Variation in the availability and utilization of dissolved organic nitrogen by the smooth cordgrass, *Spartina alterniflora*

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Abstract

North American Atlantic coast salt marshes are dominated by the highly-productive foundation species, *Spartina alterniflora*. Dissolved organic nitrogen (DON) has been suggested to be an important source of nitrogen (N) for *S. alterniflora*, however, little is known about the availability or utilization of this N pool. The dissertation objectives were to: (1) investigate latitudinal differences in the availability and utilization of DON, (2) investigate seasonal variation in DON availability, (3) determine the effects of DON fertilization and, (4) determine the importance of shoot N uptake by *S. alterniflora*.

DON availability was quantified in eight field sites from Maine to Florida. DON concentrations decreased with decreasing latitude, which I attribute to latitudinal differences in microbial activity. Due to greater DON availability, and similar measured rates of DON and NH_4^+ uptake in mesocosm experiments, I suggest that high-latitude *S. alterniflora* ecotypes rely more upon DON as a N source than mid- or low-latitude plants.

In Virginia Coast salt marshes, DON availability did not vary significantly among sites, but concentrations were lower in May than September. Nitrogen availability was spatially heterogeneous, and microbial activity is suggested to control the availability of DON at regional scales.

DON and NH_4^+ fertilization increased plant N content and productivity, resulting in selective grazing upon N-rich plants. I suggest eutrophication will not universally affect grazing in all *S. alterniflora* growth forms equally, and that N-fertilization does not increase grazing in short-form *S. alterniflora* in mid-Atlantic salt marshes.

Shoot uptake by *S. alterniflora* was found to supply 24% of seasonal plant N demand in mesotrophic salt marshes, with 30% from DON. The importance of shoot uptake is largely dependant upon relative elevation and water column nutrient concentrations.

In conclusion, my research shows that the relative importance of DON is largely dependant upon its availability, which my data suggest is greater in high-latitude salt marshes due to lower rates of microbial activity. Shoot uptake, in addition to root uptake, is an important mechanism of DON and DIN assimilation for *S. alterniflora* in N-limited salt marshes.

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Chapter 1

Introduction and rationale

Background

Tidal salt marshes are a vital component of estuarine ecosystems, and are estimated to provide 10.6 trillion dollars of ecosystem services globally per year based on their functions as fishery habitat, and nutrient and physical buffers at the land sea-interface (Costanza et al. 1997). Along the North American Atlantic coast, the foundation species *Spartina alterniflora* typically dominates the intertidal habitat due to its ability to tolerate anoxic conditions in the rhizosphere and to competitively exclude other species (Mitsch and Gosselink 1993, Pennings and Bertness 2001). *S. alterniflora* primary productivity along the latitudinal gradient from 45° to 28° has been shown to increase with decreasing latitude (Turner 1976, Kirwan et al. 2009), and it has been suggested that increases in productivity correspond to the length of the growing season and/or to temperature (Kirwan et al. 2009). Three distinct ecotypes have been reported (Seliskar et al. 2002), which also correspond to cpDNA haplotypes of northern, mid, and south Atlantic types (Blum et al. 2007), although it is not known how respective ecotype/genotype differences may be responsible for the observed differences in productivity. Regardless of the latitudinal location, nitrogen (N) is the limiting nutrient in salt marshes (Valiela and Teal 1974). Microbial mineralization of organic matter is thought to be the rate-limiting step limiting N availability and primary production in salt marsh ecosystems.

My research challenges the paradigm that salt marsh plants only use the inorganic forms of N to meet their nutritional requirements. Another potential, albeit neglected source of nitrogen to marsh macrophytes, such as *S. alterniflora,* is dissolved organic nitrogen (DON). DON is a term used to characterize a large pool of organic nitrogenous

compounds that can vary significantly in size and weight. This pool of nitrogen (N) is poorly characterized in many systems, especially with regard to the labile fraction. In this dissertation, DON will refer to the small, bioavailable fraction of low C:N compounds such as amino acids and urea.

In the 1990's, studies in arctic systems revealed that pools of available inorganic nitrogen were insufficient to meet the demands of the observed productivity. Chapin *et al*. (1993) demonstrated preferential organic nitrogen uptake by a non-mycorrhizal arctic tundra plant, and was among the first to establish direct DON use by plants. Following this experiment, Kielland (1994) demonstrated that up to 80% of an arctic plant's nitrogen may come from direct uptake of amino acids. These studies suggest that DON uptake allows plants to bypass the mineralization process which may be slow in some environments. Since these first studies, other work in the arctic tundra (Chapin et al. 1993, Kielland 1994, Schimel and Chapin 1996), grasslands (Streeter et al. 2000, Weigelt et al. 2003), and arctic salt marshes (Henry and Jefferies 2003b) have confirmed the importance of DON utilization to sustain observed levels of primary production.

One might expect that direct DON uptake would also be an important process in North American salt marshes due to slow decomposition of organic matter in saturated, anoxic soils. My M.S. and early doctoral research demonstrated that both *S. alterniflora* and *Phragmites australis* take up DON in both short-term laboratory studies, and *in situ* at rates proportional to those in laboratory experiments (Mozdzer et al. in review-b). From these studies, I concluded that DON uptake has the potential to supply up to 24% of *S. alterniflora* N demand, and 47% of *P. australis* N demand in mid-Atlantic salt marshes (Mozdzer et al. in review-b).

Although the mechanism for plant uptake of DON has been demonstrated, pools of DON are poorly quantified, and it is not known how DON uptake by *S. alterniflora* may vary along latitudinal scales. In particular, dissolved free amino acid (DFAA) and urea concentrations, which make up the majority of the bioavailable fraction of DON, have been poorly characterized by marsh ecologists. The only study that reported DFAA concentrations in vegetated salt marsh sediments was Gardner and Hanson (1979), where it was reported that total dissolved free amino acid (DFAA) concentrations were up to 9 μM in short form *S. alterniflora* sediments in Georgia. Mozdzer et al (in review-b) reported concentrations of 12.8 μM for DFAA and 7.8 μM for urea-N in mid-Atlantic salt marshes, which are approximately 30% of the bioavailable N pool. DFAA were almost an order of magnitude higher in arctic salt marshes dominated by the arctic marsh grass, *Puccinellia phryganodes,* where concentrations reached as high as 80 μM (Henry and Jefferies 2002). While these values represent the extreme ranges of potential availability in tidal salt marshes, they also suggest that a latitudinal gradient may exist in the availability of DON in temperate tidal marshes. Due to seasonal changes in microbial activity, I hypothesize the DON concentrations will decrease seasonally due to greater heterotrophic activity and high plant N demand. It is known that dissolved inorganic N (DIN) concentrations vary seasonally, and that this is attributed to increases in heterotrophic decomposition of organic matter with increases in temperature (Howarth 1993). Additionally, changes in land use, coastal development, and eutrophication influence N inputs and N cycling in salt marshes throughout North America (Hopkinson and Giblin 2008). For example, over a ten-year period, changes in land use have resulted in increases in all measured water column nutrients, with the greatest increases in

the form of DON (Verity 2002) . It has also been suggested that the increase in DON concentrations may be in the more labile form of amino acids in atmospheric deposition (Cornell et al. 1995).

Given the increases in water column N concentrations, one might expect foliar uptake of N to include both organic and inorganic forms. Shoot uptake of nutrients is an important process in submerged aquatic plants, and can provide 50% of plant N demand in *Thalasia testudinum* (Lee and Dunton 1999) and *T. hemprichii* (Stapel et al. 1996), and even 100% of the N demand for *Phyllospadix torreyi* (Terrados and Williams 1997). Surprisingly, little work has been done on salt marsh plants. Bouma et al. (2002) concluded that foliar uptake by *Spartina anglica* was low, and at most could supply 10% of plant N demand. However, European salt marshes are high in elevation (French and Reed 2001), which could account for the unusually low inundation time (2.4 hr day^{-1}) (Bouma et al. 2002). North American Atlantic coast tidal marshes typically have elevations decimeters below mean high tide (Morris et al. 2005), and periods of inundation can vary greatly depending upon the tidal range and location within the marsh platform. For example, estimates from Drake et al (2008) indicated that tall *S. alterniflora* in Massachusetts may be submerged for 8.9 hr day^{-1}. I hypothesize that due to increases in inundation time, shoot uptake may be an important process for North American *S. alterniflora.* Flumes studies in mid-Atlantic salt marshes indicated that *S. alterniflora* salt marsh ecosystems remove enough N to support 100% of the *S. alterniflora* biomass, but the mechanisms for removal were not investigated (Wolaver and Zieman 1984). No one has investigated if shoot uptake may be an important

process in *S. alterniflora* salt marshes. It is possible that the removal of DON and DIN from the water column may be attributed to shoot uptake (stem + leaves).

Objectives

The overall objective of the work presented in this dissertation was to determine the availability and utilization of DON and DIN on both regional and latitudinal scales. Specific objectives include:

1. To characterize pools of available N along the Atlantic coast of North America to determine if there are latitudinal differences in the availability and utilization of DON by *S. alterniflora*.
2. To investigate seasonal variation in DON availability in mid-Atlantic salt marshes to determine how this pool of N may fluctuate seasonally.
3. To determine the effects of DON fertilization of on a *S. alterniflora* salt marsh.
4. To determine the importance of foliar N uptake by *S. alterniflora*.

Guide to dissertation

The dissertation is divided into six chapters. Each chapter is written as an individual manuscript to be submitted for publication in peer-reviewed journals. Chapter 1 is the introduction and places the context for the research. In Chapter 2, I investigate the availability and use of DON along a latitudinal gradient from Maine to Florida using a combination of mesocosm experiments, field measurements, and laboratory studies. In

Chapter 3, I investigate the spatial and temporal variation in DON and dissolved inorganic N (DIN) availability in mid-Atlantic salt marshes. Chapter 4, which was originally designed as a fertilization experiment to investigate potential differences in DIN vs. DON fertilization evolved into a completely different manuscript. Due to an intense effect of grazing on both DON and DIN treated plants, the results of this experiment are presented as evidence of top-down control by grazing insects in mid-Atlantic salt marshes. In Chapter 5, I evaluate shoot (shoot + leaf) uptake of both DIN and DON from the water column in mid-Atlantic salt marshes. A numerical model was developed to integrate the experimentally-determined uptake rates to determine the seasonal importance of the process. In the final chapter, Chapter 6, I synthesize the results of chapters two through four, and draw several conclusions about the importance of DON utilization as well as suggest areas where more research is needed.

Chapter 2

Latitudinal variation in the availability and utilization of dissolved organic nitrogen in Atlantic coast salt marshes

Abstract

North American Atlantic salt marshes are generally considered to be nitrogen (N) limited systems, and plants within these marshes are thought to only use the inorganic forms of N. Recent research has demonstrated the ability of *S. alterniflora* to take up dissolved organic nitrogen (DON) directly. To determine the availability of DON in *S. alterniflora* marshes along the North American Atlantic coast, porewater was sampled from 8 field sites from Maine (44°N) to Florida (30°N). To determine if a latitudinal gradient exists in the utilization of both DON and NH_4^+, replicated mesocosm experiments were performed on *S. alterniflora* plants at three field sites (MA, VA, SC) representing three distinct ecotypes and cp DNA genotypes. I report that DON availability deceases with decreasing latitude along the North American Atlantic Coast. The relative availability of DON pools is largely dependant upon rates of microbial activity which can change by an order of magnitude in North American Atlantic coast salt marshes. Although *S. alterniflora* from all sites assimilated DON, plants from the southern most site had 50% greater NH_4^+ assimilation rates, suggesting that low-latitude *S. alterniflora* rely more on NH_4^+ as a N source. In contrast, in high latitude salt marshes, due to greater pools of available DON, and similar uptake rates of DON and DIN, I suggest the high-latitude *S. alterniflora* relies more upon DON as a N source. By combining availability and utilization of DON, this study creates a better understanding of nutrient utilization within salt marshes of the North American Atlantic coast.

Introduction

A central hypothesis within biogeography suggests that ecosystem-level productivity and species richness increase with decreasing latitude (Turner 1976, Hawkins et al. 2003, Hillebrand 2004, Kirwan et al. 2009). Intertidal salt marshes along the North American Atlantic coast are colonized and dominated by monocultures of the foundation species, *Spartina alterniflora* (Mitsch and Gosselink 1993, Pennings and Bertness 2001). Kirwan et al (2009) suggested that latitudinal trends in North American salt marsh productivity correspond to temperature and/or length of the growing season. Recent research demonstrated that *S. alterniflora* from mid-Atlantic salt marshes may use dissolved organic nitrogen (DON) as a nitrogen (N) source (Mozdzer et al. in review-b). However, it is not known how the importance of this process varies along the latitudinal distribution of *S. alterniflora.* The dominance of a single foundation species along a large latitudinal range provides a unique opportunity to study how biogeography, biogeochemistry, and genetic ecophysiological variation interact to influence ecosystem-level productivity.

In general, high-latitude salt marshes experience a colder, shorter growing season, resulting in slow organic matter decomposition, and peat accumulation (Pennings et al. 2009). Additionally, high-latitude salt marshes can also be severely impacted by winter-time ice scour, further limiting primary production. Conversely, low-latitude salt marshes experience a longer, warmer growing season, greater salinities due to increased evaporation, and increased herbivore pressure (Pennings and Bertness 2001, Pennings et al. 2009), and are generally more productive than high-latitude marshes (Turner 1976, Mendelssohn and Morris 2000, Kirwan et al. 2009). Geographic barrier to gene flow and

environmental variation along the North American Atlantic coast has resulted in at least three distinct ecotypes of *S. alterniflora.* High-latitude ecotypes exhibit greater shoot N concentrations, lower stem density, and smaller canopy size than those of southern marshes (Seliskar et al. 2002). The described ecotypes in high, mid, and low-latitude salt marshes correlate well with the recently identified genetic lineages (cpDNA haplotypes) of *S. alterniflora* (Blum et al. 2007) with distinct northern, mid, and southern Atlantic coast groups. No research has investigated how ecotypes/genetic variation may be responsible for the observed productivity gradients.

Productivity along this latitudinal gradient is primarily attributed to bottom-up forces. However, there is also evidence for the potential of top-down control in eutrophied salt marshes (Chapter 4, Silliman and Zieman 2001, Bertness et al. 2008). In general, temperate salt marshes are considered to be nitrogen (N)-limited systems (Valiela and Teal 1974), and addition of N results in increased salt marsh productivity regardless of the latitudinal location (Valiela and Teal 1974, Gallagher 1975, Chalmers 1979, Mendelssohn 1979, Dai and Wiegert 1997, Mozdzer et al. in review-a). While N is the primary nutrient limiting production, physio-chemical factors including salinity (Haines and Dunn 1976, Bradley and Morris 1991), H_2S concentration (King et al. 1982, Bradley and Morris 1990), and flooding (Mendelssohn and Seneca 1980, Kathilankal et al. 2008) can also affect *S. alterniflora* productivity. In addition to nutrient and physio-chemical controls, there is evidence for genetic control of productivity, density, biomass, and plant height (Seliskar et al. 2002). Acknowledging these potential differences, the current paradigm in salt marsh ecology is that salt marsh ecosystems are N-limited

ecosystems, with microbial mineralization of organic N to inorganic NH_4^+ as the key processes limiting N availability and primary production.

Recent research in mid-Atlantic salt marshes suggests that *S. alterniflora* can bypass the rate-limiting mineralization step and assimilate organic nutrients directly prior to mineralization (Mozdzer et al. 2004, in review-b). Mozdzer et al reported that this process has the potential to constitute up to 24% of plant N-demand. Direct use of dissolved organic nitrogen (DON) has been demonstrated to be an important N source in a variety of ecosystems ranging from arctic tundra (Chapin et al. 1993, Kielland 1994, Schimel and Chapin 1996), arctic salt marshes (Henry and Jefferies 2003b), boreal forests (Nasholm et al. 1998), grasslands (Streeter et al. 2000, Weigelt et al. 2003), and temperate coastal lagoons (Tyler et al. 2001). Temperate salt marshes are similar to these ecosystems in that nitrogen mineralization is low and/or unable to meet the nitrogen demand of the system, coupled with an accumulation of slowly decomposing organic matter. However, the literature is lacking on the availability of the potentially important pools of dissolved free amino acid (DFAA) and urea-N concentrations. Only two studies have reported concentrations in temperate salt marshes: DFAA-N concentrations were up to 9 μM-N in short form *S. alterniflora* sediments in Georgia (Gardner and Hanson 1979), and concentrations were up to 12.8 μM DFAA-N in a mid-Atlantic *S. alterniflora* marsh (Mozdzer et al. in review-b). At the northern extreme, DFAA in arctic salt marshes dominated by *Pucinellia phrygranodes* were up to 80 μM (Henry and Jefferies 2002). While these values represent the latitudinal extents of plant dominated salt marshes, they suggest a possible gradient in the availability of DON.

Due to differences in *S. alterniflora* productivity along latitudinal scales, and the uncertainty of ecotypic adaptation on ecophysiological processes, we hypothesized *a priori* that there would be gradients in both the availability and utilization of DON. Specifically, we hypothesized that (1) DON concentrations would be greater in high-latitude salt marshes due to slow organic matter mineralization, and that (2) high-latitude *S. alterniflora* ecotypes would rely more upon this N source. In this chapter we present the results of *in situ* short-term mesocosm experiments designed to assess the importance of DON and DIN utilization, and quantify N pools along a latitudinal gradient. These studies were combined with estimates of microbial activity in order to create a better understanding of how N availability and ecotypic variation interact to control DON uptake over this latitudinal range.

Methods

Plant N assimilation rates

Three well-established field sites were chosen forming a latitudinal, ecotypic, and biogeographic provincial gradient (Hayden and Dolan 1973, 1976, Seliskar et al. 2002). These sites included: Plum Island Ecosystems (PIE) Long term Ecological Research (LTER), Virginia Coast Reserve (VCR) LTER, and North Inlet (NIN) National Estuarine Research Reserve (NERR) (Figure 2-1). *S. alterniflora* originating from these biogeographic provinces, also correspond to distinct ecotypes (Seliskar et al. 2002) and *cp* DNA genotypes (Blum et al. 2007) of *S. alterniflora*. PIE is at the northernmost extent, and component of the Acadian biogeographic province. This site is characterized

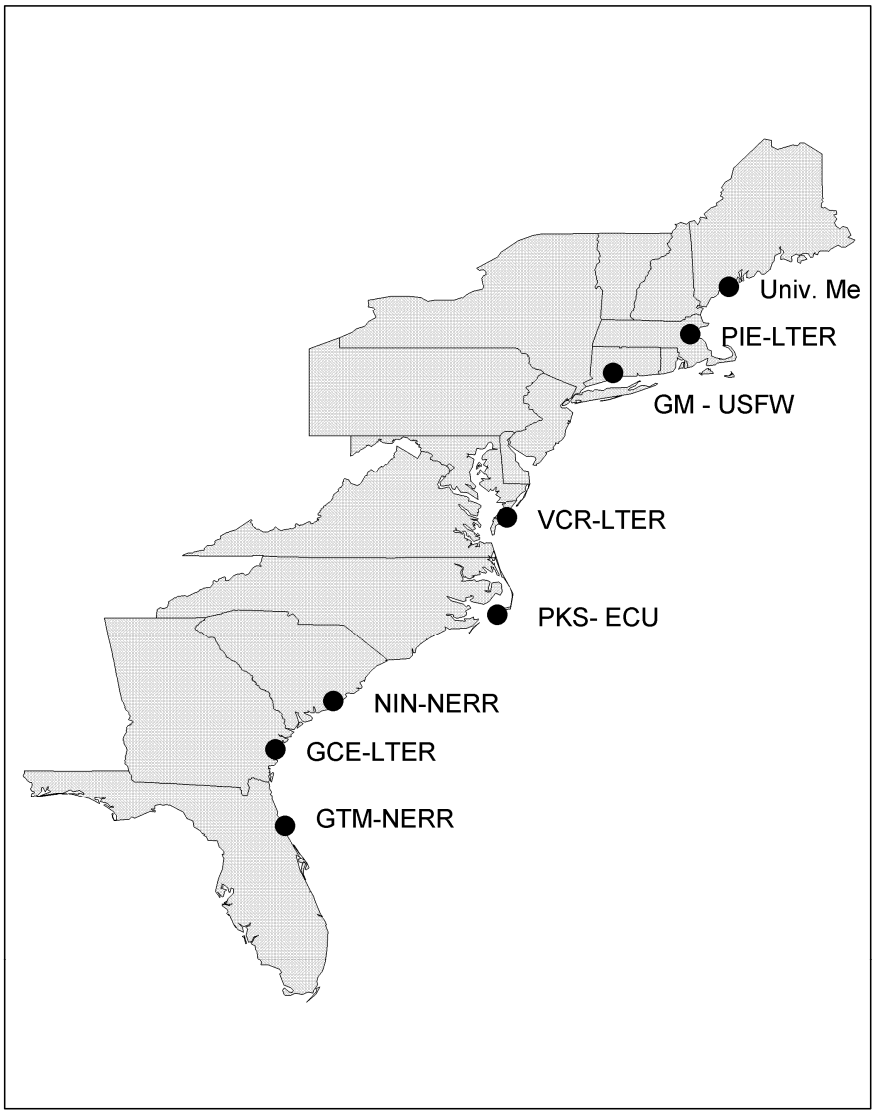

Figure 2-1. Location of the field sites along the North American Atlantic coast.

as a typical New England salt marsh which experiences a short growing season, long winters, and frequent disturbances from ice scour and wrack deposition. VCR is a typical mid-Atlantic salt marsh system, in the Virginian biogeographic province characterized by an intermediate growing season, with cold winters and occasional ice scour. NIN, within the Carolinian biogeographic province, is characterized by large expansive monotypic stands of *S. alterniflora,* with near year-round plant growth and high primary production.

To quantify *in situ* plant N uptake, ^{15}N was used as tracer in short-term mesocosm experiments. Mesocosms were used since tidal flushing of the rhizosphere with each tide makes tracer studies impractical in salt marshes. Using a 10-cm *i.d.* x 35cm long section of PVC, an intact plug of *S. alterniflora* was carefully withdrawn from each field site without disturbing the aboveground biomass. The intact plant + sediment section of marsh was extracted to a depth of at least 30 cm which is beyond the active root zone of *S. alterniflora.* Each unit was placed in an individual 5 liter container, filled with ambient creek water to a depth of 10 cm, to keep the bottom of the core saturated to maintain anoxic conditions. Mesocosms were placed in an area exposed to full sunlight and were exposed to ambient light and temperature conditions. The experiments were conducted early in the growing season when plants were actively growing and began in the low-latitude site, NIN (May 30-June 1, 2006), followed by VCR (5-7 June, 2006), and finally PIE (19-21 June, 2006). Since plant growth begins earlier in the low-latitude sites, we staggered the experiments to account for differences in the start of the growing season.

One of three treatments, $^{15}N\text{-}NH_4$, $^{13}C^{15}N$ glycine, or control (DI water) was randomly assigned to one mesocosms at each field site with five replicates per treatment.

Each N treatment received a total of 30 μmols ^{15}N dissolved in 14 ml of DI water. N treatments were added through a series of 7 equally spaced injections throughout the core, adding 2 ml per injection using a high resolution water sampler (Berg and McGlathery 2001) in reverse to equally distribute the ^{15}N. Since ambient concentrations of amino acids are already saturated relative to microbial utilization (Hanson and Gardner 1978), introducing labeled amino acids should not change the heterotrophic metabolism of these amino acids in our short-term experiment.

The experiment was terminated after 48 hours with the cutting of the aboveground biomass at the sediment surface. The stems and leaves were carefully washed under tap water, wiped dry, promptly frozen and stored until they could be freeze dried. Using a garden hose, sediments from the belowground biomass were rinsed off with tap water, and a sub-sample of live roots and rhizomes were selected from the mesocosm core. Only live, turgid roots were selected to ensure that assimilation of N was due to active uptake. After the experiment the roots were blotted dry, frozen immediately and freeze-dried with the aboveground tissue. Additionally, dead biomass including roots and rhizome material was also collected to serve as a sorption + microbial assimilation control for both roots and rhizome material. All plant parts, stems, leaves, roots, and rhizomes were analyzed separately to assess assimilation and movement of the tracer throughout the plant. Plant biomass from each part of the plant was ground to a fine powder using a ball mill, and the samples were analyzed by UC Davis Stable Isotope Facility on an Europa Integra continuous flow mass spectrometer for their ^{15}N and ^{13}C isotope ratios as well as elemental C and N analysis. Assimilation of stable isotopes was

determined by comparing stable isotope ratios in control plants to N-treated enriched ones using the following equations:

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

$$^{15}N_{assimilated} = ([\%^{15}N_{trt} - \%^{15}N_{control}]/100) \times N_{total} \times 10^{6} \quad (2)$$

Where N_{total} = the total amount of N (g) in each tissue fraction; %N is the elemental N content; DM is the dry mass (g) of the representative biomass; $^{15}N_{assimilated}$ is the amount of ^{15}N (μg) assimilated into the representative biomass, $\%^{15}N_{trt}$ is the ^{15}N concentration of N treated mesocosm at the end of the 48 hour experiment; $^{15}N_{control}$ is the concentration of the control plants at natural abundance for stem and leaf material at each site. Belowground, dead biomass was used as the control for root and rhizome to control for process of simple sorption onto the plant material. These calculations allowed for the determination of ^{15}N assimilated throughout the plant. N uptake rates were determined using the equation below:

$$N_{uptake} = (^{15}N_{root} + ^{15}N_{rhizome} + ^{15}N_{stem} + ^{15}N_{leaves})/ (DM_{root} \times t_{exp}) \quad (3)$$

where N_{uptake} is the total plant N uptake rate normalized to the dry mass of the roots in μg ^{15}N g^{-1}dw root; $^{15}N_{root}$ is the amount (μg) of N recovered in the roots, $^{15}N_{rhizome}$ is the amount (μg) of N recovered in the rhizomes, $^{15}N_{stem}$ is the amount (μg) of N recovered in the stems, $^{15}N_{leaves}$ is the amount (μg) of N recovered in the leaves, and DM_{root} is the dry mass (g) of total root biomass. Since only a subsample of both roots and rhizomes were analyzed to ensure a conservative estimate of root uptake, total belowground biomass (roots + rhizomes) was estimated using the model of Gross et al. (1991) assuming an

equal distribution to both root and rhizome. Our experimental design was factorial, with 3 latitudinal locations, and 2 nutrient treatments.

