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A COMPARISON OF THE ELEMENTAL COMPOSITION OF LEAF TISSUE OF *SPARTINA PATENS* AND *SPARTINA ALTERNIFORA* IN LOUISIANA'S COASTAL MARSHES

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□ *Elemental concentrations in leaf tissue can identify limiting conditions in crops and can be useful in managing and restoring marshes. Coastal management and restoration plans frequently include* Spartina spp. *because they are common plants in coastal wetlands across North America. Researchers frequently compare results of stoichiometric studies among* Spartina spp. *to corroborate results, although their stoichiometry may not be comparable. We compare the stoichiometry of paired samples of* Spartina patens *and* Spartina alterniflora *collected across Louisiana. Overall differences in stoichiometry between species, seasonal changes, and effects of porewater chemistry were quantified. Manganese (Mn) concentrations and calcium (Ca) concentrations were higher in* S. alterniflora *and the difference in [Ca] increased seasonally. Sodium concentrations were similar, except during prolonged inundation. Short flooding durations decreased carbon (C):nitrogen (N) without increasing [Mn] or flooding stress in both species.*

Keywords: *spartina patens*, *Spartina alterniflora*, tissue testing, marsh, wetland, management, restoration, stoichiometry, stress indicators

INTRODUCTION

Coastal management and restoration plans frequently focus on *Spartina spp.* because they are common plants in coastal wetlands across in North America. On the coast of the Gulf of Mexico, marshes are managed to promote productivity because productive marshes have higher rates of vertical accretion (Nyman et al., 2006). Marshes with high rates of vertical accretion

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are better able to keep up with relative sea level rise. Live root material contributes to the strength of marsh soils (McGinnis 1997), thus increasing resistance to erosion (Nyman et al., 1995). Other management plans involve manipulating conditions to eradicate *Spartina spp.* where they have become weedy invasives, such as in Washington, Oregon, and California. *Spartina spp.* are also commonly planted to stabilize banks of created marshes and terraces along the Gulf Coast and on the Atlantic Coast.

The success of management and restoration plans involving *Spartina spp.* depends greatly on understanding the range of conditions under which plants can survive. Management and restoration success also depends on the ability of professionals to identify when physical parameters such as salinity or water levels are outside the tolerance range for target species, and thus when conditions need to be manipulated to ensure that conditions remain within tolerance ranges for plants. Although the tolerance ranges for *Spartina spp.* are fairly well-understood, managers lack accurate and inexpensive methods of determining which factors limiting productivity.

Several potential indicators have been developed for identifying limited production in wetland plants and some environmental factors that cause it. Many of these require specialized equipment, are time-consuming, or are expensive, which makes them difficult to use over large spatial or temporal scales. For example, changes in above-ground biomass can be used to identify sites that differ in productivity (e.g., Burdick et al., 1989, Ewing et al., 1997), as can shoot elongation (Ewing et al., 1997). Once differences in productivity have been established, managers can infer which factors cause these differences from environmental monitoring data. Salinity stress and nutrient limitation can also be identified directly by measuring leaf spectral reflectance, carbon dioxide uptake, leaf expansion, and leaf proline concentration (Ewing et al., 1995, 1997). Unlike other methods of identifying limiting factors, tests to determine elemental composition of plant tissue are practical tools for managers because they are inexpensive and commercially available and collecting samples for such tests requires little time.

Limiting conditions in agricultural crops are commonly determined by measuring elemental concentrations in plant tissue. For example, concentrations of nitrogen (N) in the leaf tissue of rice < 2.8–3.6% indicate that plant growth is limited by low N availability and that fertilization may improve production (Brandon and Wells, 1986). Similarly, concentrations of manganese (Mn) > 4000 ppm in the leaf tissue of rice indicates that productivity is limited by Mn toxicity (Adriano, 1986). Some guidelines for diagnosing nutrient limitation and salinity stress in wetland plants have been developed as well. N:phosphorus (P) ratios >16 in plant tissue have been used to diagnose P-limitation (e.g., Koerselman and Meuleman, 1996). Carbon (C):N and sodium [Na] have been used to diagnose N-limitation and salinity stress, respectively, in *Spartina patens* (Tobias et al., 2010).

Guidelines for interpreting leaf tissue stoichiometry that have been previously developed may not apply to all wetland species, however, because even when different species experience the same nutrient availability, their tissue chemistry can vary widely (McJannet et al., 1995). For example, in several *Carex spp.* ranges of [N] and [P] in leaf tissue did not even overlap (Güsewell and Koerselman, 2002). Despite the understanding that stoichiometry is species-specific, congeneric references are often necessary because of the lack of information about stoichiometry for many species of management concern. The lack of information on the differences among congeneric species growing in the same conditions means that basing management decisions on the stoichiometry of congeneric species would be inaccurate. This would be particularly true if nutrient uptake mechanisms differ in their susceptibility to commonly managed stressors such as salinity, anoxia, or nutrient availability. For example, *Spartina alterniflora* and *S. patens* may have different nutrient requirements, and thus different concentrations of some elements in their leaf tissue, even when plants grow in the same location, because they are adapted to different environments. *S. alterniflora* is found in more flooded (Bertness, 1991) and more saline marshes than *S. patens* (Visser et al., 1998, 2000) so it is reasonable to suspect that these species might have different mechanisms for tolerating stressful conditions and therefore may have different nutrient requirements. In fact, *S. alterniflora* is more salinity tolerant and shows higher ion selectivity than *S. patens* (Hester et al., 2001).

