioindicator for flooding stress in marshes would allow managers to identify directly areas where conservation plans need to be altered to increase marsh productivity. Both the effectiveness of an indicator and its cost effectiveness should be considered when choosing an indicator for the success of a marsh creation project (Short *et al*., 2000). An ideal bioindicator would rapidly identify factors that induce stress in marsh plants (Ewing, McKee, and Mendelssohn, 1997) and would be simple and inexpensive enough to use regularly and across a large area—ideally, an entire coastline. Several methods for estimating productivity exist. For example, managers can use changes in aboveground biomass to identify sites that differ in productivity (*e.g*., Burdick, Mendelssohn, and McKee, 1989; Ewing, McKee, and Mendelssohn, 1997). Plant biomass is a practical indicator because it integrates many biogeochemical processes and physiological responses (Ewing *et al*., 1995). However, this method of estimating productivity requires intense sampling over a short period of time; thus it is too costly to be used regularly or over a large area. Shoot elongation varies with plant growth (Ewing, McKee, and Mendelssohn, 1997), but this technique requires

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repeated visits to sites and locating previously tagged stems. Also, while these techniques may identify areas where productivity is limited, they cannot directly identify the factors that limit production.

Identifying the causes of limited production can improve management plans by suggesting possible remedies. For example, in a marsh where low N availability limits production, treatment plans designed to lower salinity will not increase production unless they also increase N availability. Methods such as leaf spectral reflectance, carbon dioxide uptake, leaf expansion, and leaf proline concentration can be used to identify limiting factors because these metrics vary with salinity stress or nutrient starvation (Ewing *et al*., 1995; Ewing, McKee, and Mendelssohn, 1997). Although these methods can be used to identify limiting factors directly, they are too costly for use on large geographic or temporal scales.

Recent research to diagnose limited production in wetland species has focused on nutrient limitation or salinity stress; however, flooding stress is at least as important for controlling production of *S. patens* as either of these factors. *Spartina patens* is generally more productive at higher elevation and lower salinities in Louisiana marshes (Broome, Mendelssohn, and McKee, 1995). In a greenhouse experiment, *S. patens* was most productive when drained slightly (water depth = −10 cm) and least productive when constantly flooded (water depth = +20 cm; Spalding and Hester, 2007). In some locations, soil hypoxia may be the primary factor controlling nutrient uptake and growth of *S. patens* (Bandyopadhyay *et al*., 1993). For example, dieback in Louisiana's coastal marshes that are dominated by *S. patens* and *S. alterniflora* was found to be caused by submergence rather than high salinity, and aboveground biomass of *S. patens* was higher when it was grown with less flooding (Webb, Mendelssohn, and Wilsey, 1995).

Manipulative studies that link physical attributes of created marshes that can be controlled during construction with biological characteristics of plants are necessary to improve the design of created marshes (Streever, 2000). To address this need, an experiment was conducted to (1) examine the effects of flooding on biomass of *S. patens* and on biomass partitioning between above- and belowground portions of the plant and (2) develop stoichiometric indicators of biomass limitation resulting from flooding stress. This paper also proposes guidelines for using these elements to diagnose limitation of production as a result of flooding stress. Each of these elements was determined via inductively coupled plasma (ICP) mass spectrometry analysis, which is inexpensive, commercially available through university agricultural extension offices, and commonly used to detect mineral deficiencies or toxicities in agricultural crops. This experiment focuses on *S. patens* because it is the most common plant in coastal Louisiana marshes (Chabreck, 1970) and occurs throughout the Gulf of Mexico and Atlantic coastal marshes and because the authors have developed stoichiometric indicators of biomass limitation resulting from nutrient limitation and salinity stress (Tobias *et al*., 2010).

One hypothesis in this experiment was that more prolonged and consistent flooding resulting from a marsh platform built lower than the local marsh platform would be associated with decreased biomass. Numerous studies have examined the effects of flooding on aboveground biomass of *Spartina* spp. (*e.g*., Bandyopadhyay *et al*., 1993; Broome, Mendelssohn, and McKee, 1995; Morris, 2007; Visser, Sasser, and Cade, 2006). Flooding has been shown to reduce aboveground production in *S. patens* (Webb, Mendelssohn, and Wilsey, 1995). Relatively few studies have attempted to quantify belowground productivity in *Spartina* spp., and most of these have focused on the effects of nutrient additions on root productivity. These studies have generally concluded that although N fertilization may increase aboveground biomass, it either decreases (Swarzenski *et al*., 2008; Valiela, Teal, and Persson, 1976) or has no effect on belowground production (Darby and Turner, 2008b; Wigand *et al*., 2004). Even fewer studies have been conducted on the effects of flooding on the belowground biomass of *Spartina* spp., and these have generally found that flooding inhibits root productivity (Howes *et al*., 1981; Nyman *et al*., 1995; Valiela, Teal, and Persson, 1976). None of these studies has specifically manipulated flooding levels, but they have reported observations of relative flooding levels in a field setting.

A second hypothesis was that elemental concentrations—[Mn], [Fe], [Ca], or [Mg]—in leaf tissue could be used as an indicator of flooding stress. These elements vary in the tissues of wetland plants with changes in flooding, but previous studies have not developed guidelines for interpreting concentrations in leaf tissue as a means of improving restoration and management practices. Also, critical values for flooding stress in agricultural crops have not been published. Elemental concentrations need not indicate deficiency or toxicity to be useful as an indicator of stressful conditions; in fact, elements that can indicate a reversible, moderately stressed state are more useful for management than indicators of acute toxicity that would not respond to management actions. Mn and Fe in soil become mobile, and thus more available to plants, in acidic soils and under anoxic, reducing conditions. Plants passively take up Mn and Fe from soil in acidic conditions and translocate them to growing leaf tissue. In a greenhouse experiment with a mixture of sand and soil, the Mn and Fe content of leaf tissue was higher in *Leersia oryzoides* plants that experienced flooding than those that did not (Pierce *et al*., 2009). Leaf tissue [Mn] of *S. alterniflora* grown in anoxic soils was more elevated than in plants grown in aerated soil (Bandyopadhyay *et al*., 1993). Rice (*Oryza sativa*) may develop Fe toxicity as a result of low redox potential (E_h), acidic soil conditions, or both (Fageria *et al*., 2008). The Ca content of tissue also increases in plants grown under drained conditions for *Cladium jamaicense* (Lissner *et al*., 2003). Flooding stress has been shown to reduce leaf Mg content in *Panicum hemitomon* (McKee and Mendelssohn, 1989). Mn and Mg content of *S. alterniflora* tissue has been reported to correlate with plant productivity across a gradient from well drained marsh to poorly drained marsh, while [Ca] was not correlated to productivity (DeLaune and Pezeshki, 1988). Also, more productive stands of *S. alterniflora* contained higher [Mn] (DeLaune, Reddy, and Patrick, 1981).