Analysis of variance (ANOVA) was used to test for effects of N-treatment, location (biogeographic province), as well as interactions between N-treatment and biogeographic province. When significant differences were observed by ANOVA, post hoc tests were performed. All statistical analysis was performed in SAS, Version 9.2 (Cary, NC).

N Availability

Eight field sites were selected along the North American Atlantic coast to determine how availability of DON varies over the latitudinal range from Maine to Florida (Table 2-1). Sites included three LTER sites (PIE-LTER, VCR-LTER, and Georgia Coastal Ecosystems (GCE-LTER), two National Estuarine Research Reserves (North Inlet (NIN) and Guana-Tolomato-Matanzas (GTM)), and two other sites, Great Meadows salt marsh in the Stewart B. McKinney National Wildlife Refuge (Fairfield University (FU) and Pine Knoll Shores, PKS, - East Carolina State University. These sites were approximately equally distributed throughout the three biogeographic provinces. At these field sites, porewater was sampled in August 2006 using porewater equilibrators at 10 and 20 cm depths in the mid and high marsh corresponding to short and intermediate growth form *S. alterniflora*. We assume that if nutrients pass the equilibrator membrane, then these concentrations of nutrients are plant available and are representative of long term-pools. Collected porewater was filter-sterilized (GHP, 0.2

Table 2-1. Environmental characteristics at each of the study sites adapted from Blum et al 2002.

				Climate					
				Temperature (ºC)					
		Coordinates		Mean daily high		Mean daily low		Tide range	Salinity
Site	Province	Lat	Long	Jan	Jul	Jan	Jul	(m)	(psu)
U Maine	Acadian	44	69	-0.9	26	-11.4	14.6	2.8	31
PIE-LTER	Acadian	42.4	70.5	2.1	27.7	-5.8	18.4	1.5	27
GM-USFW	Virginian	41.1	73.6	2.8	26.1	-5.6	21.1	1.1	20
VCR-LTER	Virginian	37.5	74.8	7.7	30.2	-2.2	20	1.3	28
PKS-ECU	Virginian	35.7	75.9	11.3	29.2	2.6	22.1	1	21
NIN-NERR	Carolinian	32.5	80.4	13.2	31.2	4.9	24.1	1.2	28
GCE-LTER	Carolinian	31.4	81.3	15.4	32.8	3.4	22.4	2.1	28
GTM-NERR	Carolinian	30	81.2	22.3	32.6	11.8	22.3	1	28

μm, Pall), and frozen for subsequent analyses. Water from the PIE and NIN site was collected as part of a long term experiment conducted by J.T. Morris, and was also filter-sterilized (GHP, 0.2 μm, Pall). NH_4^+ data from PIE and NIN were through datasets LTE-MP-Porewater and LTE-MP-NIN-Porewater from http://ecosystems.mbl.edu/pie/data.htm. NH_4^+ for the remaining samples were analyzed using a Lachat QuickChem 8500 (Loveland, Colorado) using QuickChem method 31-107-06-1-B. Urea-N was determined using a modification of the methods of Mulvenna and Savidge (1992) and Goeyens et al. (1998). Dissolved free amino acids (DFAA) were determined on a Dionex ICS 3000 (Sunnyvale, CA) using the AccQ-Tag chemistry package from Waters Corporation (Milford, MA). To correct for N content with amino acids containing more than one N (*e.g.* arginine, histidine, and lysine), total N concentrations for these amino acids were normalized to the total DFAA-N content. Statistical analysis (one-way ANOVA) was conducted in SAS to investigate how N availability varies among salt marshes in each marsh zone among the biogeographic provinces.

Estimation of Microbial Activity

To estimate rates of microbial mineralization at each site, five 7.6 cm *i.d,* 40 cm deep cores were taken from each field site on August 4, 2006. Plant shoots were cut at the sediment surface, and intact sediment cores were sealed immediately to maintain anaerobic conditions and shipped on ice-packs overnight to our lab in Charlottesville, VA. The following morning, sub-cores were taken at 2, 5, 10, 20, 30 cm in depth from the sediment surface. Sub-cores were obtained by drilling a hole in the side of the PCV

using a hole-cutter drill bit, followed by extraction of the sediment using a de-tipped 10 cc plastic syringe (Herlihy and Mills 1985). The sub-core was immediately capped with a serum stopper.

Each sub-core was injected with 100 μCi of ^{14}C acetate (Specific Activity 50 mCi $mmol^{-1}$)in 25 μl of sterile DI water, and recapped. Acetate was chosen since it is the preferred C substrate by sulfate-reducing bacteria, the main heterotrophs in salt marsh sediments (Hansen 1993). The sub-cores were incubated for 2 hours at room temperature, 22 ±1 ºC. The contents of the sealed sediment core were transferred into in 50 ml erlenmeyer flasks and slurried with 20 ml of deionized water. The erlenmeyer flask was capped with a rubber septum and the microorganisms were killed by the addition of 2ml of 2N H_2SO_4 to the slurry. Mineralized ^{14}C acetate from the bacterial biomass was trapped as $^{13}CO_2$ in 0.1 ml phenethylamine, which was subsequently placed into liquid scintillation cocktail, and $^{14}CO_2$ mineralization from heterotrophic production was measured on a Beckman LS 6500 Multi Purpose Scintillation Counter. Relative microbial activity is reported as disintegrations per minute (dpm) $^{14}CO_2$ per cc sediment. Statistical analysis was conducted in SAS (V9.1 Cary, NC) using ANOVA to investigate provincial differences in relative microbial activity by site, depth, and the interaction.

Results

Plant N assimilation

Plant N assimilation rates varied significantly by site (biogeographic province) ($F_{2,29}$=15.54, $p<0.0001$) and N treatment ($F_{1, 29}$=4.26, $p=0.0500$) (Figure 2-2); with a

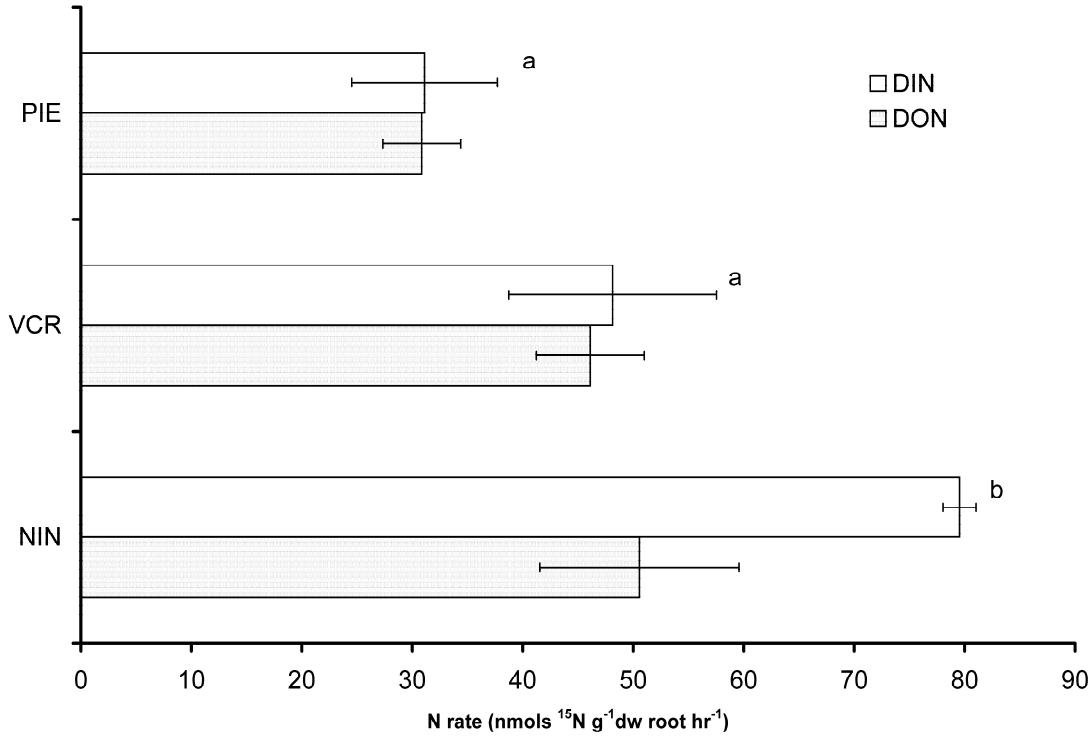

Figure 2-2. Effects of N-treatment on calculated ^{15}N assimilation rates normalized to g $^{-1}$dw root hr^{-1} ± SE by site, n=5 per N-treatment. Significant differences at α=0.05 are indicated by different letters.

marginally significant interaction of site and N-treatment ($F_{2,29}$=2.95, p=0.0717). Since PIE *S. alterniflora* had significantly less biomass than either the VCR or NIN plants ($F_{2,44}$= 45.66, p<0.001) (Table 2-2), plant uptake rates were normalized to g dw root mass. The amount of the ^{15}N incorporated into the plant biomass varied by site ($F_{2,29}$=25.69, p<0.0001), however there was no significant effect of N treatment on ^{15}N assimilation ($F_{1,29}$=2.31, p=0.1415) (Figure 2-3). NH_4^+ uptake rates varied significantly by site ($F_{1,14}$=13.35, p=0.0009), and post hoc tests indicated rates were the greatest at the southern-most site, NIN, and were lower at both VCR and PIE site (Figure 2-1). The greatest measured uptake rates of NH_4^+ at NIN correspond to the greatest incorporation of the ^{15}N tracer with 77.7 ± 10% recovered in the plant biomass (Figure 2-3). Significantly less $^{15}NH_4^+$ was taken up at each site with increasing latitude, which corresponded to lower NH_4^+ uptake rates at the VCR and PIE site (Figure 2-3).

DON uptake did not vary significantly among the three latitudinal sites ($F_{2,14}$=2.735, p=0.1055) (Figure 2-2). However, rates at VCR and NIN tended to be greater when compared to those at PIE. More than 49% of the amended ^{15}N DON was recovered in the plant biomass in the VCR and NIN sites, whereas only 18% was recovered in PIE plants ($F_{2,14}$=11.48, p=0.0016) (Figure 2-3). All plants assimilated ^{13}C in DON treatments, but enrichment was much lower than ^{15}N enrichment (data not presented). Since our experiments were at the tracer level, the ^{13}C signal is not as sensitive due natural variation ^{13}C, and physiological ^{13}C fractionation. Therefore, we use estimates of microbial activity described below to estimate the amount of mineralization that may have occurred prior to assimilation.

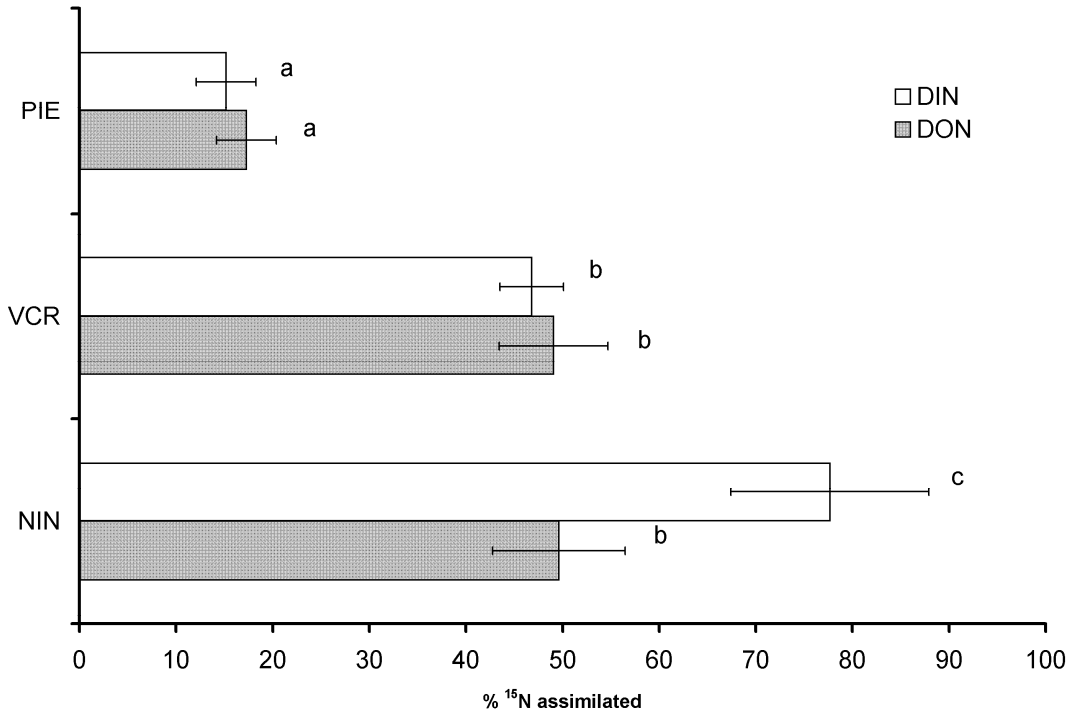

Figure 2-3. Percent of the ^{15}N treatment assimilated by *S. alterniflora* plants at each site over the 48 hr experiment ± SE. Significant differences at α=0.05 are indicated by different letters.

Table 2-2. Plant biomass and N content of *S. alterniflora* plants from mesocosm experiment. Aboveground biomass of *S. alterniflora* within the mesocosm consisting of 81 cm^2 area of marsh, leaf: stem ratio is the leaf mass (g):stem mass (g), and aboveground N is the %N content of *S. alterniflora* shoots. There is a significant effect of site ($p<0.0001$) for all variables, and different letters indicate significant differences among different sites.

Site	Abovground Biomass (g)	Leaf:Stem (g:g)	Aboveground N (% N)
PIE	2.08 ± 0.12 a	1.14 ± 0.06 a	1.61 ± 0.05 a
VCR	5.37 ± 0.28 b	0.82 ± 0.03 b	1.35 ± 0.05 b
NIN	5.06 ± 0.35 b	0.77 ± 0.04 b	1.11 ± 0.04 c

These estimates are conservative since the processes of sorption of the ^{15}N onto the root and rhizome surface was corrected for by using dead biomass for the control treatment in our calculations. Mean dead belowground material was enriched by 12% and 18% above natural abundance for glycine and NH_4^+ respectively at all sites. Using dead roots and rhizome material, our estimates remove the process of sorption of the ^{15}N onto the plant material in addition to an undefined amount of microbial processes. As such, our estimates provided may be underestimates and are solely indicative of plant uptake processes.

Different patterns of N translocation post assimilation were observed among the three sites. While the majority (~85%) of assimilated N was recovered belowground (Figure 2-4), aboveground N translocation varied by site. PIE *S. alterniflora* plants had significantly more N recovered in its leaf tissues than either VCR or NIN (Figure 2-4)($F_{2,29}$=15.38, p<0.0001). The greater amount of N assimilated into the leaf tissue may be attributed to the greater leaf to stem ratio in PIE *S. alterniflora* when compared to plants at either VCR or NIN (Table 2-2). Even though the high-latitude plants at PIE had lower biomass ($F_{2,44}$=49.2, p<0.0001), they had significantly greater N content ($F_{2,44}$=29.3, p<0.0001). N content generally decreased with decreasing latitudinal location (Table 2-2). Belowground, only rhizome translocation varied by site ($F_{2,29}$=3.97, p<0.0313), with greater ^{15}N found in the VCR & PIE sites than NIN (Figure 2-4).

N Availability

Sites were pooled by biogeographic province and marsh type since we were primarily interested in how plant-available N varied across the broad latitudinal range.

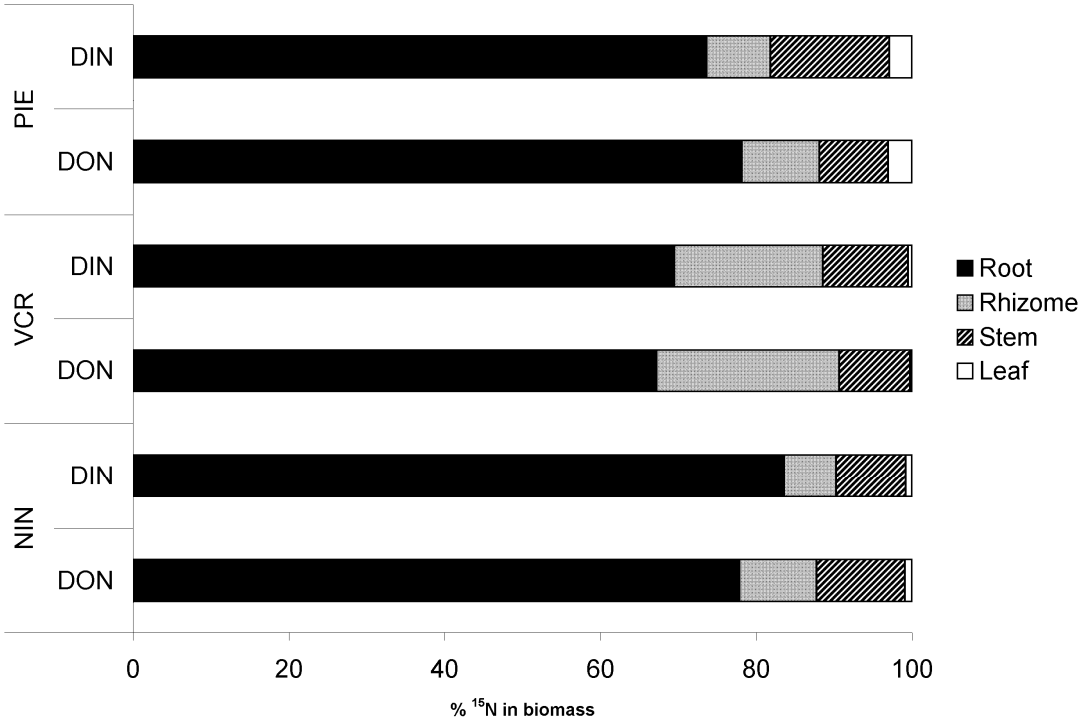

Figure 2-4. Location of assimilated ^{15}N in plant biomass at the conclusion of the 48 hour experiment.

Individual amino acid concentrations ranged from nanomolar to individual micromolar concentrations, and are presented as total DFAA-N. Combined plant available DON concentrations (DFAA-N + Urea-N) , hereafter DON were at least an order of magnitude lower than NH_4^+ concentrations at depths below 10cm. Porewater nutrient data for 20cm are presented in Appendix A. DON data, and the ratio of DON were log transformed to satisfy ANOVA assumptions.

In the low-marsh zone, Figure 2-5, NH_4^+ availability did not vary significantly by province ($F_{2,27}$=2.92, p=0.072), however, both DFAA-N ($F_{2,27}$=8.62, p=0.00014) and urea-N availability ($F_{2,27}$=4.05, p=0.029) decreased in each biogeographic province, with salt marshes in the Virginian biogeographic as exhibiting intermediate DFAA and urea N concentrations. DON was greater in the Acadian and Virginia biographic provinces ($F_{2,27}$=9.81, p=0.0012), however, the ratio of DON:NH_4^+, a proxy for the relative availability of DON was significantly greater in the Acadian province ($F_{2,27}$=7.20, p=0.0034)

In the high-marsh zone, sediments in the mid-latitude Virginian province had significantly greater NH_4^+ availability than in the Carolinian province (Figure 2-6) ($F_{2,28}$=4.31, p=0.024). Similar to the patterns observed in the low marsh, DFAA-N concentrations decreased by province with decreasing latitude, and differences among provinces were significant ($F_{2,25}$=5.64, p=0.0017). Salt marshes in the Virginian province were intermediate in concentration between the high- and low-latitude provinces. No significant differences in urea-N availability were found among high elevation salt mashes in the three biogeographic provinces ($F_{2,28}$=2.19, p=0.13). DON varied significantly by province, with the greatest availability in the Virginian and Carolinian

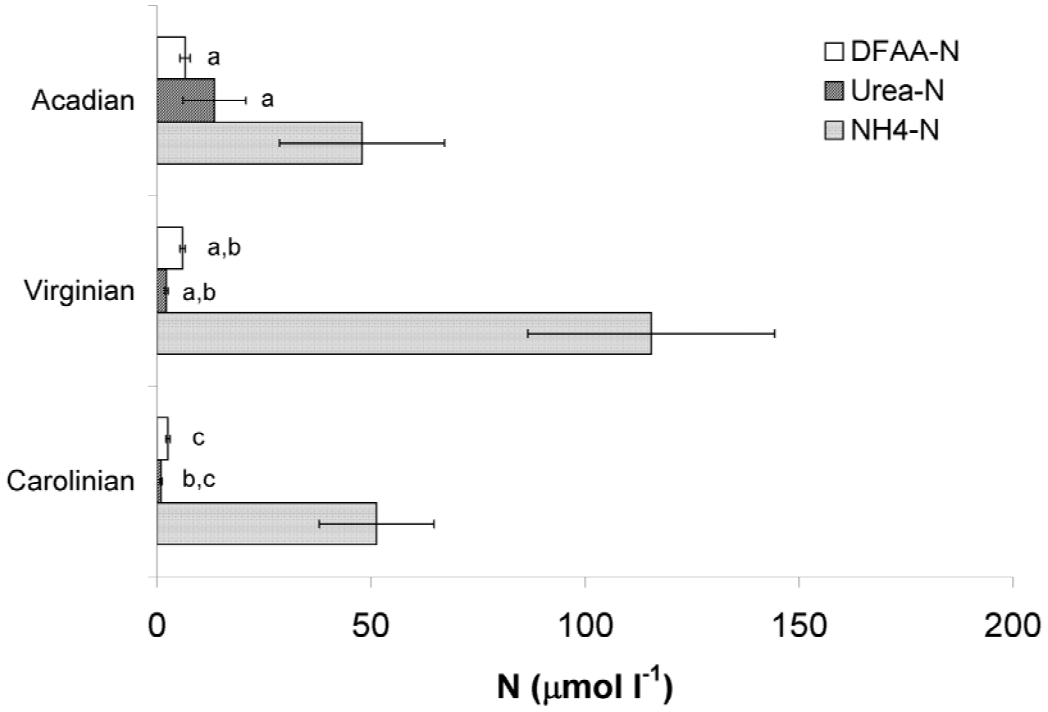

Figure 2-5. Mean porewater N availability ± 1 SE of low marsh *S. alterniflora* vs. biogeographic province at the 10cm depth. Significant differences at the p=0.05 level are indicated by different letters.

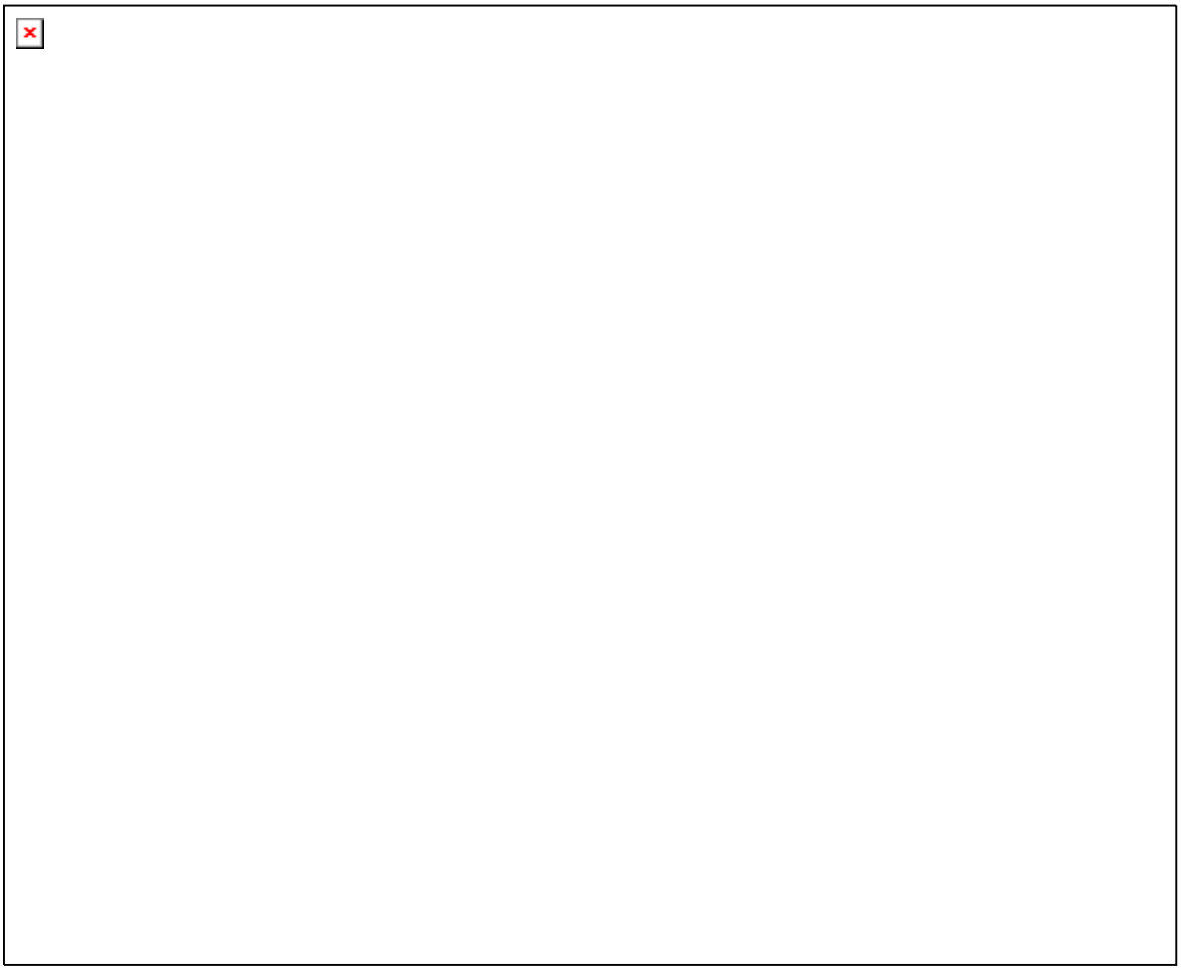

Figure 2-6. Mean porewater N availability ± 1 SE of low marsh *S. alterniflora* vs. biogeographic province at the 10cm depth. Significant differences at the p=0.05 level are indicated by different letters.

provinces ($F_{2,28}$=8.60, p=0.0014). The ratio of DON:NH_4^+ did not vary significantly in the high marsh among biogeographic provinces (p=0.39)

Microbial activity and DON uptake

We observed a significant provincial gradient in relative microbial activity among our field sites (Figure 2-7). Two-way ANOVA indicated that microbial activity varied significantly with site ($F_{8,59}$=23.77, p<0.0001), depth ($F_{4,59}$=4.88, p<0.0023), and there was a significant interaction by of site and depth ($F_{2,59}$=2.47, p<0.0258). Post-hoc tests indicated that rates at all sites were significantly different at α=0.05, with NIN exhibiting the greatest rates of microbial activity, VCR was intermediate, and PIE with the lowest rates. Post-hoc tests investigating differences in microbial activity over depth indicated that rates decreases significantly with depth All sites had similar microbial activity at the 30 cm depth, which is below the active root zone in *S. alterniflora* (Figure 2-7). Elemental C:N ratios of sediment samples collected from the same site in 2007 did not vary significantly among sites (p=0.27) or by depth (p=0.34), and did not follow the trends in microbial activity for any site (data not presented).

