

Nitrogen uptake by the shoots of smooth cordgrass *Spartina alterniflora*

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ABSTRACT: The smooth cordgrass *Spartina alterniflora* is the foundation species in intertidal salt marshes of the North American Atlantic coast. Depending on its elevation within the marsh, *S. alterniflora* may be submerged for several hours per day. Previous ecosystem-level studies have demonstrated that *S. alterniflora* marshes are a net sink for nitrogen (N), and that removal of N from flooding tidal water can provide enough N to support the aboveground biomass. However, studies have not specifically investigated whether *S. alterniflora* plants assimilate nutrients through their aboveground tissue. We determined *in situ* foliar and stem N uptake kinetics for ¹⁵NH₄⁺, ¹⁵NO₃⁻, and ¹⁵N-glycine by artificially flooding plants in a mid-Atlantic salt marsh. To determine the ecological importance of shoot uptake, a model was created to estimate the time of inundation of *S. alterniflora* in 20 cm height intervals during the growing season. Estimates of inundation time, shoot mass, N uptake rates, and N availability from long-term data sets were used to model seasonal shoot N uptake. Rates of aboveground N uptake rates (leaves + stems) were ranked as follows: NH₄⁺ > glycine > NO₃⁻. Our model suggests that shoot N uptake may satisfy up to 15% of the growing season N demand in mid-Atlantic salt marshes, with variation depending on plant elevation and water column N availability. However, in eutrophic estuaries, our model indicates the potential of the plant canopy as a nutrient filter, with shoot uptake contributing 66 to 100% of plant N demand.

KEY WORDS: Foliar uptake · Stem uptake · Ammonia · Glycine · Nitrate · DON

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INTRODUCTION

North American Atlantic coast salt marshes are dominated by the foundation species *Spartina alterniflora* (Mitsch & Gosselink 1993, Pennings & Bertness 2001). The ability of *S. alterniflora* to form stable communities and to outcompete other species is based on its tolerance to the physiological stresses associated with diurnal submergence in tidal water, and reduced anoxic soils (Bertness 1991). As an intertidal plant, *S. alterniflora* may become completely submerged during a tidal event, depending on the marsh surface elevation and variable plant height throughout the growing season. Productivity within salt marshes is generally thought to be limited by nitrogen (N) availability (Valiela & Teal 1974), and the harsh physio-chemical conditions in the rhizosphere that may limit N uptake (King et al. 1982, Chambers et al. 1998).

Although intertidal salt marsh grasses may be submerged for several hours per day with water containing bioavailable N, the extent to which shoot N uptake (leaf + stem) from the water column contribute to N demand is unknown. Subtidal seagrasses assimilate N through their shoots, although uptake varies dramatically among species. For example, shoot uptake of N can account for 50% of plant N demand in *Thalassia testudinum* (Lee & Dunton 1999) and *Thalassia hemprichii* (Stapel et al. 1996). At the extreme, *Phyllospadix torreyi* can satisfy 100% of its N demand through foliar uptake since its roots act solely as an anchor (Terrados & Williams 1997). The contribution of shoot N uptake to plant N demand is largely dependent on N concentration in the overlying water column (Stapel et al. 1996). Although nutrient concentrations may be one to several orders of magnitude greater in the sediments than in the water col-

umn (Burdige & Zheng 1998, McGlathery et al. 2001), shoot uptake may be a more energetically efficient alternative. Similar anaerobic conditions in the rhizosphere can also limit root N uptake in *Spartina alterniflora* (Bradley & Morris 1990, Chambers et al. 1998, Mendelssohn & Morris 2000); thus, shoot uptake may be an adaptation of intertidal salt marsh plants.

Flume studies in Atlantic Coast marshes have demonstrated that salt marsh ecosystems are predominantly a net sink for NH_4^+ , NO_3^- , and NO_2^- (Wolaver 1981, Wolaver & Zieman 1984, Wolaver et al. 1984, Whiting et al. 1989, Childers et al. 1993), and that they can also be a source (Wolaver et al. 1984, Childers et al. 1993) or sink (Wolaver & Zieman 1984, Childers et al. 1993) of dissolved organic nitrogen (DON). Wolaver & Zieman (1984) showed that N removal from flooding tides could supply up to 100% of the N needed to support the biomass in tall-form *Spartina alterniflora* and 30 to 105% of the N needed for intermediate-form *S. alterniflora* in a Virginia (USA) salt marsh; however, the contribution of shoot uptake to removal was not investigated. The study of Bouma et al. (2002) is frequently cited as showing that shoot uptake by *Spartina anglica* can, at most, contribute ~10% to plant N demand. This estimate was based on the premise that plants were only submerged for 2.4 h d^{-1} and that uptake rates were constant and did not vary over time with substrate concentrations. The limited periods of tidal inundation reported by Bouma et al. (2002) may be attributed to the fact that Northern European salt marshes are typically high in elevation (French & Reed 2001) and are inundated only during the highest stages of a tidal cycle. North American Atlantic coast tidal marshes generally have elevations that are decimeters below mean high tide (MHT), and periods of inundation can vary greatly depending on the tidal range and location within the marsh platform (Morris et al. 2005). For example, estimates from Drake et al. (2008) indicate that tall *S. alterniflora* in Massachusetts is inundated nearly every tide and may be submerged for 8.9 h d^{-1} , whereas the short form is infrequently inundated and only for ~3.0 h d^{-1} when averaged over both spring and neap cycles.

We investigated the potential contribution of shoot (leaves + stems) N uptake from tidal water to plant N demand in mid-Atlantic *Spartina alterniflora*. We measured uptake rates of $^{15}\text{NH}_4^+$, $^{15}\text{NO}_3^-$, and ^{15}N -glycine separately for both stem and leaf tissues to determine the uptake kinetics of inorganic and organic N in shoots. We then created a numerical model to estimate how shoot N uptake may satisfy the seasonal N demand of *S. alterniflora* using modeled vegetation growth and inundation, long-term water quality data, and experimentally determined uptake rates.

