

# Glutamine synthetase partitioning in native and introduced salt marsh grasses

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**ABSTRACT:** Plants with higher glutamine synthetase (GS) activity in photosynthetic tissues than below-ground structures (high leaf:root [L:R] GS activity) show growth advantages over plants with a low L:R GS activity ratio. The benefits of a high L:R GS activity ratio are well documented in agricultural systems, but little is known about the ecology of GS partitioning in natural systems. To determine the ecological significance of GS partitioning, we measured above- and below-ground GS activity in *Spartina* grasses field-collected from a Maine salt marsh and others raised in a growth chamber from seed. The more stress-tolerant, faster growing *S. alterniflora* had a higher L:R GS activity than *S. patens* in chamber- and marsh-grown plants throughout the growing season. Additionally, we compared GS partitioning in native and introduced subspecies of *Phragmites australis*. While we did not find a significant difference between the subspecies, the L:R GS activity in both native and introduced reeds was among the highest reported. Our results indicate that high L:R GS activity corresponds with observed stress tolerance, growth and competitive ability in both natural and agricultural systems.

**KEY WORDS:** Nitrogen metabolism · *Spartina* · Salt marsh · Glutamine synthetase · *Phragmites australis* · Native *Phragmites* · Invasive plants · Enzyme partitioning

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## INTRODUCTION

In New England salt marshes, the mean high waterline separates 2 species of cordgrass (genus *Spartina*, Poaceae). Above the mean high water line is a lawn of *Spartina patens* (Aiton) Muhl.; below is a monoculture of *Spartina alterniflora* Loisel. (Bertness & Ellison 1987). Primary productivity is N-limited for both *S. patens* and *S. alterniflora* (Mann 1977) and the species' border is maintained by a balance of competition and physical stress (Bertness & Ellison 1987, Pennings & Callaway 1992). In the absence of *S. patens*, *S. alterniflora* performs best in the high marsh, while stress restricts *S. patens* from the low marsh (Bertness & Ellison 1987). When the *S. patens* zone is amended with N, *S. alterniflora* outcompetes *S. patens* (Emery et al. 2001). Under ambient N, *S. patens* is the stronger competitor, yet is less productive than *S. alterniflora* (Emery et al. 2001).

Over the past century, *Phragmites australis* (Poaceae) has expanded its role in the salt marsh plant

community. Historically a minor component of salt marsh vegetation, the recent expansion is largely due to the introduction of an aggressive European genotype (Saltonstall 2002), now recognized as *P. australis* ssp. *australis* (Cav.) Trin Ex Steud. The native North American reed (now subspecies) *P. australis* ssp. *americanus* Saltonstall, P.M. Peterson and Soreng is less aggressive and does not form the dense monocultures typical of introduced *P. australis* (Saltonstall et al. 2004, League et al. 2006). Introduced *P. australis* is faster growing and more salt tolerant than the native (Vasquez et al. 2005), outcompetes the native in salt marshes, has higher tissue protein content and lower tissue C:N (Packett & Chambers 2006), has higher photosynthetic rates (Mozdzer & Zieman 2010) and better capitalizes on N increases than *P. australis* ssp. *americanus* (Saltonstall & Stevenson 2007).

The salt marsh imposes a high N demand on vegetation, native or otherwise. Salinity stress elicits the production of nitrogenous osmoprotectants at the expense

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of growth (Colmer et al. 1996, Hester et al. 2001), requiring a high nitrogen use efficiency (NUE) to balance stress tolerance and growth (Stewart & Rhodes 1978). *Spartina alterniflora* is more stress tolerant than *S. patens* (Maricle et al. 2007), but stress increases its N requirement (Bradley & Morris 1992). To tolerate the low marsh environment, *S. alterniflora* has a higher N-uptake rate, NUE and biomass accumulation than *S. patens* (Drake et al. 2008). *S. alterniflora* is likewise more salt tolerant than introduced *Phragmites australis* (Vasquez et al. 2006); however, once established, *P. australis* can outcompete *S. alterniflora* under field salinities and has greater N-uptake rates, tissue N concentration (Farnsworth & Meyerson 2003), shoot biomass, root biomass (under controlled conditions: introduced *P. australis* > native *P. australis* > *S. alterniflora*), a higher affinity for N at low and high concentrations and more readily uses dissolved organic N (Mozdzer et al. 2010).