Estimates of microbial activity allow us to scale the potential mineralization of organic nitrogen which we relate to potential mineralization in the mesocosm experiment. In another study in Georgia, USA, maximum rates of heterotrophic utilization of alaline in vegetated salt marsh sediments were 8.32 pmoles cm^{-3} hr^{-1} at 10cm depth in short form, and 23.4 pmoles cm^{-3} hr^{-1} at 20cm depth in tall from *S. alterniflora* sediments, (Hanson and Gardner 1978). In such a case, if we assume these rates are constant throughout the mesocosm, we can expect anywhere from 0.97 to 2.72 μmols of the initial 30μmols ^{15}N

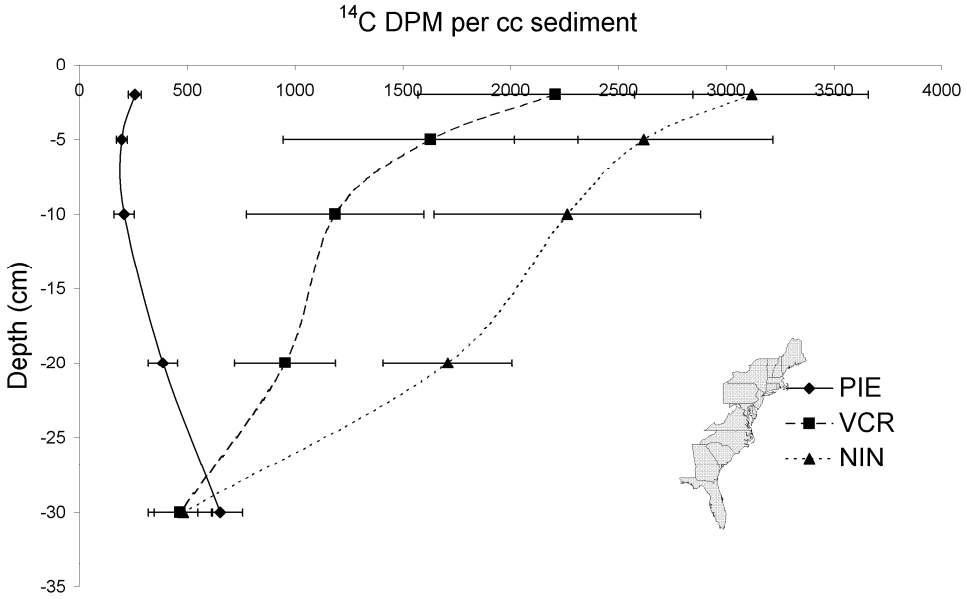

Figure 2-7. Relative microbial activity with depth at each field site expressed as ^{14}C disintegrations per minute (DPM) ± SE (n = 4 cores per site). Microbial activity varied significantly with depth ($p<0.0001$) and by site ($p=0023$).

glycine to have been mineralized to NH_4^+ during the 48-hour experiment ((8.32 or 23.4pmoles cm^{-3} hr^{-1})×48 hr × (2430 cm^{-3} sediment)/10^6 = 0.970 to 2.72 μmoles mineralized glycine N). This estimate assumes ^{15}N glycine was the sole source of heterotrophic metabolism, and gives a theoretical maximum. While some of the ^{15}DON was likely mineralized prior to assimilation, in such a case, this can at most account for at most 9% of the starting concentration. More importantly, this estimate is conservative since it does not account for differences in heterotrophic activity that may change by an order of magnitude with depth (Figure 2-7, Hanson and Gardner 1978). Even assuming such high rates of heterotrophic amino acid metabolism, at most 15% of the ^{15}N-glycine assimilated in the plant may have been mineralized prior to assimilation at the NIN (2.72 μmols mineralized / 18.11 ± 1.78μmols recovered in the plant) and VCR (2.72 μmols mineralized / 19.21 ± 1.08 μmols recovered in the plant) sites. Due to lower overall assimilation at PIE, microbial mineralization may potentially account for 35% of the DON assimilated, (2.72 μmols mineralized / 7.62 ± 1.31 μmols recovered in plant) (Figure 2-3). However, since microbial activity at PIE is an order of magnitude lower than NIN (Figure 2-7), we assume that nearly all DON uptake at PIE is attributed to plant processes alone and not mineralized prior to assimilation.

Discussion

We suggest that DON may be an available N source for *S. alterniflora*, and that the contribution of this pool varies with latitude. High-latitude salt marshes had the

greatest pools of available DON, corresponding to plants that have similar uptake rates of DON and DIN, suggesting a greater dependence of DON as a N source. While microbial activity and mineralization are generally regulated by temperature, ecotypic differences in both N uptake rates and the form of N used may potentially be attributed to differences in productivity at latitudinal scales. Ecotypes originating from high-latitude sites may have adapted to greater organic N availability, and may rely more upon DON as a N source to sustain their N requirements.

The use of DON by *S. alterniflora* in salt marshes is consistent with other ecosystems that are characterized by a slow organic matter decomposition that result in pools of available DON. While the DON uptake by *S. alterniflora* may not be as important as in arctic or boreal forest ecosystems (up to 80% of plant N demand) (Chapin et al. 1993, Kielland), our results are consistent with previous estimates of 24% for mid-Atlantic salt marshes (Mozdzer et al. in review-b). DON uptake is likely more important in high-latitude ecosystems such as arctic tundra (Chapin et al. 1993), boreal forests (Kielland 1994), and arctic salt marshes (Henry and Jefferies 2003a) where temperature imposes constraints on mineralization. We also suggest that temperature effects on mineralization play are an important influence on the relative importance of DON use along the Atlantic coast latitudinal gradient we studied. For the high-latitude salt marshes, DON utilization may be a more ecologically important process due to greater DON availability and similar measured rates of DON and DIN uptake. The similar uptake rates of DIN and DON suggest that high-latitude genotypes may be more dependant upon DON as a N source.

With decreases in latitude, the relative importance of DON uptake decreases due to decreases in DON availability. Our synoptic data show that porewater DON was strongly correlated with latitude (Figure 2-8). We suggest that mid latitude salt marshes in the Virginian province are intermediate in regards to the potential for DON use based upon intermediate DON availability and similar uptake rates of DON and DIN. Based upon the available pools of DON and similar DON and DIN uptake rates, our results are consistent with a previous study indicating that DON may account for up to 24% of plant N-demand in mid-Atlantic salt marshes (Mozdzer et al. 2004, in review-b). In the low-latitude site, DIN uptake rates were about double high-latitude sites, and *S. alterniflora* may plants rely more upon DIN as a N source. Although rates of DON uptake were the greatest within the Carolinian province, pools of DON were are not sufficient to sustain the plant N demand. This suggests that in low-latitude salt marshes, DON may not be as an important contribution to plant N demand and contributes less than the 24% demonstrated for mid-Atlantic salt marshes (Mozdzer et al. in review-b).

Differences in DIN uptake rates may be attributed to ecophysiological adaptations to the form of N available and to differences in N demand within a biogeographic province. The gradient in DIN uptake rates from high to low-latitude salt marshes corresponds well documented increases in productivity (Turner 1976, Kirwan et al. 2009). The greater productivity observed in low and mi-latitude salt marshes results in greater plant N demand.. To illustrate differences in plant N demand, we estimate the aboveground N demand using the regression of latitudinal origin and biomass from Kirwan et al. (2009) and our aboveground N content data, which suggests that mid and low-latitude plants require 14% to 20% more N than high-latitude *S. alterniflora*

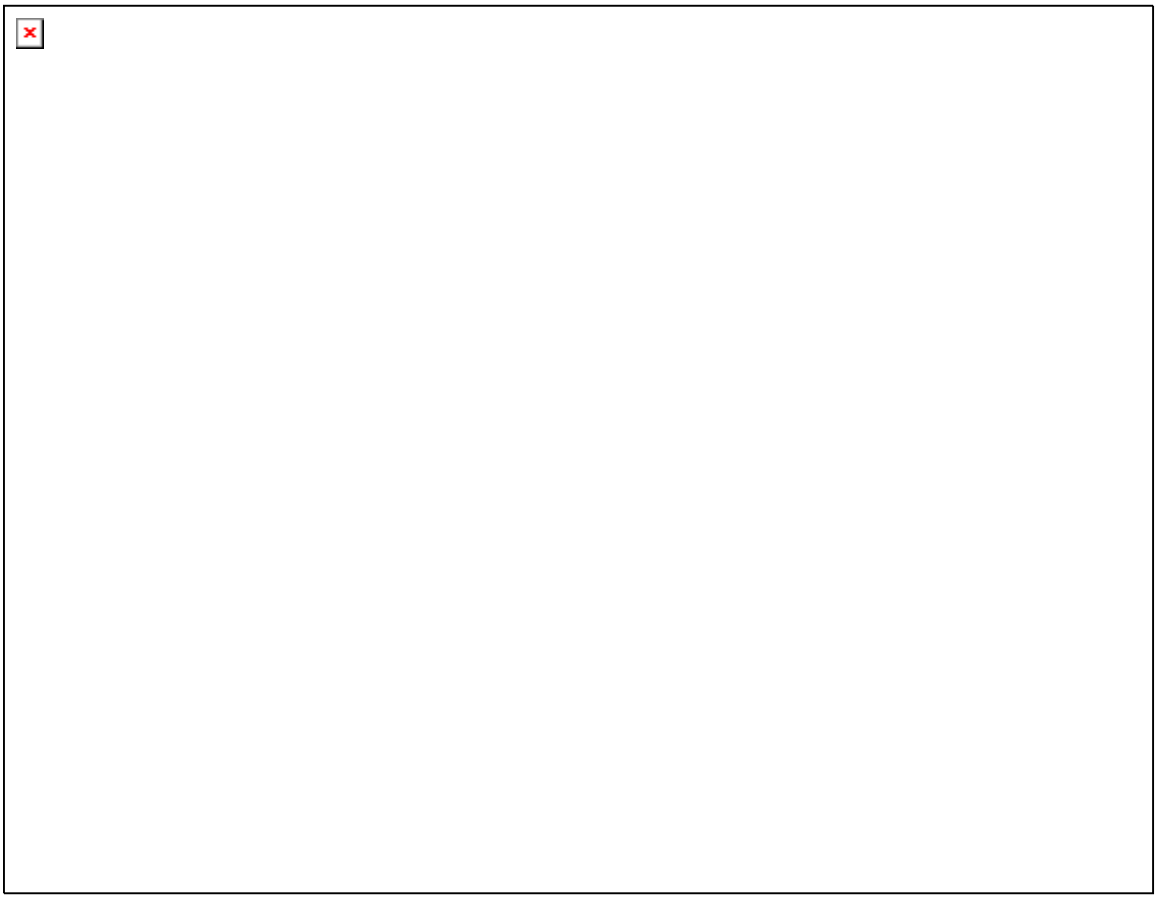

Figure 2-8. Relationship between bioavailable dissolved organic nitrogen (DON) vs. latitude of sampling site. Each point is an independent measurement of DON, which was log-transformed in to satisfy ANOVA assumptions, $P<0.0001$.

respectively. Although high-latitude *S. alterniflora* may have 40% greater shoot N content, differences in productivity result in a greater N demand at mid- and low-latitude locations. Depletion of the ^{15}N tracer from the mesocosm clearly illustrates the greater growth and N demand in low- and mid-latitude salt marshes. Due to a greater N demand, and lower shoot N concentrations, our data also suggests a greater N limitation of *S. alterniflora* with decreasing latitude.

Differences in translocation of the assimilated N also suggest differences in N demand for *S. alterniflora* growth in the three ecotypes. Although the majority of the assimilated N was in belowground tissue for all sites and treatments, high-latitude *S. alterniflora* translocated more N to its leaf tissue than either VCR or NIN. The rapid translocation to the leaf may be attributed to faster growth in the shorter growing season at high-latitudes, and the need to quickly move N to the photosynthetically active tissue. More research is suggested to specifically investigate differential strategies for N translocation among the three *S. alterniflora* ecotypes and the ecological implications.

If DFAA and urea-N concentrations remain as high in the rhizosphere as bulk porewater estimates suggest, *S. alterniflora* has the potential to compete well with microorganisms and use DON as a N source. While a portion of the ^{15}N-glycine was inevitably mineralized prior to assimilation, our calculations suggest that this may only be only 9% of the starting material. In Georgia salt marshes, DFAA are saturating relative to microbial activity with turn over times of days to weeks (Hanson and Gardner 1978). It has been suggested that pools of DFAA form since bacterial removal is less than production (Gardner and Hanson 1979). This may potentially be attributed to the fact that DFAA are poor substrates for sulfate reducing bacteria (Hansen 1993, Hansen

and Blackburn 1995), which are the predominant heterotrophs in salt marsh sediments (Howarth 1993) and the availability of NH_4^+. While plant productivity may be limited by N, productivity within the microbial community is limited by phosphorus (Sundareshwar et al. 2003). As such, in high latitude salt marshes, where heterotrophic activity is lower, this may results in greater pools of DON due to lower consumption by heterotrophic organisms. Since pools of DON in salt marsh sediments are in excess to microbial utilization, pools of DON are available for plant use.

Relative microbial activity seems to be responsible for the availability of the different N forms along latitudinal scales. These results are consistent with the observed decreases in denitrification in coastal watersheds attributed to temperature (Schaefer and Alber 2007). Since our experiments were conducted at room temperature, temperature should not be a confounding factor for our estimates of mineralization and may even result in an overestimate for our measurements at PIE. A more likely explanation may be differences in microbial abundance and sediment temperatures. Salt marshes in the Acadian province are flooded diurnally with cold water which may limit the relative abundance and activity of the heterotrophic community. In general, as microbial activity increases with decreasing latitude, less DON is available for plant use, which corresponds to and greater reliance upon DIN to satisfy plant N demand in low-latitude salt marshes.

Differences in sampling technique have the potential to yield very different results. Our methodology for analyzing N pools are representative of long-term availability based upon the equilibration method. Previous studies in Georgia salt marshes reported higher DFAA concentrations (up to 9 μM) near the southern extent of the Carolinian province in short form *S. alterniflora* (Gardner and Hanson 1979),

however, there were based on centrifuged sediment cores. Centrifuged cores inevitable may overestimate both DON and DIN due to the severing of roots and rhizomes. Mozdzer (in review-b) reported up to 12 μM DFAA amino acid in mid-Atlantic salt marshes using a high-resolution water sampler, however, this results in a bulk standing stock estimate. The problem with bulk measurements is that they may mask the fine scale fluctuations that may be more indicative of processes in the rhizosphere (Long et al. 2008). The next critical step is to investigate the availability of these nutrients in the rhizosphere. Based on our measurements of DON concentrations, and the long turnover times of DFAA in anoxic salt marsh sediments, we suggest that *S. alterniflora* plants have the potential to use organic nutrients. However, more research is needed to quantify specific pools of organic nitrogen as well as to investigate competition between plants and microorganisms in the rhizosphere.

We suggest that care should be given when interpreting data from a single site and applying the results the same species which may vary along latitudinal scales. For example, if we investigated the relative importance of DON only our low-latitude site, NIN, we may have concluded that DON uptake is not an important process in salt marsh systems due to low relative availability and high heterotrophic activity. On the other hand, our conclusions would be dramatically different for the high-latitude site, PIE, where DON constitutes a greater fraction of N availability, similar uptake rates of DON and DIN, and heterotrophic activity that is an order of magnitude lower.

By combining N availability, N utilization, and microbial activity, we present a more complete understanding of the factors controlling primary production in Atlantic coast salt marshes. We suggest there is the potential that DON uptake by *S. alterniflora*

in high-latitude salt marshes will comprise more than 24% of plant N demand previously reported in mid-latitude salt marshes. Conversely, in low-latitude marshes, where pools of bioavailable DON are lower, the contribution to total plant N demand by DON may be even lower. The availability of the pools of DON is largely dependant upon rates of microbial activity which we show can change by an order of magnitude in North American Atlantic coast salt marshes.

Chapter 3

Seasonal and Regional Variation in the availability of dissolved organic nitrogen in mid-Atlantic Salt marshes.

Abstract

N availability is generally thought to limit productivity of *S. alterniflora* salt marshes. Recent studies have reported the importance of DON as a N source for *S. alterniflora*, however, little is know about the relative availability of this pool of nutrients at regional or temporal scales. To investigate regional and temporal differences in DON availability, porewater nutrient concentrations in four Virginia salt marshes were measured at 10, 20, and 30cm in depth in the low, mid, and high marsh, corresponding to the tall, intermediate, and short form *S. alterniflora*. Temporal variation was quantified by measuring nutrient availability these sites in May and September. I found that dissolved free amino acid (DFAA) and urea-N concentrations, were an order of magnitude lower than NH_4^+ at depths beyond 10cm. In the active root zone (~10cm), DON (DFAA-N + urea-N) availability was greatest in May, and I attribute the decrease in September to increased plant and microbial demand. While DFAA concentrations decreased, urea-N concentrations increased from May to September, which I attribute to greater invertebrate activity. I suggest that regional temperature regulated heterotrophic activity is largely responsible for the lack of significant differences among sites and the greater available pools of DON in May. If I assume that seasonal patterns at the VCR-LTER are consistent within all Atlantic coast salt marshes, DON may comprise an important source of N early in the growing season, when NH_4^+ concentrations are limited by lower cumulative rates of microbial activity.

Introduction

Salt marshes are among the world's most productive ecosystems (Odum 1961, Mitsch and Gosselink 1993), and are known for their high primary production attributed to the tidal pulsing of the marsh environment (Odum et al. 1995). Traditionally viewed as a nitrogen (N) limited system (Valiela and Teal 1974), recent research has demonstrated the importance of dissolved organic nitrogen (DON) in supplying the N demand of foundation species *Spartina alterniflora* in mid-Atlantic salt marshes (Mozdzer 2005). DON availability in temperate salt marshes varies latitudinally (Chapter 2), and only two studies have reported dissolved free amino acid (DFAA) availability in salt marsh sediments (Gardner and Hanson 1979, Mozdzer et al. in review-b). Previous studies reported concentrations ranging from nanomolar to low micromolar concentrations of total DFAA-N, however, no research exists on how this pool of nutrients may vary spatially or seasonally within an ecosystem. In unvegetated Chesapeake Bay sediments, DFAA concentrations ranged from 1 - 300 μM, with the highest values found just below the surface (Burdige and Martens 1990). In fjords in Denmark, concentrations of DON in sediments were as high as 3 mM and up to 82% of the DON was in the bioavailable form of dissolved combined amino acids, DFAA, and urea (Guldberg et al. 2002) However, little is known about the dynamics of DON in salt marsh sediments (Weston et al. 2006), especially with regard to the labile plant available part of the pool.

DON is a term used to characterize a large pool of organic nitrogenous compounds that can vary dramatically in size and weight. In this chapter, DON will refer

to the small, bioavailable fraction of low C:N compounds such as amino acids and urea. DON is often found in ecosystems where organic N mineralization rates are slow and/or ecosystems are characterized by accumulations of slowly decomposing organic matter. In theses systems DON has been found to support the N demand of plants in arctic tundra (Chapin et al. 1993, Kielland 1994, Schimel and Chapin 1996), arctic salt marshes (Henry and Jefferies 2003b), boreal forests (Nasholm et al. 1998), grasslands (Streeter et al. 2000, Weigelt et al. 2003), and temperate coastal lagoons (Tyler et al. 2001).

Intertidal salt marshes at the Virginia Coast Reserve (VCR) are dominated by monocultures of *S. alterniflora* throughout the low and mid marsh elevations. Based upon the relative elevation of the marsh, *S. alterniflora* can be found in one of three phenotypic growth forms: tall, intermediate, and short. These growth forms correspond roughly to relative elevation of *S. alterniflora* on the marsh platform. Productivity of these growth forms is attributed primarily to physio-chemical differences in of the rhizosphere (Mendelssohn and Morris 2000). However, Chapter 5 suggests that shoot N assimilation from tidal waters may stimulate primary production in the intermediate and tall form of *S. alterniflora* (Chapter 5). In general, horizontal movement of water through the sediments is limited in high elevation on the marsh platform (Osgood and Zieman 1993b), resulting in limited water flow, and high rates of evapotranspiration. Evapotranspiration in the high marsh may result in hyper-saline soils which may limit *S. alterniflora* production (Haines and Dunn 1976, Bradley and Morris 1991). Since these sediments experience less horizontal flushing, they are prone to accumulation of H_2S which may also limit *S. alterniflora* productivity (King et al. 1982, Bradley and Morris 1990). At lower elevations on the marsh platform, sediments are more regularly flooded

reducing the stresses associated with both salinity and sulfide, increasing the productivity of *S. alterniflora.* Maximum productivity in *S. alterniflora* is at the lowest elevations in the marsh (Morris et al. 2002), where tidal flushing is the greatest, and sediments are the most biochemically oxidized (Mendelssohn and Morris 2000).

In addition to differences in plant phenotype based upon marsh platform elevation and physio-chemical controls, salt marshes within the Virginia Coast Reserve-Long Term Ecological Research site (Figure 3-1) also fall into one of three distinct environments: mainland, lagoonal, and barrier island. The salt marshes on the southern end of Hog Island provide an example of how a large over-wash event and different patterns in recolonization result in salt marshes of various ages in a relatively small area. Walsh (1998) identified marshes of age from 1 to over 150 years in age, and through correlation analysis suggested that younger marshes varied more annually with respect to physio-chemistry, and consistently had lower pore water nutrients concentrations when compared to older, more developed and stable marshes. This Hog Island salt marsh chronosequence, and the different salt marsh types at the VCR-LTER present a unique setting to examine how DON availability may vary based upon differences in marsh age or location. The primary difference between mainland and barrier island marshes is their geological origin. Barrier island marshes form over sand flats, or may begin from a storm overwash events. Whereas mainland marshes, such as those in Upper Phillip's Creek, may be on the order of hundreds to thousands of years old.

In this chapter, I investigate how DON availability varies spatially among sites, as well as within the three salt marsh zones at each site. I also examined how pools of nutrients may vary seasonally within the VCR-LTER system to create a better

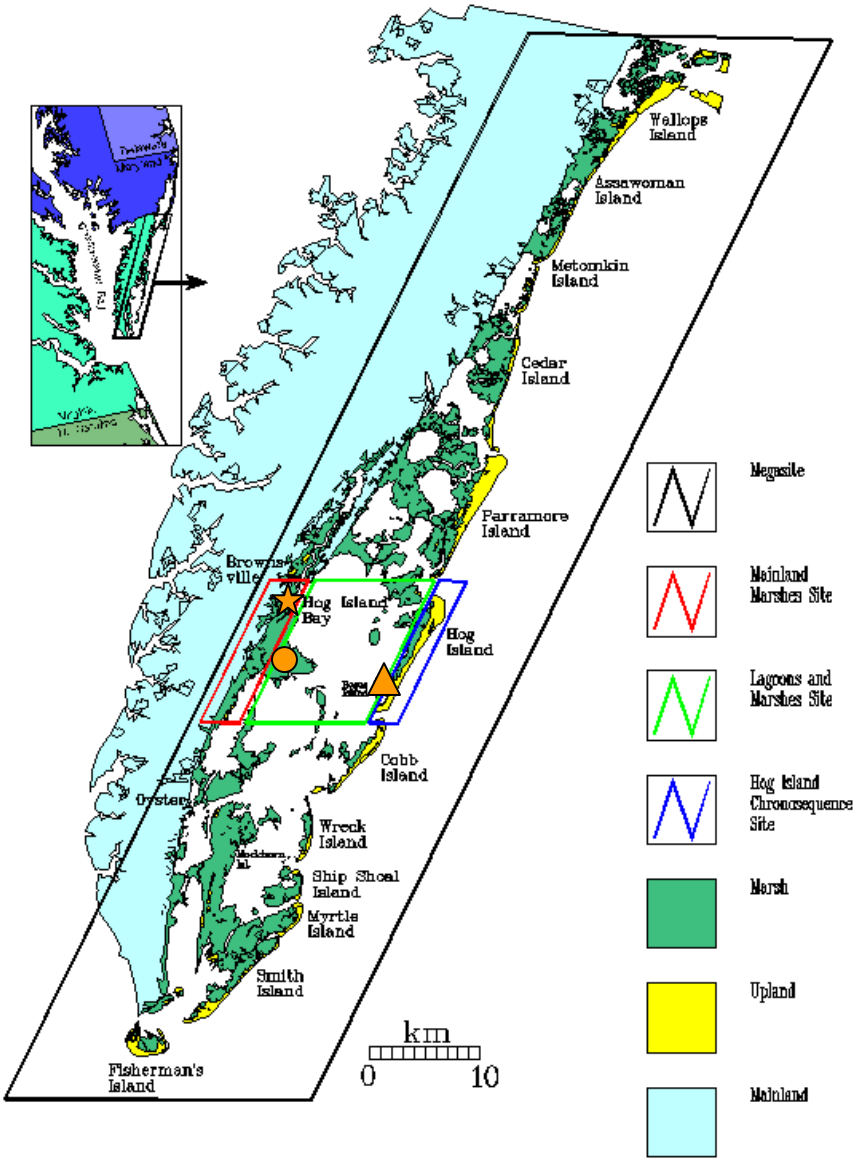

Figure 3-1. VCR-LTER Mega site. Upper Phillips Creek, UPC is identified by the star, Fowling Point, FP, is identified by the circle, and south Hog Island sites, SH, is identified by the triangle.

understanding of the relative availability of this pool of nutrients in the context of plant availability and utilization.

