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CARBON, NITROGEN, SODIUM AND PHOSPHOROUS CONCENTRATIONS AND RATIOS IN SPARTINA ALTERNIFLORA (LOISER) FROM SITES OF VARIOUS FRESHWATER AND NUTRIENT INPUTS IN COASTAL LOUISIANA WETLANDS

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CARBON, NITROGEN, SODIUM AND PHOSPHOROUS CONCENTRATIONS AND RATIOS IN *SPARTINA ALTERNIFLORA* (LOISER) FROM SITES OF VARIOUS FRESHWATER AND NUTRIENT INPUTS IN COASTAL LOUISIANA WETLANDS

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An integral part of this research was the pair of doctoral candidates in Dr. Nyman's lab, Vanessa Tobias and Amy Scaroni. Thank you to Vanessa (whose doctoral research this thesis is based on) for help with field work, sample collecting and answering any statistics/geography/biology questions I threw her way. Thank you to both Vanessa and Amy, also, for all of the moral support, expert advice and kayaking breaks that made a year of hard research go by quickly and relatively smoothly for an undergraduate student who was, at times, way in over her head.

The members of my thesis panel, Dr. John Andrew Nyman, Dr. Michael Kaller and Dr. Sharon Weltman, for their endless revisions, dedication of time out of their busy personal lives into this thesis and for their kind support. I would like to especially thank Dr. Nyman, my research advisor, for pushing me to make this thesis the best that I could; for encouraging me to keep reading one more paper, make just one more revision and to just look at one more possible correlation. He dedicated a lot of his own time into this project and his genuine interest and enthusiasm about this topic was not only encouraging, but also contagious.

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SUMMARY

Spartina alterniflora (Loiser) is an important grass in wetlands throughout the Gulf and Atlantic coast. Its ability to grow in waters as salty as seawater make it a valuable component in wetland restoration while its root system, once established, collects sediments and maintains marsh elevation – keeping the marsh from sinking.

A topic of interest in coastal Louisiana wetland restoration is whether diverting rivers can help stem the rapid conversion of wetlands into open water. By studying the differences in chemical concentrations and ratios in leaf tissue (which are directly related to plant health) from areas of different salinity levels, scientists can better understand how freshwater affects the health of this important grass and can, therefore, better predict how beneficial large-scale restoration projects, such as diverting the Mississippi River, will really be to coastal wetlands.

Similar research on the affects of salinity on the health of *S. alterniflora* has been done in other parts of the country and for this paper we looked specifically at some research done in Connecticut and Chesapeake Bay. While results from these areas were interesting and helpful in developing some of our project's statistical analyses techniques, it is important to explore this area of interest in coastal Louisiana wetlands as well since each wetland ecosystem is very distinctive from all others and one cannot be managed the same way as another.

In the end, our research matched some of the data found in the research from Connecticut, but did not match any of the data found in the research from Chesapeake Bay. This shows just how unique each of these wetland systems is. Overall, we concluded that salinity and nutrient patterns in a coastal marsh landscape, , can be determined from chemical analyses of leaf tissue. This may help restoration planners evaluate the success of freshwater and sediment diversions in Louisiana. Our results also indicate that, while *S. alterniflora* responds to different

factors similarly throughout its range on the Atlantic and Gulf coasts, differences occurred in our research results because salinity levels and availability of nutrients vary between the areas.

ABSTRACT

Throughout marshes along North America's Atlantic and Gulf coasts, *Spartina alterniflora* (Loiser) is critical to wetland structure, productivity, and vertical accretion. This cordgrass can grow across a wide range of salinity, though it is often out-competed in fresher systems by other, sometimes invasive, flora. Previous studies along the Atlantic coast have addressed the response of the carbon, nitrogen, sodium and phosphorous concentrations and ratios of *S. alterniflora* tissue to variation in salinity and nutrient availability.

We tested the hypotheses that *S. alterniflora* in Louisiana has C:N ratios that are unaffected by salinity, as in Connecticut, that the tissue Na levels increase with salinity, as in Chesapeake Bay, and that *S. alterniflora* in coastal Louisiana has N:P ratios that are rarely indicative of P-limited growth, as in Chesapeake Bay. We collected *S. alterniflora* from marshes along Louisiana's coast that differed in freshwater and nutrient inputs and measured chemical content in leaf tissue. Chemical concentrations were determined via CHN and ICP analyses. Pore-water chemistry, determined colormetrically, was measured to verify freshwater and nutrient availability.

We concluded that C:N ratios of *S. alterniflora* in Louisiana were unaffected by salinity, like Connecticut's, but we failed to detect a relationship between Na and salinity. We found no evidence of P-limitation probably because N content in Louisiana is roughly 50% of that in Chesapeake Bay. These results indicate that *Spartina alterniflora* responds similarly on both coasts, but that salinity and nutrient availability differ between the coasts.

INTRODUCTION

Spartina alterniflora (Loiser) is a perennial deciduous grass that is prominent in Louisiana's coastal wetlands (Chabreck 1970). Its ability to grow in sea water makes it an especially important pioneer species in the wetlands along Louisiana's southern coast (Travis et al. 2006). This plant plays a crucial role in the structure of wetlands by collecting sediments from water around its roots, eventually building up over time and helping to create surface areas where other wetland flora and fauna can thrive (Fang 2002, Travis et al. 2006).

The loss of Louisiana's coastal wetlands plants is thought to be largely due to salt water intrusion (GAO 2007), so part of *S. alterniflora*'s allure is that it is able to thrive in saline areas (Hopkinson and Schubauer 1984). An area of interest to many wetland scientists at the moment is how diverting freshwater and nutrients into areas of subsided wetlands can slow loss of the wetlands (Lane and Day 1999). This research focused in on nutrient concentrations since previous research has shown that C:N and N:P ratios can be used to identify nutrient limitation (Day et al. 1989). The goal of this research paper is to provide the science community with a well thought out study on various biogeochemical aspects and trends in *S. alterniflora* in hopes that shedding light upon some of the basic physical science of wetlands will prove beneficial for current and future restoration projects. Specifically, this study will delve into correlations between sites of varying saltwater and freshwater inflow throughout the coastal wetlands of Louisiana and the chemical and nutrient contents found in the *S. alterniflora* at these sites.

Previous research in this topic has been conducted throughout areas ranging from the Chesapeake Bay (Stribling and Cornwell 2001) to Connecticut, along the coast of the Long Island Sound (Anisfeld and Benoit 1997). However, we are unaware of similar studies in coastal

Louisiana. Since coastal Louisiana ecosystems vary from those found in the Chesapeake Bay and Connecticut, we felt it was important to do some of this research in Louisiana's own wetlands.

In this study, we focused on the correlations between: [1.] pore-water salinity and *S. alterniflora* leaf tissue molar carbon to nitrogen ratios (C:N), [2.] leaf tissue sodium, [Na], versus leaf tissue molar C:N, [3.] time of year during the growing season (May 25 through December 13) versus leaf tissue molar C:N, [4.] leaf tissue [Na] and pore-water salinity, [5.] leaf tissue phosphorous, [P], versus time of year during growing season, [6.] leaf tissue [Na] by month for all sites, and [7.] molar nitrogen to phosphorous ratios (N:P) by month for each of the seventeen sites in wetlands along southern coastal Louisiana. The samples used for correlations throughout the growing season were collected from May 25 through December 13. The samples collected from November 1 through December 13 were used in correlations looking at the end of the growing season.

We hypothesized that salinity would affect these chemical and nutrient contents in *S. alterniflora*. Our null hypothesis was that salinity would not affect these contents in the plants. By the end of the project, we realized that our experiment had gone deeper into the basic physical science and biogeochemical processes of this wetland cordgrass than we had originally intended. Some contents did appear to be potentially correlated with salinity levels, but there were also interesting correlations between some of the actual nutrient and chemical contents themselves, as well as interesting correlations between all of the above and the time of year in which the samples were taken. Overall, our research led us to more - very interesting - correlations and trends than our original hypothesis had projected.

STUDY AREA

In this study, we collected *S. alterniflora* samples throughout the coastal wetlands of southern Louisiana. Our four specific sites were: [1.] Four League Bay, located southeast of the Atchafalaya Bay (including both fresh and saltwater plots), [2.] Marsh Island, located south of Vermilion Bay (including both fresh and saltwater plots), [3.] Rockefeller Wildlife Refuge, located in eastern Cameron Parish (including only saltwater plots) and [4.] Cameron Prairie, located southeast of Calcasieu Lake (including only saltwater plots). For a map of the freshwater and saltwater plots sampled, see Figure 1 at the end of this paper.

No samples were collected at the freshwater plots at Rockefeller Wildlife Refuge (Dyson Bayou in Unit 6 of the refuge) because *S. alterniflora* was absent, probably because it was out-competed by other freshwater flora in this area. The saltwater plots were located in Muskrat Bayou, located southeast of Dyson. Similarly, *S. alterniflora* was only found at the saltwater plots in Cameron Prairie as well. Four League Bay's freshwater plots were located on an island that Vanessa Tobias, a doctoral candidate in the School of Renewable Natural Resources at Louisiana State University, dubbed "Grasshopper Island" and the saltwater plots were in an area called Little Blue Hammock Bayou (located southwest of Lost Lake). Marsh Island's saltwater plots were located at the west corner of the island (to the south of Vermilion Bay) while the freshwater plots were located closer to the eastern side of the island (south of West Cote Blanche Bay).

All four of the sites chosen for this research are located throughout the wetlands along the coast of Louisiana, in various proximities from the mouth of the Atchafalaya River, which carries waters from the Mississippi River as well, via the Old River Control Structure (Bourne 2000). This area contains 40% of the wetlands in the lower 48 states of the United States and is an area

of heightened reconstruction efforts (GAO 2007). Any significant amount of freshwater inflow being provided to these areas comes from the Atchafalaya River, which receives 30% of the Mississippi River at the Old River Control Structure, the point where the two rivers meet (Bourne 2000). Cameron Prairie and Rockefeller Wildlife Refuge, the two sites furthest away from the mouth of the Atchafalaya, represent the saltier sites in this research while the sites closer to the mouth, Four League Bay and Marsh Island, represent the fresher sites. *S. alterniflora* did not actually occur at Cameron Prairie in the 1960's, but the vegetation at this site changed during the late 1900's because of salinity increases associated with the Calcasieu Ship Channel (Fogarty 1965).