The purpose of this paper is to improve the accuracy of stoichiometry as a tool for management and restoration by quantifying differences in leaf tissue chemistry between *S. alterniflora* and *S. patens*. To accomplish this, we asked a series of questions. (1) Does the leaf tissue chemistry differ between these species, and if it does, which elements are different? (2) How does porewater chemistry affect the leaf tissue chemistry of each species? (3) Are there seasonal patterns leaf tissue chemistry?

Currently, it is common practice to use the results of studies of either species to corroborate results. By quantifying the differences in chemistry for plants growing in the same conditions, this paper shows which elements can be directly compared between these species and which differ. We focus on C:N, [Na], [Mn], and calcium [Ca] because these elements have been previously used to identify causes of limited production in *S. patens* (Tobias et al., 2010, Tobias, 2010). We also include potassium [K] and Na:K because maintaining a high Na:K is an essential factor for salinity tolerance in halophytes (Maathuis and Amtmann, 1999). N, K, and Ca have also been identified as being important indicators of limitation in agricultural crops and have been included in diagnosis and recommendation integrated systems (e.g., Walworth and Sumner, 1987). We report concentrations of other elements as well, however, because they may be of interest for purposes other than ours. We examine seasonal patterns because some nutrient

concentrations in plant tissue and other indicators of limiting factors change during the growing season as a result of changing requirements for growth (Ewing et al., 1997). Thus, the seasonal timing of comparisons may change how elemental composition should be interpreted.

MATERIALS AND METHODS

We collected leaf tissue samples from *S. alterniflora* and *S. patens* growing in the same location in intermediate to saline marshes across the coast of Louisiana. Samples were taken at Cameron Prairie National Wildlife Refuge, Rockefeller Wildlife Refuge, Marsh Island Wildlife Refuge, and Fourleague Bay. At each location, we sampled at two sites: one fresher and one more saline. At each site, three plots that selected haphazardly. Following Penfound and Hathaway's (1938) classification system for coastal marshes, fresher sites were chosen to include species that indicated intermediate marsh such as *Sagittaria lancifolia* and *Scirpus olneyi* and more saline sites were chosen to include species that indicate brackish marsh such as *Spartina alterniflora*. The purpose of sampling intermediate and saline marshes was to collect data spanning the range of conditions that support *S. patens* and *S. alterniflora* simultaneously. One set of paired tissue samples was taken seasonally at each plot during the growing season (spring, summer, and fall) from May 2007 to November 2008.

We collected porewater samples at 10 cm below the marsh surface at each plot using a syringe connected to a piece of rigid tubing. The tubing was sealed at the end and holes were drilled along the sides to approximately 2 cm from the end. We pre-filtered porewater using a piece of nylon stocking fitted over the end of the tubing to exclude large soil particles. We measured salinity, conductivity, and pH of porewater using a handheld meter (YSI Model 63; YSI Inc., Yellow Springs, OH, USA). For nutrient analysis, water samples were filtered using 0.45 μm nylon syringe filters to remove sediment. These water samples were transported to the lab on ice and kept cold until nutrient analyses could be performed. The concentrations of ammonia-nitrogen were determined using the Nessler method and reactive phosphorus (orthophosphate) using the ascorbic acid method (Clesceri et al., 1998).

Leaf tissue of each species was collected from leaves originating in the top 10–15 cm of the stems of plants. Care was taken to harvest tissue samples from plants growing near each other at each site to ensure that samples from each species were from plants growing in similar soil conditions. Samples were only taken from plants growing away from the edge of a bayou or lake because nutrient dynamics and plant biomass different than those of interior marshes (Mendelssohn, 1979; Mendelssohn and Morris, 2000). An edge was defined as being the area adjacent to a water body

where vegetation was visibly different from the adjacent marsh (usually 3–5 meters).

Leaf tissue samples were placed in zip-top bags and stored them on ice until we returned to the lab. Samples were rinsed to remove soil and salt that may have been present on leaf surfaces before drying them at 60°C to a constant weight. Tissue samples were then ground using a coffee grinder (Black and Decker Smartgrind; Stanley Black & Decker; Towson, MD, USA) or Wiley Mill. Between samples, the grinders were cleaned with compressed air to remove particles. We submitted dried and ground tissue samples to the LSU AgCenter's Soil Testing and Plant Analysis Laboratory (STPAL, Baton Rouge, LA, USA) to determine their elemental composition. Carbon and nitrogen content was determined using dry combustion by CHN Analyzer. Concentrations of all other elements were determined using inductively coupled plasma mass spectrometry (ICP) analysis.