If flooding stress limits belowground biomass, it would also be expected that belowground biomass would be negatively associated with indicators of flooding stress in leaf tissue. The ratio of live belowground biomass to live aboveground biomass (root:shoot ratio) was examined because it has been shown to

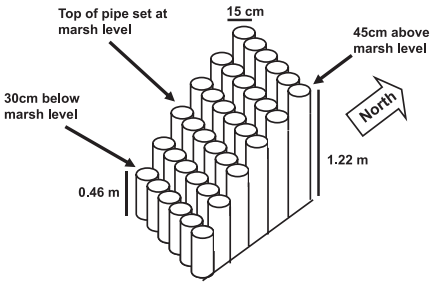

Figure 1. Shape, size, and orientation of marsh organs. Organs consist of six rows and six columns of 15-cm-diam PVC pipe. Heights of rows are 123, 107, 91, 76, 61, and 46 cm from the bottom of the pipes. Marsh organs are set into the pond sediment such that the top of the fourth row is at local marsh level. Note: Diagram is not to scale.

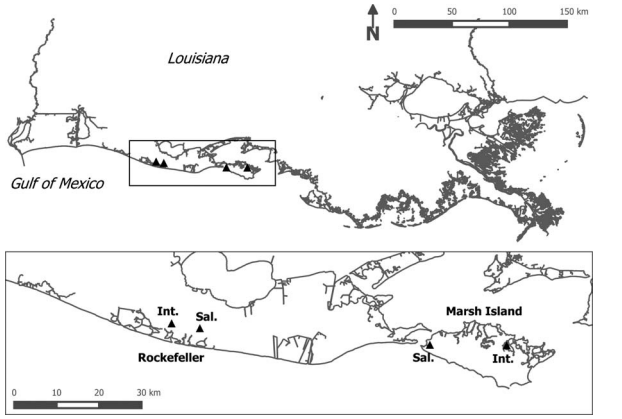

Figure 2. Locations of marsh organ installations (triangles) in intermediate (Int.) and saline (Sal.) marshes.

increase with prolonged inundation (Knox, 1986). The relationships between belowground biomass and leaf tissue, rather than root tissue, chemistry, because comparisons with leaf tissue would allow managers to infer which factor(s) limit belowground production more easily. Previous work has suggested the use of leaf tissue chemistry as a tool for identifying factors limiting aboveground production (Tobias *et al*., 2010), and it is important to understand how management decisions based on these tools will affect belowground production as well. Understanding how leaf tissue chemistry correlates with belowground productivity gives managers a tool for evaluating and improving management plans that aim to increase marsh stability or combat the effects of altered marsh hydrology.

METHODS

Manipulating flooding stress traditionally has utilized greenhouse studies (*e.g*., Howard and Mendelssohn, 1999) or three levels of flooding in the field (*e.g*., Webb, Mendelssohn, and Wilsey, 1995), but this experiment used a field-based technique that creates six levels of flooding stress (Morris, 2007). These installations are termed "marsh organs" because they resemble the pipes on a pipe organ. Marsh organs were constructed from thirty-six 15.2-cm-diam polyvinyl chloride (PVC) pipes bolted together for stability. Each marsh organ consisted of six rows of six pipes in each row (Figure 1). The pipes were cut to lengths of 122, 107, 91, 76, 61, and 46 cm. For the purposes of this paper, rows are defined as the set of six pipes of equal elevation within a marsh organ. Rows were identified by number such that row 1 was the tallest (*i.e*. least flooded) and row 6 was the shortest (*i.e*. most flooded). Columns are defined as a set of contiguous pipes consisting of one pipe of each elevation within a marsh organ. Columns were identified by letters such that column A was to the west and column F was to the east. Water could enter or exit the pipes through three pathways: from the bottom of the pipe, over the top of the pipe, or through holes in the sides of the pipe where the pipes were bolted together.

Marsh organs were installed at four locations in coastal Louisiana in the summer of 2007 (Figure 2). Locations were selected to represent a range of nutrient and salinity conditions experienced by *S. patens* in Louisiana's coastal marshes. Marshes at Marsh Island Wildlife Refuge receive fresh water, nutrients, and sediment from the Atchafalaya River. Soils at Rockefeller Refuge developed without direct riverine influences and depend entirely on rainfall for fresh water. Within both refuges, two locations were selected that differed in salinity. Following Penfound and Hathaway's (1938) classification system for coastal marshes, one marsh organ within each refuge was installed in a saline area where the surrounding marsh was dominated by *S. alterniflora* and another organ was installed within each refuge in an intermediate marsh where the surrounding marsh was dominated by *S. patens* and contained some *Sagittaria lancifolia*, *Typha domingensis*, or both at each refuge. All four organ locations were selected to be near stations where water level and water salinity data are recorded hourly (CPRA, 2015).

Marsh organs were installed in shallow ponds or lakes within marshes and were oriented so that the tallest pipes were to the north to maximize sun exposure for all pipes. Organs were dug into the soil to a level such that the fourth row from the top of the organ was even with the level of the local marsh. This resulted in row 1 being approximately 46 cm above local marsh elevation and row 6 being approximately 30 cm below local marsh elevation. Each marsh organ was adjusted to ensure that the rows were level after installation. Pipes were filled with local marsh soil to the top. Each pipe was planted with approximately 10 stems of *S. patens* collected from the adjacent marsh. Half of the pipes from each marsh organ were harvested in summer 2008, 12 months after planting (columns B, D, and F), and half in fall 2008, 15 months after planting (columns A, C, and E).

Soil *redox potential* (E_h) was measured to ensure that that soil elevation treatments could induce different levels of stress on plants. E_h was measured at 10 cm below the soil surface using platinum electrodes, a calomel reference electrode (accumet), and a pH/mV/temperature meter (Oyster 10, Extech Instruments, Waltham, Massachusetts, U.S.A.). Because of limited space inside the pipes, only one electrode of each type could be inserted into the soil. Changes in soil elevation inside

the pipes and the depth of pond water relative to the top of each pipe were measured at the time of each harvest. Hourly water level data were obtained from water level loggers at Coastwide Reference Monitoring System (CRMS; CPRA, 2015) sites near marsh organs (stations 0523, 0530, 0608, and 0610; LDNR, 2008). Distances between CRMS stations and marsh organs ranged from 0.2 to 6.9 km. In summer 2008, both the pond surrounding the marsh organ at the Rockefeller Refuge intermediate marsh site and the location of the nearest water level recorder completely dried out for at least 2 weeks. The data from the intermediate marsh organ at Rockefeller Refuge were therefore excluded from any statistical analyses of the effects of elevation on biomass or leaf tissue chemistry for the summer harvest. The leaf tissue chemistry was used as a test dataset for predictions made by potential chemical indicators because it was grown under conditions that would not cause flood stress.

