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# Effects of Freshwater and Nutrient Input on Chemical Concentrations in *Spartina alterniflora* (Loisel)

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*Spartina alterniflora* (loisel) is critical to wetland structure, productivity, and vertical accretion in marshes worldwide. Previous studies along the Atlantic coast have addressed responses of carbon (C), nitrogen (N), sodium (Na), and phosphorus (P) concentrations and ratios in *S. alterniflora* tissue to variation in salinity and nutrient availability, but data are lacking from Louisiana wetlands. *Spartina alterniflora* were collected from sites with a range of freshwater and nutrient availability along Louisiana's coast and measured chemical contents in leaf tissue. The C/N ratios in leaf tissue of *S. alterniflora* in Louisiana were unaffected by salinity, but the researchers also failed to detect a relationship between Na and salinity. There was no evidence found of P limitation. These results indicate that *Spartina alterniflora* responds similarly on both coasts, but that salinity and nutrient availability differ between coasts.

**Keywords** Eutrophication, marsh restoration, nutrients, pore water, salinity, *Spartina*

## Introduction

*Spartina alterniflora* (loisel) is a perennial grass that is the second most common emergent plant in coastal wetlands (Chabreck 1970). It also is an especially important pioneer species on newly exposed sediments (Travis, Proffitt, and Edwards 2006). This plant plays a crucial role in estuarine productivity (Deegan, Peterson, and Portier 1990) and in helping marshes maintain surface elevation in the face of global sea level rise and subsidence (Nyman et al. 2006). The loss of Louisiana's coastal wetlands is thought to be partly caused by saltwater intrusion (GAO 2007). Part of *S. alterniflora*'s allure for use in restoration plans is that it is able to thrive in saline areas (Hopkinson and Schubauer 1984). The most important factors affecting *S. alterniflora* productivity include direct and interacting effects of soil anoxia, soluble sulfide ( $S^{2-}$ ), and salinity on plant nitrogen (N) uptake and assimilation (Mendelssohn and Morris 2000).

Coastal Louisiana wetlands contain 40% of the wetlands in the lower 48 states of the United States and are an area of heightened reconstruction efforts (GAO 2007). The most recent analyses indicated that the coast loses 24 square miles of emergent wetland annually, which is roughly the size of a football field every 45 min (Louisiana Coastal Wetlands Conservation and Restoration Task Force 2003; Barras, Bourgeois, and Handley

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1994). Saltwater intrusion is a major contributor to wetland loss in some parts of Louisiana (Boesch et al. 1994), and there are numerous projects designed to enhance wetland productivity by restoring river inflow. Thus, understanding how salinity affects the growth and overall health of some of the important, native, pioneer species in Louisiana's coastal wetlands is critical.