Differences in the overall elemental composition of the leaf tissue of the two species were collected on the same day, at the same site using a multivariate paired t-test. For this test, we only used data from sites where we collected both *S. alterniflora* and *S. patens*, resulting in 54 pairs of tissue samples. To perform this test in SAS (version 9.2, SAS Institute Inc., Cary, NC, USA), we used the MANOVA option in PROC GLM to test for differences between species, while treating each pair of samples as a block. Differences between individual elemental concentrations were explored using the ANOVA tests which are also produced by the code for the multivariate t-test.

Seasonal comparisons of the concentrations of Na, Mn, and K as well as ratios of C:N and Na:K were made a priori, and were thus made independently of the results of paired t-tests. The effects of each discrete sampling period were considered separately, rather than pooling data by season over both years because weather patterns were extremely different between the two years of this study. Spring flooding on the Mississippi River was extremely high in spring 2008 and in fall 2008, storm surge from Hurricane Ike inundated all of our study sites with saline water to a depth of approximately 2.5 m. Pearson correlation coefficients (PROC CORR) were used to explore relationships between porewater chemistry and elemental concentrations in leaf tissue.

RESULTS

Overall, the leaf tissue chemistry of *S. alterniflora* and *S. patens* collected on the same day, at the same site was different ($F_{(15,39)} = 46.58$, $P<0.0001$). Concentrations of Ca, C, Mg, Mn, N, P, K, and Zn differed by species (Table 1). Molar C:N ratio was also different between species. Ca, Mg, Mn, N, P, K, and zinc (Zn) were significantly higher in *S. alterniflora* than in

TABLE 1 Comparisons of individual elemental concentrations in *S. alterniflora* and *S. patens* leaf tissue. All ratios are molar; units for elemental concentrations are given

	Model		Species		Block	
Element	$F_{54,53}$	p	$F_{1,53}$	p	$F_{53,53}$	p
Al (ppm)	1.36	0.1349	.	.	.	.
B (ppm)	1.08	0.3912	.	.	.	.
Ca (%)	1.93	0.0091	54.27	< 0.0001	0.94	0.5923
C (%)	1.70	0.027	17.45	0.0001	1.41	0.1084
Cu (ppm)	2.91	< 0.0001	0.09	0.7693	2.96	< 0.0001
Fe (ppm)	1.62	0.0407	0.22	0.6421	1.65	0.0364
Mg (%)	2.66	0.0002	61.86	< 0.0001	1.55	0.0579
Mn (ppm)	2.24	0.0019	35.67	< 0.0001	1.61	0.0427
N (%)	7.24	< 0.0001	60.83	< 0.0001	6.23	< 0.0001
P (%)	3.33	< 0.0001	55.01	< 0.0001	2.36	0.0011
K (%)	2.13	0.0032	7.06	0.0104	2.04	0.0052
Na (ppm)	3.13	< 0.0001	3.98	0.0511	3.30	< 0.0001
S (%)	2.65	0.0003	2.76	0.1028	2.65	0.0003
Zn (ppm)	4.50	< 0.0001	111.12	< 0.0001	2.49	0.0006
C:N	4.89	< 0.0001	88.68	< 0.0001	3.31	< 0.0001
N:P	2.31	0.0013	0.02	0.8910	2.35	0.0011
Na:K	1.37	0.1288	.	.	.	.

S. patens, while [C] and C:N ratio were significantly higher in *S. patens* than *S. alterniflora* (Table 2). Concentrations of aluminum (Al), boron (B), copper (Cu), iron (Fe), Na, and sulfur (S) did not differ between species. Molar N:P and Na:K also did not vary by species.

TABLE 2 (LS)Mean elemental concentrations by species. All ratios are molar; units for elemental concentrations are given

	Spartina alterniflora		Spartina patens	
	Mean	Std. Error	Mean	Std. Error
Al (ppm)	67.13	12.75	113.33	12.75
B (ppm)	5.20	0.61	6.71	0.61
Ca (%)	0.42	0.02	0.22	0.02
C (%)	44.33	0.18	45.39	0.18
Cu (ppm)	1.62	0.19	1.55	0.19
Fe (ppm)	118.87	10.80	111.74	10.80
Mg (%)	0.31	0.01	0.19	0.01
Mn (ppm)	172.35	9.89	88.85	9.89
N (%)	1.42	0.03	1.07	0.03
P (%)	0.13	0.00	0.09	0.00
K (%)	0.80	0.03	0.67	0.03
Na (%)	1.05	0.04	1.15	0.04
S (%)	0.48	0.05	0.59	0.05
Zn (ppm)	9.21	0.31	4.64	0.31
C:N	38.72	1.41	57.46	1.41
N:P	25.71	0.70	25.58	0.70
Na:K	2.48	0.16	3.22	0.16

TABLE 3 Pearson product-moment correlations and p-values describing the relationships between porewater chemistry and stoichiometry of leaf tissue for *S. alterniflora*