MATERIALS AND METHODS

Study sites. The field study was conducted within the Virginia Coast Reserve (VCR) Long-Term Ecological Research (LTER) Site in a salt marsh near Oyster, Virginia, between the Oyster Harbor (OH) and Ramshorn Channel Creek (RC). Long-term nutrient concentration data were available for these two sites and for Cob Mill Creek (CM). Together, CM, OH, and RC represent a nutrient gradient representing mainland salt marshes that receive terrestrial groundwater inputs to low nutrient lagoonal salt marshes. Salt marshes along the eastern shore of Virginia are predominantly meso-tidal, and the tidal range at Oyster Harbor is ~1.5 m. Field experiments to determine *in situ* N uptake rates were conducted on intermediate growth form (60–130 cm) *Spartina alterniflora* between 12–14 June, 2007. Plants were 56.3 ± 0.8 cm tall at the time of N treatment, and attained a height of ~100 cm by the end of the growing season (August).

Plant N assimilation rates. Methods were adapted from Bouma et al. (2002). Nitrogen uptake experiments were conducted daily between 10:00 and 14:00 h to minimize any potential diel variation. This time period corresponded to low tide and allowed us to flood the plants artificially and uniformly. Each experimental unit consisted of an *in situ* mesocosm (2.7 ± 0.1 plants mesocosm^{-1}) where plants were artificially flooded. A 15×15.25 cm (length \times internal diameter, i.d.) section of PVC was placed around the *Spartina alterniflora* plants and inserted into the sediment to a depth of ~5 cm. An agar solution (15 g l^{-1}) just above solidification temperature (~36°C) was poured into the PVC collar to a depth of ~5 cm. Immediately following the addition of agar, a tall, clear acrylic cylinder, (100 \times 10 cm i.d.) was immediately placed over the vegetation, through the unsolidified agar, and inserted ~5 cm into the ground to prevent tipping. Solidified agar formed a seal from the soil surface, and the cylinder was filled with 10 l of synthetic seawater (24.99 g NaCl_2 , 4.16 g Na_2SO_4 , 0.79 g KCl, 11.3 g $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$, 1.35 g $\text{CaCl}_2 \cdot 2\text{H}_2\text{O l}^{-1}$). This volume of water completely submerged the plants. Labeled ^{15}N ($^{15}\text{NH}_4\text{Cl}$, $^{15}\text{NO}_3$, and ^{15}N glycine (>99.8% ^{15}N); Cambridge Isotope Labs, Cambridge, MA) was added to the volume of water to adjust the starting N concentration to either 1, 5, 10, or 100 μM . There were 60 individual replicate mesocosms ($n = 5 \times 3 \text{ N treatments} \times 4 \text{ N concentrations}$). However, when leakage exceeded 10% of the initial volume, the replicate was discarded. This resulted in $n = 4$ for several N treatment and concentration combinations and in a corrected total of 53 replicates that were used to determine N uptake rates. ^{15}N depletion in the mesocosm, which was calculated as the total amount of ^{15}N assimilated into the plants, never exceeded 5% of the

starting concentration in any treatment. Control plants that were used to determine natural $\delta^{15}\text{N}$ were harvested daily throughout the duration of the experiment (15 plants d^{-1}). Three plants were pooled together by part (leaf or stem), ground, and analyzed for ^{15}N as described below. No change in $\delta^{15}\text{N}$ content was observed throughout the duration of the experiment, and mean ^{15}N content of pooled samples ($n = 19$) was used in the ^{15}N uptake rate determination.

To ensure a well-mixed medium, each mesocosm was aerated using a battery powered pump. After 1.5 h exposure of plants to the labeled ^{15}N , the experiment was terminated with the removal of the tall acrylic cylinder. Plants were cut at the surface of the agar, and were immediately rinsed in the field in 3 successive 10 l containers filled with identical synthetic seawater (no nutrients) to remove any adsorbed label. *Spartina alterniflora* was carefully blotted dry in the field, placed on ice, and transferred to the laboratory for processing. In the laboratory, plants were separated into leaf and stem portions, and freeze dried. Plant material was homogenized to a fine powder using a ball mill, and these samples were analyzed at the UC Davis Stable Isotope Facility on a Europa Integra continuous flow mass spectrometer for ^{15}N determination.

N uptake rates were determined using the modified equations of Bouma et al. (2002), where:

$$N_{\text{total}} = (\%N/100) \times \text{DM} \quad (1)$$

$$^{15}\text{N}_{\text{assimilated}} = [(\%^{15}\text{N}_{\text{trt}} - \%^{15}\text{N}_{\text{control}})/100] \times N_{\text{total}} \quad (2)$$

$$V_{\text{upt}} = ^{15}\text{N}_{\text{assimilated}} / (\text{DM} \times t_{\text{exp}}) \quad (3)$$

where N_{total} is the total amount of N (g) in the leaf or stem sample; %N is the concentration of N in the leaf or stem sample (g N g DM^{-1}); DM is the dry mass (g) of the sample; $^{15}\text{N}_{\text{assimilated}}$ is the amount of ^{15}N (g) assimilated; $\%^{15}\text{N}_{\text{trt}}$ is the ^{15}N content (mass basis) after a 1.5 h exposure to ^{15}N treatment; $\%^{15}\text{N}_{\text{control}}$ is the ^{15}N natural abundance (mass basis) in plants not exposed to ^{15}N treatments; V_{upt} ($\text{g N g DW}^{-1} \text{ h}^{-1}$) is the uptake rate of ^{15}N from flood water normalized to leaf or stem mass; and t_{exp} is the time of exposure to the ^{15}N treatments (1.5 h). Our model does not incorporate translocation of N from leaf to stem due to the short time course of the experiment.