While traveling through these areas during our days in the field, the amount of wetland loss that each of the areas had experienced was shocking. Houses that were lucky enough to still be on somewhat stable land were built up on high stilts and had clearly been raised to progressively higher stilts throughout their lifetimes. The roofs of the less fortunate houses could be spotted out in the middle of the open water in some of these marshes.

Coastal Louisiana is converting to open water at an alarming rate every year. The most recent analyses indicated that the coast loses twenty-four square miles of wetland per year- roughly the size of a football field every 45 minutes (LCWCRTF 2003, Barras et al. 1994). Saltwater intrusion is a major contributor to wetland loss (Courville 1997), so understanding how salinity affects the growth and overall health of some of the important, native, pioneer species in Louisiana's coastal wetlands - such as *S. alterniflora* – is critical if scientists are to be properly armed to conquer this problem. This research was designed to focus in on this problem by selecting sites from areas of varying salinity levels.

METHODS

To analyze how varying salinity levels affect the chemical and nutrient contents in *S. alterniflora*, plant samples were clipped from areas of high salinity and areas of low salinity at regular intervals throughout the grass' growing season. Each sample was taken from the upper 12 inches of the plant from the same location in the marsh each time. Most plants were located further into the marsh, as opposed to the outer rim along the open water, so as to control our experiment as much as possible and avoid any unexpected possible affects of more fluctuating salinity levels on plant tissue. However, during the later part of the growing season some of our freshwater areas did not have as much *S. alterniflora* as earlier months did; in these instances we did sometimes collect from plants located along the edge of the open water, if there was any *S. alterniflora* to choose from at all.

Pore-water salinity readings were also taken at each site, when available, to verify salinity levels (to see a record of each sites pore-water salinity measurements, look at the end of this paper at Table 1). After each sample was rinsed with DO (dissolved oxygen) water and dried in a drying oven for at least three days (or until samples were suitably dry), the tissue was ground up to a fine powder in a grinding machine and then stored in a 20mL vial with a rubber-coated sealed lid. To determine the chemical and nutrient contents of each of the tissue samples, Carbon, Nitrogen and ICP (inductively coupled argon plasma photospectrometer) tests were later performed on each of the dried and ground samples in the Chemical and Nutrient Analyses Laboratory in the Agronomy Department at Louisiana State University.

For the Carbon and Nitrogen tests, 0.15g of sample was weighed out from each of the thirty-three vials. Each sample was weighed on a previously zeroed aluminum foil sheet and the sample weight was recorded into a computer logsheet. The aluminum foil was then manually

folded up to contain the sample in an air-tight little purse and then placed into the rotating chamber of a Leco CN analyzer for dry combustion. Standards containing ground peach leaf tissue with known elemental concentrations were also sent through the Leco CN analyzer to check readings.

For the ICP tests, 0.5 g was weighed out from each of the thirty-three viles. Each sample was weighed on a previously zeroed piece of wax paper and the sample weight was recorded into a computer logsheet. Each sample was than manually transferred to a glass test tube. Underneath a fume hood, each sample was acid digested by adding 5mL of concentrated HNO_3 to each test tube. After 50 minutes, we added 3mL H_2O_2 to each test tube and moved all test tubes to a heat block, which was also under the fume hood. Each test tube was heated until the acid evaporated (approximately 2.75 to 3 hours). After cooling, DO water was then added to each test tube to dilute the content and then analyzed via ICP. Samples of ground peach leaf tissue were also sent through the entire process to act as standards.

After the C:N and ICP analyses were performed on all of the samples, the computer program SAS (version 9.1, SAS Institute Inc., Cary, NC, USA.) was used to estimate means and standard deviations, and test for linear correlations. By comparing our results to those found in other parts of the United States, we were better able to understand how our data corresponded and differed from wetlands around the country.

STATISTICAL ANALYSIS

I relied on my advisor for statistical analysis. We used simple linear regression to test for a relationship between leaf tissue C:N ratios and porewater salinity, and between leaf tissue [Na] concentrations and porewater salinity using Proc Reg of SAS (version 9.1, SAS Institute Inc., Cary NC, USA). We believed this was a regression situation rather than a correlation situation because we selected field sites to span a wide range in salinity. To test for an effect of time, we analyzed the data as a two-way analyses of variance (ANOVA) with replication using Proc Mixed of SAS (version 9.1, SAS Institute Inc., Cary NC, USA). In that model, main effects were area/site (depending on your terminology) and time. The model was unbalanced because two to six plots were sampled within the area/sites at different times. We also generated least-squared means and least-squared standard errors (Proc Mixed).

RESULTS

We found a significant positive relationship between molar C:N with pore-water salinity throughout the growing season (Figure 2) and at the end of the growing season (Figure 3). However, we felt that the relationship of molar C:N to pore-water salinity might not be the most reliable measure of salinity exposure because of the ever-changing nature of pore-water salinity. There was no significant correlation between molar C:N and leaf tissue [Na] content (Figure 4 and Figure 5). Overall, our C:N averaged 32(std) with no apparent change over time (see Table 2 and Figure 6).

Analyses indicated that leaf tissue [P] (%) did not differ among the three time periods (P=0.1.61) (Figure 9), nor did leaf tissue [Na] change over time (P=0.9979) (Figure 10). We also concluded that there was no significant change in leaf tissue molar N:P over time (P=0.0717) (Table 3) and the overlapping standard error bars in our molar N:P over time graph supports this statistical analysis (Figure 11).

DISCUSSION

Bradley and Morris (1992) found a negative relationship between C:N and salinity in nitrogen-starved plants, but we found a positive relationship. We felt that one possible reason for this difference could be that pore-water salinity in our field experiment was more dynamic than in their greenhouse experiment. Leaf tissue [Na] content is probably less dynamic and more representative of long term salinity conditions undergone by the plant and would, therefore, be a better indication of salinity conditions throughout the growing season than pore-water salinity. Furthermore, the fact that we still failed to detect a clear trend between pore-water salinity and leaf tissue [Na] content (Figure 7 and Figure 8), also supports the idea that that pore-water salinity is not the most reliable measure of salinity conditions.

A possible explanation for our positive relationship between C:N and salinity could be that N becomes less available as salinity increases (Mendelssohn and Morris 2000). And, even though we did not find a positive relationship between C:N and leaf tissue [Na] content, we still did not find a negative relationship as Bradley and Morris's (1992) research had found. Perhaps, a difference in soil content could explain the varying results between our sites and Bradley and Morris (1992). Research done in Connecticut marshes found no effect of salinity on tissue molar C:N either (Anisfeld and Benoit 1997). Hopkinson and Schubauer (1984) found that primary production in *S. alterniflora* was N-limited, which would also support our results. Another research paper we came across found that there was an increase in salinity over the season (Schroeder et al. 1990), so it would be reasonable to assume that perhaps we did not get a significant increase in leaf tissue [Na] content over time because our small sample size prevented us from detecting the effect.

In conclusion, we accepted our hypothesis and reject the null hypothesis. We found instances where plant tissue chemical and nutrient contents were affected by salinity levels, but our statistical analysis led us to believe that they were spurious. We also looked more closely at correlations between some of the actual chemical and nutrient contents themselves than perhaps our original hypothesis had intended. It was interesting to see such big variations in chemical and nutrient contents throughout research in different areas of the country. In future research, it might be interesting to see if the variations in nutrient and chemical contents correlate with differences in each ecosystem's physical components (such as soil content, flow patterns, etc). Also, it would be great to see future research in this field tie the biogeochemical components covered in this paper into actual wetland restoration projects.

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Table 1. Pore-water salinity (ppt) present at each of the study sites for times 1 (5/25/07 – 6/2/07), 2 (7/10/07 – 8/22/07), and 3 (11/1/07 – 12/13/07).

SITE	pore-water salinity in (ppt) from TIME 1	pore-water salinity in (ppt) from TIME 2	pore-water salinity in (ppt) from TIME 3
Four League Bay			
Fresh	7.8	5.1	N/A
Salt	N/A	11.2	12.6
Marsh Island			
Fresh	N/A	3.5	N/A
Salt	N/A	9.6	5.2
Rockefeller Wildlife Refuge			
Salt	17.9	N/A	17.7
Cameron Prairie			
Salt	N/A	N/A	15.4

Table 2. Molar C:N present at each of the study sites for times 1 (5/25/07 – 6/2/07), 2 (7/10/07 – 8/22/07), and 3 (11/1/07 – 12/13/07).

SITE	molar C:N from TIME 1	molar C:N from TIME 2	molar C:N from TIME 3
Four League Bay			
Fresh	30.3	30.7	N/A
Salt	N/A	33.2	31.5
Marsh Island			
Fresh	N/A	28.8	N/A
Salt	N/A	35.5	27.6
Rockefeller Wildlife Refuge			
Salt	32.8	N/A	40.3
Cameron Prairie			
Salt	N/A	N/A	29.6

Table 3. Molar N:P present at each of the study sites for times 1 (5/25/07 – 6/2/07), 2 (7/10/07 – 8/22/07), and 3 (11/1/07 – 12/13/07).

SITE	molar N:P from TIME 1	molar N:P from TIME 2	molar N:P from TIME 3
Four League Bay			
Fresh	3.67	3.81	N/A
Salt	N/A	4.62	6.82
Marsh Island			
Fresh	N/A	5.18	N/A
Salt	N/A	3.76	4.81
Rockefeller Wildlife Refuge			
Salt	4.19	N/A	5.42
Cameron Prairie			
Salt	N/A	N/A	6.61

Figure 1. Map of study area. Saltwater sites are marked with boxes while freshwater sites are marked with circles.