Methods

Site Description

Three salt marsh field sites were identified within the Virginia Coast Reserve-Long Term Ecological Research site along a transect from the mainland to the barrier islands to investigate how DON availability may vary both seasonally and regionally (Figure 3-1). Mainland salt marshes, are physically connected to the Delmarva peninsula; barrier island salt marshes are found in the low energy habitats on the back-side of barrier islands; and lagoonal salt marshes are located in the shallow coastal lagoons between the mainland and barrier islands. The mainland site, Upper Phillip's Creek (UPC) is the primary mainland field site of the VCR-LTER. The lagoonal marsh, located on Fowling Point (FP), is a new active field site located within the footprint of the VCR-LTER flux tower. Two barrier island sites were located on the southern end of Hog Island, where previous studies identified areas of marsh of different age attributed to over wash events establishing a chronosequence. I selected the marshes identified by Walsh (1998) as 13 year and 150+ year field sites at the time of his study, which would approximately correspond to approximately 25 and 150+ years at the time of my study. These are identified as SHY13 and SHY150 respectively (Figure 3-2).

Within each field site, porewater equilibrators were deployed in the tall, mid, and short growth forms of *S. alterniflora.* Porewater equilibrators are consisted of 20 ml

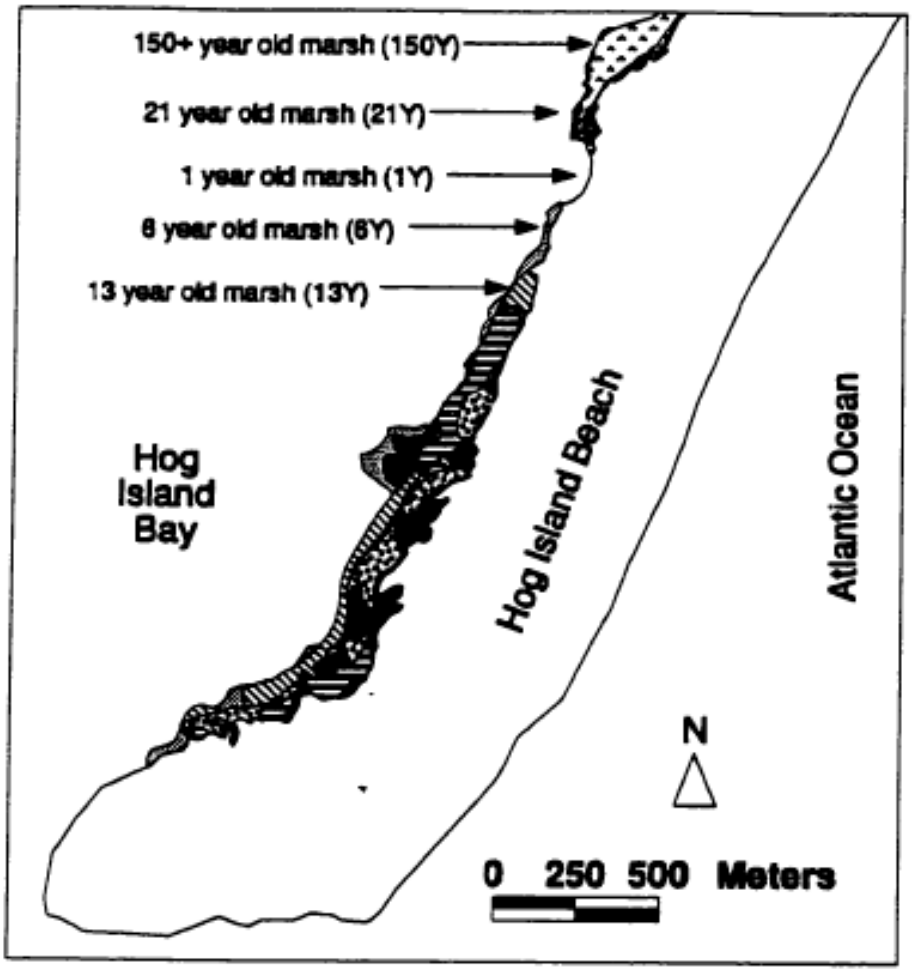

Figure 3-2 Overlay of different aged marshes along the Hog Island chronosequence taken directly from Figure 2.2 of Walsh (1998). Sites sampled on Hog Island in this study were sites 13Y and 150Y, identified at SHY13 and SHY150.

glass scintillation vials (GPI Thread 24-400, Wheaton), fitted with a 0.2 μm HT Tuffryn membrane (Pall, Gelman), which is kept in place by an open-top screw cap (open-top black phenolic screw caps, GPI Thread 24-400, Wheaton). A 60 cm section of 1.5" schedule 40 PVC was used to position the equilibrators at the proper depth. Using a 1 1/8" hole saw bit, holes were drilled into the PVC 10-cm apart, leaving approximately 5cm below the deepest hole. Five-cm sections of thin wall 1" PVC were inserted into 1.5" schedule 40 PVC, which kept the vial in place and the membrane of the equilibrator flush with the sediment surface. Equilibrators were located at 10, 20, and 30 cm below the sediment surface with the deployment of three replicate equilibrators in each of the three *S. alterniflora* growth forms. Since *S. alterniflora* growth forms have been interpreted differently, I describe tall form as plant greater than 1.3 m at end of year (EOY), intermediate form plants are greater than 0.6m but less than 1.3m, and short form is stunted and does not exceed 0.6m in height at EOY. At the PC site, equilibrators were not deployed in the tall form since it only occurs in a vary narrow zone at the creek bank.

To capture differences in seasonality, porewater nutrient concentrations were measured in May and September of 2007, corresponding to the beginning and end of the growing season. Equilibrators were deployed on April 18, 2007 and collected on May 24, 2007. Equilibrators were also deployed on August 22 and collected on Sept 4, 2007. Equilibrators of this design need ~ 30 days to equilibrate (Thomas 2004). The collected equilibrators were immediately sealed in the field with parafilm, placed in a rack on ice, and brought to the lab in Oyster for processing. Collected samples were filtered sterilized using a syringe filter (Acrodisc, GHP 0.2μm). A non filtered subsample was used to determine sulfide concentrations using the methods of Cline (1969). Samples were

immediately frozen until analyzed for NH_4^+, TDN, DFAA, and Urea-N. NH_4^+ and soluble reactive phosphorus (SRP) concentration was determined on a Lachat QuickChem 8500 (Loveland, Colorado) using QuickChem method 31-107-06-1-B. Total dissolved nitrogen was measured as NO_x-N on the Lachat QuickChem 8500 (Loveland, Colorado) after a persulfate digestion in sealed ampoules. Urea was determined using a modification of the methods of (Mulvenna and Savidge 1992) and (Goeyens et al. 1998). Dissolved free amino acids (DFAA) were determined on a Dionex ICS 3000 (Sunnyvale, CA) using the AccQ-Tag chemistry package from Waters Corporation (Milford, MA).

In this chapter, I will only discuss the pools of DFAA-N, urea-N, NH_4-N, and DON (DON= urea-N + DFAA-N). Data not presented, including SRP, H_2S, TDN are reported in Appendices B and C. Analysis of variance (ANOVA) was conducted in SAS (Version 9.1, Cary, NC). When variables did not meet ANOVA assumptions (DON and DON: NH_4^+), they were log-transformed. Since nutrient concentrations may vary by an order of magnitude by depth, these variables were analyzed by a three-way ANOVA to investigate how availability changes by site, plant growth form (marsh zone), and by month (May vs. September) for each depth. A second analysis, was conducted using only the measurement at 10 cm since this is the depth associated with the rhizosphere of *S. alterniflora.* These data were analyzed by salt marsh zone (tall, intermediate, short *S. alterniflora*) in a two-way ANOVA examining differences in site (UPC, FP, SH13, SHY150) and season (May & September).

Results

Porewater Nutrient Availability

10cm Depth

The 10cm depth is representative of the active root zone for *S. alterniflora.* At this depth, porewater DFAA-N concentrations did not vary by site, but varied by zone and month (Table 3-1). Post hoc test indicated greater DFAA concentrations in May vs. September, and greater availability in the high marsh zone corresponding to short form *S. alterniflora.* Urea-N concentrations did not vary by site, zone, or month, but had significant interactions of Site×month, and zone×month, with greater availability in September vs. May (Table 3-1). Total DON availability did not vary by site, but varied significantly by both *S. alterniflora* zone and month with significant interactions among all sources (Table 3-1). Post hoc test indicated greater DON availability in the intermediate and short form *S. alterniflora* zones than in the tall form zone. NH_4^+ availability varied significantly by site, zone, moth, and all potential interactions illustrating the variability of inorganic N availability in salt marshes within a relatively small regional range. These results suggest that the availability of local pools of DON do not vary significantly within a large geographical area.

20cm Depth

Lower in root zone for *S. alterniflora,* porewater DFAA-N concentrations did not vary by site or zone, but varied only by month (Table 3-2), with greater DFAA concentrations in May vs. September. Urea-N concentrations did not vary by site, zone, or month, but had significant interactions of Site×month, and zone×month, with greater availability in

Table 3-1. Results of ANOVA for porewater nutrient availability at 10cm depth for NH4+, DFAA-N, Urea-N, and DON.

Variable	Sources	df	MS	F	P
NH_4^+	Site	3	19706	4.17	0.0107
	Zone	2	31728	6.71	0.0027
	Month	1	139748	29.57	<.0001
	Site × Zone	5	42255	8.94	<.0001
	Site × Month	3	18502	3.92	0.0141
	Zone × month	2	31378	6.64	0.0029
	Site ×Zone × Month	5	11064	2.34	0.056
	Error	47	4725		
DFAA-N	Site	3	7.1	1.4	0.2596
	Zone	2	19.1	3.8	0.0308
	Month	1	80.5	15.8	0.0002
	Site × Zone	5	23.9	4.7	0.0015
	Site × Month	3	8.3	1.6	0.196
	Zone × month	2	0.7	0.1	0.8691
	Site ×Zone × Month	5	19.7	3.9	0.0051
	Error	47	5.1		
Urea-N	Site	3	2.8	1.5	0.2291
	Zone	2	2.5	1.3	0.2723
	Month	1	2.6	1.4	0.2502
	Site × Zone	5	4.7	2.5	0.0441
	Site × Month	3	12.4	6.5	0.0009
	Zone × month	2	16.0	8.4	0.0007
	Site ×Zone × Month	5	2.9	1.5	0.2055
	Error	48	1.9		
DON	Site	3	8.1	1.3	0.2945
	Zone	2	32.8	5.2	0.0093
	Month	1	115.1	18.2	<.0001
	Site × Zone	5	16.0	2.5	0.0416
	Site × Month	3	22.6	3.6	0.021
	Zone × month	2	19.4	3.1	0.0563
	Site ×Zone × Month	5	30.5	4.8	0.0012
	Error	47	6.3		

Table 3-2. Results of ANOVA for porewater nutrient availability at 20cm depth for NH4+, DFAA-N, Urea-N, and DON.

Variable	Sources	df	MS	F	P
NH_4^+	Site	3	37206	3.5	0.0221
	Zone	2	11668	1.1	0.3409
	Month	1	77481	7.3	0.0095
	Site × Zone	5	174840	16.5	<.0001
	Site × Month	3	2444	0.2	0.8747
	Zone × month	2	32343	3.1	0.0566
	Site ×Zone × Month	5	15879	1.5	0.2082
	Error	48	10602		
DFAA-N	Site	3	30.3	2.7	0.0583
	Zone	2	21.2	1.9	0.1651
	Month	1	233.9	20.7	<.0001
	Site × Zone	5	51.0	4.5	0.0021
	Site × Month	3	23.0	2.0	0.1224
	Zone × month	2	12.2	1.1	0.3479
	Site ×Zone × Month	5	47.9	4.2	0.0031
	Error	44	11.3		
Urea-N	Site	3	2.0	1.6	0.1925
	Zone	2	2.2	1.7	0.1882
	Month	1	2.8	2.2	0.1439
	Site × Zone	5	7.4	5.9	0.0002
	Site × Month	3	5.0	4.1	0.012
	Zone × month	2	2.4	1.9	0.1589
	Site ×Zone × Month	5	10.9	8.8	<.0001
	Error	48	1.2		
DON	Site	3	21.3	1.5	0.2227
	Zone	2	12.3	0.9	0.422
	Month	1	309.5	22.1	<.0001
	Site × Zone	5	49.7	3.6	0.0088
	Site × Month	3	15.3	1.1	0.3626
	Zone × month	2	13.6	1.0	0.388
	Site ×Zone × Month	5	86.9	6.2	0.0002
	Error	44	14.0		

Table 3-3. Results of ANOVA for porewater nutrient availability at 30cm depth for NH4+, DFAA-N, Urea-N, and DON.

Variable	Sources	df	MS	F	P
NH_4^+	Site	3	285741	9.5	<.0001
	Zone	2	148843	4.9	0.0117
	Month	1	182061	6.0	0.0181
	Site × Zone	5	100130	3.3	0.0125
	Site × Month	3	74115	2.5	0.0757
	Zone × month	2	9211	0.3	0.7389
	Site ×Zone × Month	5	25024	0.8	0.5368
	Error	45	30236		
DFAA-N	Site	3	11.5	3.7	0.0197
	Zone	2	11.1	3.6	0.0379
	Month	1	91.7	29.2	<.0001
	Site × Zone	5	6.0	1.9	0.1127
	Site × Month	3	2.2	0.7	0.5554
	Zone × month	2	3.5	1.1	0.3423
	Site ×Zone × Month	4	9.2	2.9	0.0327
	Error	41	3.1		
Urea-N	Site	3	4.7	2.1	0.1181
	Zone	2	3.1	1.3	0.2709
	Month	1	1.5	0.7	0.4215
	Site × Zone	5	10.3	4.5	0.002
	Site × Month	3	22.8	9.9	<.0001
	Zone × month	2	5.3	2.3	0.1098
	Site ×Zone × Month	5	18.7	8.2	<.0001
	Error	46	2.3		
DON	Site	3	2.0	0.3	0.7963
	Zone	2	3.2	0.6	0.5822
	Month	1	90.5	15.4	0.0003
	Site × Zone	5	23.5	4.0	0.0048
	Site × Month	3	13.1	2.2	0.0997
	Zone × month	2	12.2	2.1	0.1391
	Site ×Zone × Month	4	46.1	7.8	<.0001
	Error	41	5.9		

September vs. May (Table 3-2). Total DON availability did not vary by site or zone, but varied significantly by month (Table 3-2). Post hoc tests indicated greater DON availability in May vs. September. NH_4^+ availability varied significantly by site and month.

30cm Depth

Below the active root zone for *S. alterniflora*, porewater DFAA-N concentrations varied by site, zone, and moth (Table 3-3). Post hoc tests indicated greater DFAA concentrations in May vs. September, and greater concentration in the short and intermediate form *S. alterniflora* zone. Urea-N concentrations did not vary by site, zone, or month, but had significant interactions of Site×month, and zone×month, with greater availability in September vs. May (Table 3-3). Total DON availability did not vary by site or zone, but varied significantly only by month (Table 3-2). Post hoc tests indicated greater DON availability in May vs. September. NH_4^+ availability varied significantly by site and month.

Within site variation

South Hog Y13

Porewater DFAA-N availability was greater in May than September (Figure 3.3 and 3.4) ($F_{1,47}$=5.96, p=0.0205) with no significant differences in DFAA-N over depth (p=0.42) or by plant zone (p=0.42), in May at the 10 cm depth. Urea-N varied significantly by zone ($F_{2,52}$=14.26, p<0.0001) with lower availability in the intermediate

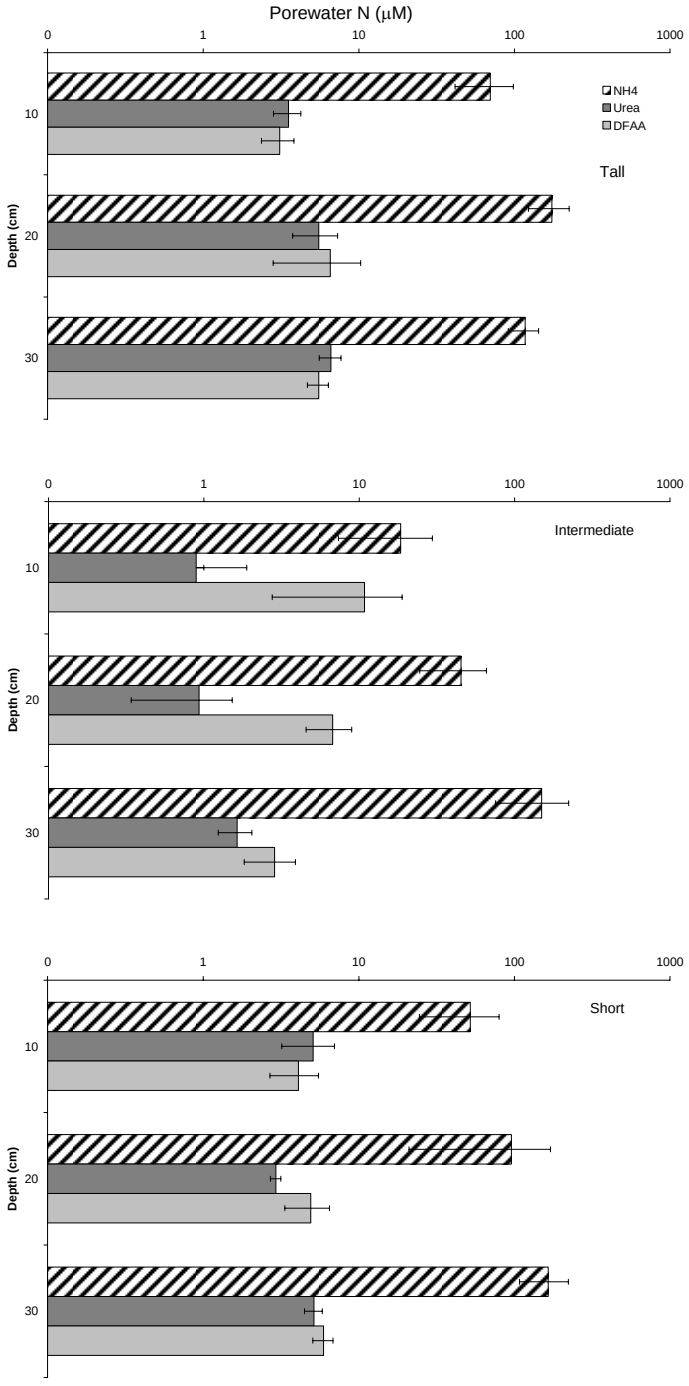

Figure 3-3. Depth profile bioavailable porewater N at the South Hog Y13 site in May 2007 for (a) tall, (b) intermediate, and (c) short form *S. alterniflora*. Mean NH_4-N, urea-N, and DFAA-N ± SE are presented from an n=3 for each marsh zone and depth. Note that the X-axis is log transformed.

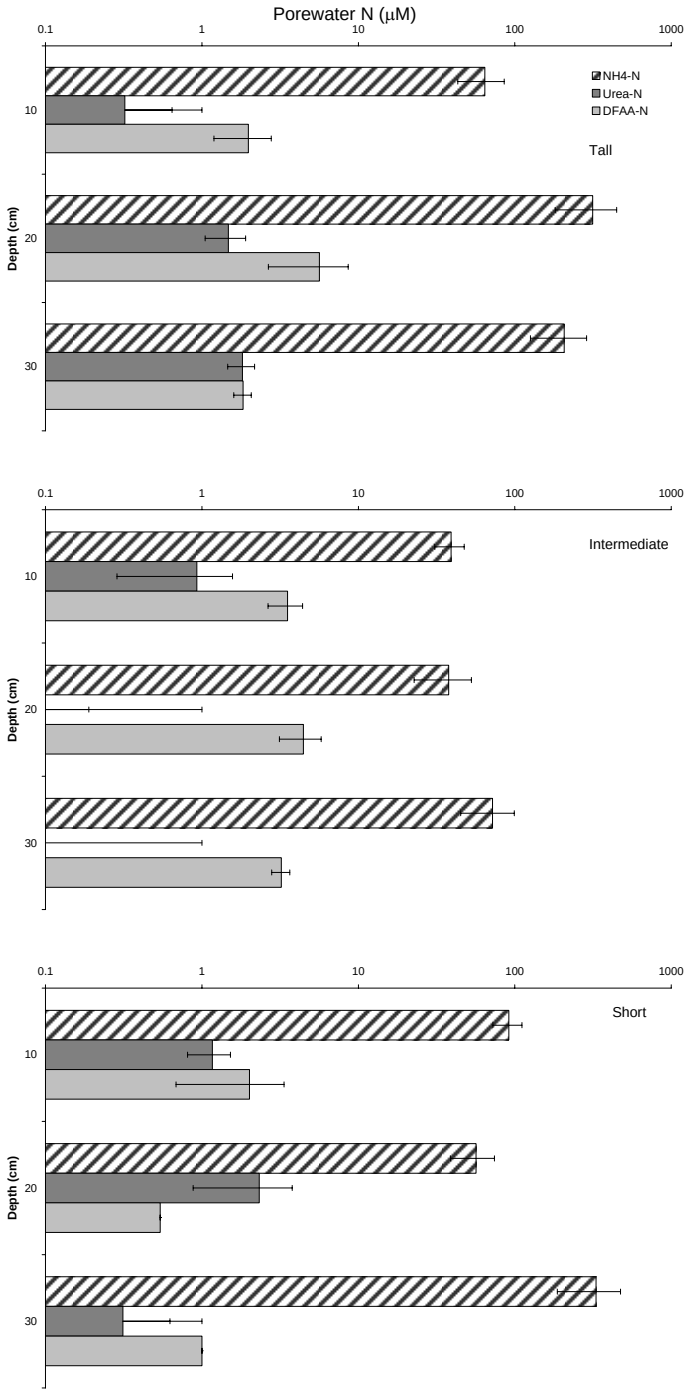

Figure 3-4. Depth profile bioavailable porewater N at the South Hog Y13 site in Sept 2007 for (a) tall, (b) intermediate, and (c) short form *S. alterniflora*. Mean NH_4-N, urea-N, and DFAA-N ± SE are presented from an n=3 for each marsh zone and depth. Note that the X-axis is log transformed.

zone than either the tall or short form zone. Urea-N also decreases later in the growing season ($F_{1,52}$=39.24, p<0.0001). Urea-N concentrations did not vary by depth ($F_{2,52}$=0.87, p=0.42). DON varied by month with a 50% reduction from May to September ($F_{1,47}$=23.15, p<0.0001) and by month by *S. alterniflora* zone (month × zone interaction) ($F_{4,47}$=2.69, p=0.0491) with greater DON availability in the tall and intermediate *S. alterniflora* zones in May. DON comprised anywhere from 4% to 36% of the N pool in the tall and intermediate form respectively at 10 cm in depth. NH_4-N was one to two orders of magnitude greater than DON at depths beyond 10cm. NH_4-N availability was significantly greater in the tall form zone ($F_{2,52}$=4.79, p=0.0145), and increased with depth in all *S. alterniflora* zones ($F_{4,47}$=2.80, p=0.0409).