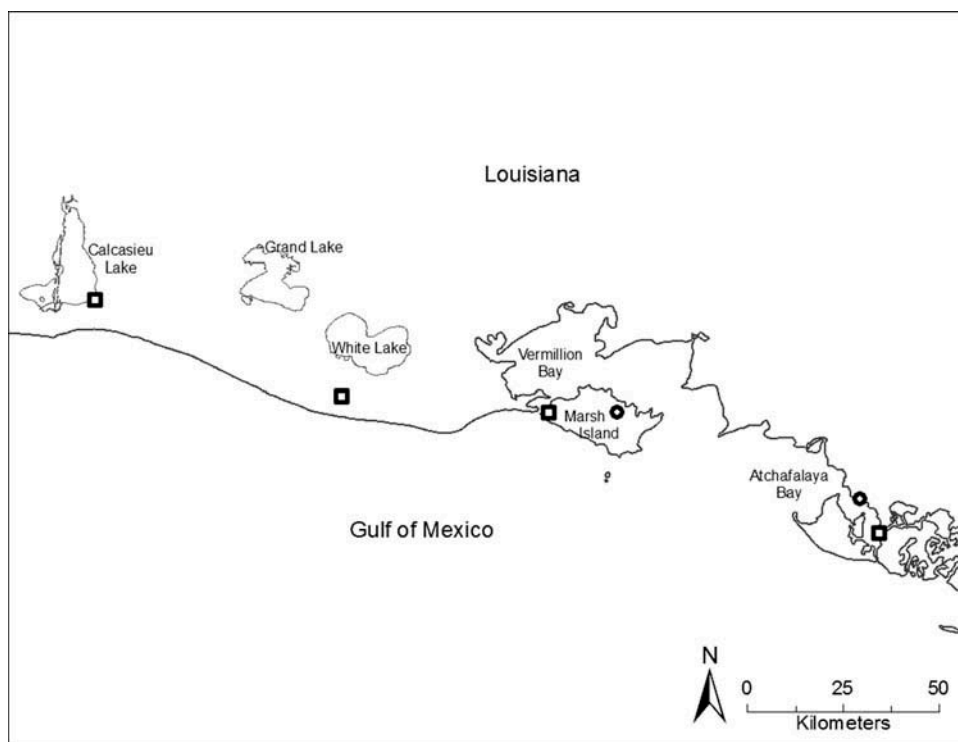
The goal of this research article is to document carbon/nitrogen (C/N) and nitrogen/phosphorus (N/P) ratios of *S. alterniflora* in coastal Louisiana and to compare those ratios to ones from plants from other coasts. We are especially interested in relationships between salinity and nutrient content. It can be hypothesized that salinity affects these chemical and nutrient contents in *S. alterniflora*. This study focuses on chemical concentrations in *S. alterniflora* tissue because previous research has shown that chemical concentrations in plant tissue can be used to identify nutrient limitation in phytoplankton (Day et al. 1989) and in numerous agricultural crops (e.g., Bailey, Sushnahan, and Beattie 1997; Campbell 2000). The possibility of using leaf tissue chemistry to identify nutrient limitation in *S. alterniflora* has been the subject of few studies. One reason more research in this area is needed is because there is no data reported concerning the effects of river inflow on nutrient concentrations in coastal Louisiana.

Primary production in *S. alterniflora* is limited by N (Hopkinson and Schubauer 1984), and N becomes less available as salinity increases (Mendelssohn and Morris 2000). In Chesapeake Bay coastal marshes, *S. alterniflora* is limited by N also, except rarely late in the growing season when there is evidence of P-limited growth (Stribling and Cornwell 2001). Nitrogen concentration of *S. alterniflora* tissue increased with salinity and the C/N decreased with salinity in marshes at North Inlet, South Carolina (Ornes and Kaplan 1989), but field data ranged from 20 to 50 ppt and lacked low salinity levels that are common in Louisiana's coastal marshes. In Connecticut marshes that differed in tidal restriction and salinity, Anisfeld and Benoit (1997) found no difference in C/N ratios of *S. alterniflora*; salinity at those sites ranged from <10 to <35 psu, which is similar to that in Louisiana. In Texas, Alexander and Dunton (2006) found that wastewater reduced C/N ratios in *S. alterniflora*, probably because of increased N availability and decreased salinity stress.

Restoring river flow to coastal marshes is one tool for coastal marsh restoration projects that is available to coastal wetland managers (Lane and Day 1999). Not only does the freshwater inflow affect the salinity levels that marsh plants are exposed to, but increased sedimentation in river outflow can also help with coast vertical accretion (Nyman et al. 2006). Neither C/N nor N/P have been used to detect the effects of the salinity reduction and nutrient increase.

## Methods

*Spartina alterniflora* tissue samples and pore-water samples were collected from four areas of Louisiana coastal wetlands (Figure 1). These areas were chosen to span a wide range of freshwater and nutrient inflow from rivers. Two of our areas, Fourleague Bay and Marsh Island State Wildlife Refuge, were located within the portion of coastal Louisiana where average annual water salinity is 5 ppt (Nyman et al. 2009) because of influence from the Atchafalaya River. The Atchafalaya River, which is managed to convey approximately 30% of the combined flow of the Mississippi River and Red River, is the fifth largest river in North America in terms of discharge. Cameron Prairie National Wildlife Refuge and Rockefeller State Wildlife Refuge, the two areas furthest away from the mouth of the Atchafalaya, represent low riverine inflow. The soils here developed without direct riverine influence because they are on the Chenier Plain and freshwater inflow at these sites is



**Figure 1.** Map showing locations of study sites where pore water salinity was measured and leaf tissue was collected during 2007. More saline sites are marked with boxes, whereas fresher sites are marked with circles.

primarily from rainfall. Salinity at Cameron Prairie National Wildlife Refuge and adjacent marshes averages 12.8 ppt (Nyman et al. 2009). *Spartina alterniflora* did not occur at Cameron Prairie prior to the 1960s but subsequently became common because of salinity increases associated with the Calcasieu Ship Channel (Fogarty 1965).