Although all of the marsh organs were completely submerged by storm surge from Hurricane Ike on September 13, 2008, minimal damage to the installations was observed after the hurricane. One exception was that the saline marsh organ at Rockefeller Refuge had tilted slightly and visual inspection indicated that soil elevation had been lost via undercutting in some of the pipes in this organ. It was unclear whether undercutting had occurred at other sites; therefore, soil elevations measured at time of fall harvest may not have been representative of the growing conditions during fall.

Care was taken to select only *S. patens* for planting; however, a few pipes included other species at the time of harvest. Stems were sorted according to species and whether they were alive or dead. Live *S. patens* tissue was rinsed to remove any soil or salt on the leaf surface. All tissue was oven-dried to a constant weight and the dry weight was recorded as aboveground biomass. After weighing, samples of leaf tissue were taken from *S. patens* plants. Leaf tissue was selected from the top 15 cm of the plant only, and no stems were included in tissue samples. Leaf tissue samples were ground in a coffee grinder (Smart-Grind, Black & Decker, Towson, Maryland, U.S.A.) and submitted to the Louisiana State University AgCenter's Soil Testing and Plant Analysis Lab (STPAL, Baton Rouge, Louisiana, U.S.A.) to determine C, N, P, K, Na, Mn, Fe, Mg, and Ca concentrations in leaf tissue. The STPAL used dry combustion by a Leco N analyzer to determine N and C content and an ICP mass spectrometry analysis to determine concentrations of all other elements.

All sediment was washed from roots with tap water. Root masses were broken apart to remove sediment that was trapped between roots. Roots were considered "clean" when water passing through them remained clear for several minutes. All material (root and peat) that was retained by a 2-mm (Fisher Scientific no. 10) standard test sieve was then sorted. Washed roots were placed in a tub filled with 3-4 cm of water. Live roots were separated from dead roots and peat material with tweezers and a magnifying glass. Live roots were defined as those that were turgid, had root hairs attached, and were light orange to white in color. Roots that were gray in color, squishy, retained no root hairs, or showed a combination of these symptoms were considered dead, as was the partially decomposed material that comprised the peat. Any live rhizomes and stem material that was below the soil surface when the plants were harvested were also considered to be live belowground biomass. Live biomass was dried to a constant weight at 60°C and weighed.

A subsampling method was developed to reduce sorting time for large root samples. After rinsing as described above, all large pieces of live material were separated from the sample. These were dried and weighed as previously described. When subsequent sorting yielded a minimal amount of live root material (less than approximately 0.5 g wet live root material in 1 hour), the remaining roots were mixed in the tub to produce a uniform mixture of live and dead material. This mix was then separated into 16 pieces of uniform area. Two of these pieces were randomly selected to be completely sorted. For these two "subsamples" both the live and dead portions of the subsample were dried and weighed. The remaining seven-eighths of the mixed live and dead material was dried and weighed together. To calculate the amount of live root biomass of the unsorted portion of a sample, the average proportion of live:dead material in the two subsamples was multiplied by the dry weight of the unsorted mixed portion. To calculate the total live belowground biomass of the pot, the calculated live biomass of the unsorted portion, the live biomass of the two subsamples, and the biomass of the large pieces of live material that were originally sorted out of the sample were added together.

The root:shoot ratio for each pipe was calculated by dividing the total live belowground biomass for each pipe by the live aboveground biomass of all species. All species were included rather than only *S. patens* because it was not possible to identify roots to species. Also, pipes with species other than *S. patens* were extremely rare, and when present, there were only a few stems.

Statistical analyses were performed using SAS (SAS Institute, Inc., Cary, North Carolina, U.S.A.) and R (R Core Team, 2015). Linear mixed effects models were used to determine the effect of elevation on above- and belowground biomass as well as selected elemental concentrations in leaf tissue (lmer in R package lme4; Bates *et al*., 2015a,b). These models included a random effect for location (organ). The random effect controls for any potential effects of installing marsh organs at several sites; marginal and conditional R^2 values presented approximate the fit of the model with only the fixed effects and with both the fixed and random effects, respectively. Associations of elemental concentrations in leaf tissue with aboveground biomass, belowground biomass, and soil elevation were tested using Pearson correlation coefficients, (SAS PROC CORR). Correlations were estimated separately for each season because previous analysis showed that the elemental composition of leaf tissue differed seasonally (Tobias, 2010). Elements that were highly correlated with biomass and soil elevation were considered potential indicators of flooding stress. Regression trees (rpart in R package rpart; Therneau, Atkinson, and Ripley, 2015) were used to identify potential values of selected elements that best separated flood-limited plants from those that were not flood-limited by their biomass for summer samples. Regression trees with random effects were also fit (REEMtree in R package REEMtree; Sela and Simonoff, 2011). Regression trees partition the data at values of each selected element, creating clusters that minimize the variance (sums of

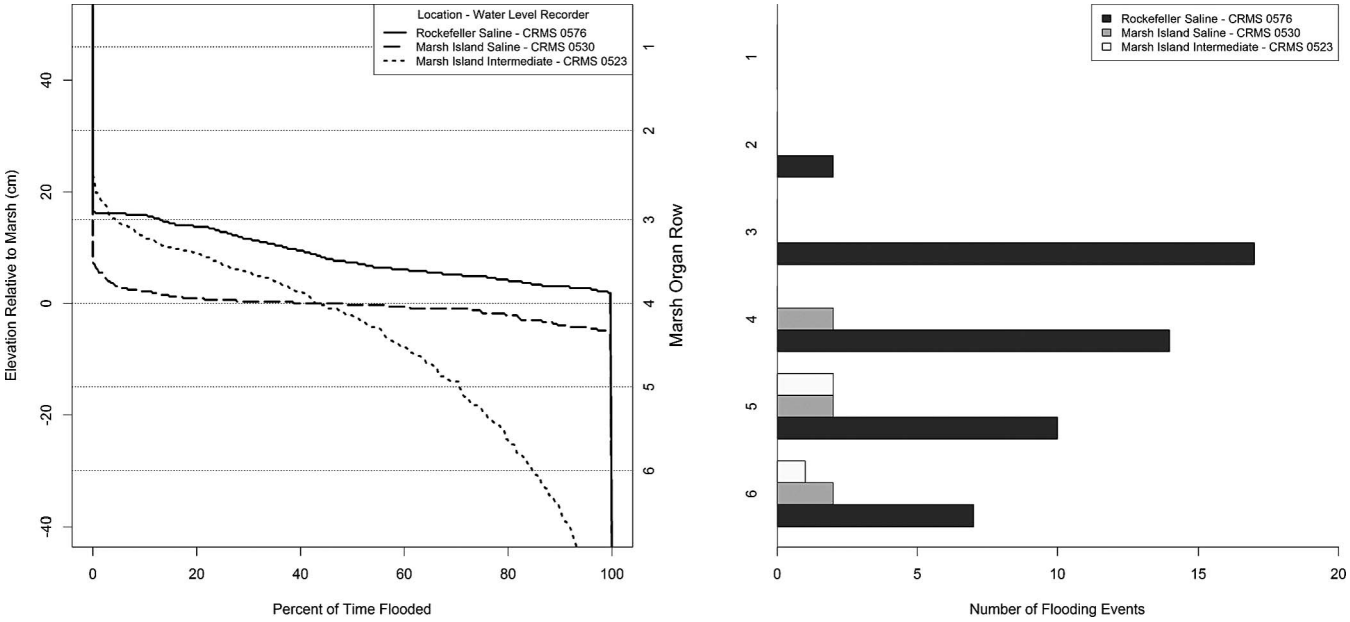

Figure 3. Hydroperiod (duration and frequency of flooding) at elevations corresponding to heights of marsh organ pipes, calculated with the nearest available water level data from Coastwide Reference Monitoring System (CRMS) recorders.