To model how uptake rates will change as a function of N concentration, maximum N assimilation rates (V_{max}) and the half-saturation constant (K_m) for N were determined from uptake rates calculated using Eq. (3), using Proc NLIN in SAS (Version 9.1, Cary). Parameter estimates were obtained using the measured uptake rates at each concentration in the Michaelis-Menten equation (Eq. 4). From these parameters, it is possible to estimate the N uptake rate at the N concentrations in the flood water (see Fig. 1).

$$V = (V_{\text{max}} \times S) / (K_m + S) \quad (4)$$

V is the experimentally determined N uptake rate ($\text{g N g DW}^{-1} \text{ h}^{-1}$) at a given nutrient concentration, S ($\mu\text{mol l}^{-1}$), V_{max} is the modeled maximum uptake rate ($\text{g N g DW}^{-1} \text{ h}^{-1}$), and K_m is the experimentally determined half-saturation constant ($\mu\text{mol l}^{-1}$).

A total of 250 plants was harvested in May and August 2006 to derive a relationship between plant height and shoot mass. Plant heights were between 7 and 40 cm ($n = 95$) in May, and between 23 and 98 cm ($n = 155$) in August. We pooled both sampling dates to create a relationship between plant height and mass. A subsample ($n = 160$) of these plants was used to determine the allocation of leaf and stem plant mass in 20 cm intervals in 5 plant size classes: 0–20, 0–40, 0–60, 0–80, and 0–100 cm. This allocation was used to estimate the fraction of the mass submerged when plants were only partially inundated.

To determine the ecological significance of shoot uptake, we constructed a numerical model that compares shoot uptake rates with total N demand as a function of flooding duration, bioavailable N (NH_4^+ , NO_3^- , DON) concentration in the water column, and vegetation growth. We calculated inundation duration, plant mass distributions (stem vs. leaf), and uptake rates for each of the 5 plant height categories (0–20, 21–40, 41–60, 61–80, and 81–100 cm). Since the elevation of the plants on the marsh platform directly influences patterns of inundation, we estimated the amount of N demand satisfied by shoot uptake for intermediate-form *Spartina alterniflora* at surface elevations of -20 and $+20$ cm relative to MSL. These elevations correspond to the growth range of intermediate-form *S. alterniflora* at our study site, and roughly to the theoretical growth range predicted for a 1.5 m tidal range (Mckee & Patrick 1988). We do not present estimates for shoot uptake for the tall form (>1.5 m in height) which grows at lower elevations along the creek bank because our allometric relationships between stem and leaf mass are only valid to a 100 cm height.

We determined periods of inundation by simulating water level (W) through time (t) with a simple sine function that represents tidal fluctuations:

$$W(t) = A \sin\left(\frac{2\pi}{\lambda}t\right) - \eta \quad (5)$$

where A represents half of the tidal range (0.75 m), $\lambda = 12.5$ h, and η is the elevation of the marsh surface relative to MSL (m). In the model, plant height (h) increases at a constant rate throughout the growing season ($1.95 \times 10^{-4} \text{ m h}^{-1}$), which is equivalent to 1 m over the entire growing season (214 d). Inundation of a plant segment occurs when $W(t)$ exceeds the height of a given plant segment, and periods of inundation, $I(t)$, are evaluated hourly. Plants take up N from the water column at rates proportional to the concentration of N according to

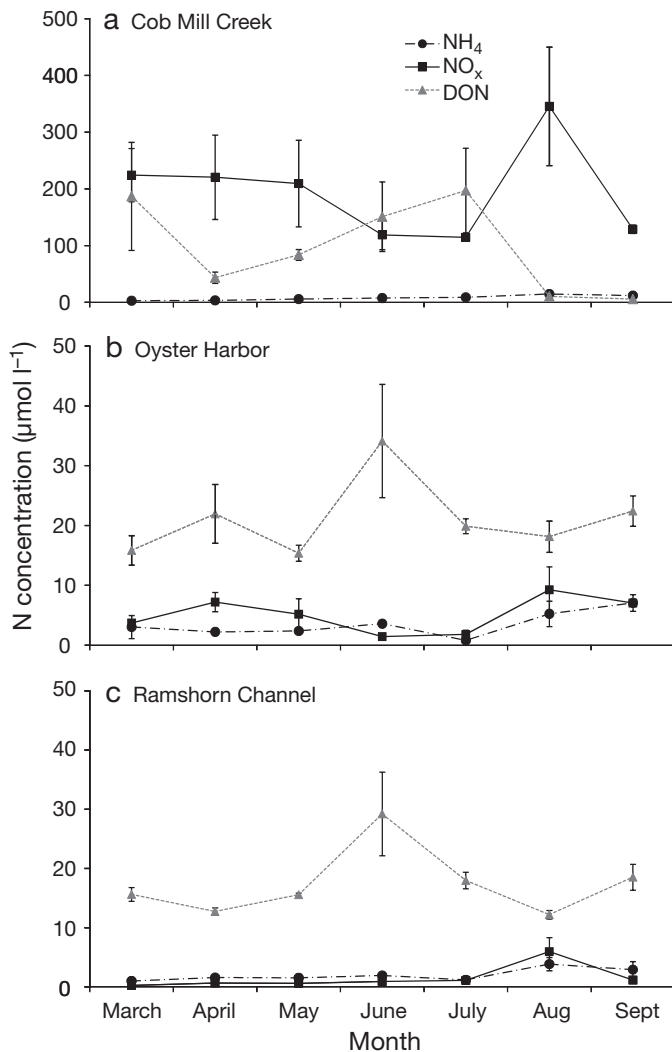


Fig. 1. NH_4^+ , NO_x , and DON availability ($\mu\text{mol l}^{-1}$; mean \pm SE) for (a) Cob Mill Creek, (b) Oyster Harbor, and (c) Ramshorn Channel Creek from long-term water quality data (2004–2008) accessed from the LTER dataset VCR99057 and VCR08143. DON was estimated by subtracting NH_4^+ and NO_x data from reported total dissolved nitrogen (TDN) values

Eq. (3) (see Table 1), where V_{\max} ($\text{g N g shoot mass}^{-1}$) is the uptake rate at saturation and K_m is the Michaelis-Menten constant. We modeled the uptake of NH_4^+ , NO_3^- , and DON in each segment of the plant according to separate values of V_{\max} for leaf and stem tissues, and long-term measurements of mean monthly concentrations of each N species along the mainland to the lagoonal marsh transect from 2004–2008 (VCR-LTER datasets VCR99057 and VCR08143; www.vcr.lter.virginia.edu) (Fig. 1). The 3 sites represent a nutrient gradient from terrestrial groundwater N inputs to low nutrient, lagoonal salt marshes and thus reflect differences in N availability in the water column. Seasonal (March to October) mean water column N (NH_4^+ +