South Hog Y150

Porewater DFAA-N availability was significantly greater in May than in September (Figure 3.5 and 3.6) ($F_{1,53}$=5.29, p=0.0274). DFAA-N did not vary significantly with depth (p=0.57) or by plant zone (p=0.08). Less DFAA were available in low marsh in September (zone × month interaction) ($F_{1,53}$=4.46, p=0.0186). Urea-N varied significantly by zone ($F_{2,53}$=4.28, p=0.021) with the intermediate form > short form > tall form . Urea-N also varied significantly monthly by plant zone, with lower availability in the tall form in September (zone × month interaction) ($F_{1,53}$=11.50, p=0.0001). Urea-N concentrations did not vary by depth (p=0.47). Thirty five percent more DON was available in May vs. September ($F_{1,53}$=5.00, p0.037) and monthly by *S. alterniflora* zone ($F_{2,53}$=4.72, p=0.015) with greater DON availability in the

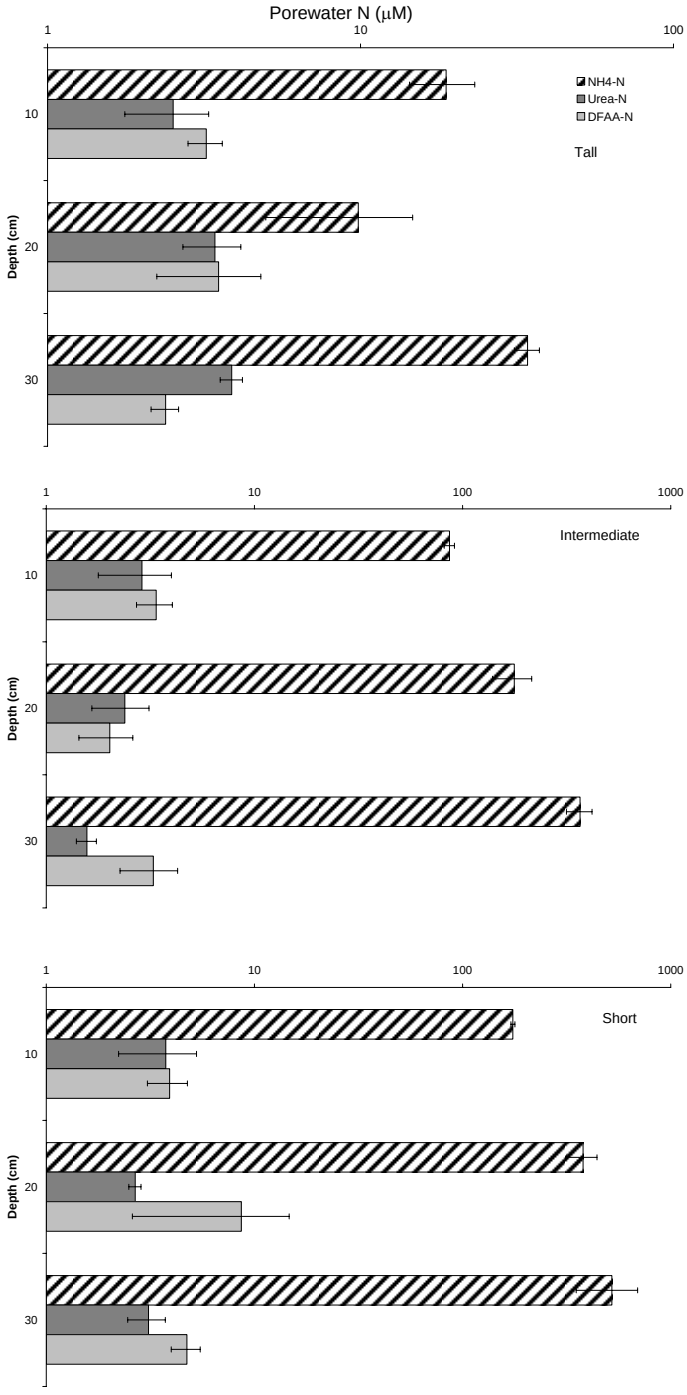

Figure 3-5. Depth profile bioavailable porewater N at the South Hog Y150 site in May2007 for (a) tall, (b) intermediate, and (c) short form *S. alterniflora*. Mean NH4-N, urea-N, and DFAA-N ± SE are presented from an n=3 for each marsh zone and depth. Note that the X-axis is log transformed, and figure a is on a different scale than figures b and c.

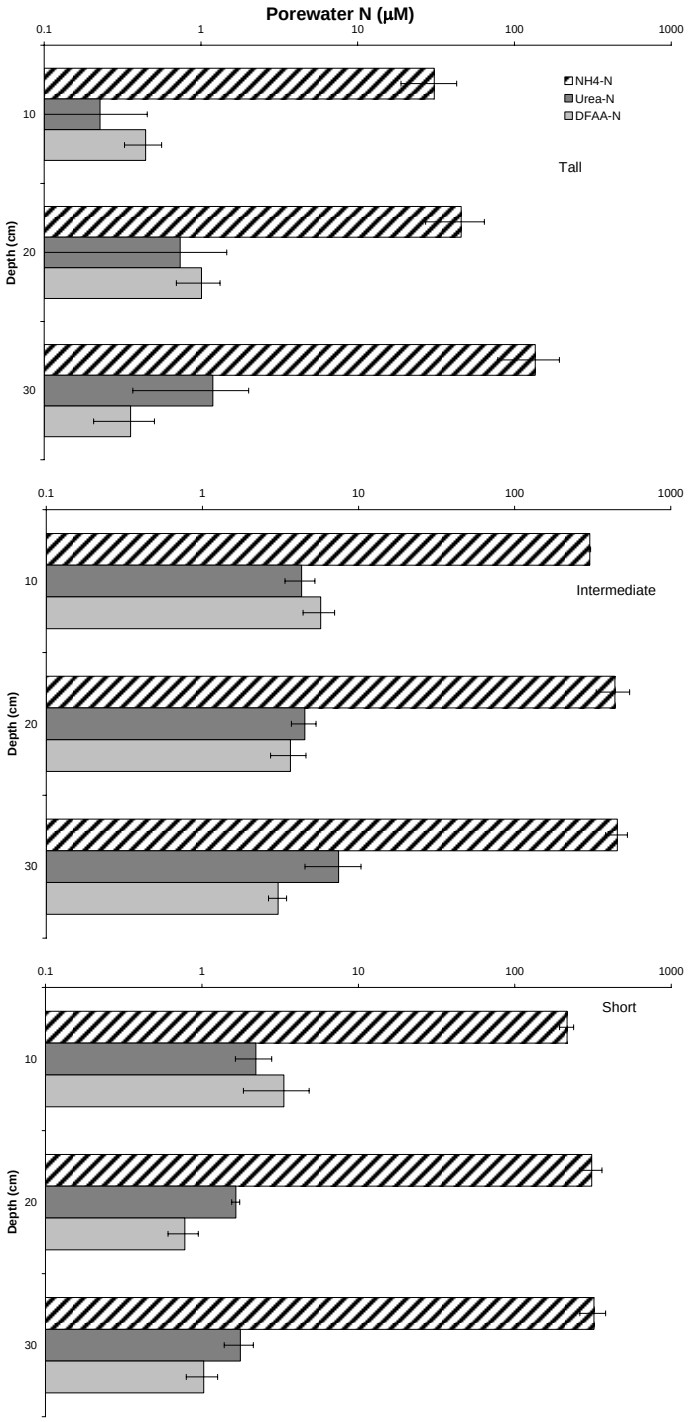

Figure 3-6. Depth profile bioavailable porewater N at the South Hog Y150 site in September 2007 for (a) tall, (b) intermediate, and (c) short form S. alterniflora. Mean NH4-N, urea-N, and DFAA-N ± SE are presented from an n=3 for each marsh zone and depth. Note that the X-axis is log transformed.

intermediate>short>tall form zones. NH_4-N was one to two orders of magnitude greater than DON greater at depths beyond 10cm, and was significantly greater in the tall form and intermediate form than the short form ($F_{2,52}$=38.56, p<0.0001). NH_4-N increased with depth ,and by month in all *S. alterniflora* zones ($F_{2,52}$=11.62, p=0.0001).

Fowling Point

Porewater DFAA-N availability was significantly lower in September than May at Fowling Point (Figure 3.7 and 3.8) ($F_{1,52}$=22.76, p<0.0001), and did not vary significantly among *S. alterniflora* zones (p=0.61) or by depth (p=0.49). Urea-N did not vary by *S. alterniflora* zone (p=0.33), but more urea-N was available in September ($F_{1,52}$=7.86, p=0.0082). More DON was available in May vs. September ($F_{1,52}$=7.50, p=.0096), however, contrary to the SH Y13 site, DON did not vary by marsh zone (p=0.85) or by depth (p=0.34). NH_4-N was one to two orders of magnitude greater than DON greater at depths beyond 10cm, and was significantly greater in the intermediate > tall > short form ($F_{2,52}$=32.48, p<0.0001). NH_4-N and increased with depth in all *S. alterniflora* zones and by month in September ($F_{2,52}$=6.20, p=0.0049).

Upper Phillip's Creek

Porewater DFAA-N availability did not vary significantly at UPC by *S. alterniflora* zone (p=0.20), depth (p=0.36), or season (p=0.43) (Figures 3.9 and 3.10). DFAA-N varied by depth, with maximum concentrations at the 20cm depth ($F_{1,45}$=23.30, p<0.0001). Some replicates for the 20 and 30cm depths were left out of the analysis due

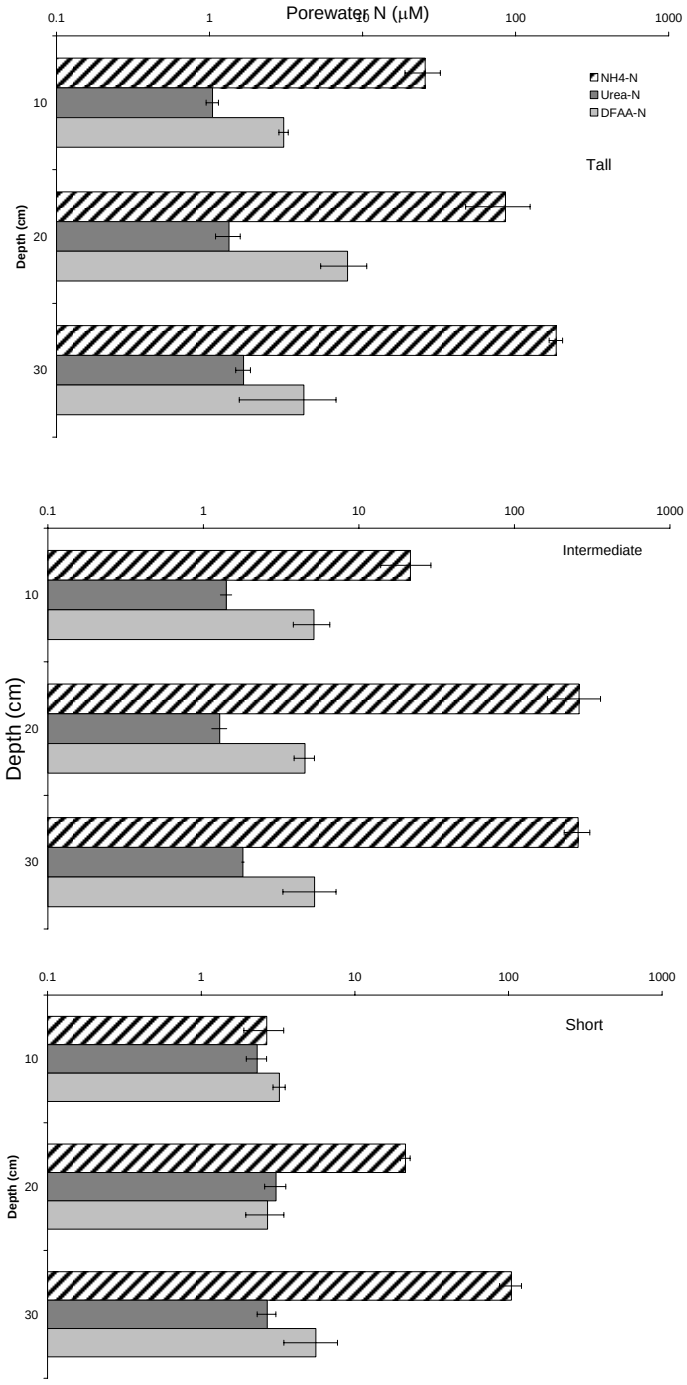

Figure 3-7. Depth profile bioavailable porewater N at the Fowling Point site in May 2007 for (a) tall, (b) intermediate, and (c) short form *S. alterniflora*. Mean NH_4-N, urea-N, and DFAA-N ± SE are presented from an n=3 for each marsh zone and depth. Note that the X-axis is log transformed.

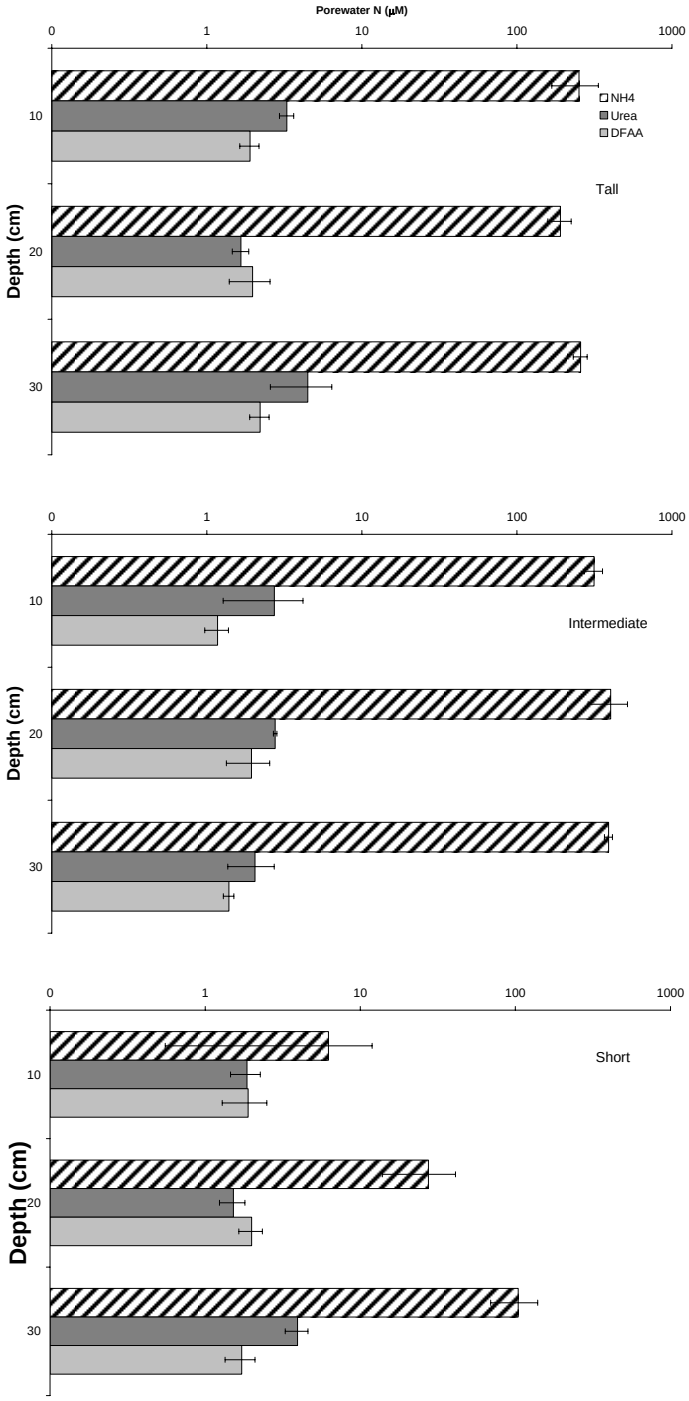

Figure 3-8. Depth profile bioavailable porewater N at the Fowling Point site in Sept 2007 for (a) tall, (b) intermediate, and (c) short form *S. alterniflora*. Mean NH_4-N, urea-N, and DFAA-N ± SE are presented from an n=3 for each marsh zone and depth. Note axis is log transformed.

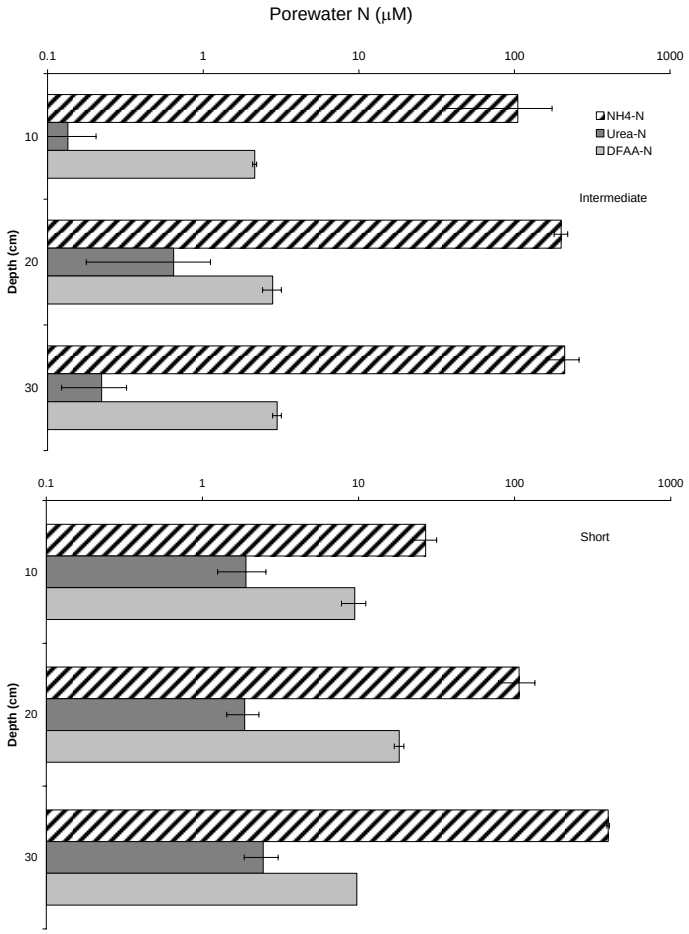

Figure 3-9. Depth profile bioavailable porewater N at the Upper Phillip's Creek site in May 2007 for (a) intermediate, (b) short form *S. alterniflora*. Mean NH_4-N, urea-N, and DFAA-N ± SE are presented from an n=3 for each marsh zone and depth. Note that the X-axis is log transformed.

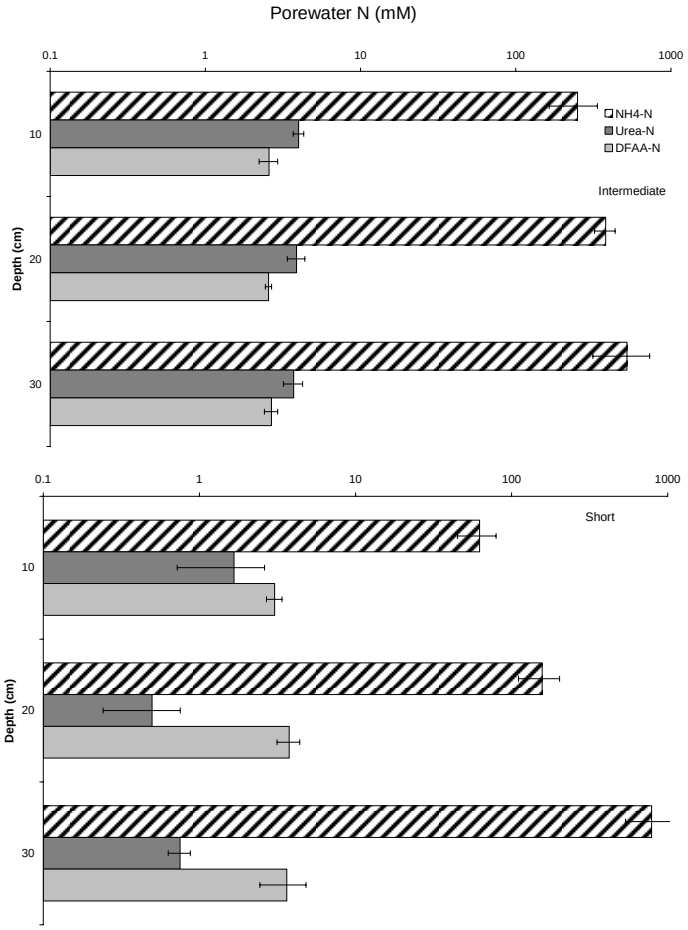

Figure 3-10. Depth profile bioavailable porewater N at the Upper Phillip's Creek site in September 2007 for (a) intermediate, (b) short form *S. alterniflora*. Mean NH_4-N, urea-N, and DFAA-N ± SE are presented from an n=3 for each marsh zone and depth. Note that the X-axis is log transformed.

to potential contamination in the DFAA and these numbers were not reliable. Urea-N varied by *S. alterniflora* zone ($F_{1,45}$=4.13, p=0.0499), with significantly more in the intermediate *S. alterniflora* zone, and concentrations increased by month ($F_{1,45}$=23.30, p<0.0001). More urea-N was available in the intermediate form ($F_{1,45}$=4.13, p0.0499). DON availability was greater in the short *S. alterniflora* zone ($F_{1,40}$=48.76, p<0.0001), and concentrations decreased by month ($F_{1,40}$=19.46, p=0.0001). NH_4-N was one to two orders of magnitude greater than DON below 10cm. NH_4-N availability did not vary by growth form (p=0.50), but increased with depth ($F_{2,45}$=14.11, p<0.0001), and was higher in September than May ($F_{1,45}$=60.72, p=0.0024).

Within S. alterniflora zone variation

In the tall form *S. alterniflora* zone, NH_4-N availability varied significantly by site ($F_{3,24}$=57.52, p<0.0001) and concentrations increased from May to September ($F_{1,24}$=7.90, p=0.012) (Figures 3-11 and 3-12). DON concentrations were greatest in May ($F_{1,17}$=4.68, p=0.031), but did not vary significantly by site (p=0.78). As a result, the ratio of DON:NH_4^+ varied seasonally ($F_{1,17}$=15.54, p=0.002), implying more DON was available to tall *S. alterniflora* plants early in the growing season. In the intermediate *S. alterniflora* zone, NH_4-N availability varied significantly by site ($F_{3,24}$=3.42, p=0.042) and concentrations were greater in September ($F_{1,24}$=18.01, p=0.0005). DON availability did not vary significantly by either site (p=0.14) or season (p=0.92), however, the ratio of DON:NH_4^+ was greater in May ($F_{3,23}$=5.72, p=0.0074) implying greater availability of DON to early in the growing season. Consistent with the tall and intermediate forms,

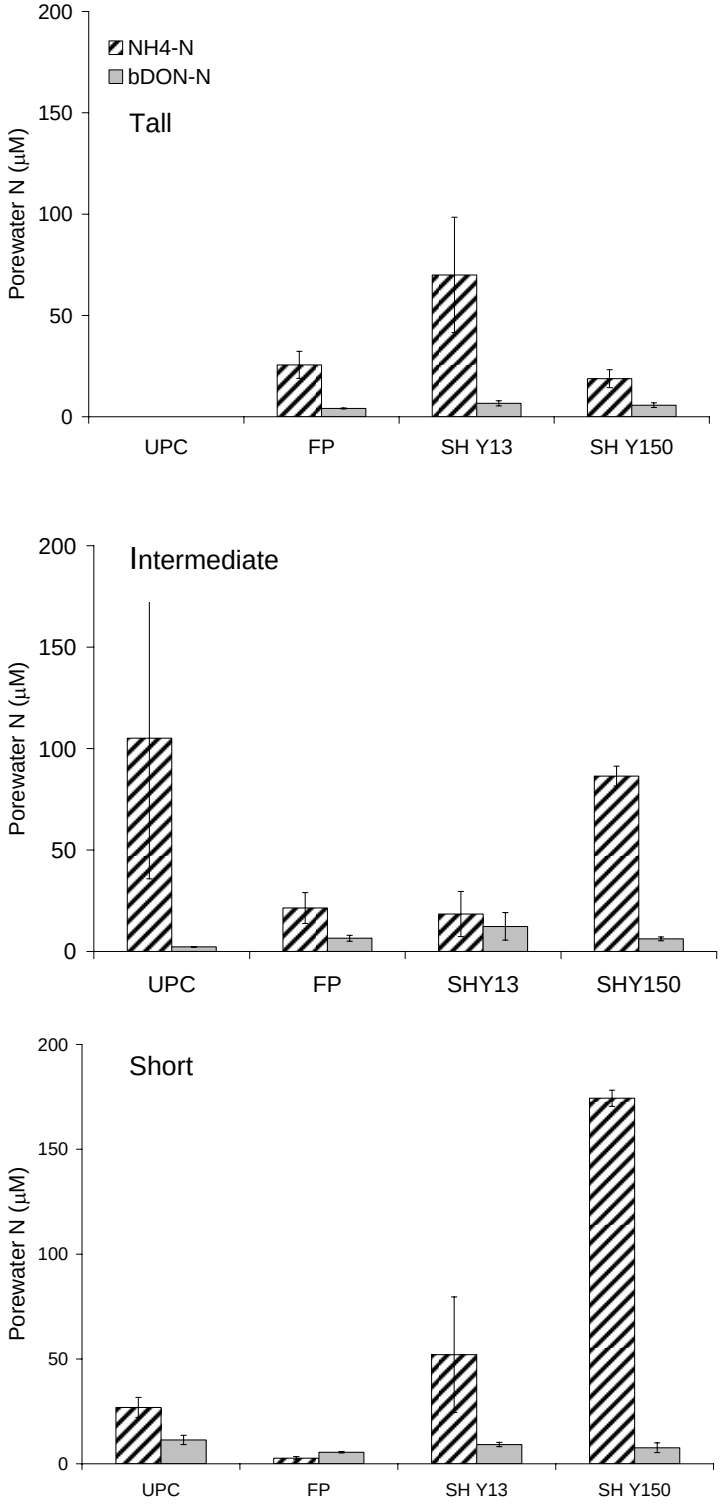

Figure 3-11. Mean porewater NH_4 and DON within VCR sites at the 10cm depth ± SE in May 2007. Figure (a) tall, (b) intermediate, and (c) short form *S. alterniflora*. n=3 for each marsh site.