squares) in biomass within each cluster. Regression trees were calculated for above- and belowground biomass for each selected element. Critical values identified by regression trees were used with the linear mixed models to determine the critical elevation that separates flood-limited *S. patens* plants from those that are limited by a factor other than flood stress.

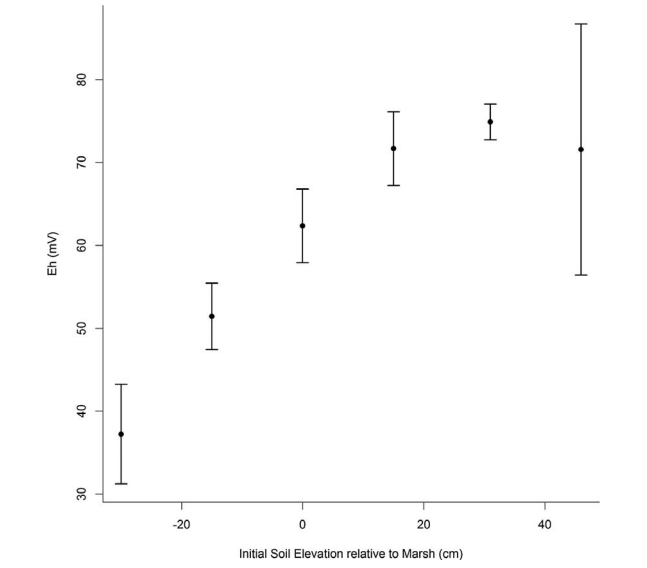

Figure 4. Mean soil E_h with 95% confidence intervals for various elevations above and below local marsh elevation measured approximately 12 months after planting.

RESULTS

The marsh organ structures produced an elevational gradient with varied hydrology at each level (Figure 3). The two tallest pipes in all marsh organs were overtopped with water rarely, if ever, whereas the shorter pipes experienced a range of flooding frequencies and durations.

Generally, E_h increased as elevation increased (Figure 4); however, the magnitude of E_h reported by the equipment appeared biased for summer samples. Although E_h appears to suggest that soils were far too oxidized to contain reduced Mn during summer sampling, visual inspection of soils showed clearly that Fe was reduced within the rooting zone. If Fe was reduced, Mn was also reduced. E_h was not used in statistical analyses, however, because of the suspected bias in E_h measurements.

Above- and belowground biomass increased with increasing initial elevation (Figure 5). At the time of the summer harvest, the soil inside most of the pipes exhibited some loss of elevation (Figure 5). The soil elevation declined most in pipes with the highest initial elevation, but these pipes also contained the highest plant biomass (Figure 5). Some pipes in the shortest row (*i.e.* pipes that were flooded 100% of the time) accumulated small amounts of unconsolidated sediment on top of the soil surface. In the fall, soil elevation loss followed a pattern similar to summer elevation loss in three out of four marsh organs. The Rockefeller Saline site had a substantial elevation loss, which apparently resulted from undermining of the pipes by storm surge from Hurricane Ike. Neither the above- nor belowground portions of the plants appeared to have been damaged by the hurricane, but the structure of the marsh organ itself appeared to have been undermined, because the north side was twisted

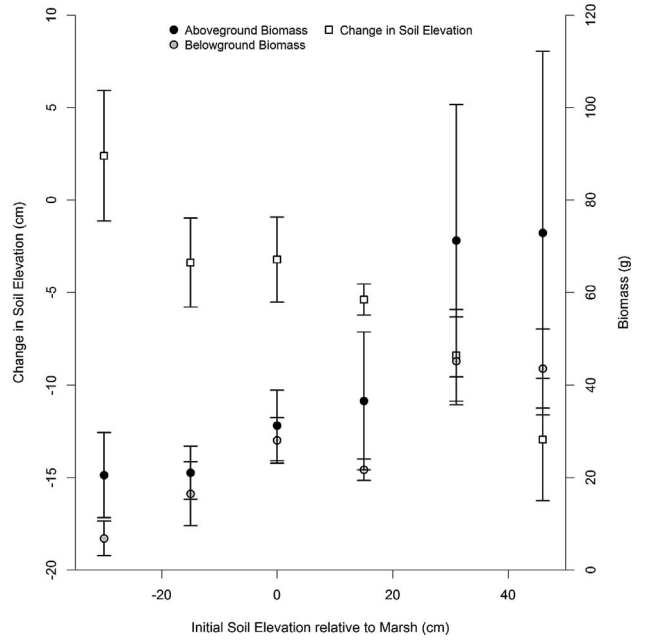

Figure 5. Mean change soil elevation and mean above- and belowground biomass (with 95% confidence intervals) for various initial soil elevations above and below local marsh elevation, measured approximately 12 months after planting.

slightly downward. No other marsh organ appears to have sustained such damage, but it was impossible to separate the effects of biogeochemical processes from possible effects of erosion for fall measurements.

To account for changes in elevation, soil elevation at the time of harvest was used rather than the initial soil elevation (*i.e.* the height of the pipes) as the dependent variable for regressions to estimate the effect of elevation on biomass. Live aboveground biomass was linearly related to soil elevation at the time of harvest in summer with the highest elevations producing the biggest biomass (Table 1). Plants harvested from the shortest pipes generally showed signs of decay, and few plants from these rows showed signs of growth. Plants grown at elevations close to marsh level or at higher elevations generally appeared healthy. Biomass at least doubled for plants grown at an initial soil elevation at or above that of the local marsh surface. Plants grown in row 1 at an initial elevation of 46 cm above marsh level clearly were not stressed by flooding; pots in this row consistently contained the highest biomass.