These four areas were selected as part of a concurrent study of factors that limit productivity and alter tissue chemistry of *Spartina patens* (Tobias 2010; Tobias, Williamson, and Nyman 2014). Within each of these four areas, two sites were selected to represent the fresh and saline ranges of *S. patens*. Only six sites were used in the study that we are reporting here because *S. alterniflora* occurred in only six of those eight sites. The sites lacking *S. alterniflora* were the freshwater sites at Rockefeller Wildlife Refuge and at Cameron Prairie, where it appeared to be outcompeted by less salinity-tolerant flora, such as *Typha* spp. and *S. patens*. At the remaining six sites, dominant species were *S. patens* or *S. alterniflora* and other species present included *Juncus roemerianus*, *Schoenoplectus* spp., *Sagittaria lanicifolia*, and *Vigna luteola*. All eight study sites were visited three times throughout the 2007 growing season: May–June, July–August, and November–December.

Leaf tissue samples clipped from *S. alterniflora* were taken from the upper 30 cm of the plant from the same general location in the marsh each time. Samples were always collected from the marsh interior and away from the streamside zone. Plant tissue samples were stored in plastic bags and kept on ice until they were brought to the laboratory. We measured salinity in samples of porewater collected from 10 cm below the soil surface when available.

In the laboratory, each plant tissue sample was rinsed with deionized water and oven dried. The tissue was ground up to a fine powder in a Wiley Mill. We submitted dried and ground tissue samples to the LSU AgCenter's Soil Testing and Plant Analysis Lab to determine elemental composition of leaf tissue. Carbon and N content was determined using dry combustion by CHN analyzer, and other elements were determined using an inductively coupled argon plasma photospectrometer (ICP).

We performed all statistical tests using SAS (version 9.1, SAS Institute Inc., Cary, N.C., USA). Simple linear regression (PROC REG) was used to test for a relationship between leaf tissue C/N ratios and pore-water salinity and between leaf tissue [sodium (Na)] concentrations and pore-water salinity. To test for effects of time and location, the data were analyzed as a two-way analysis of variance (ANOVA, PROC MIXED) with replication. The model was unbalanced because two to six sites were sampled within the sites at different times. Also, the least-squared means and least-squared standard errors were analyzed (PROC MIXED).

Relationships were investigated between (1) pore-water salinity and *S. alterniflora* leaf tissue molar C/N ratios, (2) leaf tissue Na concentration and leaf tissue C/N, (3) time of year during the growing season (25 May through 13 December 2007) and leaf tissue C/N, (4) leaf tissue Na and pore-water salinity, (5) leaf tissue P concentrations and time of year during growing season, (6) leaf tissue Na by month for all sites, and (7) molar N/P ratios by month.

## Results

Pore-water salinity ranged from 5.1 to 17.9 psu across the study sites (Table 1). Concentrations and ratios of elements in *S. alterniflora* leaf tissue from all sites over the duration of the experiment can be found in Table 2. Nitrogen in leaf tissue averaged 1.18%

**Table 1**  
Porewater salinity at each of the study sites during spring (25 May 2007–2 June 2007), summer (10 July 2007–22 August 2007), and fall (1 November 2007–12 December 2007)

Site	Pore-water salinity (ppt)		
	Spring	Summer	Fall
Fourleague Bay			
Fresh	7.8	5.1	n.a.
Saline	n.a.	11.2	12.6
Marsh Island State Wildlife Refuge			
Fresh	n.a.	3.5	n.a.
Saline	n.a.	9.6	5.2
Rockefeller State Wildlife Refuge			
Saline	17.9	n.a.	17.7
Cameron Prairie National Wildlife Refuge			
Saline	n.a.	n.a.	15.4

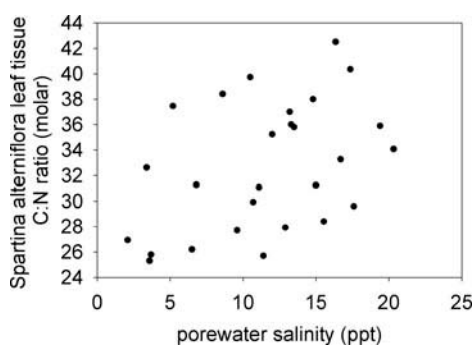
*Note.* "N.a." indicates no porewater could be extracted from a site at the time of sampling.