$\text{NO}_3^- + \text{DON}$) concentrations were: 299 ± 32 , 30 ± 3 , and $21 \pm 2 \mu\text{M}$ for CM, OH, and RC respectively. We assumed that plants can utilize only ~20% of total DON (Tyler et al. 2003), and that glycine uptake rates are indicative of uptake of all DON species. Mozdzer et al. (2010) reported no significant differences in root DON uptake at 3 different DON treatments, and we assumed that this is the same for shoot uptake. To account for fractional DON availability, we adjusted values to be 20% of reported DON, lowering seasonal mean N concentrations to 223 ± 29 , 13 ± 2 , and $7 \pm 1 \mu\text{M}$ plant available N in CM, OH, and RC, respectively.

Following the approach of Bouma et al. (2002), the fraction of shoot N demand (γ) that can be accommodated by shoot uptake can be expressed as:

$$\gamma = \int^t \int^h \frac{I(h,t) \cdot B(h,t) \cdot V(h,t)}{b(h,t) \cdot C} dh dt \quad (6)$$

where $B(h,t)$, $I(h,t)$ and $V(h,t)$ represent the shoot mass, inundation duration, and N uptake rate at a particular height on the plant through time. The aboveground plant N demand is equal to the product of its growth rate b (g h^{-1}) and the concentration of N, C , within its tissues ($0.013 \text{ g N per g leaf}$ and $0.074 \text{ g N per g stem}$). We explicitly calculated biomass and N uptake for stems and leaves in each plant height segment. Although we assumed that plant height increases linearly, with peak height and biomass being attained in September, our allometric relationships yielded a shoot mass that increases non-linearly throughout the growing season (Fig. 2). We then solved (6) numerically by calculating shoot uptake at the midpoint of each 20 cm plant segment in 1 h time steps.

RESULTS

Plant N uptake

In all treatments, leaves had greater uptake rates than stems per unit mass, and the greatest uptake rates were observed for NH_4^+ , followed by DON and NO_3^- (Fig. 3). When the experimentally determined rates were fit to the Michaelis-Menten model, V_{\max} for NH_4^+ was 500 to 800% greater than that for DON or NO_3^- , respectively (Table 1). Leaf N uptake rates were 240 to 292% greater than stem uptake rates per unit mass within the same N treatment (Table 1), which may partly be attributed to a greater surface area:volume ratio.

Plant mass and inundation time

Mass allocation in stems and leaves varies greatly as a function of plant height (Table 2, Fig. 2). In plants up to 40 cm in height, <35% of the mass was allocated to

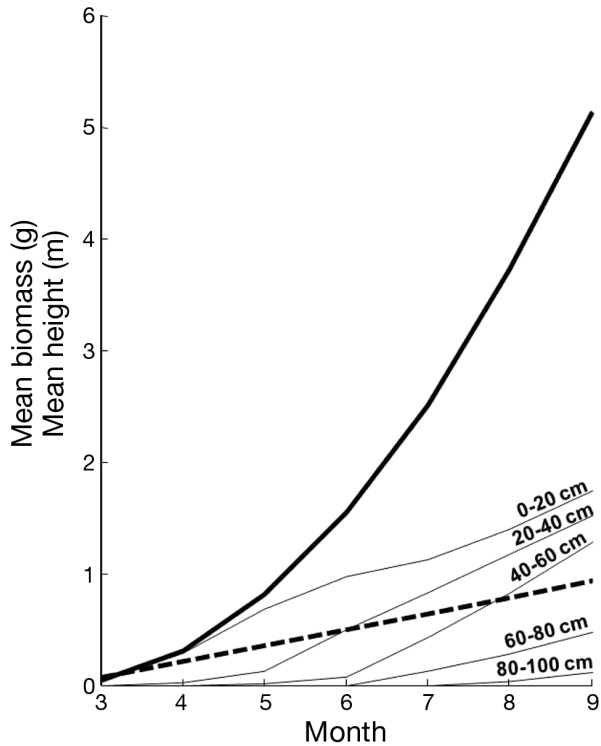


Fig. 2. Mean monthly total aboveground biomass (bold line) and plant height (dashed line) of *Spartina alterniflora* used to model N uptake rates and demand. In our model of plant growth, height increases linearly throughout the growing season, and estimates of biomass are derived from allometric relationships presented in Table 2. The thin solid lines denote biomass in 20 cm segments of the plant, which were used to calculate uptake and demand during partial inundation

Table 1. *Spartina alterniflora* uptake parameters for NH_4^+ , NO_3^- , and DON (glycine-N). Maximum uptake rate, V_{\max} , and the half saturation constant, K_m , were determined by fitting the observed uptake rates to the Michaelis-Menten equation

N treatment	Part	V_{\max} (ng N g DW ⁻¹ h ⁻¹)	K_m ($\mu\text{mol N l}^{-1}$)	r^2	n
NH_4^+	Leaf	6903 ± 1600	33.1 ± 21.6	0.83	18
	Stem	2508 ± 1101	46.3 ± 53.7	0.72	18
NO_3^-	Leaf	834 ± 162	9.6 ± 6.0	0.78	18
	Stem	343 ± 90	8.3 ± 7.1	0.72	18
Glycine	Leaf	1316 ± 134	9.7 ± 3.7	0.93	17
	Stem	449 ± 103	14.3 ± 12.4	0.71	17

Table 2. Percent of total aboveground mass in each 20 cm segment of *Spartina alterniflora*. Plants were categorized into one of 5 height classes and by mean proportion of mass in stem and leaf per unit dry weight