		Al	B	Ca	C	Cu	Fe	Mg	Mn	Mb	
Salinity (ppt)	r	−0.15	0.12	0.05	0.09	0.24	−0.40	0.16	−0.37	0.11	
	p	0.3181	0.4137	0.7511	0.5586	0.2418	0.0053	0.2739	0.011	0.5918	
ph	r	−0.16	0.00	0.27	−0.18	−0.31	−0.29	0.36	−0.14	−0.29	
	p	0.3089	0.9847	0.0793	0.2501	0.1274	0.0536	0.0175	0.3502	0.1552	
Orthophosphate (ppm)	r	−0.15	−0.10	0.23	0.17	−0.45	−0.21	0.22	−0.06	−0.40	
	p	0.3232	0.4904	0.1128	0.2512	0.0232	0.1591	0.1384	0.6933	0.045	
Ammonia-N (ppm)	r	−0.16	0.06	0.26	0.19	−0.28	−0.27	0.07	−0.12	−0.15	
	p	0.2873	0.6852	0.0711	0.1979	0.1711	0.0686	0.6388	0.4003	0.4859	
		Ni	N	P	K	Na	S	Zn	C:N	N:P	Na:K
Salinity (ppt)	r	0.04	0.27	−0.16	0.02	0.16	0.32	0.04	−0.13	0.46	0.12
	p	0.8583	0.0713	0.2807	0.9153	0.2778	0.0268	0.8127	0.3995	0.0013	0.4096
ph	r	0.10	−0.29	−0.48	−0.29	0.13	0.00	−0.40	0.42	0.10	0.56
	p	0.6506	0.0581	0.0011	0.0534	0.3971	0.9924	0.0071	0.0049	0.5105	< 0.0001
Orthophosphate (ppm)	r	0.27	−0.26	−0.24	−0.29	−0.15	0.09	−0.37	0.38	−0.06	0.08
	p	0.1974	0.0706	0.0947	0.0477	0.3192	0.5415	0.0107	0.0082	0.6798	0.5843
Ammonia-N (ppm)	r	0.03	0.39	0.02	0.23	0.31	0.64	−0.09	−0.34	0.42	−0.03
	p	0.8907	0.0064	0.9013	0.1118	0.0311	<.0001	0.5646	0.0168	0.0029	0.5843

TABLE 4 Pearson product-moment correlations and p-values describing the relationships between porewater chemistry and stoichiometry of leaf tissue for *S. patens*

		Al	B	Ca	C	Cu	Fe	Mg	Mn	Mb	
Salinity (ppt)	r	−0.07	−0.12	−0.54	−0.34	0.19	−0.07	0.24	−0.13	−0.09	
	p	0.6194	0.4289	<.0001	0.0211	0.2017	0.6568	0.1051	0.4008	0.5297	
ph	r	−0.27	−0.29	−0.27	0.08	−0.01	−0.25	0.16	−0.34	0.12	
	p	0.0811	0.0528	0.0804	0.5903	0.9701	0.1003	0.3058	0.0224	0.4199	
Orthophosphate (ppm)	r	0.17	−0.15	−0.31	0.13	−0.46	0.13	0.13	−0.11	−0.27	
	p	0.24	0.3027	0.0315	0.3606	0.001	0.3963	0.3686	0.4607	0.0686	
Ammonia-N (ppm)	r	−0.12	−0.03	−0.14	−0.55	0.02	−0.12	0.41	−0.03	−0.63	
	p	0.4114	0.8552	0.3273	<.0001	0.8796	0.3988	0.0036	0.8582	<.0001	
		Ni	N	P	K	Na	S	Zn	C:N	N:P	Na:K
Salinity (ppt)	r	−0.13	0.39	0.13	0.50	0.41	0.48	0.32	−0.33	0.53	−0.23
	p	0.3868	0.0061	0.3713	0.0003	0.0046	0.0006	0.0264	0.0232	0.0001	0.1166
ph	r	0.07	−0.11	−0.20	0.03	0.12	0.12	−0.25	0.17	0.04	0.04
	p	0.6308	0.4592	0.1903	0.8652	0.4449	0.4433	0.0994	0.2713	0.8041	0.7840
Orthophosphate (ppm)	r	−0.25	−0.05	−0.02	−0.03	−0.03	0.11	−0.15	0.14	−0.04	0.00
	p	0.0921	0.7341	0.887	0.828	0.8363	0.4453	0.3031	0.3262	0.7707	0.9799
Ammonia-N (ppm)	r	−0.75	0.61	0.36	0.74	0.65	0.77	0.46	−0.50	0.53	−0.15
	p	<.0001	<.0001	0.0112	<.0001	<.0001	<.0001	0.001	0.0003	0.0001	0.2879

TABLE 5 Correlations among porewater chemical values

		Salinity (ppt)	ph	Orthophosphate (ppm)
ph	r	0.251		
	p	0.100		
Orthophosphate (ppm)	r	0.242	0.309	
	p	0.101	0.041	
Ammonia-N (ppm)	r	0.399	0.023	0.153
	p	0.006	0.881	0.299

Higher concentrations of ammonia-N and higher salinity in porewater were associated with lower C:N in both species (Tables 3 and 4). For *S. patens*, but not *S. alterniflora*, higher salinity was associated with higher [Na] and [Mn] was negatively associated with porewater pH. Porewater salinity was negatively correlated with [Ca] and C:N in *S. patens* (Table 4). The pH of porewater was negatively correlated with [Mn] in *S. patens*. Porewater ammonia-N was negatively correlated with C:N in both species and was positively correlated with [Na] in *S. patens*. Orthophosphate was positively correlated with C:N ratio in *S. alterniflora* and negatively correlated with [Ca] in *S. patens* (Tables 3 and 4). Ammonia-N in porewater was weakly associated with porewater salinity and orthophosphate in porewater was weakly associated with pH (Table 5).

