Improving marsh restoration: leaf tissue chemistry identifies factors limiting production in *Spartina patens*

V. D. Tobias · J. A. Nyman · R. D. DeLaune · J. D. Foret

Received: 8 December 2008 / Accepted: 27 August 2009
© Springer Science+Business Media B.V. 2009

Abstract Marsh loss is a problem in many areas around the world. In order to combat the problem, scientists and managers need tools to determine its cause and evaluate the effectiveness of management techniques. Current methods for estimating productivity and identifying factors that limit productivity are too time-consuming or expensive for widespread, regular use, however. In coastal Louisiana, where *Spartina patens* (Ait.) Muhl is the most common plant, restoration seeks to slow wetland loss rates that averaged approximately 77.4 km²/year between 1978 and 2000. We used the chemical composition of leaf tissue from *S. patens* grown under controlled conditions to create a simple and inexpensive tool to identify salinity stress and nutrient limitation. By growing *S. patens* at varying nitrogen availability and salinity levels, we found that C:N ratios and Na concentrations can be used to classify factors that limit production in *S. patens*.

Keywords Wetlands · *Spartina patens* · Chemistry · Nitrogen · Salinity · Sodium

Introduction

Marsh loss is a problem in many areas of the world. In coastal Louisiana, 77.4 km²/year of marsh converted to open water between 1978 and 2000 (Barras et al. 2003). Marshes convert to open water due to many factors, including sea-level rise, sediment starvation, and changes in hydrology and soil chemistry. Fresh water and sediment input are critical factors in combating coastal marsh loss (Day et al. 2000). Mineral sediments help maintain marsh elevation by increasing soil elevation, plant production through nutrient delivery, and organic matter accumulation (DeLaune et al. 1979). Increased soil organic matter accumulation alone has also been associated with increasing marsh elevation (Nyman et al. 2006; Craft 2007). Increasing marsh elevation is essential for countering global sea-level rise and local subsidence. Determining potential causes of marsh loss is difficult because although reducing salinity and increasing nutrients can increase biomass production in *Spartina patens* (Ait.) Muhl (marsh hay...
cordgrass), a perennial wetland grass (DeLaune et al. 2005), current techniques to determine which factor limits growth are both time-consuming and expensive.

Many management techniques have been developed to combat marsh loss; however, managers often lack tools (1) to make informed decisions about which restoration technique to use or (2) to evaluate results of a technique that has been implemented. Several methods for estimating productivity currently exist; however, none is feasible for regular, widespread use for various reasons. For example, managers can use changes in above-ground biomass to identify sites that differ in productivity (e.g., Burdick et al. 1989; Ewing and McKee 1997). This method of estimating productivity requires intense sampling over a short period of time; thus it is too costly to be used regularly. Shoot elongation varies with plant growth (Ewing and McKee 1997), but using it to identify limitation requires repeated visits to sites and locating previously tagged stems. Also, while these techniques may identify areas where production is limited, they cannot identify the factors that limit production. Methods such as leaf spectral reflectance, carbon dioxide uptake, leaf expansion, and leaf proline concentration vary with salinity stress or nutrient starvation (Ewing et al. 1995, 1997). Although these attributes can be used to directly identify limiting factors, they are too costly for widespread annual use. By developing a simple, inexpensive tool to determine which factors limit plant growth across large, heterogeneous areas, we can improve the evaluation of fresh water introductions and other marsh restoration techniques.

Our objectives were to determine the feasibility of using leaf chemical characteristics to identify the factors that limit plant productivity in coastal marshes and provide a basis for interpreting nutrient ratios of samples taken in the field. In this article, we show how the leaf chemistry of *S. patens* responds to changes in salinity stress and nutrient availability under controlled nutrient and salinity conditions in a greenhouse. We use this data set to determine chemical signatures in *S. patens* leaf tissue that may be used as references to indicate factors that limit productivity in coastal marshes. We focus on *S. patens* because it is the most common plant species in coastal Louisiana (Chabreck 1970).

**Methods**

We grew *S. patens* plants in a greenhouse under varying levels of salinity and nutrients in a balanced four by four factorial design with four replications (128 experimental units). We obtained two populations of *S. patens* that differed in salinity tolerance from Mark Hester (University of New Orleans, New Orleans, LA, USA). The lethal salinity levels (50% death of above-ground tissue) for these two populations were 66 ppt for population “k” and 81 ppt for population “i” (Hester et al. 1996). We used plants from two populations with documented phenotypic differences to represent random variation rather than to investigate the effects of population on leaf chemistry. We initially grew the plants clonally in separate bedding trays containing sand, water, and commercial fertilizer (Peters 20–20–20 N–P–K).