We compared *Spartina patens* and *S. alterniflora* and the 2 subspecies of *Phragmites australis* to investigate how the location of glutamine synthetase (GS) relates to growth in salt marsh plants. GS is the rate-limiting enzyme in amino acid biosynthesis (Lam et al. 1996, Kichey et al. 2006). As part of the GS-(GOGAT) cycle, GS assimilates inorganic N ( $\text{NH}_4^+$ ) by catalyzing the amination of glutamate to glutamine (Mifflin & Lea 1977). Multiple isoforms of GS fall into 3 general categories: cytosolic  $\text{GS}_I$ , chloroplastic  $\text{GS}_{II}$  and root-specific GS ( $\text{GS}_R$ ) (Lam et al. 1996). Leaf GS is critical to amino acid biosynthesis both via the nitrate reduction/assimilatory pathway and in recovery of photorespiratory  $\text{NH}_4^+$  (Bauer et al. 1997, Cánovas et al. 2007). A recent molecular study has shown more diversity in structure and function of GS than previously recognized (Bernard et al. 2008).

Plants that preferentially increase leaf GS activity over root GS require less photosynthate for N assimilation (Schjoerring et al. 2002). Experimentally increasing leaf:root (L:R) GS activity confers a growth advantage, variously increasing photosynthetic rates (Fuentes et al. 2001), relative growth rate (Limami et al. 1999, Migge et al. 2000, Oliveira et al. 2002), stress tolerance (Hoshida et al. 2000) and tissue protein levels (Habash et al. 2001, Hirel et al. 2001, Oliveira et al. 2002, also see reviews by Andrews et al. 2004, Good et al. 2004). Transgenic plants with increased root GS activity decreased biomass production relative to controls (Limami et al. 1999, Harrison et al. 2003), indicating that the tissue-specific location of GS impacts fitness more than whole-plant GS activity.

Our first experiment compared GS partitioning in *Spartina alterniflora* and *S. patens* in the field. A second *in situ* experiment compared GS activity of *Phragmites australis* ssp. *australis* and *P. australis* ssp. *amer-*

*icanus*. For our third experiment, we raised both *Spartina* spp. in a growth chamber with 3 N treatments to test the impacts of N source on GS activity. We hypothesized that the fast-growing, stress-tolerant *S. alterniflora* would have a higher L:R GS ratio than the congeneric *S. patens* in both field and controlled conditions. In *Phragmites* ssp., we hypothesized that introduced *P. australis* ssp. *australis* would have higher L:R GS activity than *P. australis* ssp. *americanus*.

## MATERIALS AND METHODS

***Spartina* collection.** Samples of *S. alterniflora* and *S. patens* were collected from the Little River Marsh on the Wells National Estuarine Research Reserve (Wells, ME, 43° 20' 12.21" N, 70° 32' 24.25" W). We sampled on 3 occasions during the growing season of 2005: July (n = 10), August (n = 10) and October (n = 5) prior to senescence. Samples were randomly selected along a transect bordering the *S. patens* and *S. alterniflora* zones. Leaf samples consisted of all above-ground growth for each plant; rhizomes were not tested for GS activity. Plant material was rinsed in cool freshwater, separated into root and shoot and placed in liquid nitrogen ( $\text{LN}_2$ ). Soil water from the root sample was filtered onto a NaCl refractometer to determine pore water salinity. Soil temperatures were recorded using an analog soil thermometer.

Cation exchange resin bags (10 g resin) were used to determine plant-available  $\text{NH}_4^+$  in the *Spartina alterniflora* and *S. patens* zones (n = 10) for the growing season of July to October (modified from Binkley & Matson 1983). In autumn 2005, resins were air-dried, cleaned of roots and silt, and eluted in 1 N KCl. Following 18 h incubation at ambient temperature, vacuum-filtered (Whatman #1) elutions were sent to the University of Maine Soil Analysis Lab to determine  $\text{NH}_4^+$  concentrations.