Porewater salinity was lowest during summer sampling periods and higher during spring and fall sampling (Table 6). On average, pH was generally neutral to slightly acidic. Ammonia-N was substantially higher in summer and fall 2008 than in previous sampling periods. Average porewater ammonia-N for spring 2007 through spring 2008 ranged from approximately 0.8–2.7 mg/L. There were no apparent seasonal patterns in porewater concentrations of orthophosphate and concentrations of orthophosphate remained >1 mg L^{-1} throughout the study.

TABLE 6 Summary of porewater chemistry by season

		Salinity (ppt)		Ammonia-N (ppm)		Orthophosphate (ppm)		pH	
Season	N	Mean	Std. Error	Mean	Std. Error	Mean	Std. Error	Mean	Std. Error
2007									
Spring	5	13.88	2.82	0.77	0.15	5.48	2.19	6.37	0.27
Summer	7	7.73	1.37	1.06	0.37	2.63	0.99	7.12	0.21
Fall	11	15.13	1.49	2.66	0.62	5.41	1.15	8.89	1.07
2008									
Spring	10	12.65	1.22	1.80	0.68	2.32	0.65	6.52	0.13
Summer	12	11.89	1.54	7.32	2.40	6.55	1.48	6.74	0.07
Fall	9	15.44	1.02	12.42	3.28	5.54	0.93	6.91	0.10

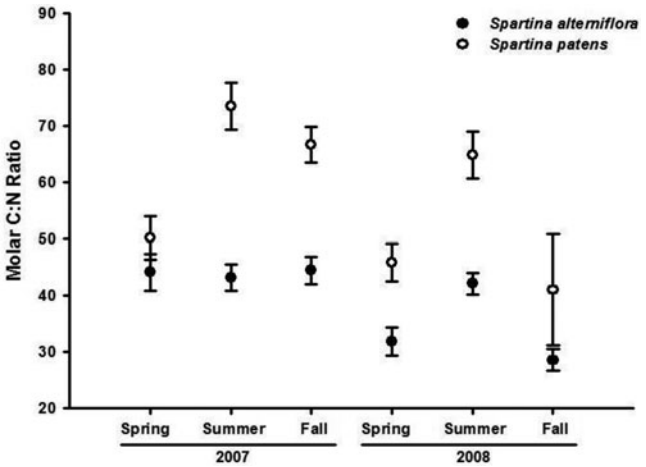

FIGURE 1 Molar C:N ratios (±1 SE) in leaf tissue of *S. alterniflora* and *S. patens* in Louisiana marshes over two growing seasons.

Average C:N ratios in *S. patens* were greater than C:N ratios in *S. alterniflora* for every sampling period (Figure 1). C:N ratios were lower in both species in spring and fall 2008 than spring and fall 2007, respectively. *S. alterniflora* [Na] was similar to [Na] in *S. patens* in 2007 but not in 2008 (Figure 2). The [Na] in both species generally followed the same seasonal pattern as porewater salinity and was substantially higher in fall 2008 than in other seasons. In most seasons, average Na:K was higher in *S. patens* than in *S. alterniflora* (Figure 3). Ratios of Na:K increased in *S. alterniflora* and decreased in *S. patens* throughout both growing seasons, causing their Na:K

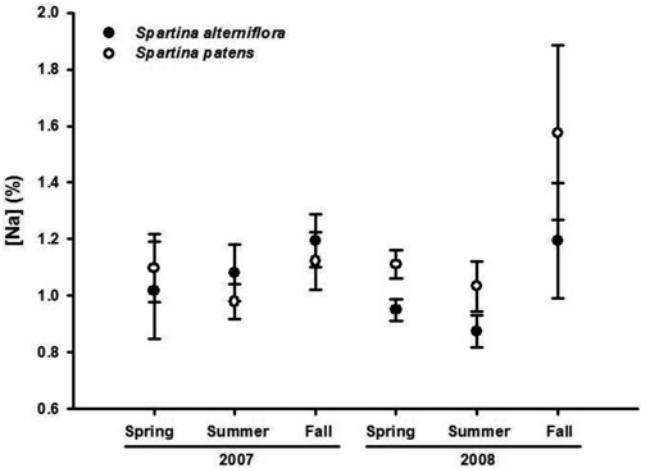

FIGURE 2 Sodium concentrations (±1 SE) in leaf tissue of *S. alterniflora* and *S. patens* in Louisiana marshes over two growing seasons.