Segment (cm)	Part	% biomass in each 20 cm segment				
		20	40	60	80	100
0–20	Stem	28.7	33.6	43.0	31.9	26.7
	Leaf	71.3	57.5	20.1	8.9	7.2
21–40	Stem		0.0	2.8	14.6	16.1
	Leaf		8.9	29.3	18.8	13.5
41–60	Stem			0.0	2.3	10.8
	Leaf			4.8	17.3	14.2
61–80	Stem				0.0	0.0
	Leaf				6.2	9.2
81–100	Stem					0.0
	Leaf					2.2

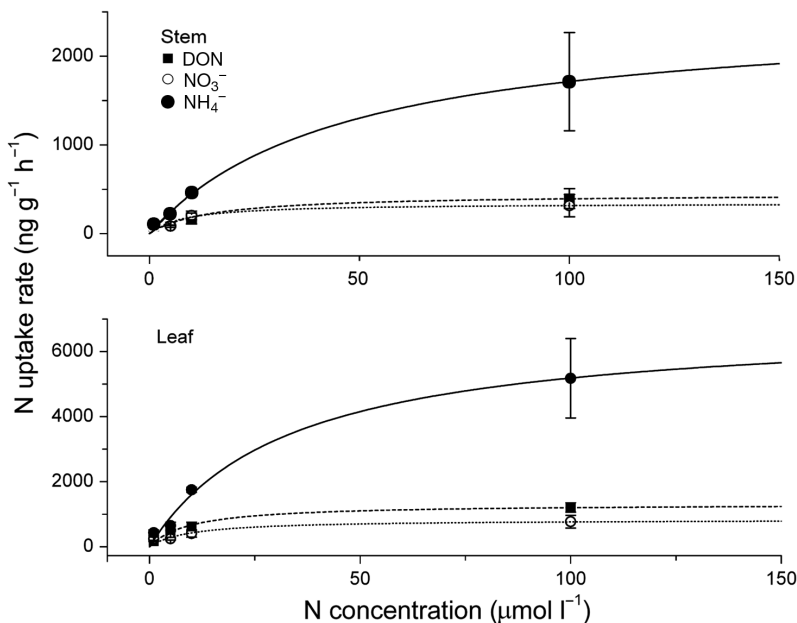


Fig. 3. Effects of ^{15}N substrate and concentration on uptake rates ($\text{ng N g DW}^{-1} \text{h}^{-1}$) on intact *Spartina alterniflora* plants. Reported values are mean $V_{\text{upt}} \pm \text{SE}$. Plotted lines are uptake rates estimated from Michaelis-Menten kinetics

the stem. However, as plant height increases, more mass is allocated to the stem to support the leaf mass. Allocation to stems increases to 46% for 41–60 cm tall plants, 48% for 61–80 cm tall plants, and 54% for 81–100 cm tall plants (Table 2). Inundation duration changes as a function of both plant height and relative elevation on the marsh platform (Fig. 4a,b). In the low marsh scenario (Fig. 4a), our model predicts that the lowest portions of the plant are submerged for $\sim 400 \text{ h mo}^{-1}$, or $\sim 6 \text{ h tide}^{-1}$. The time of inundation decreases as a function of segment height. Inundation hours total ~ 300 , 250, 200, and 100 h mo^{-1} for segments of the plant that are 21–40, 41–60, 61–80, and 81–100 cm above the marsh surface. When *Spartina alterniflora* grows to a meter in height, plants are completely submerged for only 3.3 h tide^{-1} , but $< 3\%$ of the plant's mass occurs at the tallest segment (Table 2).

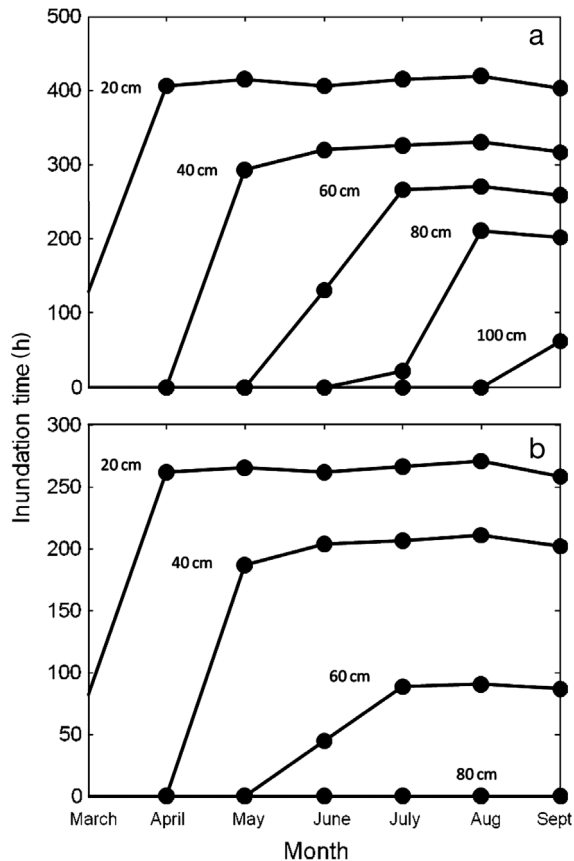


Fig. 4. Monthly predicted inundation time (h) for each 20 cm segment in intermediate-form *Spartina alterniflora* plants at elevations (relative to MSL) of (a) -20, and (b) +20 cm

Increases in marsh elevation result in a decreased average inundation time, assuming the same growth rate and structure. In the high elevation scenario (Fig. 4b), inundation times for the 0–20 and 21–40 cm segments are reduced by >40% to ~250 and 200 h mo⁻¹, respectively, and by 60% in the 41–60 cm segment to <100 h mo⁻¹.