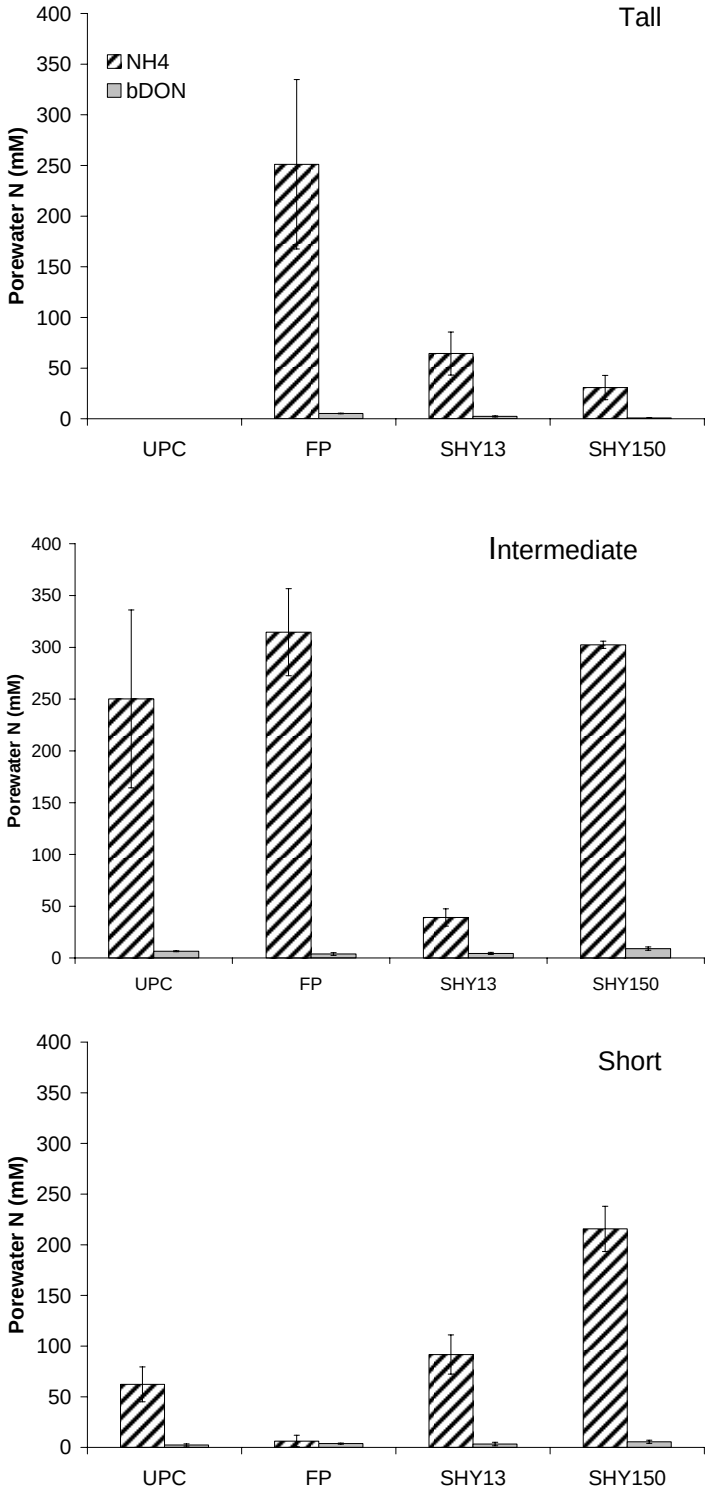

Figure 3-12. Mean porewater NH_4 and DON within VCR sites at the 10cm depth ± SE in September 2007. Figure (a) tall, (b) intermediate, and (c) short form *S. alterniflora*. n=3 for each marsh site.

NH_4-N varied by site ($F_{3,24}$=57.72, p<0.001), and concentrations were greater in September ($F_{3,24}$=7.90, p=0.012). More DON was available in May ($F_{3,24}$=3.42, p=0.042), with no significant differences among sites (p=0.378). The ratio of DON:NH_4^+ was greater in May than September ($F_{1,24}$=17.89, p=0.0006), implying greater availability early in the growing season.

Relationship of plant available bDON:DON

No distinct patterns of bioavailable DON (bDON) bBON:DON, a proxy for plant available DON was evident among sites or by plant zone (Table 3-4). Quantification of DFAA-N and urea-N is both time consuming and expensive when compared to routine analysis of NH_4-N. I had hoped to create a relationship to estimate pools of plant available DON measurements relative to DON measurements, however, but was not able to do so with my data.

Discussion

Plant available N within salt marshes of the VCR-LTER is highly variable based upon differences among site and *S. alterniflora* zone measured in this study. Among sites, NH_4^+ availability exhibits the greatest variation, and seasonal differences are consistent with previous work at these sites with higher availability in May than in September (Aiosa 1996, Walsh 1998, Tyler and Zieman 1999, Silliman and Zieman 2001). The lack of significant differences in DFAA, urea-N, and DON availability among sites at the VCR-LTER suggests spatial heterogeneity and that latitudinal patterns

Table 3-4. Composition of DON and the relationship bDON to NH_4 in May 2007 across sites. bDON:NH4 is the proportion of DFAA-N+urea-N to NH_4-N. %bDON:DON is the percentage of bDON to DON porewater. Values indicate mean ± SE. Note that bDON:NH4 was log transformed for analysis to satisfy ANOVA assumption. Statistical significance from ANOVA are presented for each variable by *S. alterniflora* zone.

Site	Tall		Intermediate		Short	
	bDON:NH4	%bDON:DON	bDON:NH4	%bDON:DON	bDON:NH4	%bDON:DON
UPC	n.a.	n.a.	0.07 ± 0.03	5.5 ± 0.9	0.46 ± 0.09	28.8 ± 6.5
FP	0.20 ± 0.071	8.0 ± 0.6	0.60 ± 0.41	5.0 ± 1.5	2.60 ± 0.90	5.7 ± 0.3
SH Y13	0.11 ± 0.026	24.0 ± 1.5	0.73 ± 0.26	20.5 ± 10.5	0.60 ± 0.47	33.7 ± 5.8
SH Y150	0.36 ± 0.121	12.7 ± 2.6	0.07 ± 0.01	3.7 ± 0.3	0.04 ± 0.01	16.0 ± 6.0
ANOVA p-value	0.20	0.0018	0.02	0.0	0.0015	0.0306

in microbial activity described in Chapter 2 control the relative availability of these N pools.

The ~50% seasonal decrease in DFAA-N may be attributed to a depletion of the pool from both plant uptake of DFAA (Chapter 2, Mozdzer et al. in review-b), and cumulative effect of higher summertime decomposition Urea-N showed opposite trends in availability, with greater availability in September. This difference may be attributed to the greater abundance and activity of resident invertebrates, *e.g. Uca spp.* and *Geukensia demissa* that release urea-N in waste material. Contrary to terrestrial ecosystem where urea-N is short lived, urea forms significant pools of bioavailable N in salt marsh sediments. In terrestrial ecosystems, urea is rapidly hydrolyzed by the urease enzyme which is ubiquitous in terrestrial soils (Mobley and Hausinger 1989). Salt concentrations in arctic salt marshes have been shown to decrease the activity of the urease enzyme (Wilson et al. 1999). Salt concentrations in mid-Atlantic salt marshes may be even higher due to greater rates of evapotranspiration, further limiting the activity of the enzyme resulting in the accumulation of urea-N in temperate salt marsh sediments.

While some of the spatial heterogeneity may come from differences in marsh zone, salt marsh sediments are extremely heterogeneous. The relative availability in NH_4-N drives the relative importance of the DON pools. Acknowledging that this study is limited by only one young marsh (~25 years old), my data suggests that marsh age may have an effect on the type of N available. The greater reported productivity in the younger SH Y13 marsh vs. SH Y150 marsh (Walsh 1998) may potentially be attributed to the greater availability and uptake efficiency of DON. DON uptake is energetically more efficient since the overall plant carbon cost of N acquisition is lowest for DFAA <

NH_4^+ <<NO_3^- (Clarkson 1986). As a result, more energy can be allocated to plant growth, rather than N acquisition. More research is needed to specifically address differences in N availability in young versus old marshes, and the potential implications for plant productivity.

Increased availability of DON in the intermediate and short *S. alterniflora* growth form zones may increase the relative dependence and utilization of this pool of N. The lower pools of DON in tall form *S. alterniflora* sediments may be attributed the greater relative heterotrophic utilization of DFAA, which decreases by almost 70% in the short form zone (Hanson and Gardner 1978). With increases in elevation on the marsh platform, physio-chemistry, and not necessarily N availability, affects productivity (Mendelssohn and Morris 2000). Associated with increases in NH_4^+ concentration is an accumulation of toxic H_2S which significantly decreases the rate and affinity for NH_4^+ uptake (Bradley and Morris 1991, Chambers et al. 1998) However, it is not known how sulfide affects DON uptake. It may be possible that DON uptake is not affected in the same way and NH_4^+. However more research is needed to investigate the interactions of H_2S on DFAA uptake rates.

I suggest that plant DON may be an important source of N in the early part of the growing season in mid-Atlantic salt marshes when plants are most actively growing and the relative abundance of DON to NH_4^+ is greater. Chapter 2 demonstrated that given a DON source, *S. alterniflora* will use it at similar rates to NH_4^+ *in situ* in both high- and mid-latitude salt marshes (Chapter 2). Thus, it is reasonable to suggest that mid-Atlantic *S. alterniflora* may up-regulate the process when the availability is greater early in the growing season. With increases in NH_4^+ availability, due to higher summertime net

decomposition, DFAA may not be an important source of N. Although bulk NH_4^+ concentrations may be high, this does not always indicative of high levels of productivity since high sulfide concentrations and low hydraulic conductivity in organic sediments may limit movement of N to the plant roots, limiting *S. alterniflora* growth (Walsh 1998, Mendelssohn and Morris 2000). Under this scenario, it is unclear how elevated H_2S will affect DON the uptake process. More importantly, research in needed to quantify plant available N in the rhizosphere since bulk porewater samples may not correctly reflect porewater nutrient concentrations in the rhizosphere (Long et al. 2008). I suggest that regional temperature regulated heterotrophic activity is responsible for the greater available pools of DON in May. If I assume that seasonal patterns at the VCR-LTER are consistent within all Atlantic coast salt marshes, I suggest that DON may comprise an important source of N early in the growing season, when NH_4^+ is limited by lower cumulative rates microbial activity.

Chapter 4

Interactions of grazing, nitrogen, herbivory, and *Spartina alterniflora* growth form in a mid-Atlantic salt marsh

Abstract

As one of the most productive ecosystem in the world, prevailing theory suggests salt marsh ecosystems are primarily controlled by bottom-up physio-chemical forcings. In recent years a new paradigm is emerging suggesting the potential for top-down control in some salt marsh ecosystems. However, the literature is conflicting with regard to the importance of top-down control on biomass and productivity. This paper presents the results of two nitrogen (N) fertilization experiments in both short and intermediate form *Spartina alterniflora* in a mid-Atlantic salt marsh that experienced different degrees of herbivory by the grasshopper, *Orchelimum fidicinium*. There was intense herbivory on the intermediate form N-fertilized plants, resulting in significant decreases in productivity and end of year biomass. Up to 79% of plant biomass may have been removed by grazing herbivores. In contrast, no significant increases in herbivory were observed in the short form *S. alterniflora* given identical N fertilization. These results suggest that nitrogen will not uniformly increase top-down forcing throughout the salt marsh, and that factors other than N content are responsible for differences in grazing among the different *S. alterniflora* growth forms.

Introduction

Salt marshes, the dominant intertidal habitat along the east coast of America, are among the most productive ecosystems in the world (Mitsch and Gosselink 1993, Pennings and Bertness 2001). These ecosystems are viewed as detritus-based food webs, with only an estimated 5-10% of carbon fixed by the marsh grasses entering the herbivorous food chain (Pennings and Bertness 2001). These biogenic systems, created and maintained by the organisms within them, are typically comprised of monocultures of halophytic plants. Salt marshes can be divided into zones along a gradient based on the salinity and plant communities. The low marsh, begins at the land-sea interface, and increases in elevation to the high marsh, which borders the upland terrestrial zone. *Spartina alterniflora* is the most common halophyte in salt marsh ecosystems along the North American Atlantic coast (Mitsch and Gosselink 1993). It varies in growth form depending on location within the salt marsh, with tallest forms in the low marsh and shortest forms in the high marsh. Salt marsh productivity is generally believed to be controlled by abiotic physio-chemical factors such as nutrient availability (Valiela and Teal 1979, Osgood and Zieman 1993a), salinity (Haines and Dunn 1976, Bradley and Morris 1991), and H_2S concentration (King et al. 1982, Bradley and Morris 1990). The abiotic forcings controlling plant primary production constitute the classical example of bottom-up control.

In the last decade, studies have documented top-down effects in some eutrophic salt marsh ecosystems (Silliman and Zieman 2001, Silliman and Bertness 2002, Bertness et al. 2008, Bertness and Silliman 2008). Grazing by invertebrates has been studied in

many salt marshes (Odum and Smalley 1959, Teal 1962, Parsons and Delacruz 1980, Vince et al. 1981, Bazely and Jefferies 1989, Silliman and Zieman 2001), and plant nutrient content is one factor that is believed to influence food choices by grazers (Valiela and Teal 1974, Bertness and Silliman 2008). Fewer studies have focused on grazing by insects (e.g., Planthoppers: *Prokelisia marginata*; Grasshoppers: *Orchelimum fidicinium*) despite the evidence of insect grazing damage in salt marsh communities. The marsh grasshopper, *O. fidicinium,* feeds almost exclusively on *S. alterniflora,* scraping material from the adaxial surface (facing the stem) of the middle portion of the leaf, and chewing the tips and sides. Grazing wounds can be from 1-15 cm long and 1-2 cm wide. Geographically, *O. fidicinium* is found in mid- and low-latitude salt marshes, with Virginia salt marshes as the northern most extent in their distribution (Wason and Pennings 2008). *P. marginata* are sap sucking insects, that feed along the middle portion of the leaves, and are found throughout *S. alterniflora's* range.

The literature is conflicting with regard to the importance of top-down control by grazing insects. An early study by Smalley (1960) estimated that *O. fidicinium* ingested less than 1% of the net primary productivity of *S. alterniflora*. More recently, Bertness et al.(2008) reported that insect grazers have the potential to remove 40-57% of the mean biomass in short form *S. alterniflora* and 66% of the mean biomass in tall form *S. alterniflora* in artificially nitrogen-enriched salt marshes. On the other hand, Johnson and Jessen (2008) found that even at a 10-fold increase in grasshopper (*Melanoplus* spp.) density, there was little loss in aboveground biomass in a New England salt marsh. Studies have shown that *P. marginata* did not exert top-down control in low-latitude Atlantic coast salt marshes (Gustafson et al. 2006), however, on Pacific Coast salt

marshes, reductions in biomass were attributed to an evolved loss of herbivore resistance (Grevstad et al. 2003). Latitudinal studies have shown that grazing pressure and the palatability of plants varies with increases in herbivore pressure with decreasing latitude (Siska et al. 2002, Pennings et al. 2009).

This paper presents the results of two independent fertilization experiments in short and intermediate growth form of *S. alterniflora* in a mid-Atlantic salt marsh. While our study was not specifically designed as an herbivory study, our methods allowed us to capture and quantify the effects of herbivory, and we present our findings as evidence that potential top-down control will not uniformly affect all *S. alterniflora* growth forms in mid-Atlantic salt marsh ecosystems as has been observed in New England salt marshes.

Methods

Two independent fertilization experiments were conducted during the growing season of 2006 on both intermediate and short form *S. alterniflora* in the lower Phillip's Creek marsh complex at the Virginia Coast Reserve VCR-LTER (Figure 4-1). An area of short *S. alterniflora* (in the high marsh (< 0.60 m height)) and intermediate *S. alterniflora* (in the middle marsh (> 0.6 m < 1.2 m)), within 50 m of each other, were chosen for the fertilization experiment. Within each zone, 9 plots were established (0.25 m^2 plots for intermediate, and 1 m^2 plots in short growth form) in a randomized complete block design (RCBD). Each of the plots (total 18 plots) received 1 of 3 treatments as follows: control, amino acid-N addition, and NH_4Cl addition, resulting in 3 replicates of each

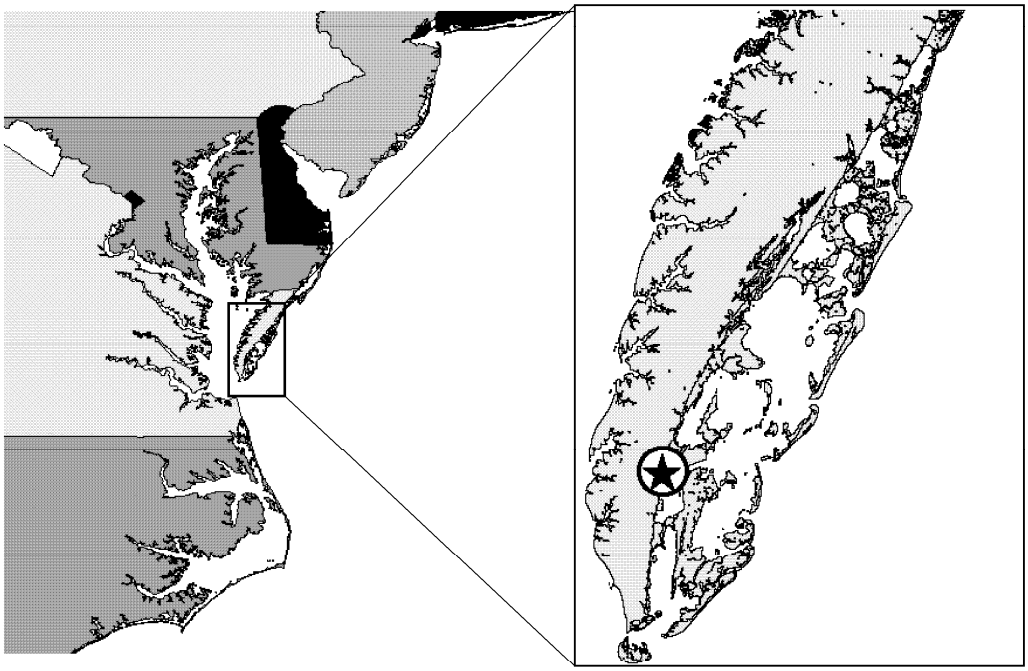

Figure 4-1. Field site location in Virginia, USA. Phillips Creek Marsh, identified by the star, is located within the Virginia Coast Reserve- Long Term Ecological Research site.

treatment in both short and intermediate *S. alterniflora*. Nitrogen applications were the equivalent of 42.6 g N yr^{-1}, which has been demonstrated to increase *S. alterniflora* productivity within the VCR-LTER ecosystem by over 400% (Silliman and Zieman 2001). Plots were enriched using 15-ml polypropylene centrifuge tubes, perforated with 16 holes, and containing the N-treatment wrapped in a nylon mesh (Williams and Ruckelshaus 1993). Fertilization was completed on May 15, June 15, and July 15, with control plots receiving empty vials.

Monthly productivity was assessed for intermediate form *S. alterniflora* only using the Morris and Haskin (1990) census method every 30 days on ~16th day of each month from May to September. In addition, in both *S. alterniflora* growth form experiments, a destructive harvest (0.0625 m^2 clip plots) was completed to estimate end of year biomass (EYB) on August 30th. Harvested plants were freeze dried, and analyzed for nitrogen content using an elemental analyzer (CE Instruments NA2500, Milan, Italy).

During the July productivity census in the intermediate form, we observed significant insect grazing and a measured decline in productivity within the N-treated plots. In order to quantify the effects of herbivory, grazing damage was recorded on August 1st, by assessing grazing on each stem within the 0.0625 m^{-2} productivity plot. The grazing damage was qualitatively ranked into one of three categories: low, moderate and heavy grazing. Low grazing was negligible, with not any visibly noticeable grazing damages. Moderate grazing included leaf shredding and/or scraping, without affecting the canopy structure of the plants. Heavy grazing included bent or missing leaves and reductions in plant height from the previous census, which resulted in changes in canopy

structure. All visible grazer damage was attributed to grasshopper grazing, since when present it is the most visibly damaging to the plants, and snail densities are low at this site.

Grasshopper densities present at this field site were quantified in a previous study in 2002 and 2003. Densities approximately 5 m^{-2}, ranging from 1 to 20 m^{-2}, in 2002 and 2003 (McGoff 2004). Snails were less than 2 m^{-2} (McGoff 2004).

Statistical analyses were completed using SAS 9.1.3 (Cary, NC). ANOVA tests were used to statistically measure the difference between all datasets. All N-treated plots were pooled due to lack of significant differences between the two N treatments. Nitrogen treated plots had n = 6 whereas control plots had n = 3. Graphs illustrate the mean values, with standard error bars to demonstrate the variance. Since we expected N to increase plant biomass and productivity *a priori*, one-tailed ANOVA was used in the short-form experiment at α=0.05. Since intermediate growth from *S. alterniflora* was impacted by grazing, these results were analyzed by two-tailed ANOVA at α=0.05. (Sokal and Rohlf 2001).

Results

Fertilization significantly increased (p = 0.05) plant tissue N content in both short (N-treated 1.85%) and intermediate (N-treated 1.55%) *S. alterniflora* when compared to control plots (1.07% and 0.96% respectively; Figure 4-2). Nitrogen content within control plots in both short and intermediate grasses were not significantly different from one another (p = 0.36). However, short-form N-treated plots had significantly higher tissue N

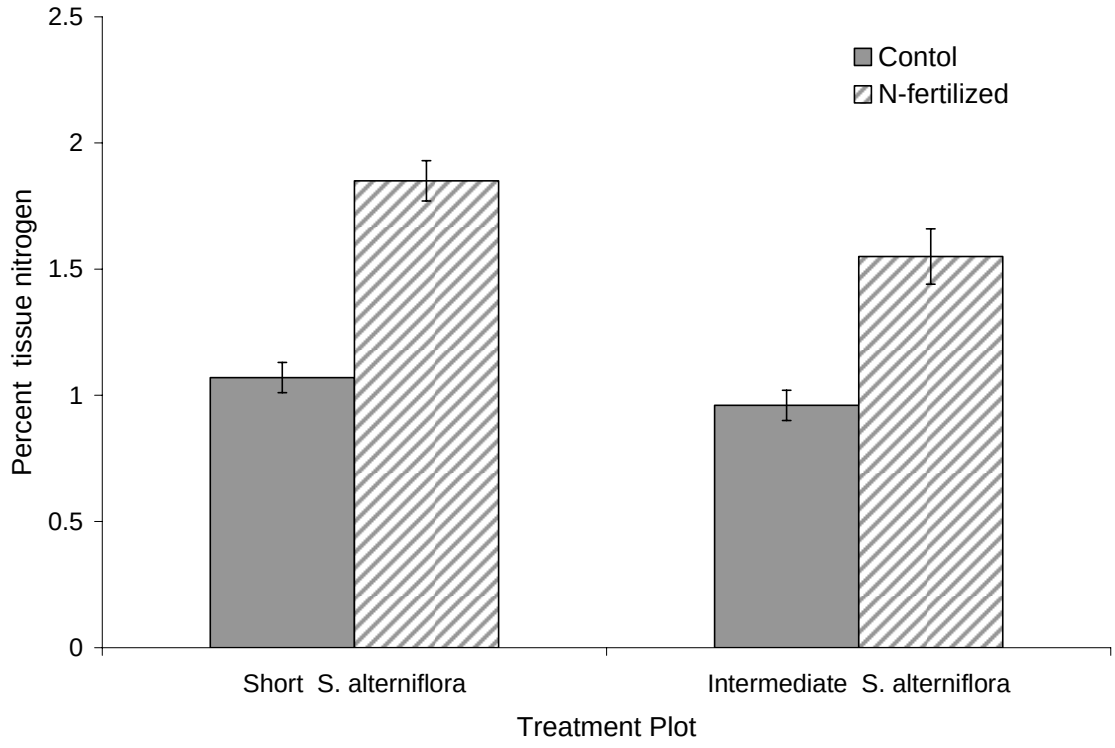

Figure 4-2. Tissue nitrogen percent in August for both short and intermediate form *S. alterniflora* in control and N-treated plots. ± 1SE. P < 0.005 for control vs. N-trt in both intermediate and short form; p = 0.36 for short vs. intermediate control plots; p = 0.05 for short vs. intermediate N-trt plots. Letters indicate significant differences.

than intermediate growth form ($p = 0.05$). The increase in plant N content reduced the C:N ratio's in both *S. alterniflora* growth forms similarly from 44.2 ± 3.3 and 40.2 ± 2.0 to 26.6 ± 1.7 and 23.2 ± 1.1 for intermediate and short forms respectively (Table 4-1).

End of year biomass (EYB) in short-form *S. alterniflora* increased significantly by 50% ($p = 0.097$; N-treated plots 478 g m^{-2}; control plots 323 g m^{-2}) (Figure 4-3). This increase in EYB in the short form resulted in it being comparable to the EYB of intermediate growth from *S. alterniflora* in this marsh. In contrast, EYB in intermediate *S. alterniflora* was reduced significantly ($p = 0.008$) by 47% in N-treated plots (247 g m^{-2} vs. control plots 447 g m^{-2}). EYB was significantly greater in N-treated plots in short-from *S. alterniflora* when comparing the two growth forms ($p = 0.003$).

Herbivory within the short-form *S. alterniflora*, was minimal (Low) in all plots, therefore these data are not presented. Intermediate-form *S. alterniflora* grazing was divided into three categories: Low, Moderate and Heavy. The two categories of Moderate and Heavy grazing were combined, indicating the "Total" amount of plants that were negatively affected by grazing. Control and N-treated plots had significantly different levels of grazing in low, moderate and total grazing categories but not in the heavy grazing category (Low: $p = 0.02$, control 74%, N-trt 15%; Moderate: $p = 0.02$, control 11%, N-trt 54%; Heavy: $p = 0.15$, control 14%, N-trt 30%; Total: $p = 0.02$, control 26%, N-trt 85%) (Figure 4-4).