We made experimental soils from a homogeneous mixture of 90% commercial play sand and 10% potter’s clay to which we added one of four combinations of 19–5–8 and 35–0–0 encapsulated...
(slow-release, non-water soluble) fertilizer. We chose specific nutrient treatments to approximate 25, 75, 125, and 200% of the nitrogen \((4.90 \times 10^{-4}, 1.46 \times 10^{-3}, 2.43 \times 10^{-3}, \text{ and } 3.89 \times 10^{-3} \text{ gN/cm}^3, \text{ respectively})\) and phosphorus levels \((2.4 \times 10^{-5}, 7.3 \times 10^{-5}, 1.2 \times 10^{-4}, \text{ and } 1.9 \times 10^{-4} \text{ gP/cm}^3, \text{ respectively})\) of unmanaged, \textit{S. patens}-dominated marshes at Rockefeller Wildlife Refuge (approximately 29° 37’ N, 92° 36’ W; Foret 2001). The average nutrient levels of these marshes at Rockefeller Wildlife Refuge were approximately 1.96 \times 10^{-3} \text{ gN/cm}^3 and 9.6 \times 10^{-5} \text{ gP/cm}^3 (Foret 2001). The actual levels of nitrogen achieved in the experimental soils were 30, 95, 130, and 205% and the actual levels of phosphorus achieved were 125, 375, 620, and 980% of nutrient levels at Rockefeller Refuge. We planted two stems of the same population (“i” or “k”) in each one-gallon pot. We placed two pots, one containing each population, in 64 14-gallon randomly arranged tubs and flooded the tubs with well water to the soil surface inside the pots. Plants were allowed to grow for 26 days before we raised the salinity level of the water in the tubs.

We raised the salinity in the tubs using Forty Fathoms marine mix (bioassay grade) in five installations over a 10-day period until the water in the tubs reached the target salinity. Target salinities were 2, 6, 18, and 36 ppt. Mean actual salinities achieved were 2, 5, 17, and 38 ppt. We replaced water lost to evapotranspiration twice weekly to keep the pots flooded to the soil surface. In order to reduce build up of salt in the soil, we poured water from the tubs over the soil surface. We collected pore water samples from a randomly selected sub-sample of 16 pots every 3–4 weeks and measured conductivity and salinity in the pore water and tub water. The experiment lasted 144 days from the time we began the nutrient treatments. Merino et al. (in press) tested the hypothesis that the response of growth to nutrient availability did not vary with salinity. They found that growth varied most in response to nutrient availability at low salinity, but did not vary at all at high salinity (Fig. 1).

At the conclusion of the experiment, we harvested above- and below-ground tissue over a 3-day period. We washed the below-ground tissue and dried both above- and below-ground tissue at 60° and weighed it to determine biomass. Because above- and below-ground biomass were linearly correlated \((R^2 = 0.981649, \; P = 0.0001)\), we added them together to estimate total biomass (Merino et al. in press). Using the average biomass of pots grown under specific nutrient and salinity conditions, we classified treatment combinations in terms of factors that limit productivity.

We classified pots into four groups by limiting factor: nitrogen, salinity, both, or neither (Table 1). Pots with nitrogen treatments \(\geq 30\% \text{ N} \) and salinities \(<10 \text{ ppt}\) were classified because neither-limited as the high biomass of plants in these treatments (Fig. 1) suggested that a factor other than salinity or nitrogen limited growth. Pots that had an average porewater salinity of \(<10 \text{ ppt}\) and nitrogen treatment of \(30\% \text{ N}\) (Fig. 1) were classified as nitrogen-limited due to their low biomass combined with low nitrogen availability. We reasoned that salinity was not limiting growth in these pots because the same salinity treatments did not limit growth in the neither-limited pots. Although biomass was too similar in plants grown at higher salinities to use it to identify limiting factors, we applied the same logic we used for the lower salinity pots. Pots with average salinities \(>10 \text{ ppt}\) and nitrogen treatments \(>30\% \text{ N}\) were classified as salinity-limited. The remaining pots \(\text{i.e.}, \text{ those with salinity } >10 \text{ ppt} \text{ and nitrogen treatment of } 30\% \text{ N} \) were classified as both-limited (Fig. 1). This classification resulted in an unequal number of pots in each limiting factor group.

We ground above-ground tissue samples from each pot using a Wiley mill to produce a homogeneous tissue sample for chemical analysis. We...
determined carbon concentration using a CHN analyzer in the lab at University of Louisiana, Lafayette. We sent ground tissue samples to the LSU AgCenter’s Soil Testing and Plant Analysis Lab (STPAL, LSU, Baton Rouge, LA, USA) to determine nitrogen, phosphorus, and sodium concentrations in leaf tissue. The STPAL used dry combustion by Leco N analyzer to determine nitrogen content. They used ICP analysis to determine concentrations of sodium and phosphorus.