Model predictions

In our low elevation scenario at ambient field N concentrations, the contribution of shoot (leaves + stem) N uptake decreases with decreasing water column N concentrations. Shoot N uptake has the potential to account for up to 22% of monthly plant N demand in CM (high N), 16% in OH (middle N), and 9% in RC (low N) (Fig. 5). The total aboveground plant N demand that can be attributed to shoot uptake (total g N from shoot uptake:total g N shoot mass) averaged over the growing season was highest in CM (15%), intermediate in OH (10%), and lowest in RC (5%). Overall, the contribution of shoot uptake to satisfy

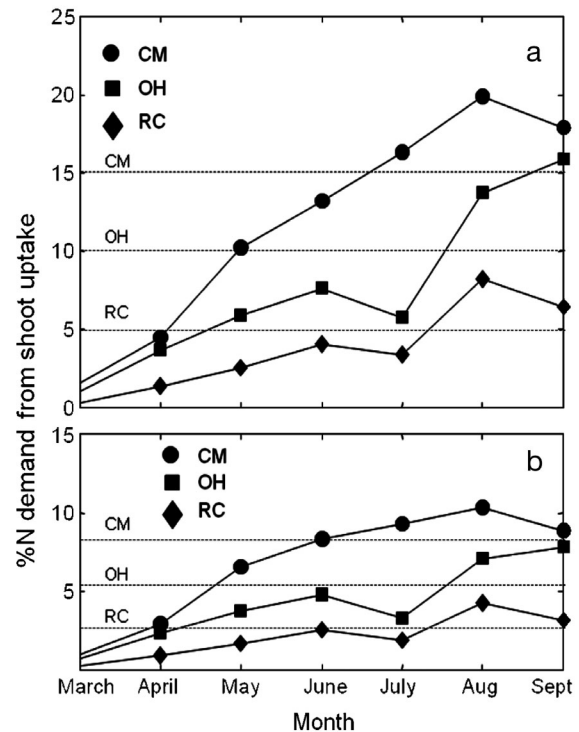


Fig. 5. Predicted monthly contribution to *Spartina alterniflora* N demand satisfied by shoot uptake in the salt marsh at the Cobb Mill Creek (CM), Oyster Harbor (OH), and Ramshorn Channel Creek (RC) (high, middle, and low N respectively) sites at (a) -20, and (b) +20 cm MSL. Dashed horizontal lines indicate the mean growing season N demand (total g N from shoot uptake:total g N shoot mass) that can be attributed to shoot uptake at each site

plant N demand is proportional to the availability of water column nutrients. For example, when NH₄⁺ concentrations dropped in July at the OH and RC sites (Fig. 1), so did the contribution of NH₄⁺ to plant N demand (Fig. 6a). Although water column nutrients may be elevated at the beginning of the growing season (Fig. 1), shoot uptake does not make a significant contribution to N demand at this time since mass is insufficient to support uptake (Fig. 2).

Shoot N uptake was primarily driven by water column NH₄⁺ concentration because uptake rates for NH₄⁺ were 4× greater than those for NO₃⁻ or DON (Fig. 6a–c). The contribution of shoot uptake varied with decreasing N availability from the CM to the OH and finally to the RC site (Figs. 1 & 5a). Over the growing season, shoot NH₄⁺ uptake alone could satisfy 8% of plant N demand at the high nutrient site CM (Fig. 6a). In contrast, NO₃⁻ contributed least to plant N demand (4%; Fig. 6c) although monthly mean NO₃⁻ concentrations in the flood water at CM (Fig. 1) exceeded Michaelis-Menten saturating concentrations (Table 1) by an order of magnitude. DON contributed a similar proportion of shoot N demand

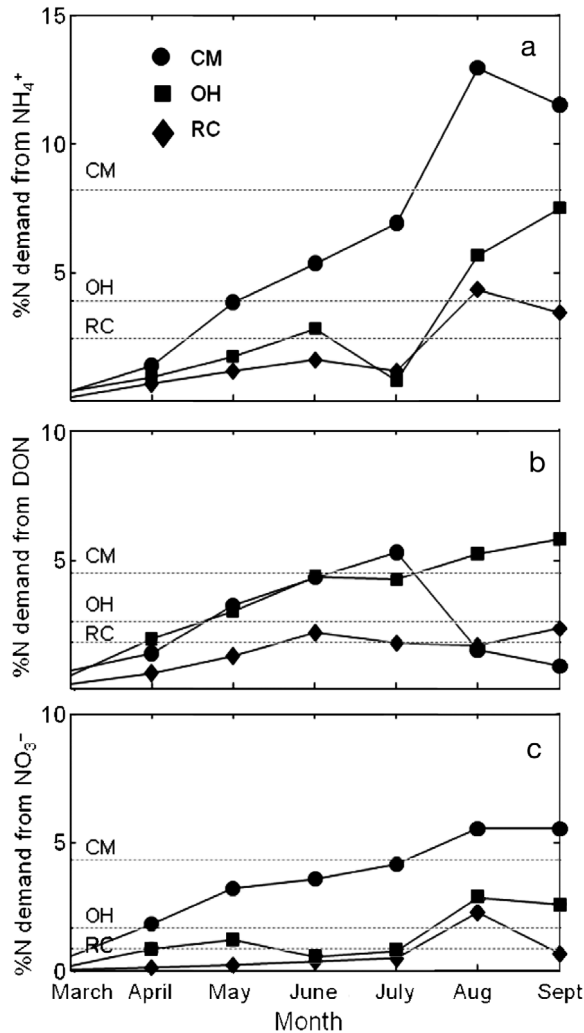


Fig. 6. Predicted monthly contribution to *Spartina alterniflora* N demand satisfied by shoot uptake of (a) NH_4^+ , (b) DON, and (c) NO_3^- at the Cobb Mill Creek (CM), Oyster Harbor (OH), and Ramshorn Channel Creek (RC) (high, middle, and low N respectively) sites at -20 cm MSL. Dashed horizontal lines indicate the mean growing season N demand (total g N from shoot uptake:total g N shoot mass) that can be attributed to shoot uptake at each site

as NO_3^- (Fig. 6b), although bioavailable concentrations were nearly an order of magnitude lower than that of NO_3^- (Fig. 1).

With a 40 cm increase in elevation (from -20 to $+20$ cm MSL), the relative importance of shoot uptake decreased by $>60\%$ (Fig. 5b). The decreased contribution of shoot uptake to seasonal plant N demand (8% of plant N demand in CM, 5% in OH, and 3% in RC) is solely attributed to shorter periods of inundation. The seasonal patterns in N uptake are identical to those described above. However, the reduction in contribution is attributed to lower periods of inundation due to a higher position on the marsh platform (Fig. 4).