Monthly productivity was assessed in intermediate-form *S. alterniflora*. The control (dashed) line illustrates productivity of the grasses through the summer, with a decline in August as the grasses start to senesce (Figure 4-5). Predicted productivity is illustrated using Silliman & Zieman (2001) estimates assuming snail densities of 48 m^2

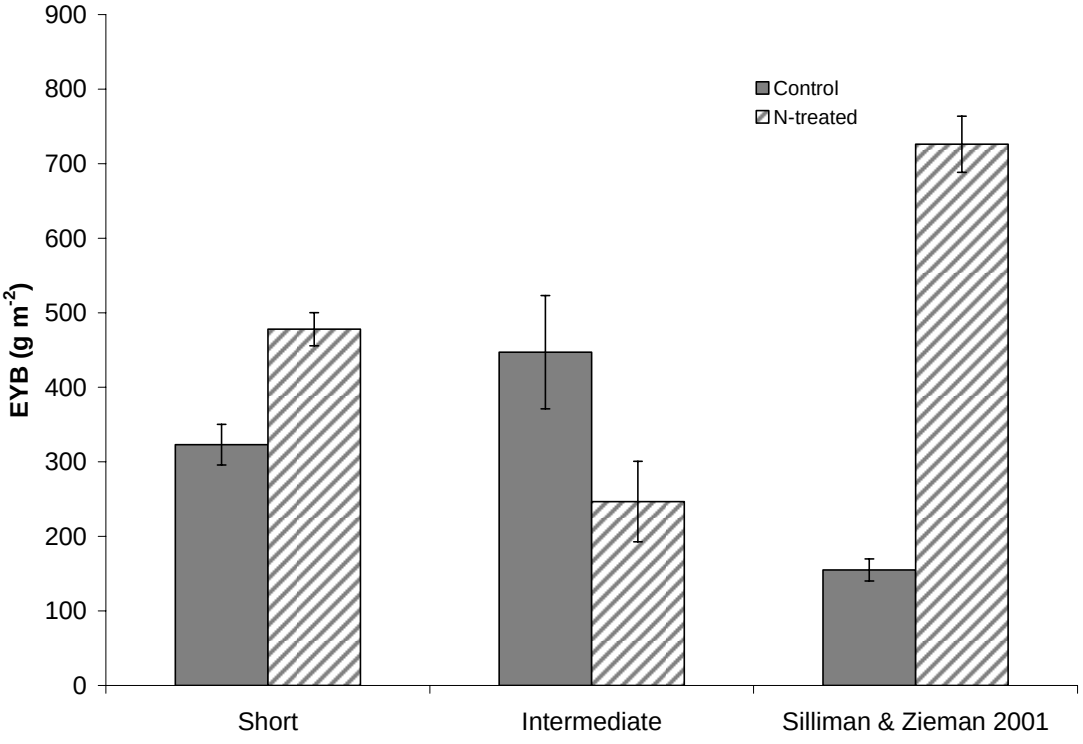

Figure 4-3. Treatment effects on end of year biomass (EYB) in g m^{-2} ± 1SE. P = 0.008 for intermediate form control vs. N-treated plots; p = 0.049 for short form control vs. N-treated; p = 0.003 N-treated plots in short and intermediate form.

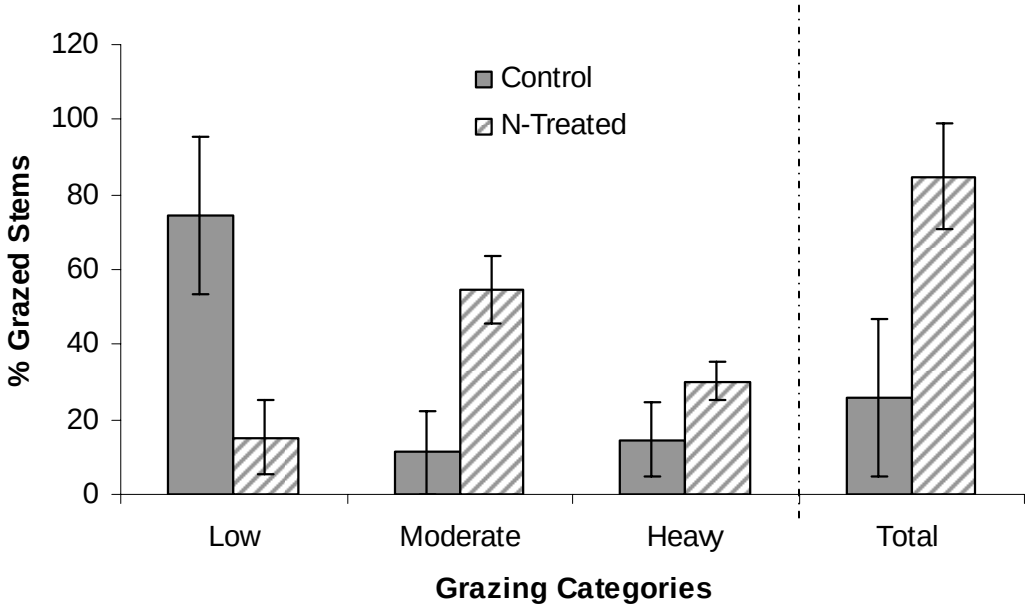

Figure 4-4. Percent of stems grazed in intermediate *S. alterniflora*. ± 1SE. Total represents the sum of moderate and heavy grazing, the vertical line illustrates the separation of this group from measured data. All grazing categories, except heavy, had significantly different control grazing versus N-treated grazing (Low p = 0.021, Moderate p = 0.024, Heavy p = 0.149, Total p = 0.02).

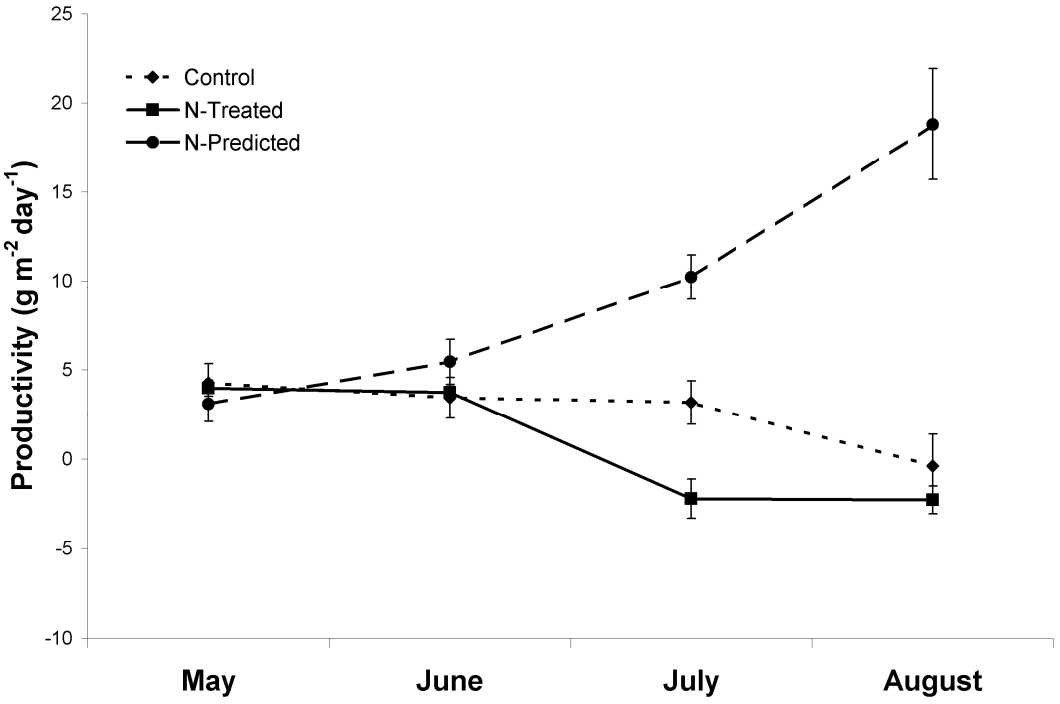

Figure 4-5. Monthly productivity (g m^{-2} day^{-1}) in intermediate form *S. alterniflora*. The upper line is predicted productivity using data from Silliman (2001), the other two lines illustrate measured productivity in control and N-treated plots. ± 1SE.

Table 4-1. Mean (± SE) C content and C:N ratio for short and intermediate form *S. alterniflora.*

	%C	**C:N**
Short *S. alterniflora*		
Control	42.3 (0.9)	39.9 (2.0)
N-fertilized	42.6 (1.1)	23.2 (1.1)
Intermediate *S. alterniflora*		
Control	42.0(0.8)	44.2(3.3)
N-fertilized	40.5 (0.5)	26.6 (1.7)

(ambient density treatment). The predicted loss in productivity is very conservative, since at this density (25× ambient) estimate, since snails are not a significant components of this marsh. Predicted productivity increased throughout the summer, peaking in August. *S. alterniflora* productivity started at approximately 4 g m^{-2} day^{-1}, in May and June during the onset of the growing season. The N-treated plots showed a strong decline in *S. alterniflora* productivity in July and August. This decline in productivity is directly attributed to the removal of biomass due to grazing. The difference between the predicted N-treated productivity and measured N-treated productivity ranges from a loss of anywhere from 14.4 g biomass m^{-2} d^{-1} in July, to a maximum loss of 21.1g biomass m^{-2} d^{-1} in August.

Discussion

There is conflicting evidence in the current literature for top-down control by grazing consumers in New England salt marshes. While Bertness et al. (2008) reported consumer control in eutrophic Rhode Island salt marshes, Johnson and Jessen (2008) concluded that even at 10 times the ambient densities, grasshoppers (*Melanoplus* sp.) demonstrated weak consumer control of *S. alterniflora* in a Massachusetts salt marsh. The literature suggests that as N content within plant tissues increases, herbivory will also increase in all *S. alterniflora* growth forms (Bertness et al. 2008). In our study, fertilizing the marsh with N significantly increased the tissue N content in both short and intermediate *S. alterniflora* and thus we would expect increases in herbivory in both growth forms. While N fertilization did result in significant grazing losses in the

intermediate form with N enrichment, we did not observe an increase in grazing with N enrichment in the short form.

While we expected N fertilization to increase both EYB and plant N content in intermediate form *S. alterniflora,* as was observed in the short form, , we observed a significant (47%) reduction of EYB relative to control plants. We attribute this dramatic reduction of approximately 200 g m^{-2} to insect grazing since periwinkle densities were less than 2 m^{-2}.. Silliman & Zieman (2001) reported a 460% increase in EYB given identical levels of N fertilization in another VCR marsh at 4 times our ambient snail densities (155 g m^{-2} to 726 g m^{-2}). If we assume no absolute maximum biomass and use this same 460% increase, our biomass could have approached 2000 g m^{-2} with N-treatments. Our control values (447 g m^{2}) are 3 times greater than Silliman and Zieman (2001) control values. Other authors (Valiela and Teal 1974, Gallagher 1975, Chalmers 1979, Mendelssohn 1979, Dai and Wiegert 1997) have reported similar marsh EYB, ranging from 348 to 471 g m^{-2} that have responded to N-fertilization by increasing EYB 650 to 1800 g m^{-2}. Restricting the reference data geographically due to different biogeographic ecotypes (Seliskar et al. 2002) and cpDNA genotypes (Blum et al. 2007), North Carolina salt marsh EYB increased 400% (to 1800 g m^{-2}) (Broome et al. 1975). Assuming a similar 400% increase in biomass due to fertilization (447 to 1786 g m^{-2}), and based upon our measured 247 g m^{-2} in N-fertilized plants, approximately 1540 g m^{-2} (86%) of biomass was consumed by grazing herbivores.

N-fertilized intermediate *S. alterniflora* was preferentially grazed by grasshoppers. Low levels of grazing in the non-fertilized control plants (~25%) is similar to values previously reported (Smalley 1960). Increased grazing in N-treated intermediate

form (~84% of plants) is likely attributed to the 50% greater nitrogen content, and we suggest that once herbivores found N-rich plants, they continued grazing these N-rich plants to optimize their foraging strategy. Grasshoppers are highly mobile grazers, unlike snails, and their foraging strategy has not yet been investigated. Such localized, severe grazing events have previously been observed in the same field site on N-fertilized plants growing in pots 2 decades earlier (R. Chambers, *pers comm.*). Instances of extreme grazing have been observed in New Jersey salt marshes where *S. alterniflora* was grazed down to bare stems (N. McGoff, *pers. observation.*).

Grasshoppers in mid-Atlantic salt marshes have the ability to remove up to 80% of aboveground biomass. Comparing our measurements to those of Silliman and Zieman (2001), grazing herbivores reduced plant productivity by at least 12.4 g biomass m^2 day^{-1} in July, and 21.1 g biomass m^2 day^{-1} in August (Figure 4-5). This theoretical loss in productivity corresponds to approximately 1000 g, or 80% of biomass directly consumed by herbivores. By using two different parameters to assess grazing loses we came to comparable results; 1000 g based on reduced productivity and 1600 g based on EYB.

Interestingly, we did not observe significant effects of grazing in the short form *S. alterniflora* given identical N-fertilization and significantly greater N content. This is contradictory to the data presented by Bertness et al. (2008) in an experiment that added approximately 70% more N to the salt marsh, which was not intended to mimic any natural scenarios. Our experimental N additions constitute the high end of potential N loading from anthropogenic sources (Nixon et al. 1996). In our experiment, total above ground N in short form *S. alterniflora* was 8.8 g N m^2 (plant N content × EYB), which according to according to Bertness et al.'s (2008) correlation model of total aboveground

N and insect damage, more than 85% of our plants should have been negatively affected by grazing. This is not the case in mid-Atlantic salt marshes, and suggests that elevating N alone does not increase the palatability or herbivory of short form *S. alterniflora* in mid-Atlantic salt marshes.

Since N fertilization did not stimulate herbivory, differences in palatability or grazer type may explain our results. Previous studies investigating the palatability of *S. alterniflora* to grazers have only investigated how *S. alterniflora* palatability varies across latitude (Pennings et al. 2001, Siska et al. 2002, Pennings and Silliman 2005, Pennings et al. 2009), but have not addressed how palatability may vary among the short, intermediate, and tall growth forms. Short form *S. alterniflora* is a stunted, stiff, tough plant, less succulent than the taller counterpart. Leaf toughness is a factor known to affect the palatability of *S. alterniflora* along latitudinal scales (Siska et al. 2002). We found it uncommon to find grasshoppers grazing the short form of *S. alterniflora* in a natural salt marsh ecosystem (*personal observation*) and it appears from this experiment that increasing the N content, or lowering the C:N ratio of the short-form *S. alterniflora* did not increase its palatability to grasshoppers in mid-Atlantic salt marshes. A change in grazer species may potentially explain the lack of short-form grazing when compared to New England salt marshes. Above 37° latitude, there is a shift in grazer dominance from *O. fidicinium* to *Conecephalus spartinae* (Wason and Pennings 2008). However, grazing patterns between the two species have not been identified. In eutrophied New England salt marshes, Bertness et al (2008) suggested that planthoppers (*Prokelisia* spp.) were primarily responsible for grazing damage, however, planthoppers exhibited little top-down control in southern salt marshes (Gustafson et al. 2006). The potential differences

in planthopper grazing may potentially be attributed to differences in palatability in low vs. high latitude *S. alterniflora* (Siska et al. 2002).

While our small-scale experiments did show a dramatic effect of herbivory, we suggest that the effects of grazing can vary greatly both spatially and temporally. Specifically, N enrichment did not increase grazing in the short-form *S. alterniflora* which was located only 50 m away from the intermediate fertilized plots. By creating artificially N-rich plants in a pristine habitat, we potentially altered normal grazing patterns and data must be interpreted cautiously. However, the data suggests that highly mobile grazers, such as grasshoppers, may optimize their foraging strategy and target localized areas of N-rich plants.

Historically, grasshoppers have been described as ingesting less than 10% of the primary productivity of a marsh (Smalley 1960, Johnson and Jessen 2008). On an ecosystem level, and over the long term, Smalley's estimate may still be a representative value. While intensity of grazing pressure and grazer density may vary from year to year, even given 10 times ambient density, grazers were found to exert little top-down control (Johnson and Jessen 2008). The VCR-LTER complex, a relatively pristine ecosystem, falls between the extremes of little consumer control in New England marshes, to high consumer control in southern salt marshes based upon latitudinal patterns in herbivory (Pennings et al. 2001, Pennings et al. 2009). However, salt marsh ecosystems are currently under great threat from anthropogenic impacts, eutrophication, coastal development, and relative sea level rise (Hopkinson and Giblin 2008). The observed contradiction in herbivore response between the two growth forms suggests that factors other than nutrients are responsible for top-down control, and that herbivore grazing is

spatially heterogeneous. Due to conflicting results in the literature, we suggest that future N fertilization studies should mimic realistic N loading scenarios to ensure that observations are applicable to future conditions. Caution should be used whenever interpreting results of small-scale N fertilization experiments due to the creation of an alternate food source in the environmental that has the potential to alter normal grazing patterns. We suggest that future studies should control for grazer density to create a realistic understanding of the complex interactions of primary producers and grazing herbivores. Finally, more research is needed, since it is not known how large scale eutrophication will affect herbivore densities and/or their predators.

Chapter 5

Importance of shoot nitrogen uptake by *Spartina alterniflora*

Abstract

The smooth cordgrass, *Spartina alterniflora*, is the foundation species in intertidal salt marshes of the North American Atlantic coast. Depending upon its elevation within the marsh, *S. alterniflora* may be submerged several hours per day. Previous ecosystem-level studies have demonstrated that *S. alterniflora* marshes are a net sink of N, and that removal of nitrogen (N) from flooding tidal water can provide enough N to support the aboveground biomass. However, studies have not specifically investigated if *S. alterniflora* plants assimilate nutrients through their aboveground tissue. To test this hypothesis, we determined *in situ* foliar and stem N uptake kinetics for $^{15}NH_4$, $^{15}NO_3$, and ^{15}N-glycine by artificially flooding plants in a mid-Atlantic salt marsh. To determine the importance of this process, a model was created to estimate time of inundation by *S. alterniflora*, in 20 cm height intervals, from April to September. Estimates of inundation time, plant biomass, N-uptake rates, and N availability from long-term data sets were used to model the seasonal importance of foliar N uptake. Aboveground N-uptake rates (leaves + stems) were the greatest for NH_4^+ > glycine > NO_3^- Our model suggests that shoot N uptake may satisfy up to 25% of seasonal N demand in mesotrophic mid-Atlantic salt marshes, and the importance of this process is largely dependant upon plant elevation and water column N availability. In eutrophic estuaries, our model suggests that shoot uptake may contribute up to 75% N demand assuming similar elevation and elevated N concentrations.

Introduction

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

Although intertidal salt marsh grasses may be submerged for several hours a day with nutrient containing tidal water, it is not known to what extent shoot uptake (leaf + stem) may contribute to *S. alterniflora* N demand. In sub-tidal estuarine environments, it is known that seagrass assimilate N through their shoots since N root uptake may be limited by oxygen availability and H_2S toxicity (Stapel et al. 1996, Lee and Dunton 1999). Shoot N uptake varies dramatically by species, and can account anywhere from 50% of plant N demand in *Thalasia testudinum* (Lee and Dunton 1999) and *T. hemprichii* (Stapel et al. 1996) to the extreme example of *Phyllospadix torreyi* that can satisfy 100% of its N demand through foliar uptake (Terrados and Williams 1997). The relative importance of this process is largely dependant upon 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 the water column (Burdige and Zheng 1998, McGlathery et al. 2001), shoot uptake may be a more energetically efficient alternative. These same anaerobic conditions in the rhizosphere also limit N uptake in *S. alterniflora* salt marshes (Bradley and Morris 1990, Chambers et al. 1998, Mendelssohn and Morris 2000) and shoot uptake may be a similar advantage to intertidal salt marsh plants.

Shoot N uptake, and the water column pool of N have been an overlooked factor which have the potential to contribute to the increased *S. alterniflora* productivity in the low marsh. Historically, differences in N growth form have been attributed to differences in sediment physio-chemistry and flooding regimes (Mendelssohn and Morris 2000). Changes in phenotype form the tall growth from at the creek bank, to intermediate and short form with increasing in elevation, is generally attributed to a more reduced environment, decreasing N uptake rates (Bradley and Morris 1990, Chambers et al. 1998, Mendelssohn and Morris 2000). Additionally, plants must invest substantially more energy and root biomass to ameliorate the more anoxic reduced sediments (Mendelssohn and Morris 2000), reducing their aboveground productivity. Increased tidal inundation in the low marsh, exposes the plants to tidal water longer, resulting in a greater potential for shoot N uptake to stimulate productivity.

Flume studies in Atlantic Coast marshes have demonstrated that salt marshes are predominantly a net sink for NH_4^+, NO_3^-, and NO_2^-, causing a net removal of N from the water column (Lee 1979, Wolaver 1981, Wolaver et al. 1984, Wolaver and Zieman 1984, Whiting et al. 1989, Childers et al. 1993), and they can also be a source (Wolaver et al.

1984, Childers et al. 1993) or sink (Wolaver and Zieman 1984, Childers et al. 1993) of dissolved organic nitrogen (DON). The amount of N removed from the flood tide can supply up to 100% of the N needed to support the biomass in tall form *S. alterniflora* and anywhere between 30 to 105% of the N needed for intermediate form *S. alterniflora*, however, the mechanism for removal was not investigated (Wolaver and Zieman 1984). Bouma et al (2002) is frequently cited as showing that shoot uptake by *Spartina anglica* can at most contribute approximately 10% of plant N demand. This estimate this was based upon the premise that plants were only submerged for 2.4 hours a day and constant uptake rates that did not vary by month 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 and Reed 2001), and are inundated only during the highest stages of a tidal cycle. North American Atlantic coast tidal marshes generally have elevations decimeters below mean high tide (MHT), and periods of inundation can vary greatly depending upon the tidal range and location within the marsh platform (Morris et al. 2005). For example, estimates from Drake et al (2008) indicated that tall *S. alterniflora* in Massachusetts may be submerged for 8.9 hr day^{-1} whereas short *S. alterniflora* is submerged for 3.0 hr day^{-1}.

Given the potentially long periods of tidal inundation in Virginia tidal marshes, and previous work demonstrating significant removal of N from the water column during flood events, we investigated the importance of shoot (leaves + stems) N uptake from tidal water. We measured uptake rates for $^{15}NH_4^+$, $^{15}NO_3^-$, and ^{15}N-glycine to determine the uptake kinetics of inorganic and organic N shoot uptake separately for both stem and leaf tissue. We then created a numerical model to determine the importance of shoot

uptake in satisfying *S. alterniflora*'s N demand using modeled vegetation growth and inundation, long-term water quality data, and experimentally-determined uptake rates.

Methods

Plant N assimilation rates

The field study was conducted within the Virginia Coast Reserve (VCR) Long Term Ecological Research (LTER) at a saltmarsh near Oyster, VA (Figure 5-1). Salt marshes along the eastern shore of Virginia are predominantly meso-tidal, and the tidal range at Oyster Harbor is about 1.5 m. Field experiments to determine *in situ* N uptake rates were conducted on intermediate growth form (60-130 cm) *S. 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 approximately 100 cm by the end of the growing season. Methods of this study were adapted from those of Bouma et al. (2002). Nitrogen uptake experiments were conducted daily between the hours of 10 am and 2pm to minimize any potential diel variation. This time period also corresponded to low tide and allowed us to flood the plants artificially and uniformly. Each experimental unit consisted of an *in situ* mesocosm where plants within a 78.5 cm^2 area of marsh (corresponding to 2.7 ± 0.1 plants per mesocosm) were artificially flooded during a low-tide event. A 15 cm long x 15.25 internal diameter (*i.d.*). cm section of PVC was placed around the *S. alterniflora* plants and inserted into the sediment to a depth of approximately 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 approximately 5 cm. Immediately following the addition of agar, a tall, clear acrylic

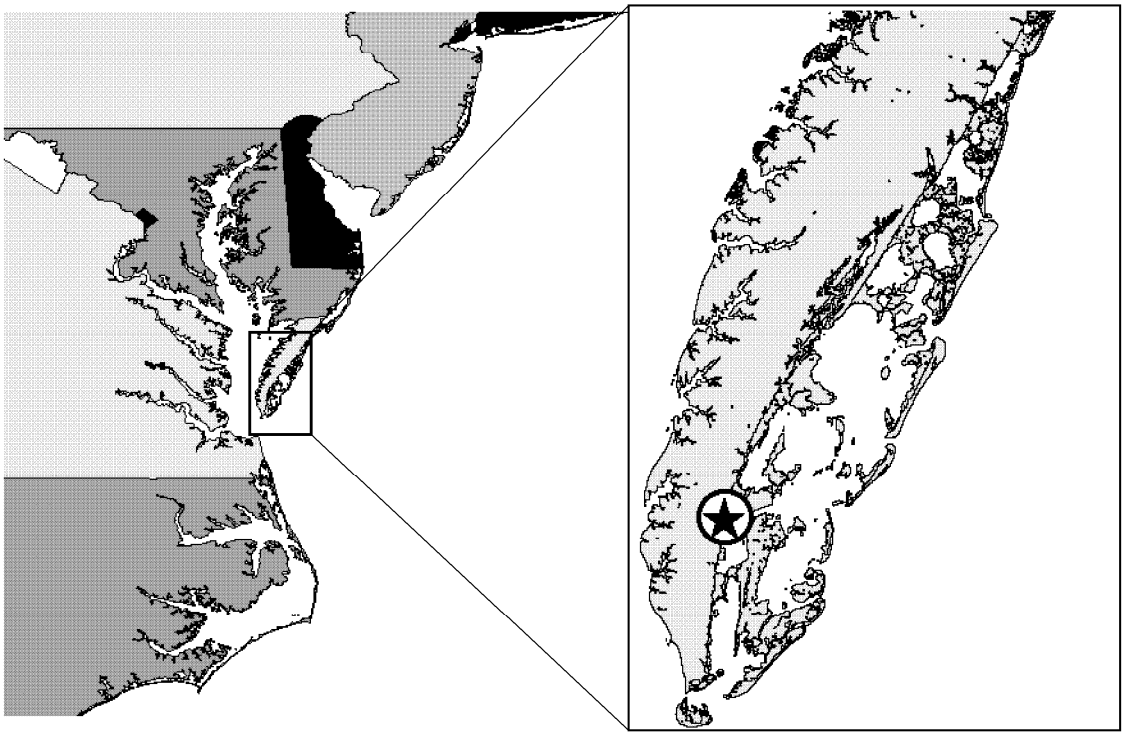

Figure 5-1. Location of VCR-LTER Oyster transect on the Eastern Shore of Virginia, USA.

cylinder, 100cm x 10 cm *i.d.* was immediately placed over the vegetation, through the unsolidified agar, and inserted about 5 cm into the ground to prevent tipping. Solidified agar formed a seal from the soil surface, and the cylinder was filled with 10 liters of synthetic seawater with the following composition (24.99 g $NaCl_2$, 4.16 g Na_2SO_4, 0.79 g KCl, 11.3 g $MgCl_2 \cdot 6H_20$, 1.35 g $CaCl_2 \cdot 2H_20$) per liter mimicking *in situ* seawater. This volume of water completely submerged the plants at this location. A concentrated ^{15}N tracer was added to the volume of water to adjust the starting N concentration to either 1, 5, 10, or 100 μM. Plants within the mesocosm were then exposed to one of three N treatments, $^{15}NH_4Cl$, $^{15}NO_3$, and ^{15}N glycine (> 99.8 % ^{15}N Cambridge Isotope Labs, Cambridge, MA) at the concentrations given above. There were 60 individual replicate mesocosms (n=5 N treatment^{-1} N concentration^{-1}). However, when leakage exceeded 10% of the initial volume, the replicate was discarded (n=7). This resulted in a corrected total of 53 replicates (n=4 for several N treatment^{-1} concentration^{-1}) used to determine N uptake rates.