Data were analyzed as a one-way ANOVA with four groups (neither-, nitrogen-, salinity-, and both-limited) using PROC MIXED in SAS. PROC MIXED has the capability to handle unbalanced sample sizes within groups, as in our analysis. We used contrasts within the ANOVAs to compare N:P ratios, C:N ratios, and Na concentrations of plants grown at high salinity with those of plants grown at low salinity. We used LSMeans to obtain a mean for each of the groups. In order to determine boundaries for the tool to evaluate limiting factors, we averaged the means of the high and low salinity groups. We used the same procedures to make comparisons between plants grown at high and low nitrogen levels. Correlations were performed using Pearson’s correlation coefficient. We determined significance for all tests using an alpha level of 0.05.

## Results

There was a significant difference in N:P ratios among the four limiting factors \( (F_{3,103} = 22.53, P < 0.0001) \). Plants that were not nitrogen-limited had lower N:P ratios than plants that were nitrogen-limited \( (F_{1,103} = 14.05, P = 0.0003; \text{Fig. 2}) \). Plants that were salinity-limited had lower N:P ratios than plants that were not salinity-limited \( (F_{1,103} = 45.90, P < 0.0001; \text{Fig. 2}) \).

There was a significant difference in C:N ratios among limiting factors \( (F_{3,104} = 12.38, P < 0.0001) \). Plants that were not nitrogen-limited had lower C:N ratios than plants that were nitrogen-limited \( (F_{1,104} = 36.69, P < 0.0001; \text{Fig. 3}) \). The mean C:N ratio for non-nitrogen-limited plants was 42.07 whereas the mean C:N ratio for nitrogen-limited plants was 69.94. The average of the mean C:N ratio overall was 56. C:N ratios of plants that were salinity-limited were not significantly different from C:N.
There was a significant difference in Na concentration among limiting factors ($F_{3,103} = 22.53$, $P < 0.0001$). Plants that were not nitrogen-limited had higher Na concentrations than plants that were nitrogen-limited ($F_{1,122} = 14.13$, $P = 0.0003$; Fig. 4). Sodium concentrations were higher in plants that were salinity-limited than plants that were not salinity-limited ($F_{1,122} = 131.75$, $P < 0.0001$). The mean Na concentration for salinity-limited plants was 1.4%. The mean Na concentration for non-salinity-limited plants was 0.8%. The average of the mean Na concentration overall was 1.1%. Sodium concentrations in plants were correlated with water salinity ($r = 0.811$, $P < 0.0001$).

**Discussion**

Biomass measurements alone could not be used to determine the cause of the limitation of production because intermediate levels of biomass developed where growth was salinity-limited, nitrogen-limited, and co-limited by high salinity and low nitrogen availability (Fig. 1). The large difference in biomass between plants grown in limited and unlimited conditions highlights the importance of determining limiting factors for improving the health of degrading marshes. Merino et al. (in press) found that maximum biomass for *S. patens* occurred when plants grew in water low in salinity and soil high in nutrients. Although previous studies appear to disagree on the growth response of *Spartina* spp. to changes in salinity, the results of our study show that the range of salinities in which tests were conducted could have influenced the results of these studies. For instance, DeLaune et al. (2005) showed that for *S. alterniflora* grown where salinity was less than 8 ppt, adding nutrients had a bigger effect on growth than decreasing salinity. Our results suggest that these lower salinities likely do not produce conditions that limit production in *Spartina* spp. A study (Foret 2001) found that *S. patens* had large differences in growth responses to salinity where salinity differed from 15 ppt to near 0 ppt. The change in growth in this study was likely due to reducing salinity stress on the plants.

N:P ratios in leaf tissue could not be used to identify nitrogen or salinity limitation because N:P ratios were affected by changes in nitrogen and salinity levels. Phosphorus content in leaves did not vary much, and was generally high. Although plants were subjected to relatively high levels of phosphorus in all treatment soils, this is unlikely to have impaired productivity because unlike nitrogen, excess phosphorus has not been shown to damage plants. Our N:P ratios (range: 20.57–104.85, mean: 44.01) were

---

**Fig. 3** Mean molar C:N ratios (±1 SE) of *Spartina patens* leaf tissue grown under various nutrient and salinity conditions. Nutrient-limited indicates low nutrients limited productivity. Salinity-limited indicates high salinity limited productivity. Neither-limited indicates plants received high nutrients and low salinity. Both-limited indicates plants received low nutrients and high salinity.