Figure 2. Molar C:N of *Spartina alterniflora* leaf tissue and pore-water salinity (ppt) in coastal Louisiana, 2007, for time 3 only (November 1-December 13).

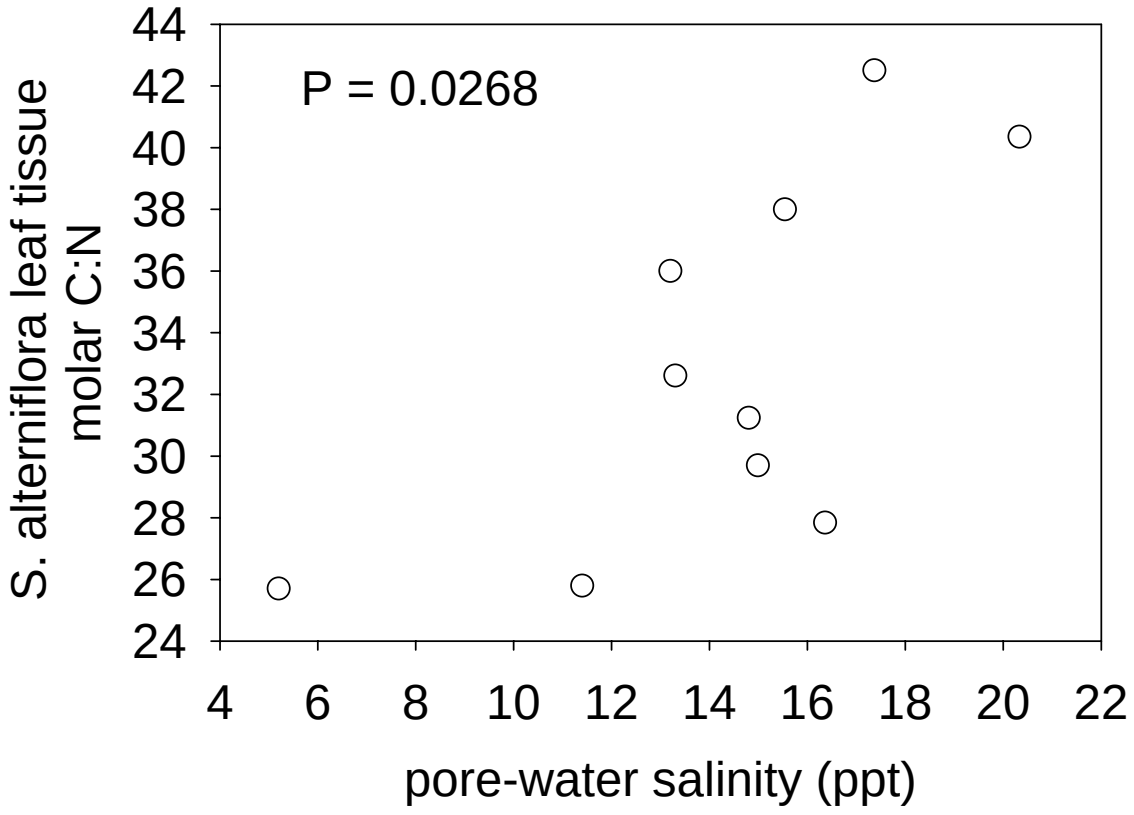


Figure 3. Molar C:N of *Spartina alterniflora* leaf tissue and pore-water salinity (ppt) in coastal Louisiana, 2007, for all times (May 25-December 13).

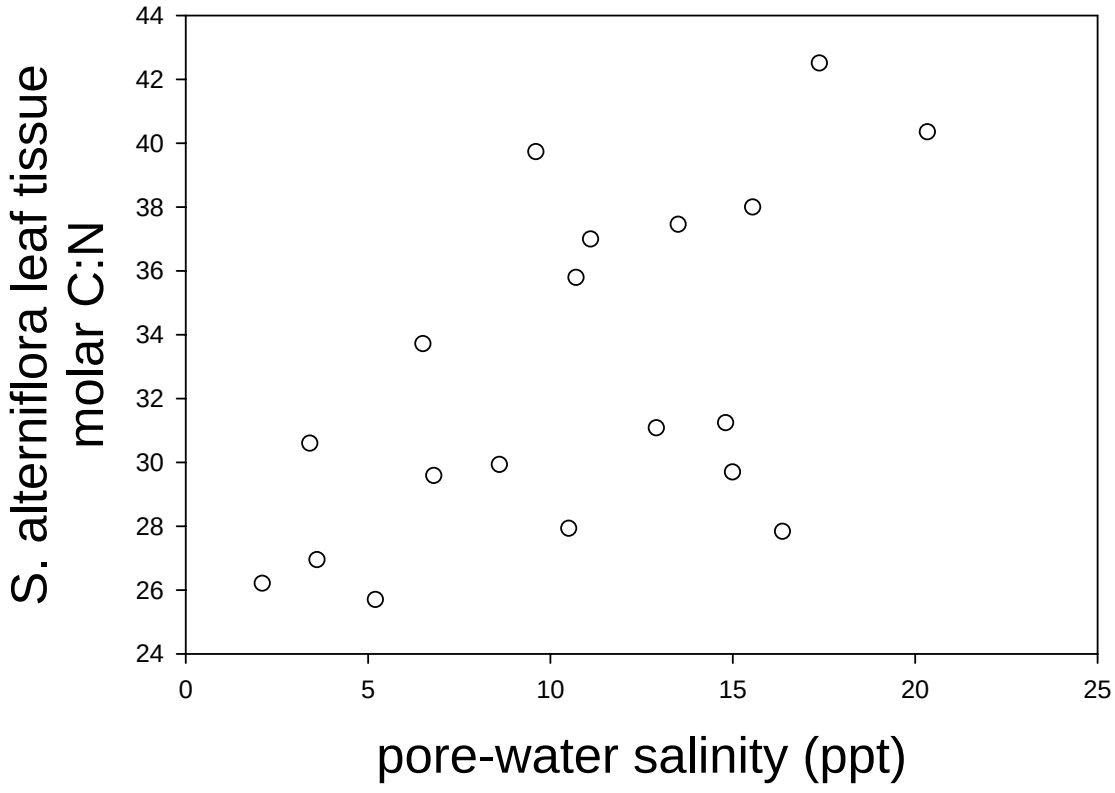


Figure 4. Molar C:N of *Spartina alterniflora* leaf tissue and leaf tissue [Na] content (ppt) in coastal Louisiana, 2007, for time 3 only (November 1-December 13).

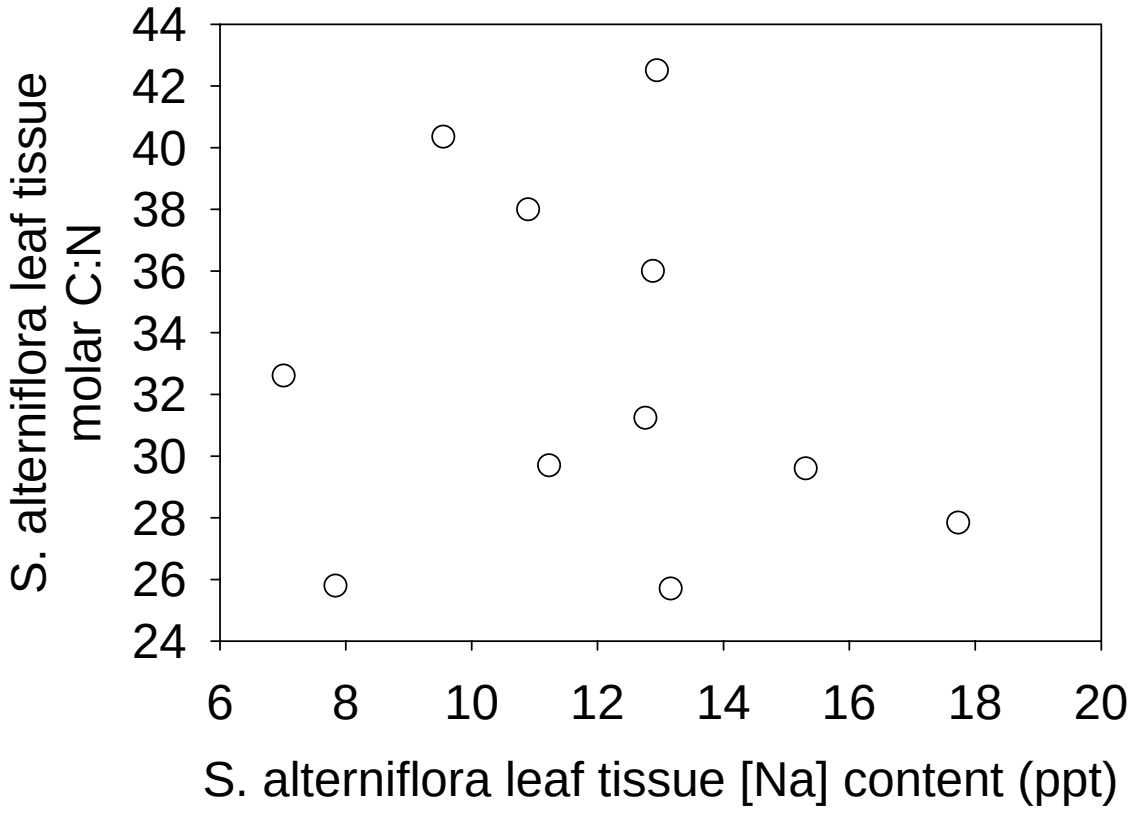


Figure 5. Molar C:N of *Spartina alterniflora* leaf tissue and leaf tissue [Na] content (ppt) in coastal Louisiana, 2007, for all times (May 25-December 13).