In the leaf tissue harvested during the summer, Mn and Ca were the only elements that correlated highly ($|r| > 0.50$) with measures of biomass and soil elevation (Table 2). In the fall, no elements correlated highly with measures of biomass and soil elevation. Correlations during fall are reported to facilitate comparisons with other studies that have reported end of season tissue concentrations. These associations are not interpreted here, however, because previous studies have shown that the elemental composition of leaf tissue in the fall is not a good indicator of growing conditions for *S. patens* (Tobias 2010).

Variances for random effects in the regression trees were small ($<10^{-6}$), so a single critical value was considered for each element-biomass combination because site-specific critical values would have added no additional predictive value. Regression trees identified [Mn] < 256 ppm as an indicator of flood limitation for aboveground biomass ($R^2 = 0.482$) and [Mn] < 247 ppm as an indicator for belowground biomass ($R^2 = 0.306$). Regression trees identified [Ca] < 0.3% as an indicator for both above- and belowground biomass ($R^2 = 0.517$ and 0.636, respectively). In all regression trees, the mean and variance of biomass were higher for the cluster with elemental concentrations above the critical value than for the cluster below the critical value (Figure 6). Using leaf tissue collected from the intermediate site at Rockefeller Refuge, which drained for at least 2 weeks before harvest, the critical value for [Ca] correctly identified 100% of plants as not flooded, and the critical values for [Mn] correctly identified all but one plant (94%; $n = 17$) as not flooded.

The relationship between soil elevation and [Mn] or [Ca] for plants harvested in the summer, as estimated by linear mixed effects models, is shown in Figure 7 as well as a demonstration of how this relationship, along with information about leaf tissue concentrations, can be used to identify marshes growing at elevations that are limited by flooding stress. Parameter estimates and model fit are given in Table 1. Dashed lines show critical values (the mean of above- and belowground indicators for [Mn] and the single indicator for [Ca]) and the predicted soil

Table 1. *Linear mixed models used to estimate the effect of soil elevation on biomass and to relate leaf tissue concentrations of Mn and Ca in* S. patens *to soil elevation. Marsh organ (site) was included as a random effect in each model. Marginal R^2 estimates the fit of the fixed effects portion of the model and the conditional R^2 estimates the fit of the combined fixed and random portions of the model.*

	Model Fit (R^2)		Fixed Effects						Random Effects		
Dependent Variable	Marginal	Conditional		Estimate	Standard Error	Chi Squared	df	*p*		*n*	Variance
Aboveground biomass (g/pot)	0.578	0.585	Intercept	25.54	1.17	221.25	1	<0.0001	Organ (intercept)	3	2.1
			Soil elevation	0.59	0.07	73.77	1	<0.0001	Residual	54	120
Belowground biomass (g/pot)	0.307	0.385	Intercept	39.77	7.34	29.38	1	<0.0001	Organ (intercept)	3	104.2
			Soil elevation	0.94	0.20	21.43	1	<0.0001	Residual	44	829.1
Log(root:shoot) ratio	0.003	0.256	Intercept	0.15	0.19	0.64	1	0.800	Organ (intercept)	3	0.09
			Soil elevation	0	0	0.19	1	−0.436	Residual	43	0.25
Elevation (cm above marsh)	0.501	0.578	Intercept	−19.8	5.30	13.94	1	0.0002	Organ (intercept)	3	41.33
			Mn (ppm)	0.09	0.01	54.79	1	<0.0001	Residual	52	226.64
Elevation (cm above marsh)	0.567	0.730	Intercept	−43.67	7.98	29.95	1	<0.0001	Organ (intercept)	3	104.4
			Ca (%)	161.86	17.31	87.39	1	<0.0001	Residual	52	172.8

Table 2. *Pearson correlation coefficients for measure of biomass and soil elevation with leaf tissue concentrations of various elements in* S. patens *leaf tissue.*

	Summer				Fall			
	Biomass (g/pot)				Biomass (g/pot)			
Leaf Tissue Concentrations	Belowground	Aboveground *S. patens*	Aboveground All Species	Soil Elevation	Belowground	Aboveground *S. patens*	Aboveground All Species	Soil Elevation
Al (ppm)								
r	−0.158	−0.205	−0.220	−0.293	−0.199	0.045	−0.012	−0.752
p	0.3709	0.2379	0.2049	0.0874	0.3287	0.8282	0.9547	<.0001
n	34	35	35	35	26	26	26	26
B (ppm)								
r	0.388	0.279	0.239	0.221	−0.296	−0.088	−0.140	−0.854
p	0.0234	0.1046	0.1664	0.2022	0.1426	0.6707	0.4937	<.0001
n	34	35	35	35	26	26	26	26
Ca (%)								
r	0.600	0.628	0.591	0.720	−0.072	0.331	0.310	−0.134
p	0.0002	<.0001	0.0002	<.0001	0.7284	0.0986	0.1235	0.5149
n	34	35	35	35	26	26	26	26
Cu (ppm)								
r	−0.019	−0.006	−0.024	−0.048	−0.412	−0.217	−0.279	−0.679
p	0.9154	0.9717	0.8916	0.7849	0.0363	0.288	0.1675	0.0001
n	34	35	35	35	26	26	26	26
Fe (ppm)								
r	−0.129	−0.078	−0.090	−0.167	−0.304	−0.131	−0.190	−0.785
p	0.4672	0.6552	0.6086	0.3376	0.1315	0.5244	0.3516	<.0001
n	34	35	35	35	26	26	26	26
Mg (ppm)								
r	0.201	0.141	0.093	−0.053	−0.295	0.023	−0.042	−0.708
p	0.2555	0.4192	0.594	0.7613	0.144	0.9118	0.8385	<.0001
n	34	35	35	35	26	26	26	26
Mn (ppm)								
r	0.575	0.584	0.551	0.672	−0.162	−0.023	−0.080	−0.460
p	0.0004	0.0002	0.0006	<.0001	0.4292	0.9105	0.6993	0.0182
n	34	35	35	35	26	26	26	26
Ni (ppm)								
r	−0.159	−0.211	−0.229	−0.266	−0.301	−0.114	−0.179	−0.822
p	0.3706	0.2233	0.1864	0.1223	0.1346	0.58	0.3815	<.0001
n	34	35	35	35	26	26	26	26
N (%)								
r	−0.118	−0.228	−0.280	−0.363	0.263	0.498	0.479	0.483
p	0.5078	0.1874	0.1029	0.0323	0.1948	0.0096	0.0132	0.0125
n	34	35	35	35	26	26	26	26
P (%)								
r	0.125	0.119	0.126	−0.046	−0.188	0.023	−0.005	0.210
p	0.4818	0.4976	0.4697	0.795	0.3565	0.9115	0.981	0.3024
n	34	35	35	35	26	26	26	26
K (%)								
r	0.235	0.351	0.353	0.427	0.088	0.212	0.258	0.661
p	0.1807	0.0385	0.0373	0.0106	0.6679	0.2992	0.2037	0.0002
n	34	35	35	35	26	26	26	26
Na (ppm)								
r	0.205	0.083	0.089	−0.082	−0.383	−0.070	−0.133	−0.658
p	0.244	0.6366	0.6102	0.6379	0.0534	0.7349	0.5179	0.0003
n	34	35	35	35	26	26	26	26
S (%)								
r	0.280	0.133	0.111	0.034	−0.554	−0.227	−0.264	−0.463
p	0.1089	0.4455	0.5256	0.8473	0.0034	0.2657	0.1918	0.0174
n	34	35	35	35	26	26	26	26
Zn (ppm)								
r	−0.096	−0.093	−0.113	−0.101	−0.225	0.089	0.039	−0.590
p	0.589	0.596	0.5187	0.5652	0.2692	0.6669	0.8511	0.0015
n	34	35	35	35	26	26	26	26
Molar C:N								
r	0.036	0.146	0.188	0.270	0.253	−0.080	0.000	−0.029
p	0.841	0.402	0.2795	0.1169	0.2323	0.7088	0.9982	0.8941
n	34	35	35	35	24	24	24	24