**Table 2**

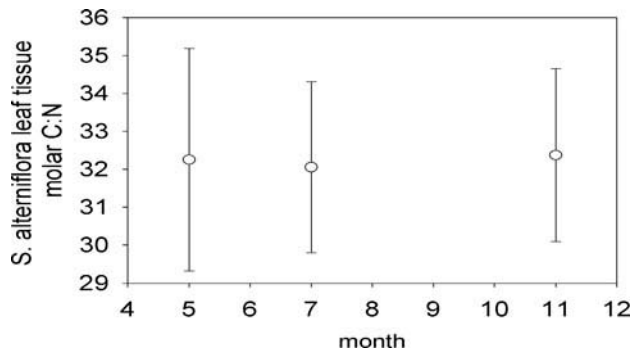
Concentrations and ratios of elements in leaf tissue of *Spartina alterniflora* collected from six sites in coastal Louisiana between May and December 2007 (porewater salinity collected at the same sites and times also is included)

Parameter	Mean	Standard deviation	Median	Minimum	Maximum
<b>Leaf tissue</b>					
Al (ppm)	80.1	85.2	47.0	4.53	367
Bo (ppm)	3.78	1.46	3.49	1.28	10.2
Ca (%)	0.393	0.161	0.349	0.137	0.838
Fe (ppm)	104	87.0	75.7	17.3	479
Mg (%)	0.326	0.122	0.318	0.135	0.567
Mn (ppm)	172	168	125	16.9	835
P (%)	0.118	0.031	0.118	0.071	0.178
K (%)	0.776	0.285	0.735	0.361	1.52
Na (ppm)	11835	3512	11325	6738	19168
S (%)	0.440	0.315	0.386	0.127	1.31
Zn (ppm)	11.4	15.2	7.76	3.93	92.0
N (%)	1.18	0.181	1.19	0.849	1.56
C (%)	43.6	1.22	43.5	41.6	46.9
C/N (molar)	32.3	4.8	31.3	25.3	42.5
C/N (mass)	37.7	5.56	36.4	29.5	49.6
Na/K (molar)	1.03	0.50	0.98	0.36	2.37
Na/K (mass)	1.75	0.85	1.67	0.62	4.03
N/P (molar)	4.78	1.27	4.78	2.658	7.838
N/P (mass)	10.6	2.80	10.6	5.86	17.3
<b>Pore water</b>					
Salinity (ppt)	11.5	5.16	12.0	2.1	20.3

and did not differ among sampling times. There was a significant positive relationship between C/N ratio and pore-water salinity ( $P = 0.0068$ ,  $R^2 = 0.2583$ ; Figure 2). The C/N ratios and leaf tissue Na were unrelated ( $P = 0.1793$ ). Overall, the C/N ratios averaged 32 with no apparent variation over time ( $P = 0.9950$ ; Figure 3). Sodium concentration

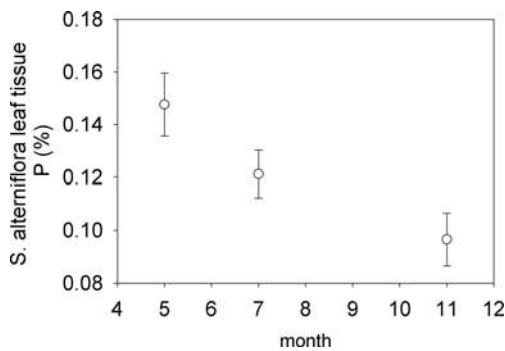


**Figure 2.** Molar C/N of *Spartina alterniflora* leaf tissue and pore-water salinity (ppt) in coastal Louisiana, 2007, for all times (25 May–13 December).

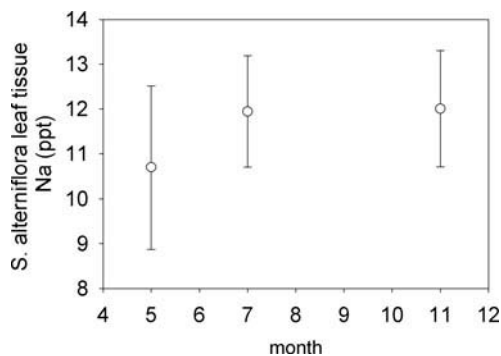


**Figure 3.** Molar C/N of *Spartina alterniflora* leaf tissue in coastal Louisiana, 2007, by month for all study sites.

(Na) was not related to pore-water salinity ( $P = 0.9840$ ). Leaf tissue P (%) differed among the three time periods ( $P = 0.0573$ ; [Figure 4](#)), but leaf tissue Na did not change over time ( $P = 0.8296$ ; [Figure 5](#)). There was no significant change in leaf tissue molar N/P over time ( $P = 0.0746$ ; [Figure 6](#)).