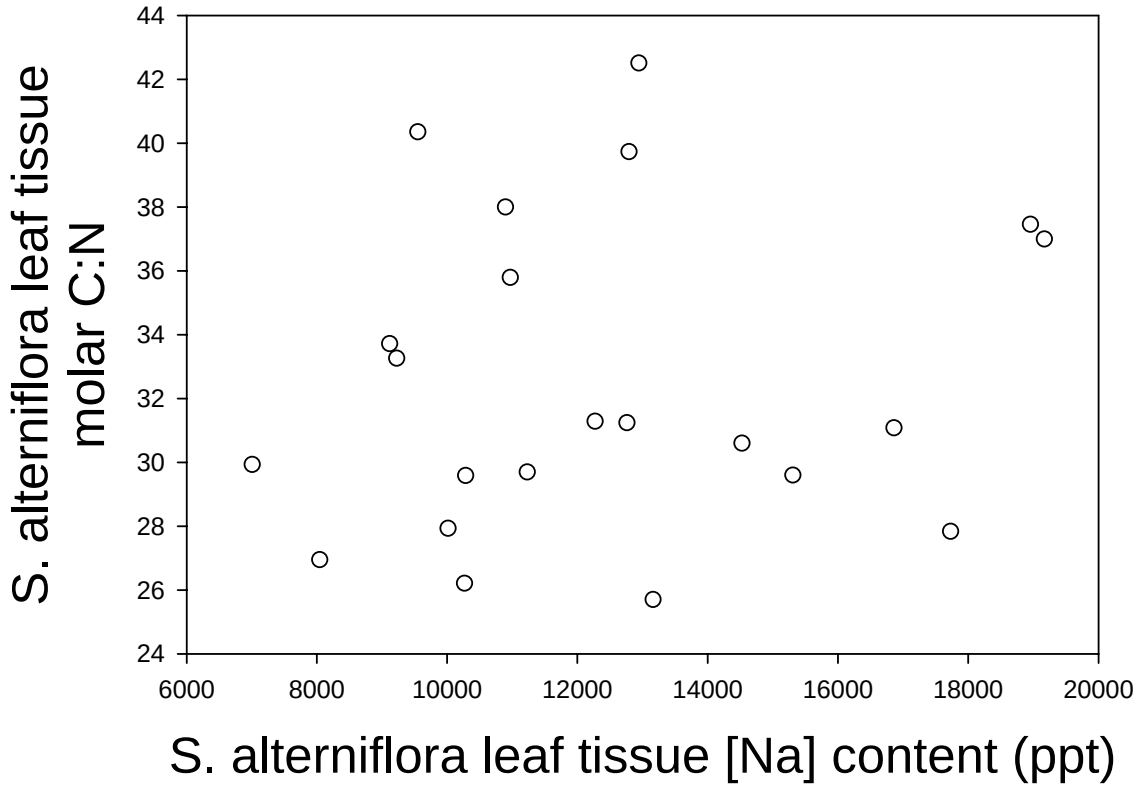


Figure 6. Molar C:N of *Spartina alterniflora* leaf tissue by month for all study sites.

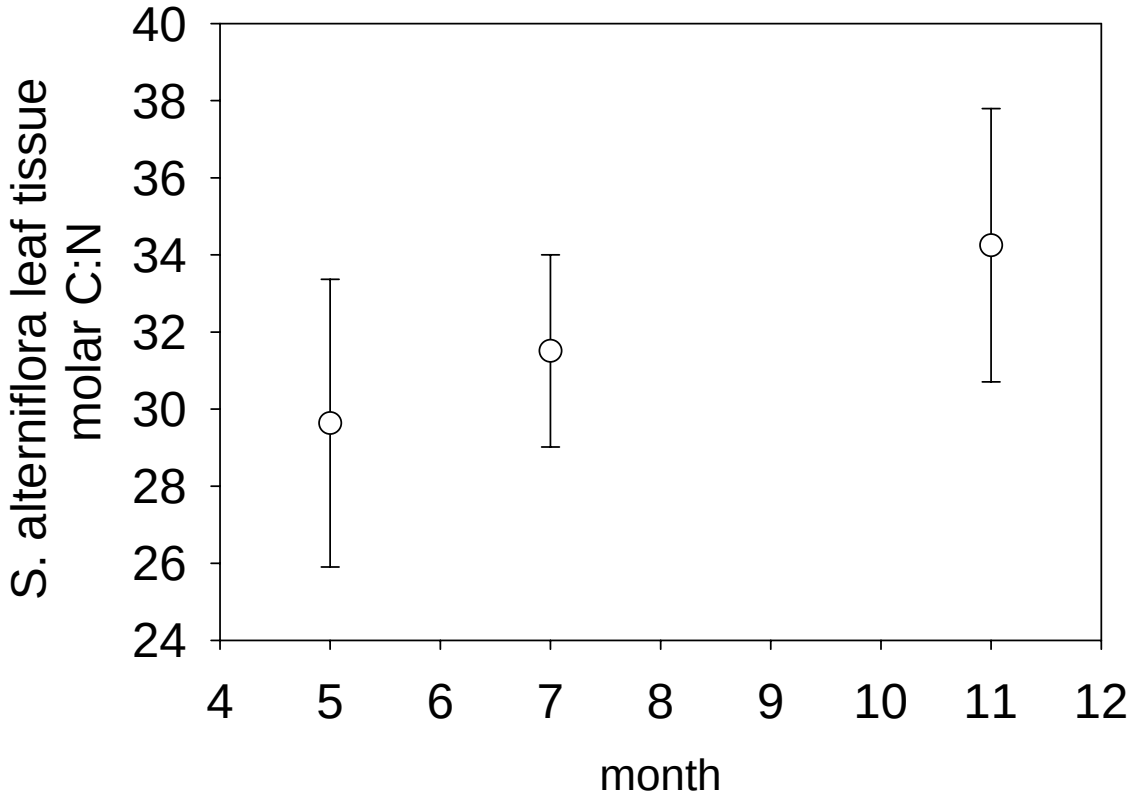


Figure 7. [Na] content (ppt) of *Spartina alterniflora* leaf tissue and pore-water salinity (ppt) in coastal Louisiana, 2007, for time 3 only (November 1-December 13).

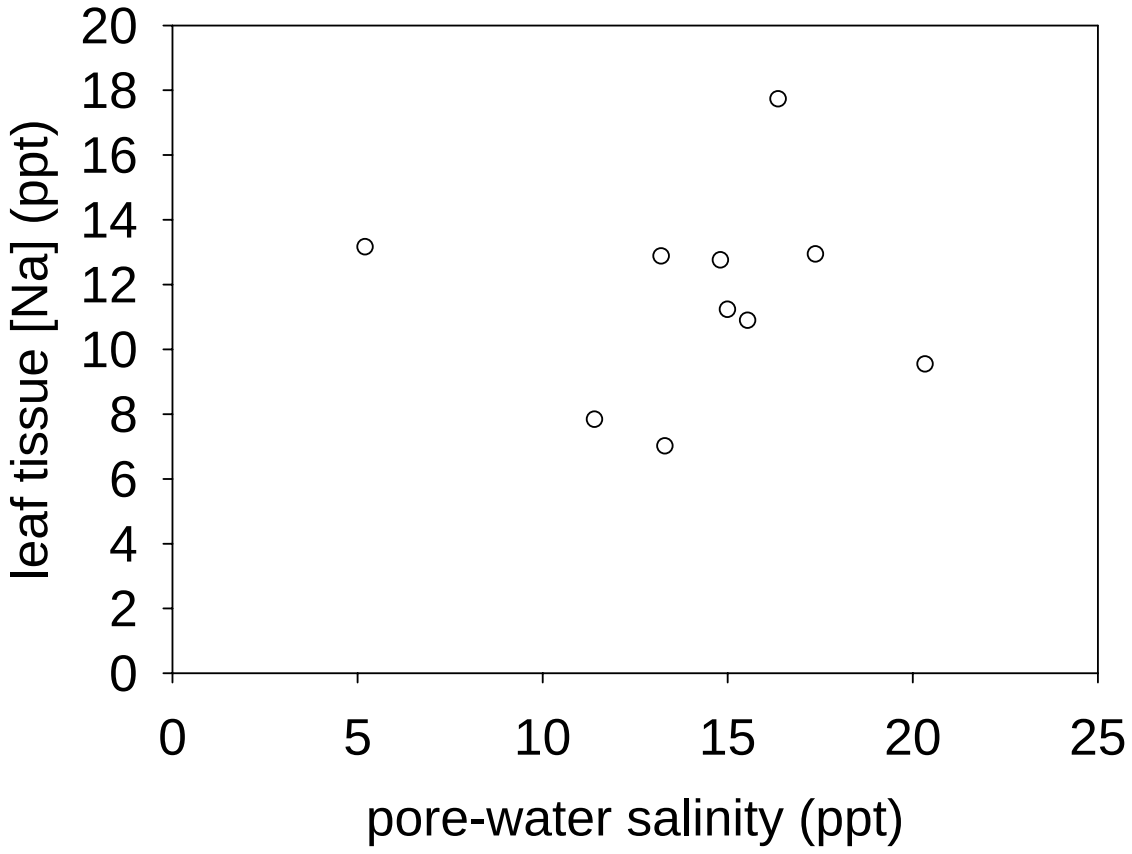


Figure 8. Pore-water salinity (ppt) and [Na] content (ppt) of *Spartina alterniflora* leaf tissue in coastal Louisiana, 2007, for all times (May 25-December 13).