***Phragmites* collection.** We harvested the 3 uppermost fully developed leaves and viable root tissue from each subspecies (n = 10) from the Libby River Marsh (Scarborough, ME, 43° 33' 48.76" N, 70° 18' 34.72" W) in late August 2004. Subspecies were distinguished using morphological characteristics and type specimens were sent to the Phragmites Diagnostic Service ([www.invasiveplants.net](http://www.invasiveplants.net)) to corroborate our identifications. Native and introduced samples were chosen haphazardly, washed in cool freshwater and immersed in  $\text{LN}_2$ . Environmental characteristics were not recorded for *P. australis*.

**Growth chamber.** For our growth chamber experiment, seeds of *Spartina alterniflora* and *S. patens* were cold-stratified at 4°C in 40% NaCl solution for 4 wk (modified from McNinch & Garbisch 2004) and then

planted in a medium of 50 % sand and 50 % (v/v) commercial potting mix. Attempts to germinate seeds on sand alone were unsuccessful. Seedlings were transplanted to individual cups and maintained under a 16 h light:8 h dark cycle of  $900 \mu\text{E m}^{-2} \text{s}^{-1}$  at  $24^\circ\text{C}$  for 5 mo. Three N treatments consisted of either 10 mM  $\text{NH}_4^+$  (from  $[\text{NH}_4^+] 2 \text{SO}_4$ ), 10 mM  $\text{NO}_3^-$  (from  $\text{KNO}_3$ ) or 5 mM  $\text{NH}_4^+$  and 5 mM  $\text{NO}_3^-$ , in a modified nutrient solution. Nutrient concentrations were chosen to minimize N limitation. Non-draining nursery trays (9 plants of each species per tray) prevented cross-contamination between nutrient treatments (2 trays per treatment). The trays were arranged randomly and the plants sat in 2 to 4 cm of freshwater throughout the experiment, to avoid differential responses to a given salinity level. Water ( $500 \text{ ml tray}^{-1} 0.5 \text{ wk}^{-1}$ ) and nutrient treatments ( $25 \text{ ml plant}^{-1} \text{ wk}^{-1}$ ) were applied from top and bottom, respectively, providing  $2.5 \text{ mg N m}^{-2} \text{ d}^{-1}$ . Mature plants were harvested in the same manner as field samples. Six samples of each species per treatment were used to determine GS and an additional 6 were dried to determine biomass.

**Enzyme analysis.** All samples were stored at  $-70^\circ\text{C}$  until processed. Leaf samples were washed in ice water ( $<4^\circ\text{C}$ ) and homogenized in liquid nitrogen using all leaf material for each plant. Viable root tissue was homogenized by the same method as leaf tissue. To extract enzymes, field samples of both *Spartina* spp. and *Phragmites australis* ssp. were ground in a chilled imidazole extraction buffer of pH 7.5 (modified from Knight & Langston-Unkefer 1988) and then clarified by centrifugation. Chamber-grown plants were treated as field samples, with one exception: enzymes were extracted in the buffer described in Long & Oaks (1990), which is compatible with assays for nitrate reductase and the GS assay used here (T. J. Knight unpubl. data). Attempts to determine nitrate reductase levels were unsuccessful on frozen tissue and were omitted from analysis (see discussion in Mendelssohn 1979). GS activity in leaf and root samples was determined by the glutamine synthetase transferase assay (Shapiro & Stadtman 1970). Each sample was assayed in triplicate and the GS activity was determined as the optical density (OD) at 540 nm.

Each sample's OD readings were averaged and standardized to GS activity (reaction product:  $\mu\text{M}$  gamma-glutamyl-hydroxamate  $\text{g}^{-1} \text{min}^{-1}$ ). An L:R GS activity ratio was calculated from the leaf and root activities for each individual plant. Species differences were analyzed by 1- or 2-way ANOVA (see Table 2) and log-transformed as necessary (Sokal & Rohlf 2003). Ratio data were compared by Wilcoxon's signed rank test, which is more suited to ratios than ANOVA (Legendre & Legendre 2004). All statistical analyses were conducted in JMP® (SAS Institute, www.sas.com).