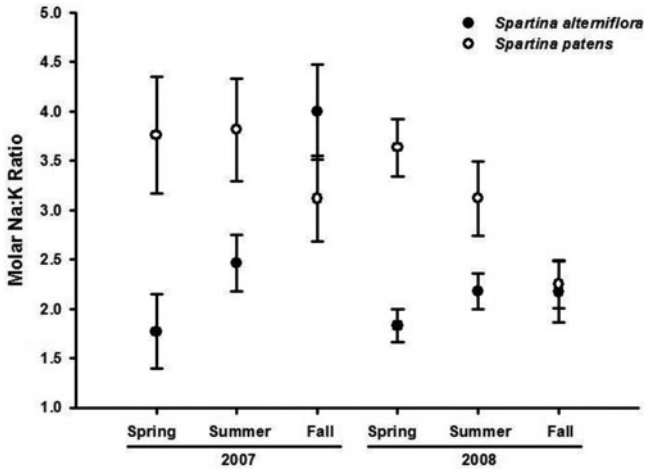

FIGURE 3 Molar Na:K ratios (±1 SE) in leaf tissue of *S. alterniflora* and *S. patens* in Louisiana marshes over two growing seasons.

ratios to converge in the fall. Ratios of Na:K were most similar in fall 2008 (Figure 3). The ratio of Na:K was higher in *S. alterniflora* and generally decreased in *S. alterniflora* throughout the growing season, except in fall 2008. The ratio of Na:K increased in *S. patens* throughout both growing seasons and the Na:K ratios of the two species converged in both fall sampling periods. Patterns in [K] mirrored patterns in Na:K ratios(Figure 4). [Na] was correlated with [K] in *S. patens* ($r = 0.552$, $p < 0.0001$) but not in *S. alterniflora* ($r = 0.086$, $p = 0.5384$). [Na] and [K] appear to be most related in *S. patens* where [Na] in leaf tissue was high (Figure 5). There appears to be a weak

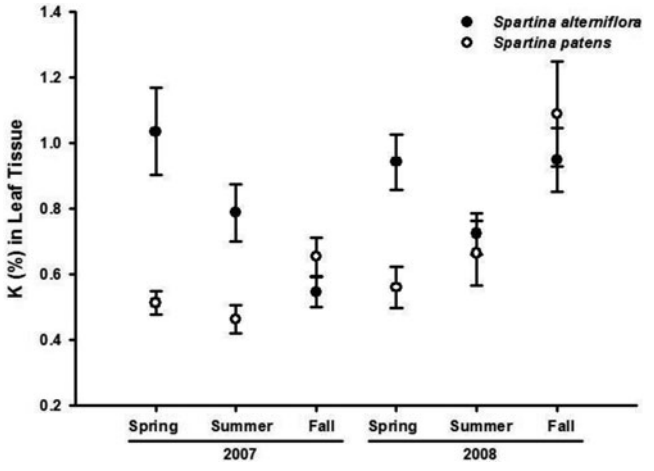

FIGURE 4 Concentrations of K (±1 SE) in leaf tissue of *S. alterniflora* and *S. patens* in Louisiana marshes over two growing seasons.

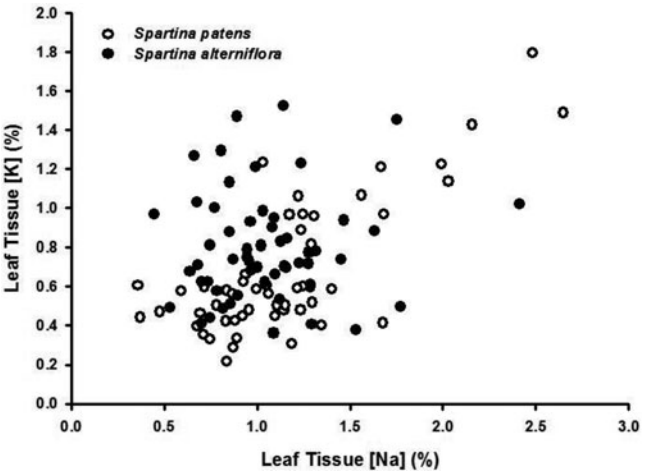

FIGURE 5 Leaf tissue [Na] and [K] of *S. alterniflora* and *S. patens*.

seasonal effect on [Mn] in *S. alterniflora*, but not on *S. patens* (Figure 6). [Mn] was always higher in *S. alterniflora* than in *S. patens*. [Mn] in *S. patens* was consistently below 140 ppm, while [Mn] in *S. alterniflora* was rarely that low. [Ca] was consistently higher in *S. alterniflora* than in *S. patens* (Figure 7). In both years, the difference in [Ca] between the two species was smaller in the spring and became larger throughout the growing season. While [Ca] consistently increased in *S. alterniflora* throughout the growing season, [Ca] decreased substantially in the fall of 2007 and in the summer of 2008, relative to their respective previous seasons.

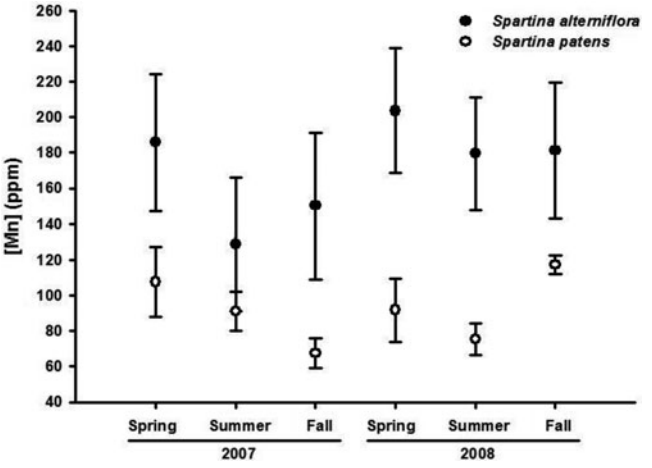

FIGURE 6 Manganese concentrations (±1 SE) in leaf tissue of *S. alterniflora* and *S. patens* in Louisiana marshes over two growing seasons.