**Fig. 4** Mean molar sodium concentrations (±1 SE) of *Spartina patens* leaf tissue grown under various nutrient and salinity conditions. Nutrient-limited indicates low nutrients limited productivity. Salinity-limited indicates high salinity limited productivity. Neither-limited indicates plants received high nutrients and low salinity. Both-limited indicates plants received low nutrients and high salinity.
somewhat higher than the ranges reported for *Spar-tina* spp. in previous studies. Foret (2001) found N:P ratios between 18 and 32 for *S. patens*. Stribling and Cornwell (2001) found N:P ratios between 7.4 and 25 (converted to molar ratios from the reported mass ratios) for *S. alterniflora*. Our highest N:P ratios occurred at our lowest salinities (Fig. 2), which could be because soils have a higher phosphate sorption capacity in freshwater than in saline conditions (Sundareshwar and Morris 1999). There are too few reports of N:P ratios from the field to determine if the high N:P ratios that we observed at low salinities are common. It is likely that such high N:P ratios would rarely develop in the field where soil P is high because unlike marsh soils, our experimental soils lacked dissolved organic matter which would compete with phosphorus for clay binding sites in addition to chloride.

C:N ratios were useful in identifying nitrogen limitation because C:N ratios varied predictably with nitrogen levels. Higher C:N ratios indicated limitation of productivity by nitrogen starvation. Our C:N ratios (range: 19.84–138.88, mean: 49.04) were within the ranges reported for *Spartina* spp. in previous studies. Foret (2001) reported C:N ratios between 40 and 120 for *S. patens*. Bradley and Morris (1992) reported C:N ratios between 30 at high salinity and 90 at low salinity for above-ground tissue of *S. alterniflora*. Our findings also agree with previous studies reporting that enhanced nitrogen decreased the C:N ratio of *Spartina* spp. leaf tissue (Foret 2001; Bradley and Morris 1992). In contrast to Foret’s findings that increased nutrient availability reduced C:N ratios only where salinity was low, in our study, C:N ratios also decreased with higher nitrogen availability where salinity was high. Our findings agree with Bradley and Morris’s (1992) finding that the internal nitrogen supply needed to maintain growth in *Spartina alterniflora* increased with increasing salinity.

Sodium concentration in leaf tissue was a useful tool for identifying salinity stress. While changes in both salinity and nitrogen levels affected sodium concentration, the effect of salinity on Na concentration was much greater than the effect of nitrogen variations on Na concentration. Plants that grew in water with higher salinity had higher sodium concentrations in their leaf tissue. Sodium concentrations in leaf tissue of other marsh species have also been shown to increase with increases in water salinity level (McKee and Mendelssohn 1989; Bradley and Morris 1991). The high correlation between leaf tissue Na and water salinity suggests that a single measurement of leaf tissue salinity is a better indicator of salinity exposure than a single measurement of water salinity due to the dynamic nature of water salinity in coastal marshes.

Our findings confirm that the chemical composition of the leaf tissue of *S. patens* can be used to determine if low nitrogen availability or high salinity limit productivity. Using a combination of the response of C:N ratios and sodium concentration in plant tissue to variations in the conditions in which the plants were grown, it is possible to distinguish plants grown under different limiting conditions (Fig. 5). This tool (Fig. 6) could eliminate much speculation about methods for improving production in degrading coastal marshes by allowing managers to more easily test their assumptions about which factors limit production. Using small samples of leaf tissue to determine leaf chemistry also has the potential to be more cost-effective than current methods for identifying limiting factors via measuring biomass since it is less time-consuming. The type of elemental analysis that we used for this study is relatively inexpensive and available through agriculture and extension offices throughout the United States. Studies are needed to confirm that this tool can
identify limiting factors under field conditions for *S. patens* and other species.

One limitation of this study is that these nitrogen and sodium signatures do not reflect changes in C:N ratios and sodium concentrations that may result from variations in flooding stress. Future experiments will identify the chemical signatures that can be used to identify marshes that are stressed by flooding and the effects flooding may have on the signatures we have already identified. A second limitation of this study is that vegetation responses to stress under constant, controlled conditions may not accurately reflect responses to natural variations in marshes. Future efforts will focus on field experiments to test whether the relationships we observed in this greenhouse experiment apply to plants growing in the field.

Acknowledgments Funding for this study was provided by and by the Coastal Restoration and Enhancement through Science and Technology Program (contract no. CREST 07-10). We thank D. Price for providing tissue samples used for tissue analysis and portions of the data used in this study. We also thank J. Merino for providing data.

References


McKee KL, Mendelssohn IA (1989) Response of a freshwater marsh plant community to increased salinity and increased water level. Aquat Bot 34:301–316

marshes. Wetl Ecol Manag. doi:10.1007/s11273-008-9124-4