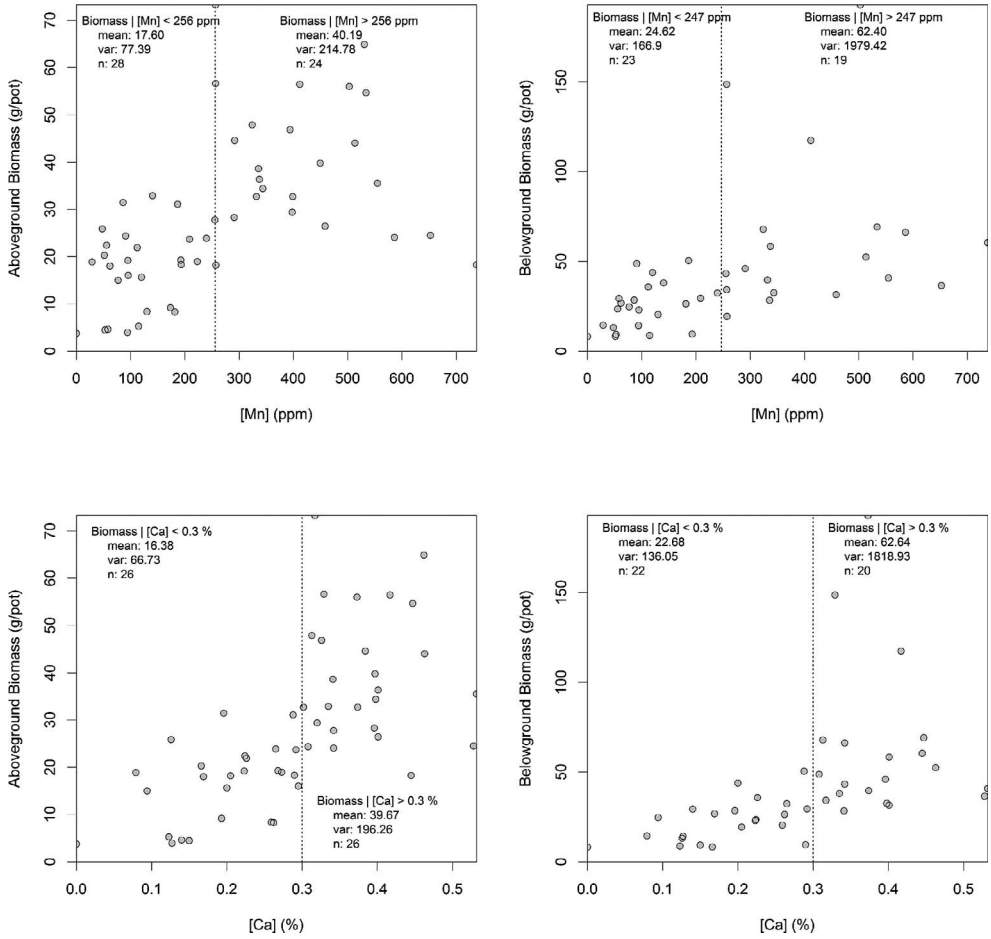

Figure 6. Results of regression tree analysis to separate flood-limited *S. patens* plants from nonflood-limited plants based on leaf tissue concentrations of Mn and Ca. Cutoff values represent the values that best separated flood-limited plants from those that were not flood limited by their biomass for summer samples. The individual data points are included to illustrate visually the difference in variation of *S. patens* biomass between the groups identified by the regression tree analysis.

elevation for plants with those leaf tissue concentrations. The dark bars around the predictions on the *y*-axis identify the 95% confidence intervals for the predicted elevations. Biomass in plants with concentrations below those shown would be limited by flooding stress; those with concentrations above would be limited by something other than flooding. The soil elevation predictions for critical values of both [Mn] and [Ca] are close to an elevation of zero (*i.e.* local marsh level), and the confidence intervals for the predictions include elevations of zero.

DISCUSSION

Within the ranges of flooding in this study, there was no evidence of an optimal elevation for *S. patens* biomass. The results of this study were similar to observations of *S. patens* in Cape Cod marshes and Chesapeake Bay marshes. In Cape Cod marshes, more frequent flooding was associated with marsh dieback and an inability of flooded *S. patens* to tolerate additional sources of physiological stress (Smith, Mederios, and Tyrell, 2012). In Chesapeake Bay marshes, aboveground growth increased with higher elevations throughout the intertidal zone (Kirwan and Guntenspergen, 2012). There is a tradeoff between higher productivity of *S. patens* and increased loss of soil elevation as the initial elevation of the created marsh platform increases. Perhaps more importantly, in marshes that have already subsided below local marsh elevation, flooding stress is too great for *S. patens* to thrive, although they may accrete some mineral sediment on top of the created marsh platform. It is important for managers to take this loss of soil elevation into account.

Results presented here support the recommendation that restoration efforts in Louisiana's *S. patens* marshes should target water levels at 15 cm below marsh elevation and that marshes with more flooding are less stable (Nyman *et al.*, 2009). The elevation of the marsh platform in stable marshes is within centimeters of the mean daily high-water level (Nyman *et al.*, 2009) and marsh elevation varies little (6 cm across 125 m of marsh; Nyman *et al.*, 1994). The large elevation losses in response to drainage that were observed suggest that managers and restoration professionals should limit the use of drainage to improve marsh productivity. Draining marsh soils