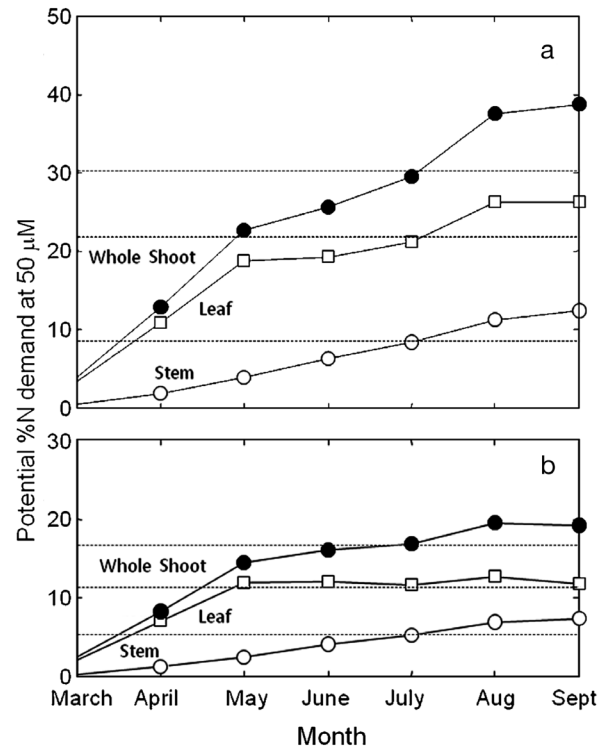


Fig. 7. Potential contribution to N demand satisfied by N uptake by whole shoot, leaf and stem tissues of *Spartina alterniflora* (N uptake rates at V_{max}) at (a) -20 cm and (b) $+20$ cm MSL. Dashed horizontal lines indicate the mean growing season N demand (total g N from shoot uptake/total g N shoot mass) that can be attributed to shoot uptake at each site

Leaf uptake is responsible for $\sim 70\%$ of total shoot uptake under both elevation scenarios assuming uptake at $50 \mu\text{M N}$ (Fig. 7a,b). The greater contribution from leaf tissues may be attributed to the greater N uptake rates in leaves than in stems (Table 1, Fig. 7a,b). Although stems do not support high uptake rates, *Spartina alterniflora* stems still contribute $\sim 30\%$ of total shoot uptake (Fig. 7).

DISCUSSION

Our model predicts that shoot uptake at ambient field concentrations has the potential to provide up to 15% of a plant's seasonal aboveground N demand in mid-Atlantic salt marshes under natural conditions. These estimates are up to 50% greater than previously reported for *Spartina anglica* from a eutrophic marsh (Bouma et al. 2002). Even at our most N-limited site ($7 \pm 1 \mu\text{M N}$), shoot uptake by *S. alterniflora* potentially supplied 5% of plant N demand, which is similar to the estimate of Bouma et al. (2002) for their most eutrophic site ($156 \pm 11 \mu\text{M N}$). For *S. alterniflora* in Virginia, the importance of shoot uptake is dependent on both the

elevation of the plants on the marsh platform, which affects the time of inundation, and available N (specifically NH_4^+) concentrations in the flooding water. Specifically, assuming marshes of similar elevation and tidal range, our model results suggest that shoot uptake has the potential to supply 34% of plant N demand in eutrophic estuaries ($\geq 50 \mu\text{M N}$) (Fig. 7). The conclusion that shoot uptake by *S. anglica* can supply at most 10% of N demand in a high-N estuarine system was based on the assumptions that flood water N concentrations were low when mass was great enough to sustain significant N uptake rates, and that inundation time was $< 2.4 \text{ h d}^{-1}$ (Bouma et al. 2002). We came to a different conclusion for *S. alterniflora* although N uptake rates were about half of those reported for *S. anglica*. Our differences in the potential contribution from shoot uptake can be attributed to differences in plant inundation time, where partial inundation may vary from 12 h d^{-1} in April to $\sim 3.3 \text{ h d}^{-1}$ of complete submergence in September. Modeling N uptake on partially submerged plants is critical since *S. alterniflora* is only submerged for $\sim 1.7 \text{ h high tide}^{-1}$ even late in the growing season when plants grow to heights of $\sim 100 \text{ cm}$. However, at this height, $> 88\%$ of a single *S. alterniflora* shoot has the potential to take up N for $\sim 8 \text{ h d}^{-1}$.

Both the water column N concentrations and the form of the available N influence the potential significance of shoot N uptake. NH_4^+ uptake is the predominant process in satisfying shoot N demand, although concentrations of NH_4^+ in the water column are lower than those of other N species. A similar pattern is seen in seagrasses where shoot N uptake is largely dependent on NH_4^+ concentrations in the water column (NH_4^+ comprises $> 80\%$ N taken up) (Stapel et al. 1996). Assuming similar proportional availability of N, DON uptake constitutes the second greatest contribution to plant N demand. Although water column NO_3^- concentration may be an order of magnitude greater than the concentration needed to saturate the uptake kinetics, NO_3^- uptake rates and the contribution of NO_3^- are the lowest. This may be attributed to the relatively high energetic cost of assimilating and reducing NO_3^- (Lambers et al. 1998). The energetic cost of using NO_3^- may be too high given the availability of DON and NH_4^+ in the water column and in the rhizosphere. This low uptake rate of NO_3^- may explain why Drake et al. (2008) concluded that foliar uptake was not an important process in a $^{15}\text{NO}_3^-$ enrichment experiment in a New England salt marsh. While large amounts of NO_3^- may not be effectively removed from the water column by *Spartina alterniflora* shoot uptake ($< 4\%$ total N demand), our results suggest that NH_4^+ and DON may be removed this way. Our results also suggest that DON uptake can account for almost 30%