## RESULTS AND DISCUSSION

Research on agricultural species connects elevated L:R GS activity to fitness advantages and our results showed similar patterns in salt marsh grasses. Our data support the hypothesis that the faster growing and more stress-tolerant species of *Spartina* would have significantly higher L:R GS activity than the slower growing congener across all field sampling events and under certain nutrient conditions in growth chamber treatments. While we are not able to accept the hypothesis that introduced *Phragmites australis* would have higher L:R GS activity than the native subspecies, both subspecies had higher values than *Spartina* spp.

In field-collected plants, L:R GS activity varied between sampling dates in both species (see Habash et al. 2001); however, *Spartina alterniflora* was consistently higher than *S. patens* (July:  $p = 0.002$ ; August:  $p = 0.0006$ ; October:  $p = 0.02$ ; species  $\times$  month:  $p = 0.017$ ; Fig. 1). Root GS activity was significantly different between species for all sampling events (July:  $p < 0.0001$ ; August:  $p < 0.0001$ ; October:  $p = 0.006$ ; Fig. 1). Leaf GS activity was only substantially different in the August samples ( $p = 0.0517$ ; Fig. 1).

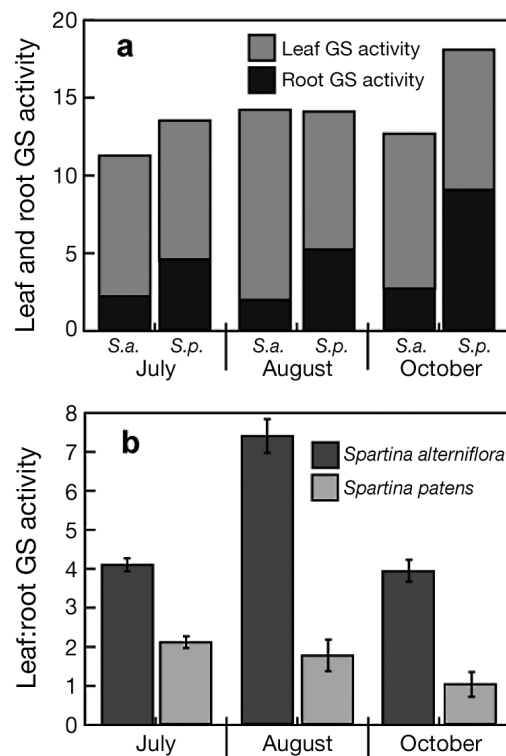


Fig. 1. *Spartina alterniflora* and *S. patens*. Glutamine synthetase (GS) activity in field-collected *S. alterniflora* (S.a.) and *S. patens* (S.p.). Results are grouped by sampling event. (a) Mean leaf and root GS activity ( $\mu\text{M}$  gamma-glutamyl-hydroxamate  $\text{g}^{-1} \text{min}^{-1}$ ). (b) Mean leaf:root GS activity ( $\pm 1$  SE)

Soil ammonium, salinity and temperature in *Spartina patens* and *S. alterniflora* zones were not significantly different at any sampling period (Table 1). Our samples were chosen from monocultures close to the boundary of *S. alterniflora* and *S. patens*, where the environmental conditions may not vary as dramatically as deeper within the vegetation zones.

Chamber-grown *Spartina* spp. had significantly different L:R GS activity in the nitrate ( $p = 0.02$ ) and control (which received both N sources;  $p = 0.005$ ) treatments. The 2 *Spartina* spp. had similar L:R GS activity when grown on  $\text{NH}_4^+$ , but this may be related to chlorosis observed in our ammonium-treated plants, making it difficult to differentiate between treatment and injury. Similar to our field experiment, species' leaf GS activities were not significantly different in any treatment, while root GS activity differed between species in the nitrate (marginally not significant,  $p = 0.078$ ) and control ( $p = 0.0003$ ) treatments (Fig. 2). Biomass varied with treatment ( $p = 0.036$ ), but not by species (Table 2), indicating that the 2 species responded similarly to N source, including decreased biomass in the nitrate treatment (Fig. 2).