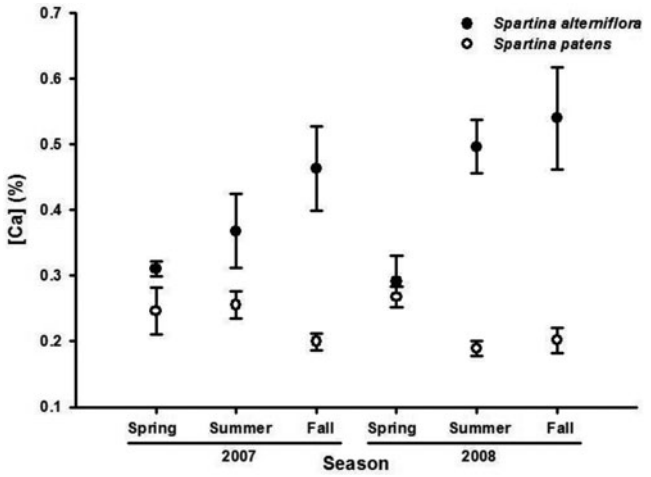

FIGURE 7 Calcium concentrations (±1 SE) in leaf tissue of *S. alterniflora* and *S. patens* in Louisiana marshes over two growing seasons.

DISCUSSION

Although Na uptake in *S. alterniflora* and *S. patens* responded similarly to changes in porewater salinity at the range of salinity where the two species occur together (0.5–19.2 ppt in our study), *S. alterniflora* is known to be more salinity tolerant than *S. patens*. [Na] in leaf tissue of *S. patens* has been shown to increase with increasing salinity, and an average [Na] of 1.1% suggests that *S. patens* growth was limited by salinity stress (Tobias et al., 2010). In contrast, although increased salinity reduces *S. alterniflora* productivity, [Na] in leaf tissue peaks for plants grown at 15–20 ppt salinity and at flooding levels similar to those experienced by plants at our sampling sites (Brown et al., 2006). Another study showed that mean [Na] in leaf tissue of *S. alterniflora* for plants growing in salinities of 10, 20, and 30 ppt was not different and [Na] was only slightly higher for plants growing in a salinity of 40 ppt (Bradley and Morris, 1991).

Higher [K] in *S. alterniflora* than in *S. patens* in paired samples may account for the similarity in [Na] that we observed between these species. *S. alterniflora* exhibits more ion selectivity than *S. patens* (Hester et al., 2001) because *S. alternisflora* is able to take up more K than *S. patens* when growing under the same conditions. When salinity is low, halophytes can accumulate high [K] in their tissue (Flowers and Colmer, 2008). When salinity is high, high K^+ availability may block the influx of Na^+ into roots (Zhang et al., 2010). The correlation of porewater salinity with [Na] and [K] in the leaf tissue of *S. patens,* but not in the leaf tissue of *S. alterniflora,* also suggests that while *S. patens* is salinity stressed, *S. alterniflora* growing in the same location is not. The correlation of [K] with [Na] in *S. patens* but not in *S. alterniflora* suggests that *S. patens* is unable to take up K without also taking up Na in

high salinity environments, while *S. alterniflora* is able to exclude Na. More research is needed, however, to describe how salinity affects Na and K uptake dynamics in these species and to identify elemental concentrations or ratios that may identify salinity limitation in *S. alterniflora*.

Although both species increase their uptake of N to improve exclusion of Na in their roots, the process of doing so may be more efficient in *S. alterniflora* than in *S. patens*. When growing under the same porewater conditions, *S. patens* incorporates less N on average into its leaf tissue than *S. alterniflora*. On average, productivity in the *S. patens* sampled in our study was limited by both low N and high salinity (mean C:N = 57.46, mean [Na] = 1.07) based on a tool to diagnose limitation of production by N-limitation and salinity stress (Tobias et al., 2010). On average, *S. alterniflora* that we collected was not N-limited, however, because the average [N] in our *S. alterniflora* (mean [N] = 1.42%, mean salinity = 10.5 ppt) was higher than the critical N concentrations reported in two separate studies. In *S. alterniflora* growing in mesocosms with 15 ppt salinity, the critical [N] was 7.3 ± 0.7 gN/kg (0.73%; Smart and Barko 1980). Similarly, another study also found that that at 20 ppt salinity critical N concentration was around 8.2 gN/kg (0.82%; Bradley and Morris, 1992).