of shoot N uptake at ambient nutrient concentrations in a relatively unpolluted Mid-Atlantic salt marsh. This finding is proportionally similar to estimates that root DON uptake can account for 24% of plant N demand in *S. alterniflora* (Mozdzer et al. 2010). Other studies have also shown that DON contributes substantially to macroalgal (Tyler et al. 2005, Tyler & McGlathery 2006), and seagrass (Vonk et al. 2008) N demand. In a scenario of estuarine eutrophication, the magnitude of the potential growth response will ultimately depend on the source, species, and concentration of allochthonous N. For example, riverine and groundwater inputs dominated by NO_3^- will unlikely benefit *S. alterniflora* production as suggested in the NO_3^- fertilization experiment in Massachusetts (Drake et al. 2008). Atmospheric N deposition, including wet and dry deposition (NH_4^+ , urea, and amino acids) may enhance shoot uptake due to the greater uptake potential of these N sources. The relative contribution of atmospheric N to salt marshes is quite variable, and can supply 1 to 40% of plant N demand (Hopkinson & Giblin 2008). Changes in land use may also alter allochthonous N inputs. For example, decadal-scale data suggest that water column N concentrations are increasing, with the greatest increases being in the form of DON (Verity 2002). Assuming that these increases in DON and NH_4^+ will continue in the future, shoot N uptake may become a more important process in satisfying plant N demand.

Marsh elevation and plant height are factors that determine the importance of shoot uptake. Our data also suggest that the enhanced productivity of both tall and intermediate growth form *Spartina alterniflora* may also be potentially attributed to shoot uptake of nutrients from the water column. Morris et al. (2002) reported a biomass optimum that increases with decreasing elevation to a certain threshold in *S. alterniflora*. We suggest that the higher productivity at lower elevation within a marsh (more frequently inundated) may also be partly attributed to shoot uptake of N from the water column. Beyond the elevation threshold, there is likely to be a tradeoff between the potential for shoot N uptake, and reduced photosynthetic capacity due to tidal inundation (Kathilankal et al. 2008). With increases in elevation on the marsh platform, the relative importance of shoot uptake decreases by almost 50%, suggesting that root uptake, controlled by physio-chemistry in the rhizosphere (Bradley & Morris 1991, Chambers et al. 1998), may become the predominant source of N. This transition at the higher elevation also corresponds to a change in plant phenotype from intermediate to the infrequently inundated short growth form *S. alterniflora*.

Biomass partitioning favors shoot uptake in low elevation *Spartina alterniflora*. Plants from lower por-

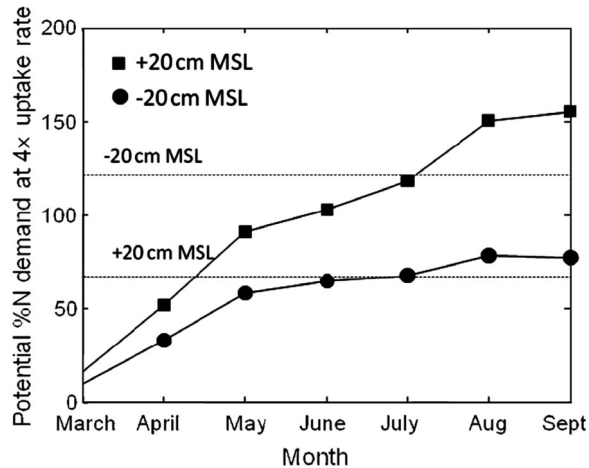


Fig. 8. Potential contribution to *Spartina alterniflora* N demand satisfied by N uptake in a eutrophic ($50 \mu\text{M N}$) brackish estuary. Low salinity is assumed to increase N uptake rates 4-fold. Dashed horizontal lines indicate the mean growing season N demand (total g N from shoot uptake:total g N shoot mass) that can be attributed to shoot uptake at each elevation

tions of the marsh tend to have lower root:shoot ratios (Gallagher 1974, Dame & Kenny 1986). One interpretation may be a greater investment in shoot mass when N can be easily taken up from an aerobic water column. Maintaining roots in an anaerobic environment is energetically costly and influences estuarine species zonation (Maricle et al. 2006). Hence, shoot N uptake may explain differences in root:shoot ratios in different *S. alterniflora* growth forms along the marsh platform.

Changes in salinity and nutrient availability may also affect shoot uptake. For example, Bouma et al. (2002) observed a 400% increase in leaf NH_4^+ uptake rates in brackish salt marshes of lower salinity (18.5) compared to salt marshes of higher salinity (31) in the Netherlands. In our model simulations, we assumed that *Spartina alterniflora* N uptake rates remain constant over naturally occurring ranges of salinity. However, salt marshes are exposed to a range of salinities that may vary with location in the estuary and proximity to freshwater input. Assuming a similar 400% increase in leaf NH_4^+ uptake rates alone in brackish salt marshes, and uptake rates measured at $50 \mu\text{M N}$ (representing a eutrophic estuary), our model indicates that shoot uptake of N can satisfy between 66 and 151% of seasonal aboveground plant demand depending on marsh elevation (Fig. 8). While these estimates need to be verified experimentally, the ability of plants to adapt to their environment suggests that rates of shoot N uptake can be enhanced significantly in brackish environments to satisfy nearly all *S. alterniflora* N demand.

CONCLUSIONS

We suggest that shoot uptake of N from the water column is a substantial source of N for *Spartina alterniflora*. Annual estimates of aboveground plant N demand acquired by shoot uptake for *S. alterniflora* (5 to 15% for this site) fall between the range for terrestrial plants (<10%) (Wilson 1992, Boyce et al. 1996, Tomaszewski et al. 2003) and seagrasses (50 to 100%) (Stapel et al. 1996, Terrados & Williams 1997, Peuke et al. 1998, Vonk et al. 2008). However, when our data and model are applied to brackish, eutrophic systems, shoot uptake appears to have the potential to meet 66 to >100% of plant N demand. The contribution of shoot uptake is directly proportional to the time of inundation (a function of marsh elevation and plant height), available N concentrations, and salinity, which may vary with location of the marsh in an estuary. Our results also suggest that the aboveground canopy of *S. alterniflora* may be an effective filter for removing nutrients from the water column, similar to seagrass dominated ecosystems.

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