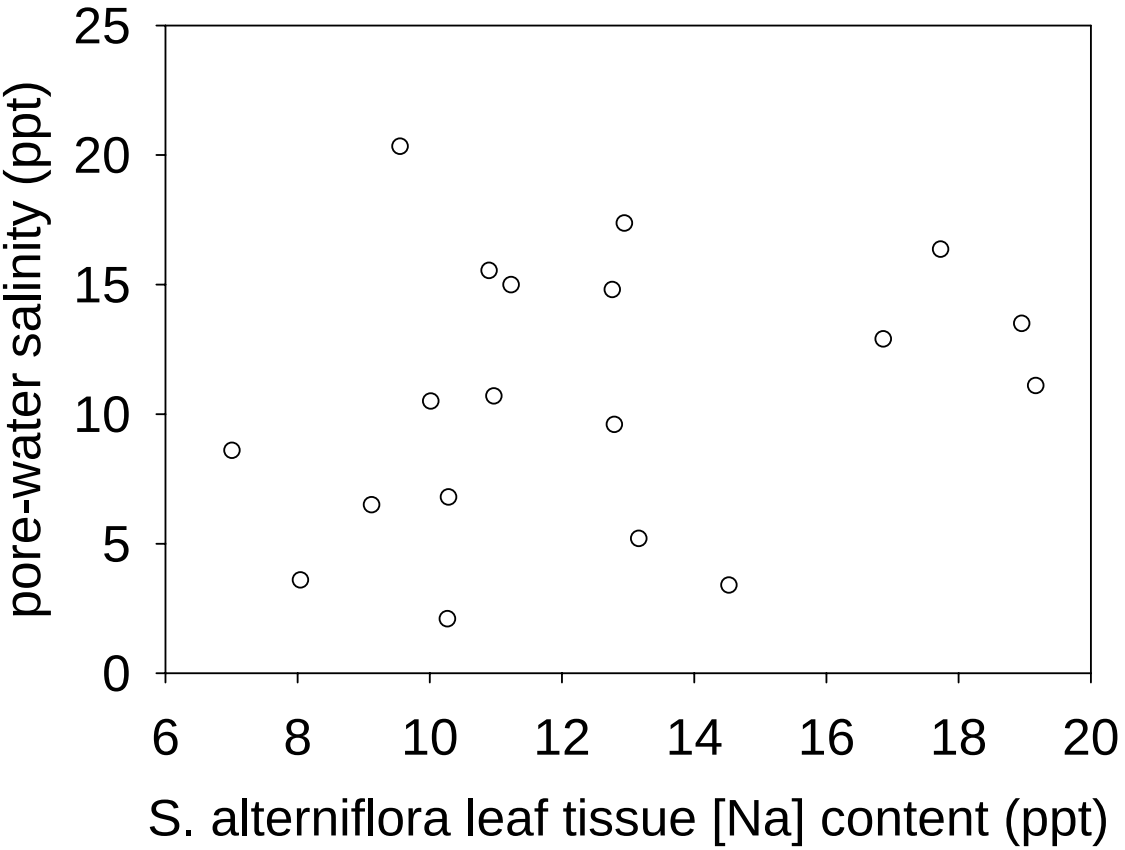


Figure 9. [P] (%) of *Spartina alterniflora* leaf tissue by month for all study sites.

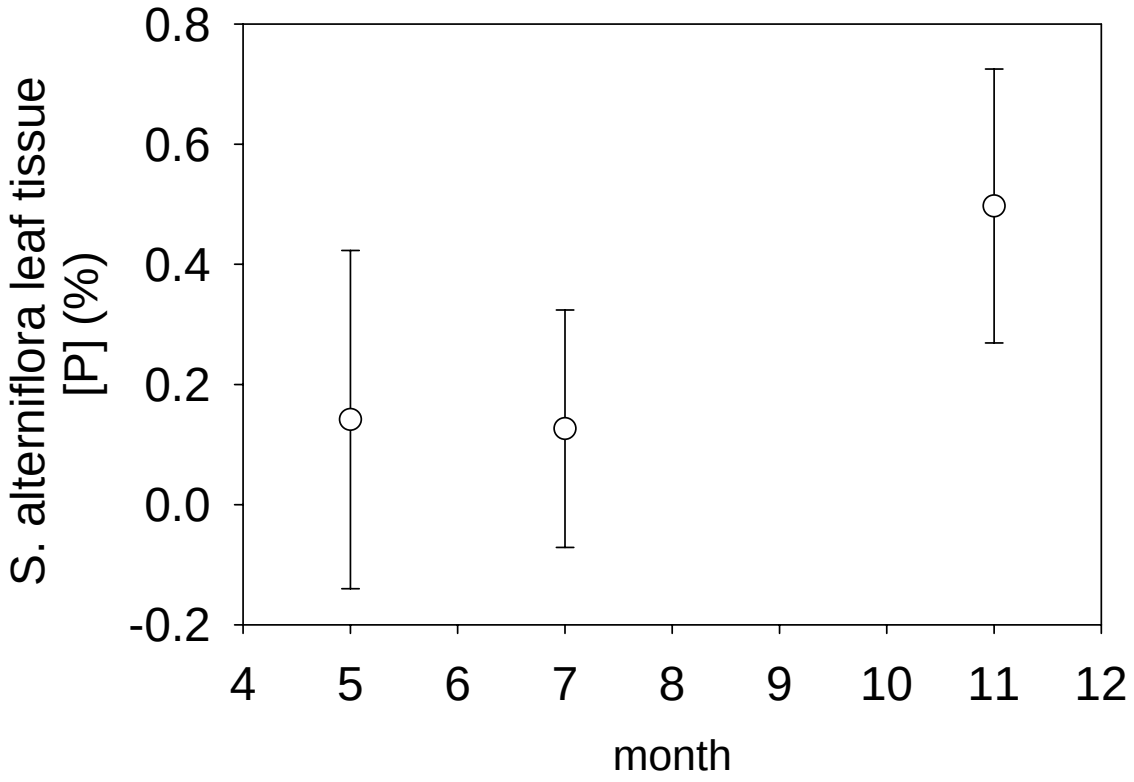


Figure 10. [Na] content (ppt) of *Spartina alterniflora* leaf tissue by month for all study sites.

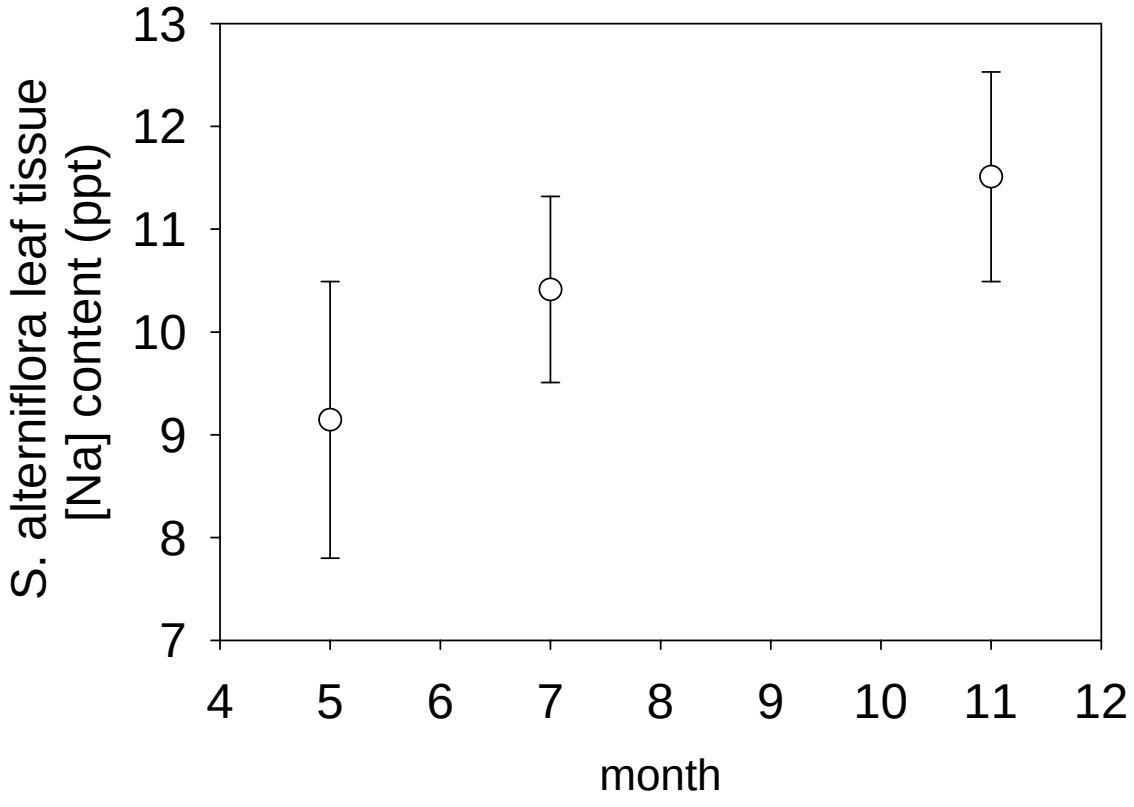
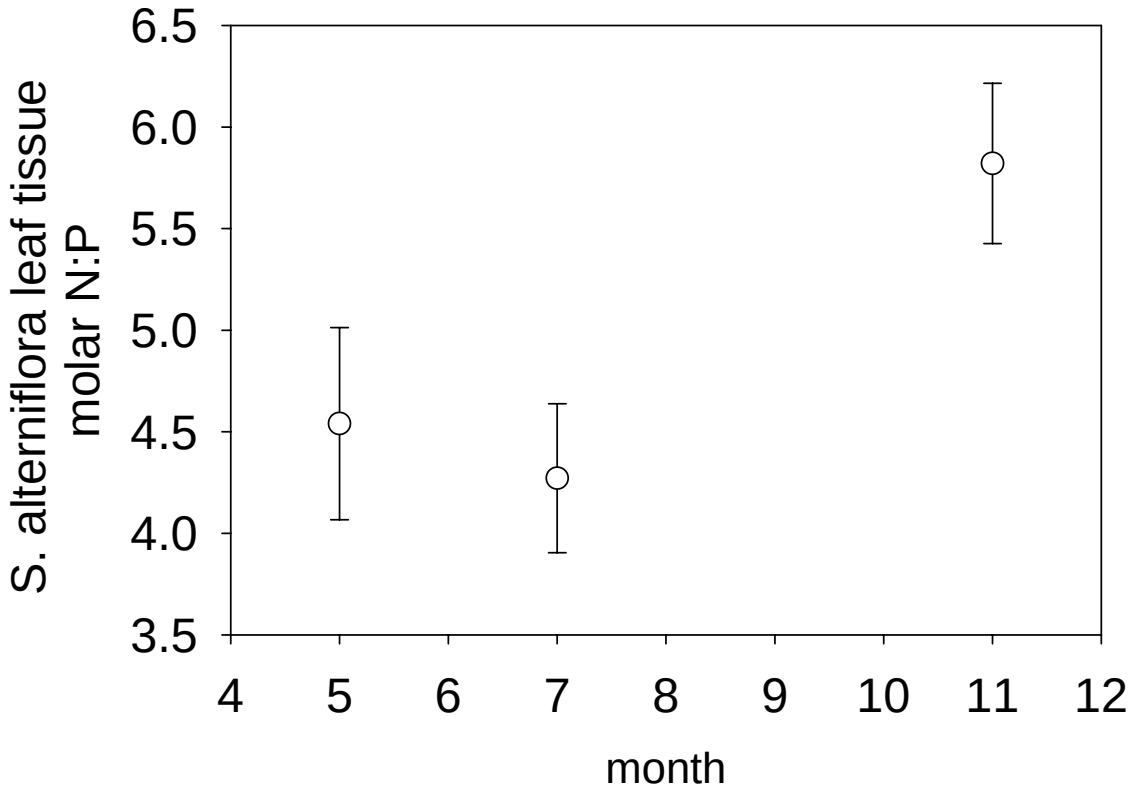