There are several reasons for *S. patens* having consistently higher C:N ratios in its leaf tissue than *S. alterniflora*. First, on average *S. patens* in our study was N-limited but *S. alterniflora* was not. Although C:N in both species was correlated with porewater ammonia and salinity, high C:N in *S. patens* was more strongly associated with low N availability than it was for *S. alterniflora* (Tables 3 and 4). We expect to see higher C:N ratios in plants that are more N-limited (Foret 2001, Tobias et al., 2010). Second, differences in N uptake are likely to be related to differences in salinity as well as N-availability because limitation of N-uptake is more susceptible to salinity stress in *S. patens* than in *S. alterniflora*. Lower C:N ratios in the fall of 2008 illustrate how plants react to flooding with high salinity water: plants may have taken up N in response to Na from saline storm surge water. *S. alterniflora* requires more nitrogen in its tissue when grown in more saline conditions (Bradley and Morris 1992). Leaf tissue did not show signs that storm surge produced flooding stress in our study plants. If plants were severely flood stressed they would be unable to take up nitrogen (Mendelssohn and Morris, 2000). Soil hypoxia may influence nutrient uptake more than salinity level for *S. patens* (Bandyopadhyay et al., 1993). The latter study was conducted using salinity levels on the low end of the range observed in the current study, however. Plants in our study were either not stressed by the relatively short duration of flooding by storm surge or they recovered quickly and were able to take up N that built up in the porewater during the floods.

Higher average [Mn] in *S. alterniflora* than in *S. patens* for each sampling period suggests that *S. alterniflora* is more flood tolerant than *S. patens*. We expect plants that are less stressed by flooding to have higher [Mn] in their

leaf tissue because [Mn] in *S. alterniflora* leaf tissue increases when marsh elevation was raised by adding sediment (DeLaune et al., 1990). Similarly, [Mn] in *S. patens* leaf tissue decreases with increased flooding in organic marsh soils, and [Mn] <223 ppm indicates that *S. patens* grew in soils that are flooded above the soil surface (Tobias, 2010). On average, *S. patens* in this study was moderately flood stressed because [Mn] was always below 223 ppm and increased flooding from Hurricane Ike did not decrease [Mn] in the leaf tissue. Plants were still able to take up N from porewater, however, which suggests that the plants were not severely stressed by flooding. For *S. patens*, although [Mn] in summer leaf tissue reflects recent flooding conditions, fall leaf tissue may not (Tobias, 2010). [Mn] <223 ppm may not indicate flooding stress in *S. alterniflora* as is does in *S. patens*, however, because production in *S. alterniflora* is stimulated by moderate flooding (Morris, 2002). Biomass measurements would be necessary to determine what [Mn] indicate flooding stress in *S. alterniflora*.

The seasonal timing of sampling is critical for accurate interpretation of leaf tissue chemistry. Previous studies have indicated that while *S. alterniflora* biomass increases from March to September (Darby and Turner, 2008), *S. patens* productivity, as measured by leaf elongation, declines after June (Ewing et al., 1997). Differences in the length and timing of the growing season for these species suggest that although the best time to take samples for tissue analysis to diagnose limitation in *S. patens* is summer (Tobias, 2010), fall may be the best time to diagnose the causes of limited production in *S. alterniflora*. Given these differences in seasonal production comparisons between *S. patens* and *S. alterniflora* early in the growing season may be more accurate than comparisons made toward the end of the growing season.

The concentration of Ca in leaf tissue may be a good indicator of recent plant productivity because [Ca] in both species closely follow patterns of seasonal biomass production. [Ca] in *S. patens* decreased sharply in fall 2007 and summer 2008, which may indicate that production had ceased between these and the previous sampling periods. [Ca] in *S. alterniflora* continued to increase throughout both growing seasons, which may indicate that *S. alterniflora* has a longer growing season than *S. patens* growing under the same conditions. Because we tested new leaves and Ca is not translocated from older plant tissue into new plant tissue as the plant grows (Jones, 1998), [Ca] in our samples reflect conditions that the plant experienced recently. [Ca] may be influenced somewhat by conditions earlier in the growing season, however, because plants with greater root biomass are more able to take up Ca. [Ca] in *S. patens* leaf tissue is unaffected by changes in redox potential (Eh; Bandyopadhyay et al., 1993) and [Ca] uptake in both species is unaffected by changes in salinity (Bradley and Morris, 1991; Bandyopadhyay et al., 1993). Thus changes in [Ca] are more likely to be caused by seasonal changes in plant production rather than by plant reactions to changes in porewater conditions caused by storm surge in Fall 2008.

Making comparisons between the leaf tissue chemistry of *S. alterniflora* and *S. patens* should be undertaken with caution because concentrations of certain elements differ significantly between these two species. When growing under the same porewater conditions, *S. patens* incorporates less N, Mn, and Ca on average into its leaf tissue than *S. alterniflora*. The time of the year in which samples were taken should be taken into account because C:N ratios, [Ca], and [Mn] exhibit seasonal patterns which may be related to seasonal changes in plant production and/or climate patterns. Large weather events such as storms, spring floods, and possibly droughts should also be taken into account because *S. patens* and *S. alterniflora* react differently to environmental conditions. The use of pulsed flooding as a management tool to reduce salinity and increase N may be beneficial to both species. Flooding events of short durations deliver N subsidies to marshes without negatively affecting N uptake in either species or increasing [Mn] in leaf tissue.

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