*Phragmites australis* subspecies were not significantly different in leaf, root or L:R GS activity (Fig. 3). Leaf GS activities in both *P. australis* ssp. were comparable to August leaf GS in *S. alterniflora* (native:  $13.09 \pm 0.65$ ; introduced:  $12.76 \pm 0.56$ ;  $p = 0.729$ ). Root GS activity was minimal in both subspecies (native:  $1.12 \pm 0.14$ ; introduced:  $0.97 \pm 0.11$ ;  $p = 0.397$ ), indicating that

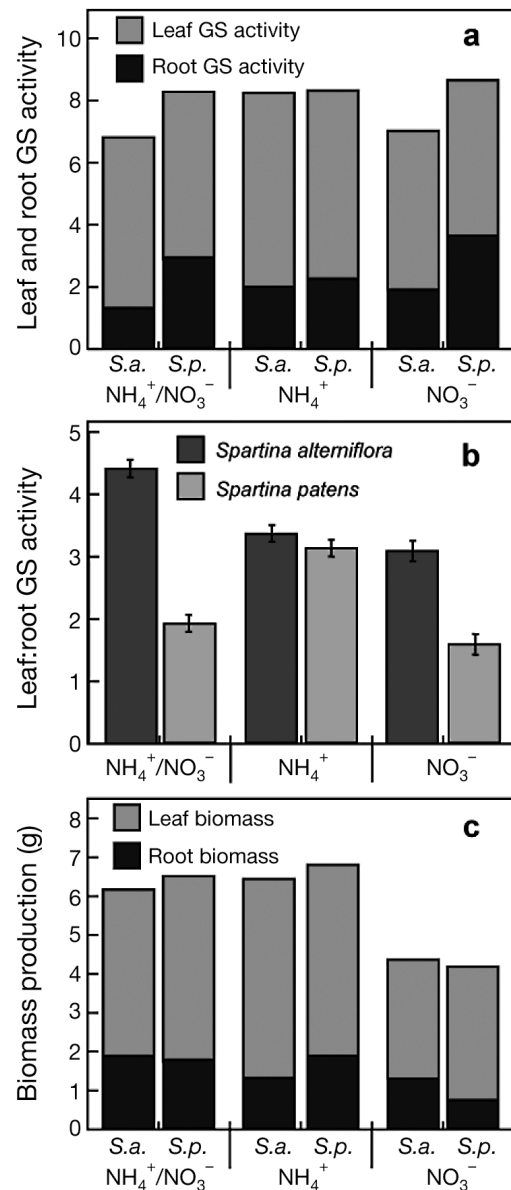


Fig. 2. *Spartina alterniflora* and *S. patens*. Glutamine synthetase (GS) activity and biomass production in chamber-grown *Spartina* grasses. *S. alterniflora* and *S. patens* samples received one of 3 N treatments:  $\text{NH}_4^+$ ,  $\text{NO}_3^-$ , or  $\text{NH}_4^+/\text{NO}_3^-$  combined. (a) Mean leaf and root GS activity ( $\mu\text{M}$  gamma-glutamyl-hydroxamate  $\text{g}^{-1} \text{min}^{-1}$ ). (b) Mean leaf:root (L:R) GS activity ( $\pm 1$  SE). (c) Biomass production

Table 1. Mean *in situ* soil temperature ( $^{\circ}\text{C}$ ) and salinity ( $\text{‰}$ ) recorded for field-collected *Spartina* grasses ( $\pm 1$  SE). Both temperature and salinity were measured at the same depth as the root sample. Soil ammonium is a season-long (July–October) measure, expressed as  $\text{mg NH}_4^+$  ( $10 \text{ g resin}^{-1}$ )