Figure 11. Molar N:P of *Spartina alterniflora* leaf tissue by month for all study sites.



APPENDIX

(SAS Data and Additional Graphs)

```
dm log 'clear';

dm output 'clear';

options ls=80;


data one;

drop num mo cu;

input num sample Al Bo Ca Cu Fe Mg Mn Mo P K Na S Zn;

cards;

208001001 1 27.815 3.479 0.338 11.718 74.873 0.328 206.802 -999.000 0.163 0.884 16335.027 0.530
11.747

208001002 2 159.362 3.708 0.252 21.786 154.931 0.257 243.886 -999.000 0.145 0.829 21331.442 0.332
17.717

208001003 3 211.841 2.783 0.317 10.268 212.254 0.190 327.695 -999.000 0.158 1.523 11421.125 0.223
12.795

208001004 4 15.911 3.079 0.300 2.820 54.515 0.246 121.970 -999.000 0.112 0.739 8703.088 0.209 6.910

208001005 5 28.889 3.261 0.271 5.365 59.753 0.221 130.335 -999.000 0.119 0.999 7719.386 0.239 10.860

208001006 6 25.773 2.678 0.325 6.153 76.629 0.275 142.843 -999.000 0.146 1.030 6738.531 0.209 11.410

208001007 7 195.045 4.143 0.246 271.804 194.252 0.261 81.566 -999.000 0.174 1.134 15421.209 0.335
91.972

208001008 8 59.691 3.263 0.406 30.598 122.001 0.391 47.675 -999.000 0.151 0.746 11606.096 0.213
17.790

208001009 9 22.821 2.659 0.286 6.533 50.208 0.237 56.138 -999.000 0.106 0.740 14523.621 0.372 10.358

208001010 10 37.571 2.779 0.360 3.543 60.270 0.260 49.379 -999.000 0.119 1.293 8042.578 0.401 10.595

208001011 11 52.558 3.255 0.223 7.316 76.588 0.200 140.354 -999.000 0.131 0.721 12271.551 0.199
10.243

208001012 12 48.753 3.591 0.169 6.953 101.786 0.174 67.438 -999.000 0.125 0.775 12791.301 0.150
8.899

208001013 13 72.026 3.935 0.485 1.120 96.732 0.333 188.085 -999.000 0.117 0.625 7007.372 0.408 6.186
```

208001014 14 367.183 4.874 0.566 1.633 479.512 0.441 320.727 -999.000 0.145 0.699 10014.890 0.383 7.951

208001015 15 159.670 3.344 0.486 1.102 102.373 0.470 78.560 -999.000 0.083 0.662 10969.007 1.085 6.875

208001016 16 47.462 3.678 0.604 -999.000 143.384 0.269 834.699 -999.000 0.131 0.478 10265.841 0.178 6.176

208001017 17 6.534 3.505 0.442 21.908 72.568 0.201 491.631 -999.000 0.178 1.072 10281.607 0.273 23.730

208001018 18 9.440 3.673 0.251 1.174 55.072 0.143 129.343 -999.000 0.141 1.134 9117.530 0.127 12.180

208001019 19 4.526 3.605 0.262 1.170 54.290 0.135 100.099 -999.000 0.149 1.298 9224.429 0.164 8.749

208001021 20 225.829 5.174 0.455 1.408 156.952 0.567 86.596 -999.000 0.100 0.692 16860.767 1.219 7.091

208001022 21 121.477 4.258 0.334 1.451 94.264 0.434 127.928 -999.000 0.098 0.781 18954.942 1.309 8.227

208001023 22 130.733 4.695 0.338 1.102 109.172 0.419 114.056 -999.000 0.125 0.783 19168.156 1.151 9.291

208001024 23 32.239 3.578 0.693 -999.000 47.218 0.554 112.935 -999.000 0.077 0.732 9547.867 0.256 3.929

208001025 24 27.019 3.260 0.568 -999.000 41.893 0.505 165.189 -999.000 0.082 0.361 10894.405 0.475 4.050

208001026 25 40.596 5.313 0.456 1.713 56.504 0.320 128.898 -999.000 0.073 0.406 12941.410 0.388 4.575

208001027 26 46.583 3.184 0.490 1.222 70.996 0.428 23.782 -999.000 0.087 0.718 12759.658 0.449 6.630

208001028 27 77.226 3.377 0.277 1.724 77.686 0.303 16.884 -999.000 0.109 0.498 17727.360 0.607 6.725

208001029 28 43.080 4.407 0.412 1.253 61.229 0.329 25.618 -999.000 0.071 0.535 11228.897 0.526 5.183

208001030 29 245.492 5.072 0.838 1.187 187.735 0.498 453.041 -999.000 0.115 0.380 15307.113 0.444 6.776

208001031 30 76.916 10.223 0.497 2.115 234.390 0.373 264.625 -999.000 0.140 0.784 13162.301 0.429 7.495

108002011 31 18.096 3.287 0.137 1.279 17.326 0.176 60.252 -999.000 1.103 0.078 12877.698 0.431 5.020

108002012 32 34.295 1.277 0.155 1.297 26.442 0.315 112.453 -999.000 1.559 0.090 7835.761 0.284 7.564

108002013 33 50.360 2.118 0.573 2.158 69.714 0.431 289.369 -999.000 1.186 0.086 7012.390 0.403 6.201

;

run;

/*Aluminum, ppm Boron, ppm Calcium % Copper, ppm Iron, ppm Magnesium % Manganese, ppm Molybdenum, ppm Phosphorus % Potassium % Sodium, ppm Sulfur % Zinc, ppm*/

proc sort; by sample; **run**;

data two;

input sample N C;

cards;

1 0.95551 42.8

2 1.3391 42.4

3 1.4669 43.3

4 1.0325 43.2

5 1.1204 44.5

6 1.3401 44.4

7 1.025 42.1

8 1.3457 43.5

9 1.1967 42.7

10 1.3811 43.4

11 1.2005 43.8

12 0.93244 43.2

13 1.2493 43.6

14 1.2804 41.7

15 1.0373 43.3

16 1.4231 43.5

17 1.2781 44.1

```
18 1.1292 44.4

19 1.1395 44.2

20 1.1479 41.6

21 0.97077 42.4

22 0.98289 42.4

23 0.92652 43.6

24 1.0087 44.7

25 0.84934 42.1

26 1.2134 44.2

27 1.3863 45

28 1.2187 42.2

29 1.2459 43

30 1.4647 43.9

31 1.103 .

32 1.559 .

33 1.186 .

;

run;


proc sort; by sample; run;


data three;

input sample date$ site$ poresalt;

cards;

1 5/25/07 BHBinland 12

2 5/25/07 BHBstreamside .

3 5/25/07 GH1 3.7

4 5/29/07 MRB1 16.7
```

5 5/29/07 MRB2 17.6

6 5/29/07 MRB3 19.4

7 6/2/07 BarreTambor .

8 6/2/07 MadisonCanal .

9 7/10/07 MIFW1 3.4

10 7/10/07 MIFW2 3.6

11 7/10/07 MISW1 .

12 7/10/07 MISW3 9.6

13 7/25/07 BHB1 8.6

14 7/25/07 BHB2 10.5

15 7/25/07 BHB3 10.7

16 7/25/07 GHI2 2.1

17 8/22/07 FLBFR1 6.8

18 8/22/07 FLBFR2 6.5

19 8/22/07 FLBFR3 .

20 8/22/07 FLBS1 12.9

21 8/22/07 FLBS2 13.5

22 8/22/07 FLBS3 11.1

23 11/1/07 MRB1 20.33

24 11/1/07 MRB2 15.54

25 11/1/07 MRB3 17.37

26 11/2/07 CPS1 14.8

27 11/2/07 CPS2 16.36

28 11/2/07 CPS3 14.99

29 11/9/07 MISW1 .

30 11/9/07 MISW3 5.2

31 12/13/07 BHB1 .