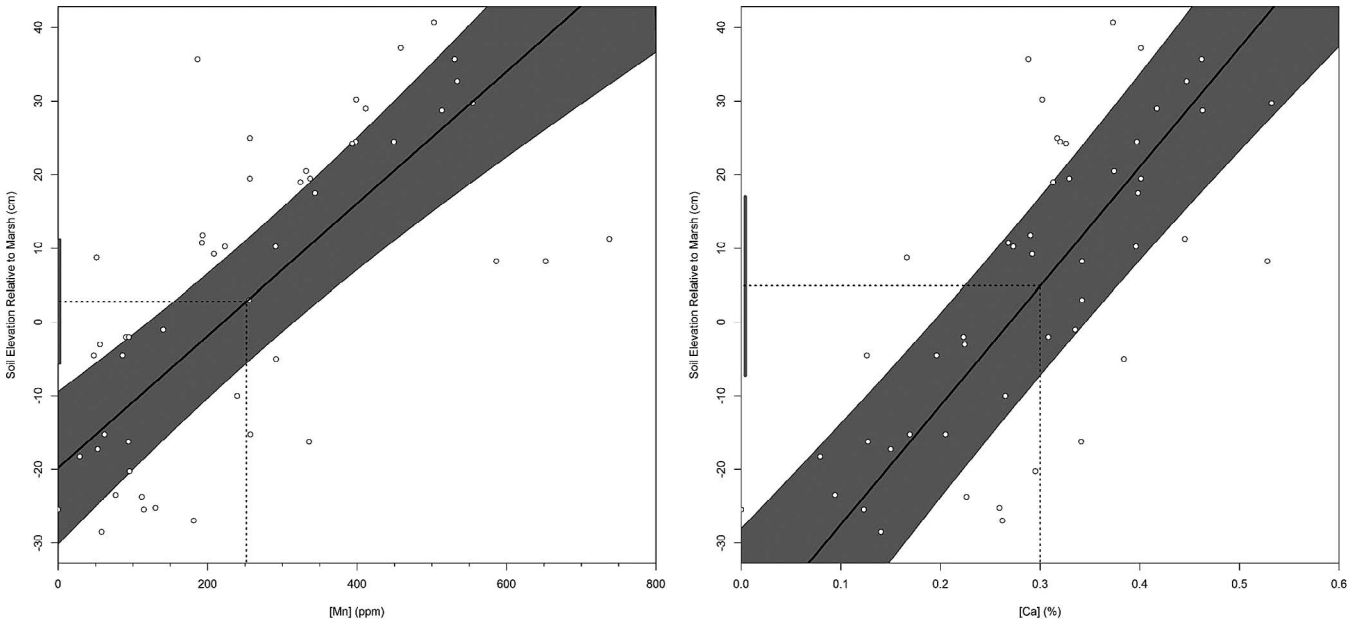

Figure 7. Relationship between leaf tissue concentrations of Mn and Ca in *S. patens* leaf tissue and the soil elevations at which the plants were grown, as estimated by linear mixed models. Shading represents 95% confidence intervals. Estimates of parameters and model fit can be found in Table 1.

increased productivity of *S. patens* in this study, but it also caused increased loss of soil elevation. In extremely drained marsh soils, *S. patens* was the most productive, but the plants in the most drained soils were unable to keep up with the rates of soil elevation loss. Short, shallow drawdowns early in the growing season may have a positive effect on production without causing the substantial losses of soil elevation that occurred in the most drained pipes. Loss of soil elevation may have been caused by a combination of factors. The soils used were highly organic, so one would expect them to oxidize upon draining. Also, as soils dried they may have compacted. It is beyond the design of this experiment to determine which might be responsible here, however. Regardless of the cause, loss of elevation after drainage of wetland soils is an important consideration for management and restoration plans.

[Mn] and [Ca] in the leaf tissue of *S. patens* harvested during the summer can be used to differentiate plants that were grown with average water levels above the soil surface from plants that were grown with average water levels below the soil surface. The relationship between [Mn] and live *S. patens* biomass was more indicative of a limiting factor relationship than the relationship between [Ca] and biomass (Figures 6 and 7). In a typical plot of growth responses to changes in nutrients, one would expect that growth would increase up to a point when the concentration of the nutrient reached an adequate level. Beyond that point, growth would no longer increase and would remain stable unless toxicity developed (Epstein and Bloom, 2005). These curves are typically developed in controlled greenhouse situations, however. In this experiment, salinity and nutrients were allowed to vary with environmental conditions; thus, when flooding was no longer limiting, biomass was controlled by these other factors. Where biomass was not limited by flooding, variation in biomass would be expected because salinity and nutrient availability vary among study sites. Production in plants with leaf tissue [Mn] > 247–256 ppm is limited by something other than flooding, such as high salinity or low nitrogen availability, as indicated by the wider variation in productivity for these plants.

Several studies have shown that in greenhouse conditions, wetland plants exhibit increased [Mn] in aboveground tissue when stressed by flooding (*e.g.*, Bandyopadhyay *et al.*, 1993; Pierce *et al.*, 2009). A few studies have shown the opposite, however, for plants grown in flooded soils that are subsequently drained. For example, the Mn content of common carpet grass (*Axonopus affinis*) and centipedegrass (*Eremochloa ophiuroides*) was also higher in plants that were drained after flooded conditions than in plants that were flooded but not drained (Bush *et al.*, 1999). Another study (Lissner *et al.*, 2003) found that *C. jamaicense* had higher [Mn] at $E_h = +600$ mV than at either +150 or −150 mV where P was not limiting. The authors suggest that the Mn^{2+} with which they amended their experimental soils was not oxidized after draining of the soil.

In this experiment it is more likely, however, that Mn^{2+} availability was primarily controlled by pH or organic matter content of the soil, rather than redox potential. Because of this, redox potential may be somewhat irrelevant to the availability of Mn in drained wetland soils (Gotoh and Patrick, 1972). At low pH, as often occurs in drained wetland soils such as the local soil used, most of the Mn in soils is expected to be soluble Mn^{2+}, which is more easily taken up by plants, rather than insoluble Mn^{4+}. Such conditions would not be likely to develop in experimental soils that are a mix of clay and sand. It is possible that Mn^{2+} was available to plants in all of the flooding treatments in the present experiment; Mn^{2+} can be available

throughout a wide range of E_h because it can make complexes with organic matter (Reddy and DeLaune, 2008, p. 425).

[Mn] in leaf tissue may have been influenced by poor root growth in the most flooded pipes. Wetland plants are adapted to flooded conditions, but extended flooding can disrupt physiological functioning and nutrition (DeLaune, Pezeshki, and Lindau, 1998). Lack of oxygen in the soil may reduce root growth and the ability of the roots to take up nutrients. Although *S. patens* roots are able to tolerate low E_h conditions once established, they are less able to grow into reduced soils than oxidized ones (Pezeshki, Matthews, and DeLaune, 1991). Even established roots that have developed extensive aerenchyma tissue cannot survive in anoxic soils indefinitely (Pezeshki, 2001). Soon after soils become hypoxic, metabolism in plant roots switches to fermentation (Drew, 1997). Even if Mn^{2+} was more available in the most flooded pipes, the roots would be unable to take it up because anaerobic respiration produces less energy than aerobic respiration, so plants lose the ability to absorb nutrients or translocate them to stems or leaves (Epstein and Bloom, 2005). Studies of Mn uptake are needed to understand the mechanisms responsible for observations in the present study.