Month	<i>S. patens</i>	<i>S. alterniflora</i>	df	F	p
<b>Soil salinity</b>					
July	$28.00 \pm 0.516$	$29.00 \pm 0.516$	9	1.875	0.1877
August	$29.90 \pm 0.348$	$30.10 \pm 0.348$	9	0.1651	0.6893
October	$13.80 \pm 2.142$	$14.80 \pm 2.142$	4	0.1098	0.7498
<b>Soil temperature</b>					
July	$17.10 \pm 0.272$	$16.60 \pm 0.272$	9	1.6917	0.2098
August	$15.00 \pm 0.189$	$15.40 \pm 0.189$	9	2.25	0.151
October	$8.00 \pm 0.424$	$7.40 \pm 0.424$	4	1	0.3466
<b>Soil ammonium</b>					
July–October	$1.59 \pm 0.21$	$1.31 \pm 0.21$	9	0.8232	0.376

Table 2. Results of 2-way ANOVAs on leaf and root glutamine synthetase (GS) activities ( $\mu\text{M}$  gamma-glutamyl-hydroxamate  $\text{g}^{-1} \text{min}^{-1}$ ), leaf:root (L:R) GS activity ratios and biomass production (g dry weight) in *Spartina* grasses from field and growth chamber studies. Values in **bold** are significant

Source	Variable	Effect	df	F	p
Field	Leaf GS	Species	1	1.83	0.184
		Month	2	1.98	0.151
		Species $\times$ Month	2	2.23	0.121
	Root GS	Species	1	0.31	0.584
		Month	2	10.46	<b>0.002</b>
		Species $\times$ Month	2	38.49	<b>&lt;0.0001</b>
	L:R GS	Species	1	36.71	<b>&lt;0.0001</b>
		Month	2	4.65	<b>0.015</b>
		Species $\times$ Month	2	4.48	<b>0.017</b>
Growth chamber	Leaf GS	Species	1	0.05	0.82
		Treatment	2	1.47	0.247
		Species $\times$ Treatment	2	0.001	0.999
	Root GS	Species	1	12.27	<b>0.0015</b>
		Treatment	2	1.56	0.227
		Species $\times$ Treatment	2	1.82	0.179
	L:R GS	Species	1	11.94	<b>0.0017</b>
		Treatment	2	1.96	0.159
		Species $\times$ Treatment	2	2.55	0.095
	Biomass	Species	1	0.004	0.949
		Treatment	2	3.77	<b>0.035</b>
		Species $\times$ Treatment	2	0.08	0.919

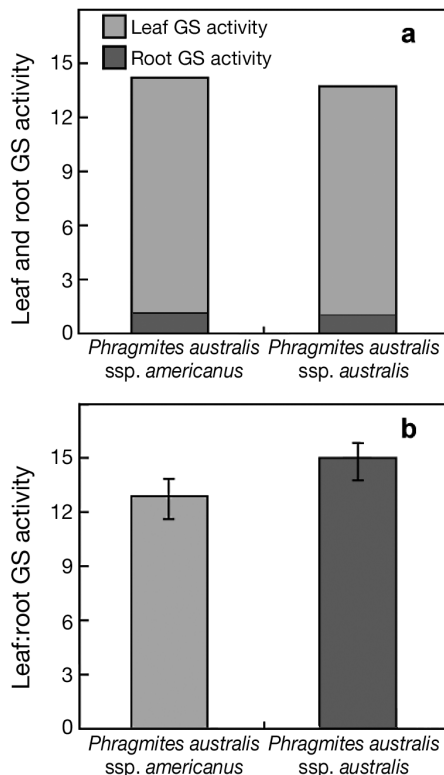


Fig. 3. *Phragmites australis* ssp. Glutamine synthetase (GS) activity in field-collected native (*P. australis* ssp. *americanus*) and introduced (*P. australis* ssp. *australis*) *P. australis* subspecies. (a) Mean leaf and root GS activity ( $\mu\text{M}$  gamma-glutamyl-hydroxamate  $\text{g}^{-1} \text{min}^{-1}$ ). (b) Mean leaf:root GS activity ( $\pm 1$  SE)

this species conducts nearly all N assimilation in photosynthetic tissue.