32 12/13/07 BHB2 .

```
33 12/13/07 BHB3 .

;

/*30 11/9/07 MISW3 5.2 (may not be reliable?  messed up graduated cylinder/bad sensor)*/

proc sort; by sample; run;

data four; merge one two three;
by sample;
/*the next two lines estimate the moles of C and N in 100 grams of tissue
the third line estimates the C:N molar ratio*/
cmoles100 = C*12.010;
nmoles100 = N*14.006;
CtoN = cmoles100/nmoles100;
namoles100 = NA*22.989;
kmoles100 = K*39.102;
NAtoK = namoles100/kmoles100;
nmoles100 = N*14.006;
pmoles100 = P*30.974;
NtoP = nmoles100/pmoles100;
/*the next x lines lump the dates into similar time periods*/
if date = "5/25/07" then time =1;
if date = "5/29/07" then time =1;
if date = "6/2/07" then time =1;
if date = "7/10/07" then time =2;
if date = "7/25/07" then time =2;
if date = "8/22/07" then time =2;
if date = "11/1/07" then time =3;
```

```
if date = "11/2/07" then time =3;

if date = "11/9/07" then time =3;

if date = "12/13/07" then time =3;

run;


proc corr;

var cton NAtoK NtoP C N Al Bo Ca Fe Mg Mn P K Na S Zn;

run;



data five; set four;

if date = 3;

run;


proc means; run;


proc reg;

model cton = poresalt; run;


proc reg;

model NAtoK = poresalt; run;


proc reg;

model cton = na;

run;


proc reg;

model cton = NAtoK;
```

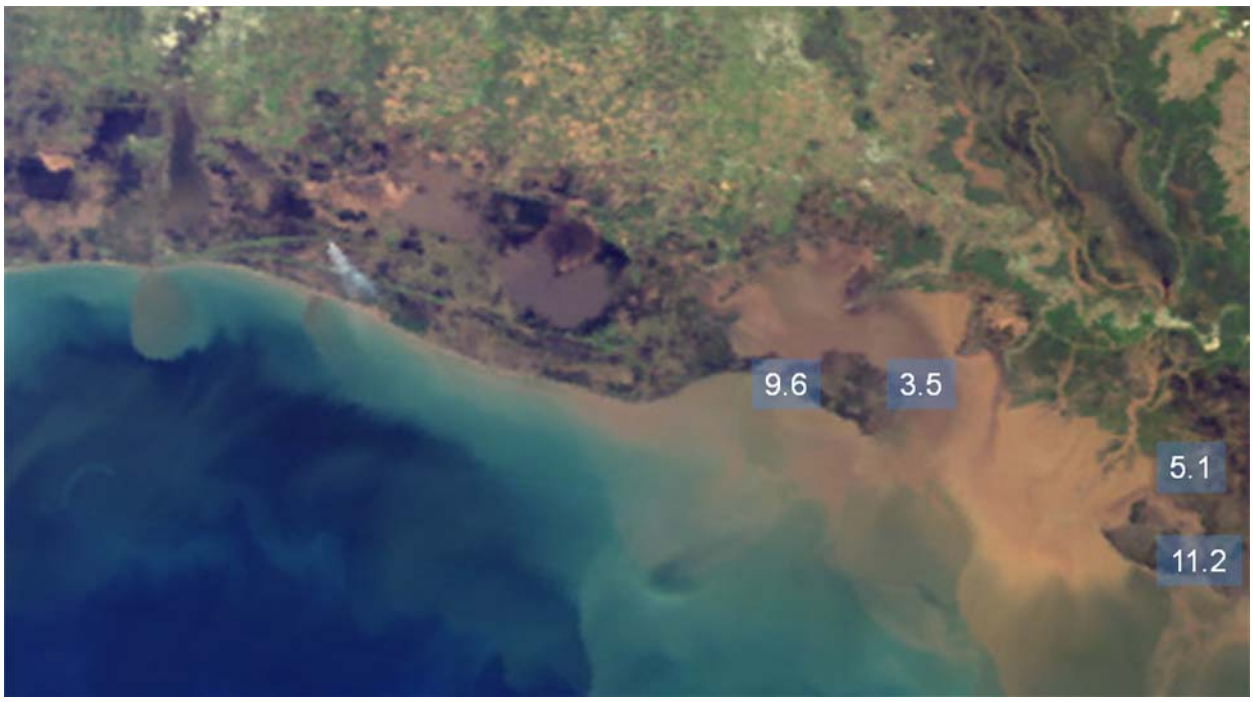
```
run;


data six; set four;
/*the next x lines lump the sites into similar areas*/
if site = "FLBFR1" then area = "FLBFR";
if site = "FLBFR2" then area = "FLBFR";
if site = "FLBFR3" then area = "FLBFR";
if site = "GH1" then area = "FLBFR";
if site = "GHI2" then area = "FLBFR";
if site = "BHB1" then area = "FLBS";
if site = "BHB2" then area = "FLBS";
if site = "BHB3" then area = "FLBS";
if site = "BHBinland" then area = "FLBS";
if site = "BHBstreamside" then area = "FLBS";
if site = "MIFW1" then area = "MIF";
if site = "MIFW2" then area = "MIF";
if site = "MISW1" then area = "MIS";
if site = "MISW3" then area = "MIS";
if site = "MRB1" then area = "RockS";
if site = "MRB2" then area = "RockS";
if site = "MRB3" then area = "RockS";
run;
proc mixed;
class area time;
model cton = time;
random area area*time;
lsmeans time;
run;
```

```
run;


proc freq;
tables time site;
run;
```