Like [Mn], [Ca] in the leaf tissue of *C. jamaicense* has also been shown to increase with increasing E_h (Lissner *et al.*, 2003). Although [Ca] was better able to predict flooding levels than [Mn], it may less useful as an indicator of flooding stress because the variation in the relationship between [Ca] and total live biomass was relatively constant across the range of [Ca] observed. If [Ca] were used as an indicator of flooding stress, the constant variation in the relationship between [Ca] and biomass would suggest that production in all plants was limited by flooding stress. This was not the case in the study presented here, as the variation in the relationship between average flooding depth and biomass becomes more variable when plants experience less flooding.

A second reason [Ca] may not be an ideal indicator of flooding stress is that it may be influenced by other factors that are unrelated to flooding. [Ca] in leaf tissue may also be controlled by N availability (Jones, 1998), salinity (Epstein and Bloom 2005), or both. Because [Ca] may be influenced by N availability, salinity, and flooding level, [Ca] may be a better indicator of overall biomass than of flooding stress alone. More productive plants with higher rates of transpiration also have higher [Ca] in their leaf tissue (Jones, 1998) which also suggests that low [Ca] could indicate general limitation of growth. [Ca] has been used to indicate the overall degree of limitation by environmental factors in diagnosis and recommendation integrated systems (Bailey, Beattie, and Kilpatrick, 1997). More research may be necessary to identify interactions between these factors and flooding.

Critical values identified for [Mn] and [Ca] in leaf tissue were useful for identifying limitation in general and limitation by flooding stress, respectively, in *S. patens* for plant tissue harvested during the summer but could not be used to identify flooding stress in plants harvested during the fall. The seasonal nature of the results of this study suggests that making comparisons among studies relating to the tissue of plants harvested at different times during the growing season may not be possible. This is not unexpected, however, because the nutritional needs and uptake of other ions change throughout the growing season for *S. patens* (Ewing *et al.*, 1995). Indicators of salinity limitation were effective only in spring and summer, whereas indicators of nutrient limitation were more effective in fall (Ewing, McKee, and Mendelssohn, 1997). Similarly, in spring, *S. alterniflora* is rarely N limited, but the demand for N increases to supply its larger biomass as it grows and to produce osmotica to block Na+ uptake during dry summers (Bradley and Morris, 1992).

Although they are based on a single growing season of observation, these cutoff values show promise as indicators of flooding stress because they correctly identified the growing conditions of nearly all samples used to test them. Further testing of [Mn] or [Mn] in combination with [Ca] rather than [Ca] alone is recommended to identify flooding stress in *S. patens* for restoration and management purposes. It is uncertain whether the results of this study could be applied to marshes outside the Gulf of Mexico. Differences between this study and previous research conducted in Atlantic Coast marshes suggest that the results of this study may only be applicable to marshes with small tidal amplitude and low mineral soil. Further studies must be done to determine whether productivity in other systems is controlled by flooding stress, as it is in Louisiana's coastal marshes.

An aboveground indicator of belowground production would provide a relatively easy and cost-effective method for understanding how management actions such as manipulating hydrology affect root biomass. In a marsh system where root biomass strongly influences soil stability and the resilience of the community to sea level rise, such as marshes along the Gulf Coast, such an indicator could help managers make more informed decisions about their methods for managing marshes. The results presented here suggest that concentrations of Mn and Ca in the leaf tissue of *S. patens* could form part of an indicator to monitor belowground productivity of marshes. Although low concentrations of these elements were associated with the smaller root biomass of plants grown at low elevations, variability in the relationship suggests that additional factors may need to be considered.

Elevations below the natural marsh platform had reduced belowground biomass. Plants grown at these elevations experience a longer duration of flooding; in many cases, this was a single constant flood event that lasted the duration of the study. Elemental analysis of leaf tissue showed that [Mn] and [Ca] in leaf tissue, which vary with flooding stress, also vary with belowground biomass in this study. Plants whose belowground biomass is limited by flooding stress take up less Ca because rates of Ca absorption are governed by the size of the root system (Loneragan and Snowball, 1969). Increasing water levels did not affect root:shoot ratio, but root:shoot ratio varied widely. This suggests that flooding affects above- and belowground biomass in a similar manner but that changes in nutrient availability, salinity, other local conditions that were not measured in this study, or a combination of factors may alter the allocation of biomass between roots and shoots; however, the interacting effects of season and location explained a very small proportion of the variation in root:shoot ratio. In contrast to results presented in the present paper, high root:shoot ratios have been observed in *S. alterniflora*

growing in unfavorable soil conditions (Knox, 1986) and high root:shoot ratios have been interpreted as evidence of flooding stress (Nyman *et al*., 1994).

The variation in the relationship between elevation and live belowground biomass suggests that factors other than depth of flooding contribute to belowground biomass, even when plants experience high levels of flooding. This contrasts with the response of aboveground biomass, which increases linearly with decreasing flooding when plants are flooded above the soil surface and varies more when water levels are lower. Also, soil elevation explained less of the variation in live belowground biomass (approximately 30%) than in aboveground biomass (approximately 58%). The contrast in effects of flooding on above- and belowground biomass suggests that increased availability of nutrients, low salinity, or both may provide some protection from flooding stress for belowground biomass that it does not provide for aboveground biomass. Elevation, and hence duration of flooding, was the dominant factor controlling aboveground biomass, and although elevation explained less of the variation in belowground biomass, it was still a significant factor.

Future studies should consider interactions of flooding with other stress factors. For example, studies using controlled levels of salinity and nutrient availability would be necessary to evaluate potential interactions between results presented in this paper and previously observed effects of salinity stress and nutrient availability on leaf tissue stoichiometry in *S. patens* (Tobias *et al*., 2010). An additional line of further study is the effect of competition with species that are better adapted to living in less flooded environments. Although *S. patens* growing in isolation produced more biomass at the highest elevations in this study, it is likely that *S. patens* would experience competition from facultative or upland species at these elevations.

CONCLUSIONS

Spartina patens biomass increases with increasing elevation, unlike *S. alterniflora*, which exhibits optimum growth at an intermediate elevation. In spite of this, management for optimum biomass production for *S. patens* marshes should target elevations at or only slightly above the natural stable marsh elevation of the daily mean high-water level, because marsh soil above this elevation tends to lose elevation faster than plants can vertically accrete to make up for elevation loss. It may be possible to use leaf tissue concentrations measured in summer of Mn, Ca, or both to identify *S. patens* plants whose biomass production is limited by waterlogging caused by low elevation. Additional research is needed to identify how factors such as salinity and nitrogen availability affect the relationship between biomass production and leaf tissue concentrations of Mn and Ca in *S. patens*, however.

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