The magnitude of GS partitioning in *Phragmites australis* subspecies exceeds any terrestrial plant identified in the literature, excluding transgenic or hybrid plants. Previous studies have found L:R GS activity ratios as high as 12:1 in transgenic tobacco (*Nicotiana* spp.) which overexpresses genes for leaf GS (T. J. Knight unpubl. data), and 21:1 in hybrid strawberries (*Fragaria* spp.; Claussen & Lenz 1999). Work on submerged marine angiosperms *Zostera noltii* and *Cymodocea nodosa* reported L:R GS activities more than 10-fold the values recorded in terrestrial plants, which corresponded to differences in their growth rates (Kraemer & Mazzella 1999), demonstrating that the fitness advantages associated with high L:R GS activity hold true across a broad range of taxa.

In the absence of a direct comparison, our observed L:R GS activities coincide with reports of fitness and growth within and between our study genera. Both subspecies of *Phragmites australis* assimilate organic N faster than *Spartina alterniflora* (Mozdzer et al. 2010). Introduced *P. australis* has faster growth rates than *S. patens* (Windham 2001) and *S. alterniflora* (Farnsworth & Meyerson 2003), and *S. alterniflora* has higher NUE, relative growth rate and biomass production than *S. patens* (Drake et al. 2008).

Prior work on GS in salt marsh plants found that GS activity changed with environmental cues. Stewart & Rhodes (1978) tested activity of N-assimilation enzymes in salt marsh species, proposing that production of

N-based osmolytes requires a high NUE. As salinities increased, leaf GS increased and root GS decreased in all species tested. In a later study, Ahmad et al. (1982) found that  $GS_{II}$  and  $GS_R$  in the halophyte *Triglochin maritima* were more sensitive to salinity than  $GS_I$ , and salinity decreases root GS activity. Following increased leaf GS activity, glutamine levels increased, followed by proline, glycine betaine and other compatible solutes. Increases in GS activity may be a direct response to physiological stress, either for osmolyte production or stress recovery (Stewart et al. 1977). Salt-sensitive root GS may offer a fitness advantage to halophytes. Our observation that root GS activities changed with time and N source, while leaf GS activity remained constant, suggests that multiple cues (temporal or environmental) affect root GS, while leaf GS is less sensitive.

Many plants (particularly C4 plants) require a low level of salinity in order to reach their photosynthetic maximum. In *Spartina alterniflora*, C:N decreases and tissue N increases with rising salinity (Bradley & Morris 1992), which also corresponds with elevated proline and glycine betaine levels (Naidoo et al. 1992) and an elevated growth rate at moderate salinities (Wang et al. 2006). *Spartina* spp. reach their optimum growth rate and stomatal conductance at salinities between 10 and 20‰ (Maricle et al. 2007). Experimental results have shown that N uptake and relative growth rate increase in *Phragmites australis* with increasing salinities (Chambers et al. 1998, Vasquez et al. 2005). Native *P. australis* increases N uptake with up to 20‰ increased salinity (Mozdzer et al. 2010). Hartzendorf & Rolletschek (2001) observed an increase in proline and glutamine concentrations and a decrease in asparagines and glutamate in *P. australis* leaf and rhizome tissue with increasing salinity. Similar changes in photosynthetic rate, tissue N, N uptake, growth rate and amino acid concentrations have been observed in plants with elevated L:R GS activity (reviewed in Andrews et al. 2004, Good et al. 2004). Inducible increases in L:R GS activity may impact the way plants avoid (osmolyte production), tolerate (salinity-resistant physiology) or recover from (increased amino acid biosynthesis) physical stress.

In conclusion, the faster growing and more stress-tolerant *Spartina* species had a higher L:R GS activity rate than its congener in both field and controlled-growth studies. Our observations coincide with reports of growth, stress tolerance and competition in these 2 species. We did not find higher L:R GS activity in the competitively dominant *Phragmites australis* ssp. *australis* than the native *P. australis* ssp. *americanus*; however, our observations may be more important than the initial question. The magnitude of GS partitioning observed in *P. australis* ssp. was among the highest

reported and may contribute to the success of this cosmopolitan species known for high relative growth rate and NUE. The relationship between our results and observations in the literature warrant further research into both the mechanism and ecology of L:R GS partitioning.

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