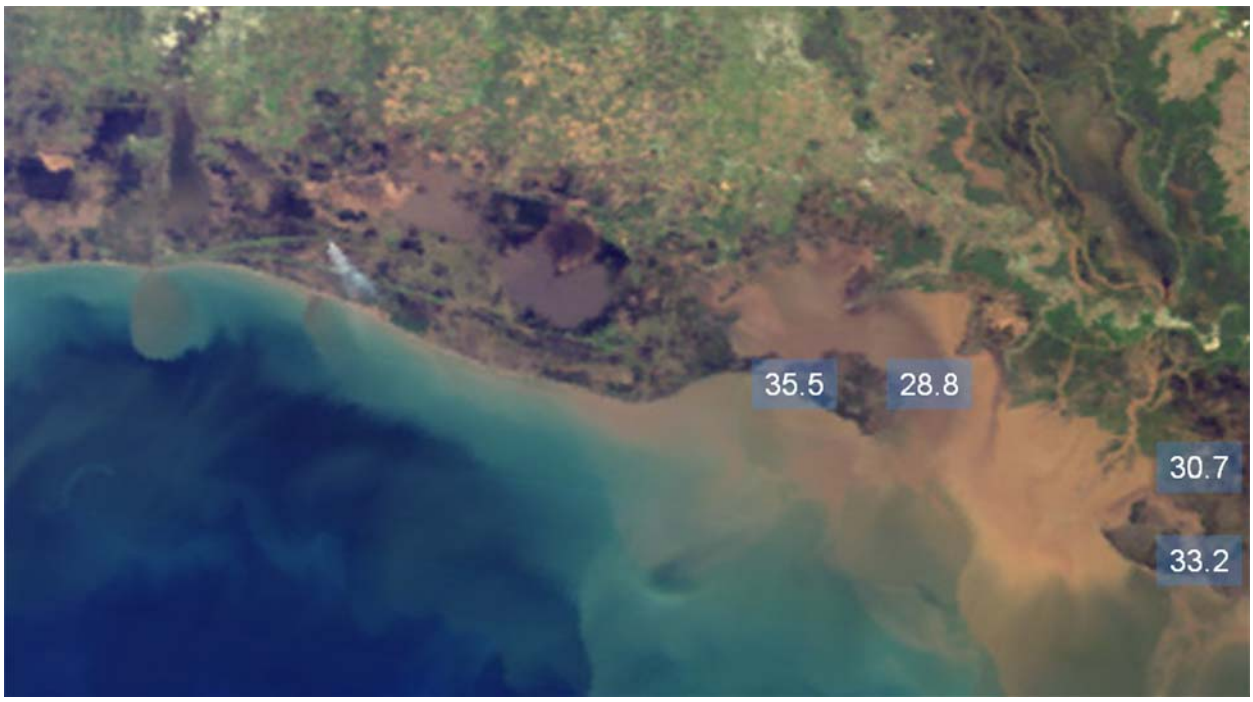
Pore-water salinity (ppt), May 2007



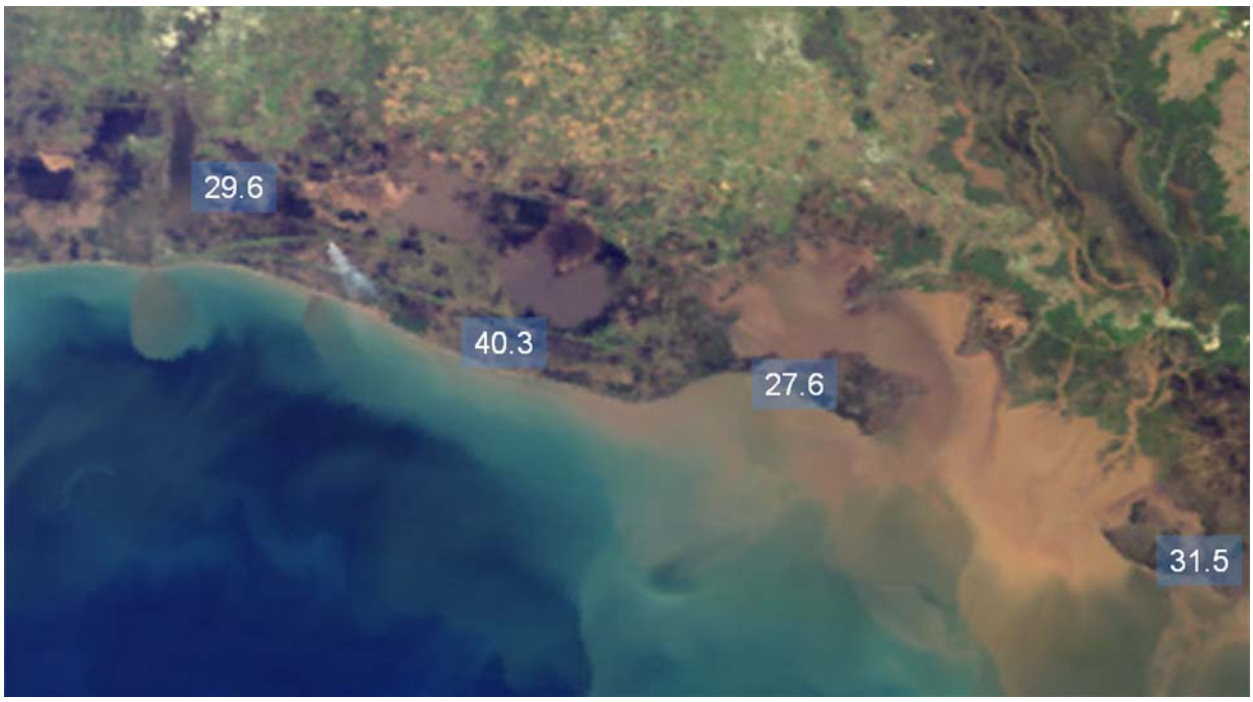
Pore-water salinity (ppt), July 2007

Pore-water salinity (ppt), Nov 2007

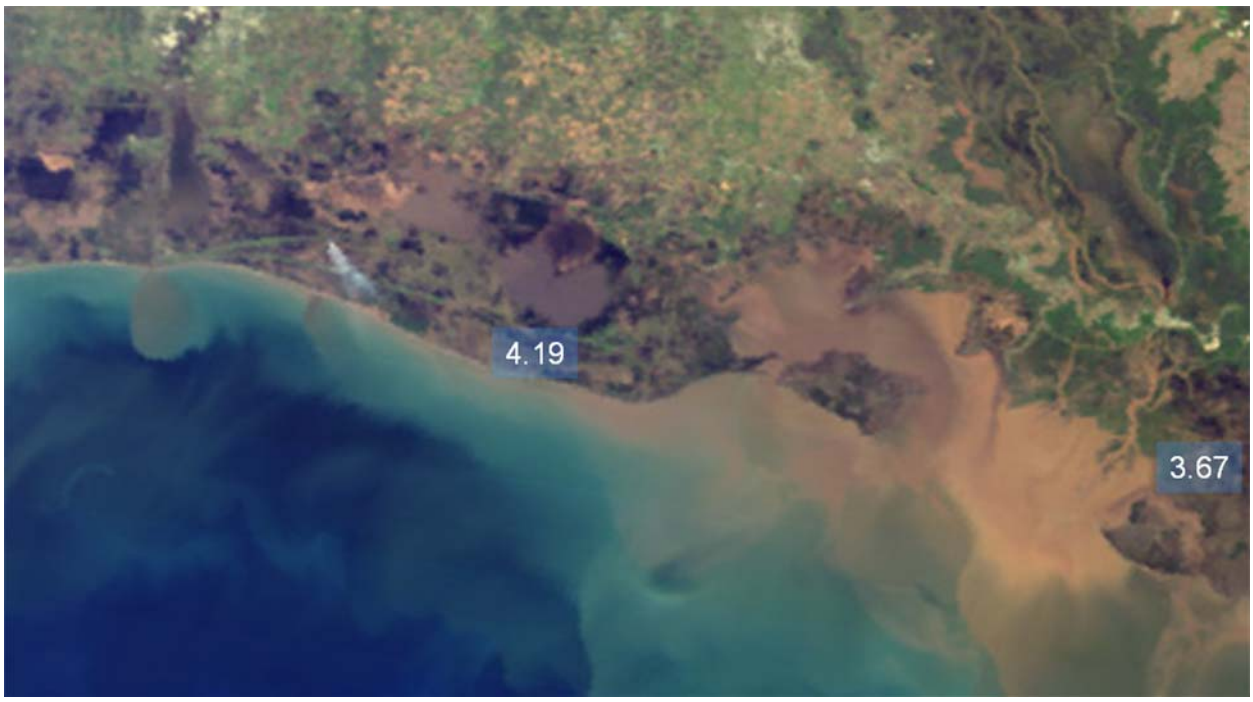
Molar C:N ratios in *S. alterniflora* leaves, May 2007.



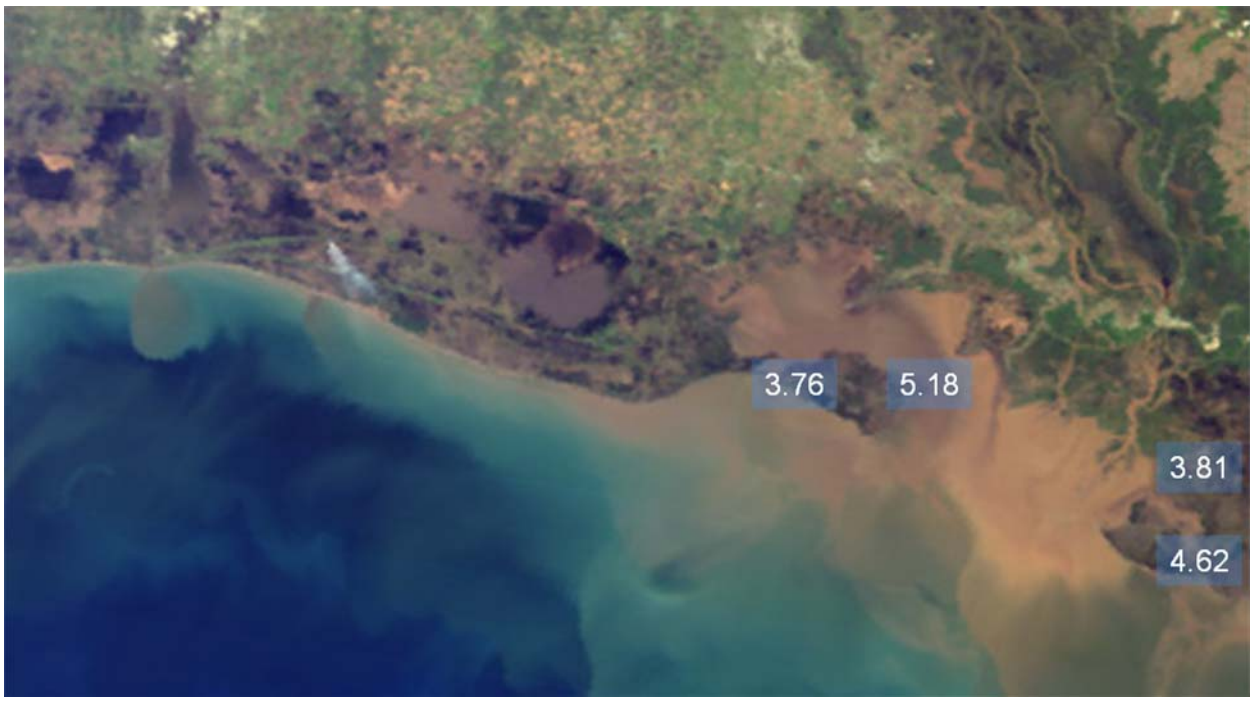
Molar C:N ratios in *S. alterniflora* leaves, July 2007



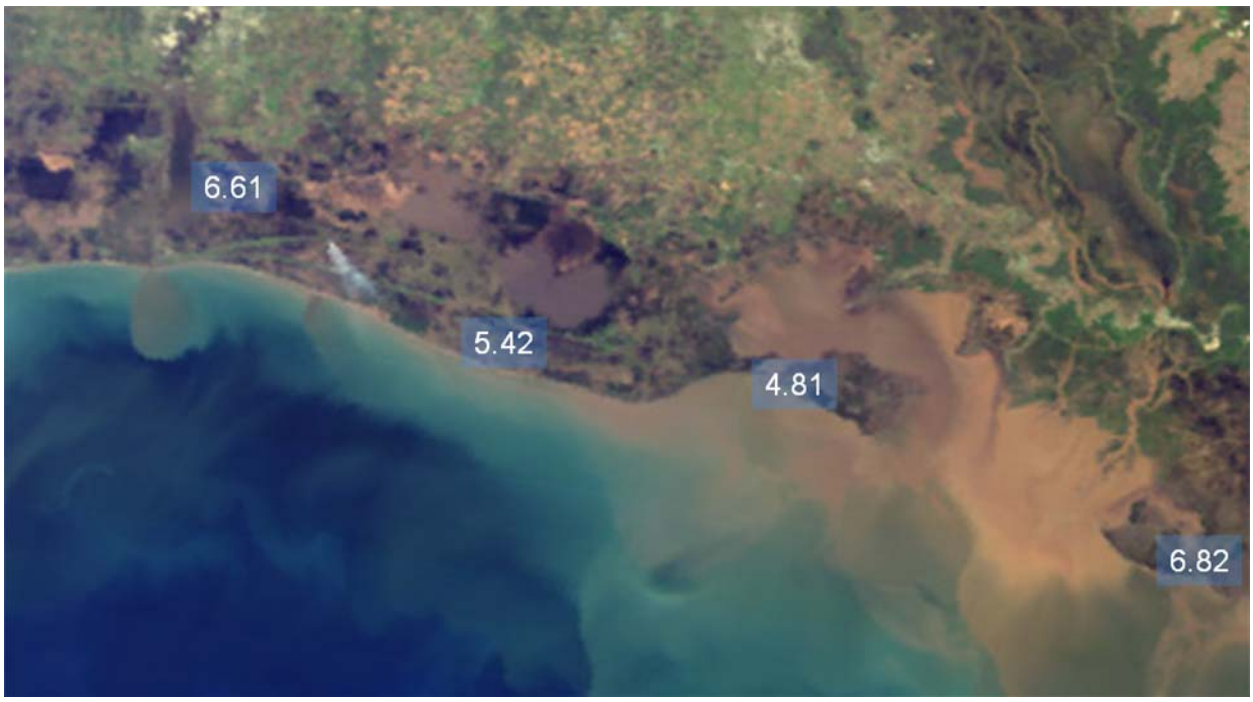
Molar C:N ratios in *S. alterniflora* leaves, Nov 2007



Molar N:P ratios in *S. alterniflora* leaves, May 2007



Molar N:P ratios in *S. alterniflora* leaves, July 2007



Molar N:P in *S. alterniflora* leaves, Nov 2007