**Figure 4.** Phosphorus (%) of *Spartina alterniflora* leaf tissue by month for all study sites.



**Figure 5.** Sodium (ppt) of *Spartina alterniflora* leaf tissue by month for all study sites.

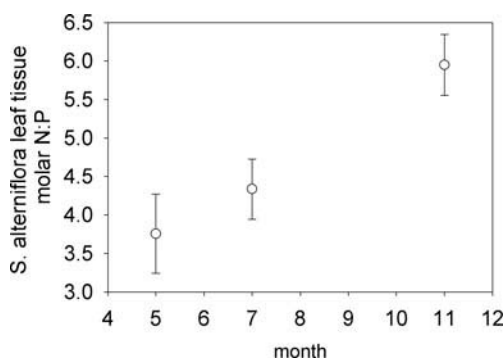


Figure 6. Molar N/P of *Spartina alterniflora* leaf tissue by month for all study sites.

## Discussion

The positive relationship we observed between leaf tissue C/N ratio of *S. alterniflora* and salinity differs from other studies, which found no relationship between these parameters in Connecticut plants (Anisfeld and Benoit 1997) or a negative relationship (Bradley and Morris 1992) between those variables in South Carolina plants. One possible reason for these differences is that pore-water salinity in our field experiment was lower than that observed by Anisfeld and Benoit (1997) in Connecticut. Salinity at the sites was lower as well as more dynamic than in the greenhouse study by Bradley and Morris (1992). It is also possible that the positive relationship between C/N and salinity at the present sites is related to differences in stress factors. While salinity and flooding stress appear to have been elevated enough to hinder N uptake, as reported by Mendelssohn and Morris (2000), salinity stress was not great enough to illicit changes in osmotic potential inside plants associated with elevated N concentration as reported by Bradley and Morris (1992).

The lack of relationship between porewater salinity and leaf tissue Na may result from leaf tissue Na being less dynamic than the salinity of porewater. If this is true, one would not expect leaf tissue Na to be strongly correlated with porewater salinity in a system with variable salinity such as that of the present study sites. Leaf tissue Na would reflect long-term salinity conditions experienced by the plant and would therefore be a better indication of salinity conditions over time. Alternatively, another possible reason that the data did not show a relationship between leaf tissue Na and porewater salinity is that *S. alterniflora* roots exclude Na from uptake to reduce internal Na (Bradley and Morris 1991). However, Na in leaf tissue of *S. alterniflora* for plants growing in salinities of 10, 20, and 30 ppt in the same study was not different and Na was only slightly greater for plants growing in a salinity of 40 ppt (Bradley and Morris 1991). This supports the interpretation that *S. alterniflora* was not likely to be stressed by high salinity at our study sites, and therefore Na would not be expected to vary with salinity in porewater.

Although C/N and N/P remained fairly constant throughout the growing season in our study, decreasing P illustrates that the seasonal timing of sampling may influence the results of comparisons among studies. The data were collected throughout the growing season, whereas Bradley and Morris (1992) reported data only from the end of the growing season. This is important 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, McKee, and Mendelssohn 1997). In the present samples, molar N/P ratios never exceeded 8, however, which suggests no evidence of P limitation.



This is not surprising, because P limitation has only been reported once in *S. alterniflora* and, even in that study, only at one nitrogen-rich site in Chesapeake Bay (Stribling and Cornwell 2001). At that site, N/P molar ratio exceeded 50 (Stribling and Cornwell 2001).

Future research should investigate whether variations in nutrient and chemical contents correlate with differences in each ecosystem's physical components, such as soil content, flow patterns, interannual variability, and river discharge, which would influence salinity and nutrient availability, interannual variability, and sea level (Morris, Kjerfve and Dean 1990). Also, future research could tie the biogeochemical components covered in this article into actual wetland restoration projects.

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