To ensure a well-mixed medium, each mesocosm was aerated using a battery powered pump. After 90 minute exposure to the ^{15}N tracer, 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 three successive 10 liter containers filled with identical synthetic sea water (no nutrients) to remove any adsorbed label. *S. alterniflora* was carefully blotted dry in the field, placed on ice, and transferred to the lab for processing. In the lab, plants were separated into leaf and stem portions, and freeze dried for sample preservation. Plant biomass was homogenized to a fine powder using a

ball mill, and these samples were analyzed at the UC Davis Stable Isotope Facility on an Europa Integra continuous flow mass spectrometer for their ^{15}N determination.

N uptake rates were determined using modified equations of Bouma et al, where:

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

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

$$V_{upt} = (^{15}N_{assimilated} \times 10^3)/ (DM \times t_{exp}) \quad (3)$$

where N_{total} is the total amount of N (g) in the biomass; %N is the concentration of N in the biomass (g N/ g DM); DM is the dry mass (g) of the representative biomass; $^{15}N_{assimilated}$ is the amount of ^{15}N (g) assimilated into the representative biomass, $\%^{15}N_{trt}$ is the ^{15}N content on a mass basis after a 90 minute exposure to ^{15}N treatment; $\%^{15}N_{control}$ is the ^{15}N natural abundance content on a mass basis of plants not exposed to ^{15}N treatments. V_{upt} (gN gdw^{-1} hr^{-1}), is the uptake rate of ^{15}N from flood water normalized to leaf or stem biomass. DM, is the dry mass (g) of the biomass, and t_{exp}, is the time of exposure to the ^{15}N treatments (90 min).

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 using Proc NLIN in SAS (Version 9.1, Cary, NC). Parameter estimates were obtained using the measured uptake rates at each concentration in the Michaelis-Menten equation (equation 4). From these parameters, it is possible to predict the N-uptake rate at any given N concentration within our range.

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

V is the experimentally determined N uptake rate (g N gdw^{-1} hr^{-1}) at a given nutrient concentration, S (μmol l^{-1}); V_{max} is the modeled maximum uptake rate (gN gdw^{-1} hr^{-1}); and K_m is the experimentally determined half-saturation constant (μmol l^{-1}).

Two hundred and fifty plants were harvested in May and August 2006 to create an allometric relationship between plant height and biomass (Appendix D). A subsample (n= 160) of these plants was used to determine the allocation of leaf and stem biomass in 20 cm intervals in one of five plant size classes: 0-20 cm, 0-40 cm, 0-60 cm, 0-80 cm, 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 N foliar uptake, we created a numerical model to compare foliar uptake with total N demand as a function of flooding duration, nutrient (NH_4^+, NO_3^-, DON) concentrations in the water column, and vegetation growth. We calculated inundation duration, biomass distributions (stem vs. leaf), and uptake rates for the 5 segments of a single plant height (0-20 cm, 21-40 cm, 41-60 cm, 81-100 cm). Since the elevation on the marsh platform directly influences inundation time, we estimate the % N demand that can be attributed to shoot uptake for intermediate *S. alterniflora* within a 40cm elevation range at elevations of -20 cm and +20 cm relative to mean sea-level. These elevations correspond to the range of intermediate growth form *S. alterniflora* in the marsh at Oyster (Appendix E), and correspond roughly to the theoretical growth range of the intermediate form given our tidal range (Mckee and Patrick 1988). We do not present estimates for shoot uptake for the tall form (> 1.5m in height) which grows at lower elevations along the creek bank since allometric

relationships between stem and leaf biomass are only valid to 100cm in height, the maximum height of intermediate form *S. alterniflora.*

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

$$W(t) = A\sin(\frac{2\pi}{\lambda}t) + \eta \qquad (5)$$

where A represents half of the tidal range (0.75m), λ =12.5 hours, and η is the elevation of the marsh surface relative to mean sea level (MSL) (m). In the model, plant height (*h*) increases at a constant rate throughout the growing season (1.95E-4 m/hr), and inundation occurs when w(t) exceeds the height of a given segment.

Plants take up N from the water column at rates proportional to the concentration of N according to the Michaelis-Menten equation:

$$V(t) = \frac{v_{\max}[S(t)]}{K_M + [S(t)]} \qquad (6)$$

where v_{max} (g N / g biomass) equals the uptake rate at saturation and K_M is the Michaelis-Menten constant (Table 5-1). We consider 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 tissue, and long-term measurements of average monthly concentrations of each N species in the Oyster sub-estuary from VCR-LTER datasets VCR99057 and VCR08143 (www.vcrlter.virginia.edu) (Figure 5-2). The three sites, Cob Mill Creek (CM), Oyster harbor (OH), and Ramshoal Channel Creek (RC) represent a nutrient gradient from terrestrial groundwater N inputs to lagoonal salt marshes and thus reflect differences in N

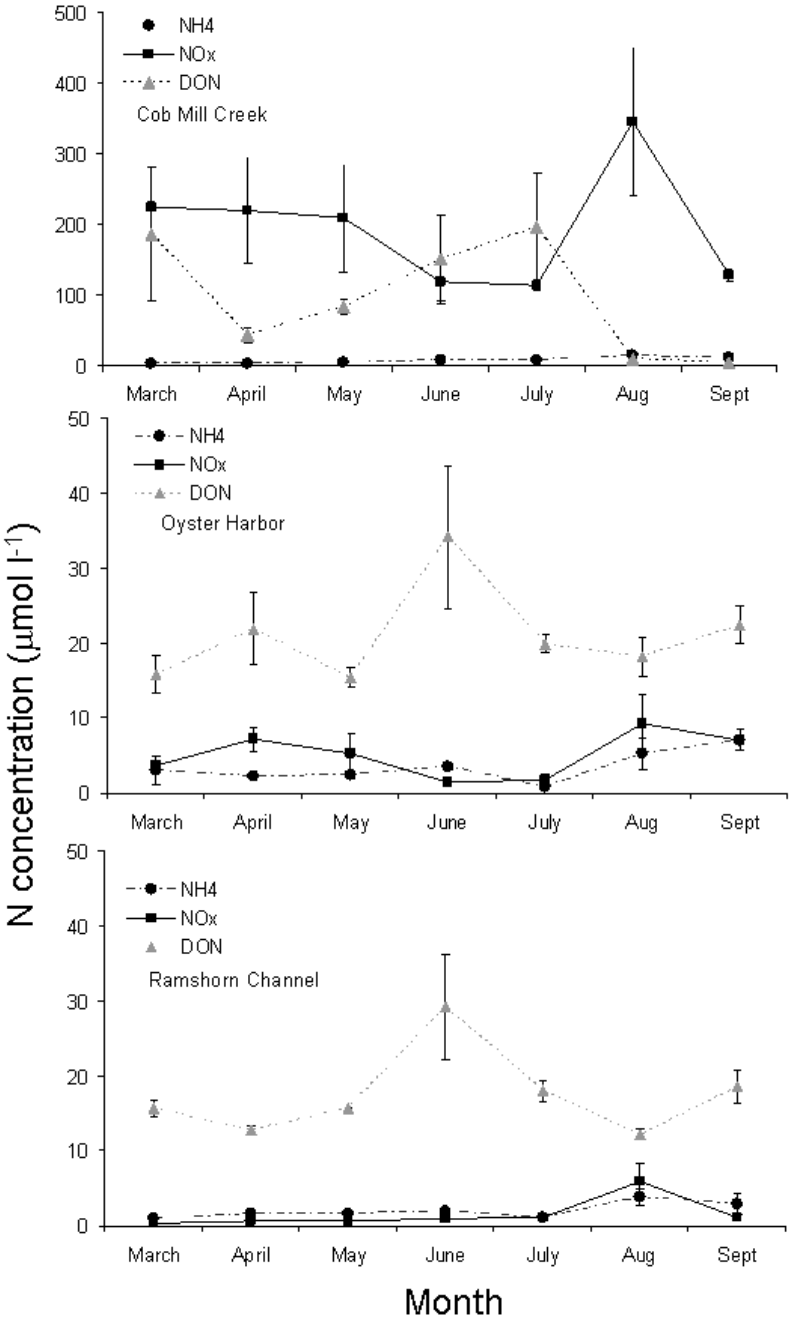

Figure 5-2. Mean NH4+, NOx, and DON availability (mol l-1) ± SE from long term water quality data for Cob Mill Creek (CM), Oyster Harbor (OH), and Ramshorn Channel Creek (RC) from 2004-2008 accessed from the LTER dataset VCR99057 and VCR08143. DON was estimated by subtracting NH4+ and NOx data from reported TDN values.

availability in the water column. We assume that plants can utilize only about 20% of total DON (Tyler et al. 2003), and that glycine uptake rates are indicative of all DON species. Mozdzer et al. (in review-b) reported no significant differences in root DON uptake given three different DON treatments, and we are assuming this is the same for shoot uptake.

The fraction of N demand (γ) that can be accommodated by foliar uptake can therefore be expressed as:

$$\gamma = \int^{t} \int^{h} \frac{I(h,t) \cdot B(h,t) \cdot V(h,t)}{bC} dhdt \qquad (7)$$

where *B(h,t)* and *I(h,t)* represent the biomass and inundation duration at a particular height on the plant through time. The plant's N demand is equal to the product of its growth rate *b* (g/hr) and the concentration, *C*, of N within its tissue (0.013 gN / g leaf and 0.074 gN / g stem). Although we assume a linear growth rate of plant height, with peak height and biomass attained in September, our allometric relationships yield plant biomass that increases non-linearly throughout the growing season. We solve (*3*) numerically by calculating uptake rates in 20 cm segments of the total plant height using time steps of 1 hr, and calculate biomass and N uptake rates for stems and leaves, and in each plant height segment separately.

Results

Plant N uptake

In all treatments, leaves had greater uptake rates than stems, and the greatest uptake rates were observed in NH_4^+, with subsequent decreases in both the DON and the NO_3^- treatment (Figure 5-3). When the experimentally-determined rates were fit to the Michaelis-Menten model, V_{max} for NH_4^+ was anywhere from 500 to 800% greater than either DON or NO_3^- respectively (Table 5-1). Leaf N uptake rates were between 240% to 292% greater than stem uptake rates within the same N treatment (Table 5-1), which may in part be attributed to a greater surface area: volume ratio when compared to stem biomass.

Plant Biomass & Inundation Time

Biomass allocation of stems and leaves varies greatly as a function of plant height (Table 5-2). In plants up to 40 cm in height, less than 30% of the biomass was allocated to the stem. However, as plant height increases, more biomass is allocated to the stem in order to support the leaf biomass. Allocation to stems increases to 46% for plants 40-60 cm tall, 48% for plants 60 – 80 cm tall, and 54% for plants 80-100 cm tall (Table 5-2). Defining relationships between stem and leaf biomass, and the proportion of biomass in each 20 cm segment is critical to understanding how partial flooding of the shoot contributes to the uptake of nutrients from the water column.

Figures 5-4 and 5-5 illustrate how inundation in each segment of an individual plant changes as plants grow taller throughout the growing season. In the low marsh scenario, our model predicts that the lowest portions of the plant are submerged for approximately 400 hr per month, or approximately 6 hr per tidal cycle. The time of inundation decreases as a function of segment height. Inundation hours total ~ 300 hrs,

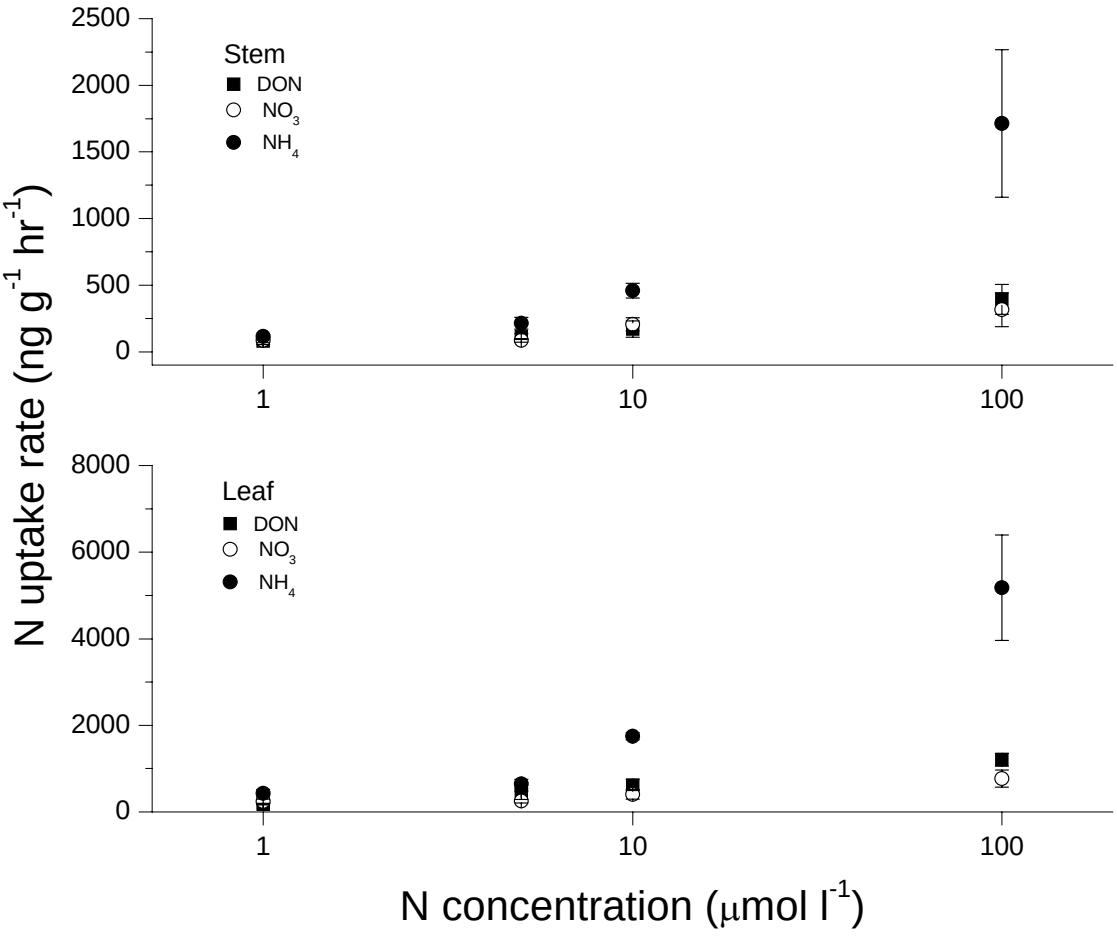

Figure 5-3. Effects of ^{15}N substrate and concentration on uptake rates (ng N gdw^{-1} hr^{-1}) on intact *S. alterniflora* plants. Reported values are mean V_{upt} ± SE.

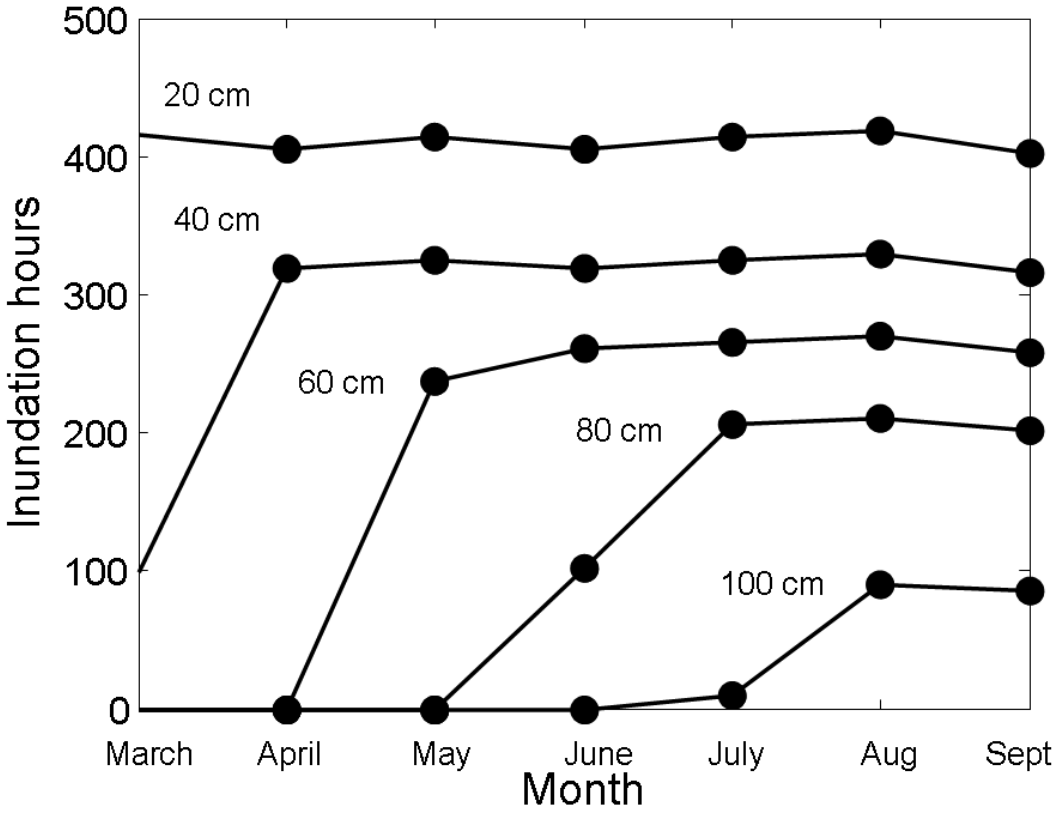

Figure 5-4. Monthly predicted inundation time (hours) for each 20 cm segment in intermediate S. alterniflora at an elevation of -20 cm relative to MSL.

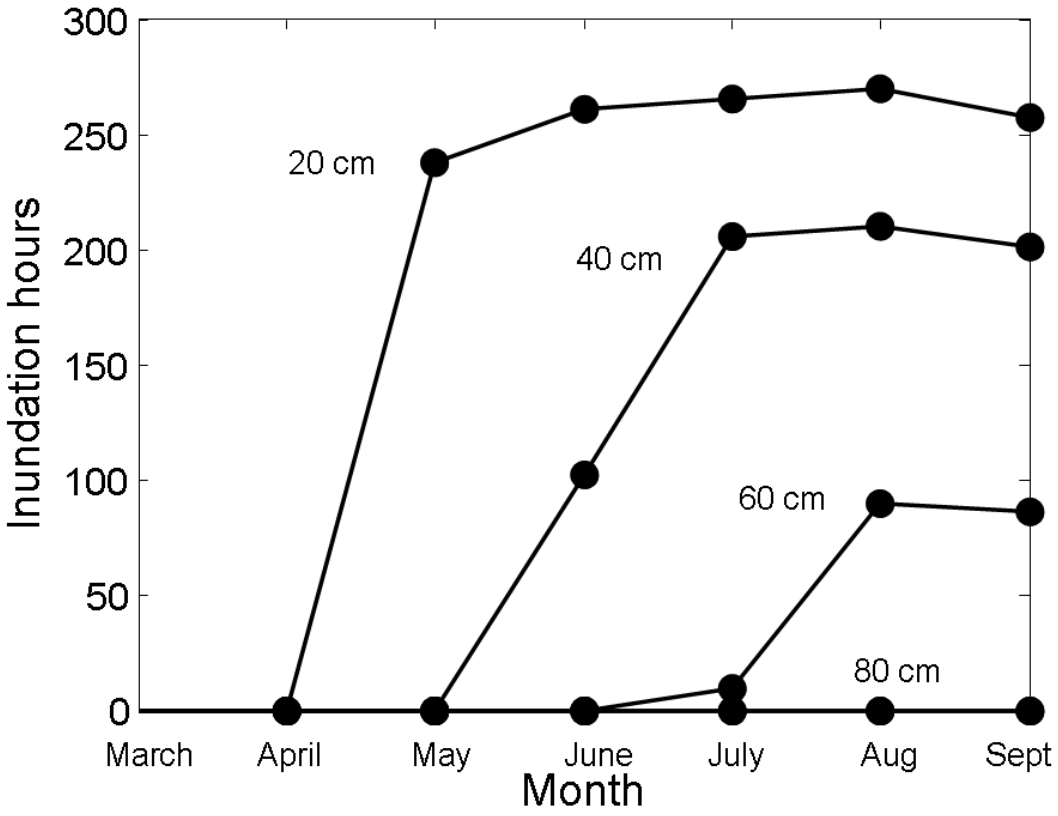

Figure 5-5. Monthly predicted inundation time (hours) for each 20 cm segment in intermediate S. alterniflora at an elevation of -20 cm relative to MSL.

Table 5-1. Percent biomass in each 20cm segment. Plants were categorized into one of five height and mean proportion of biomass in stem and leaf per unit dry weight.

		% biomass in each 20 cm segment				
segment	part	20 (cm)	40 (cm)	60 (cm)	80 (cm)	100 (cm)
0-20 cm	stem	28.7	33.6	43.0	31.9	26.7
0-20 cm	leaf	71.3	57.5	20.1	8.9	7.2
20-40 cm	stem		0.0	2.8	14.6	16.1
20-40 cm	leaf		8.9	29.3	18.8	13.5
40-60 cm	stem			0.0	2.3	10.8
40-60 cm	leaf			4.8	17.3	14.2
60-80 cm	stem				0.0	0.0
60-80 cm	leaf				6.2	9.2
80-100 cm	stem					0.0
80-100 cm	leaf					2.2

Table 5-2. 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 gdw^{-1} hr^{-1})	K_m (umol 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	0.78	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

250 hrs, 200 hrs and 100 hrs per month for segments of the plant 20-40 cm, 40-60 cm, 60-80, and 80-100 cm above the marsh surface. When *S. alterniflora* grows to a meter in height, plants are completely submerged for only 3.3 hrs per tide, but less than 3% of the plant's biomass occurs at the tallest segment, and shoot uptake occurs in the remaining 97% of the plant biomass for longer durations (Table 5-2).

Increases in marsh platform elevation decreases inundation time assuming the same rate of growth. In the high-elevation scenario, submergence of the plants is not significant until May, and inundation times for the 0-20 cm segments and 20-40 cm segments are reduced by over 40% to approximately 250 and 200 hrs per month respectively, and by 60% in the 40-60 cm segment to 100 hrs per month (Figure 5-5). Inundation within the 60-80 cm segment is negligible in this scenario.

Model predictions

In our low-elevation scenario, and at ambient field concentrations of N, the relative importance of shoot (leaves + stem) N uptake decreases with decreasing water column N concentrations, and has the potential to account for up to 35% of monthly plant N demand in CM, 27% in OH, and 13% in RC (Figure 5-6). The total N demand that can be attributed to shoot uptake (total g N from foliar uptake / total g N EYB) averaged over the growing season was the greatest in CM (25%), intermediate in OH (16%), and lowest in RC (8%). Overall, the contribution of the process to satisfy plant N-demand is proportional to the availability of water column nutrients available at these field sites (Figure 5-2). Although water column nutrients may be elevated in the beginning of the

growing season, shoot uptake does not make a significant contribution to N-demand since biomass is insufficient to support large amounts of uptake.

Shoot uptake of N was primarily driven by water column NH_4 availability, since uptake rates of NH_4^+ were 4x greater than either of the two N species, and uptake varied with ambient concentrations across the nutrient gradient (Figures 5-2 and 5-7). Seasonally, shoot NH_4^+ uptake alone can satisfy 14% of plant N-demand at CM (Figure 5-7). Even though water column NO_3^- concentrations sometimes were an order of magnitude greater than saturating concentrations (Figure 5-8 and Table 5-1), N demand from NO_3^- shoot uptake was at most 6% at CM, and less than 2% at OH and RC. Shoot uptake of DON uptake was similar in magnitude to that of NO_3^- (Figure 5-9) despite concentrations that were nearly an order of magnitude lower than NO_3 (Figure 5-2). If the N source in the water column was always saturating for all nutrients, which might occur in eutrophic systems, *S. alterniflora* N uptake rate would equal V_{max}. Under these conditions, shoot N uptake could potentially account for ~ 75% of total plant N demand in our low elevation scenario (Figure 5-10) and ~ 40% of plant N demand in our high elevation scenario.

With increases in elevation, the relative importance of shoot uptake decreased by over 60% (Figure 5-11). Shoot uptake could supply 13% of plant N demand in CM, 9% in OH, and 4% in RC. The seasonal patterns in N uptake are identical to those described above. However, the reduction in contribution is attributed to lower periods of inundation (Figure 5-5).

Figures 5-11 and 5-12 illustrate the contribution of stem and leaf tissue to plant N demand. Although leaves take up N at least twice as fast as stems for all N species, stem

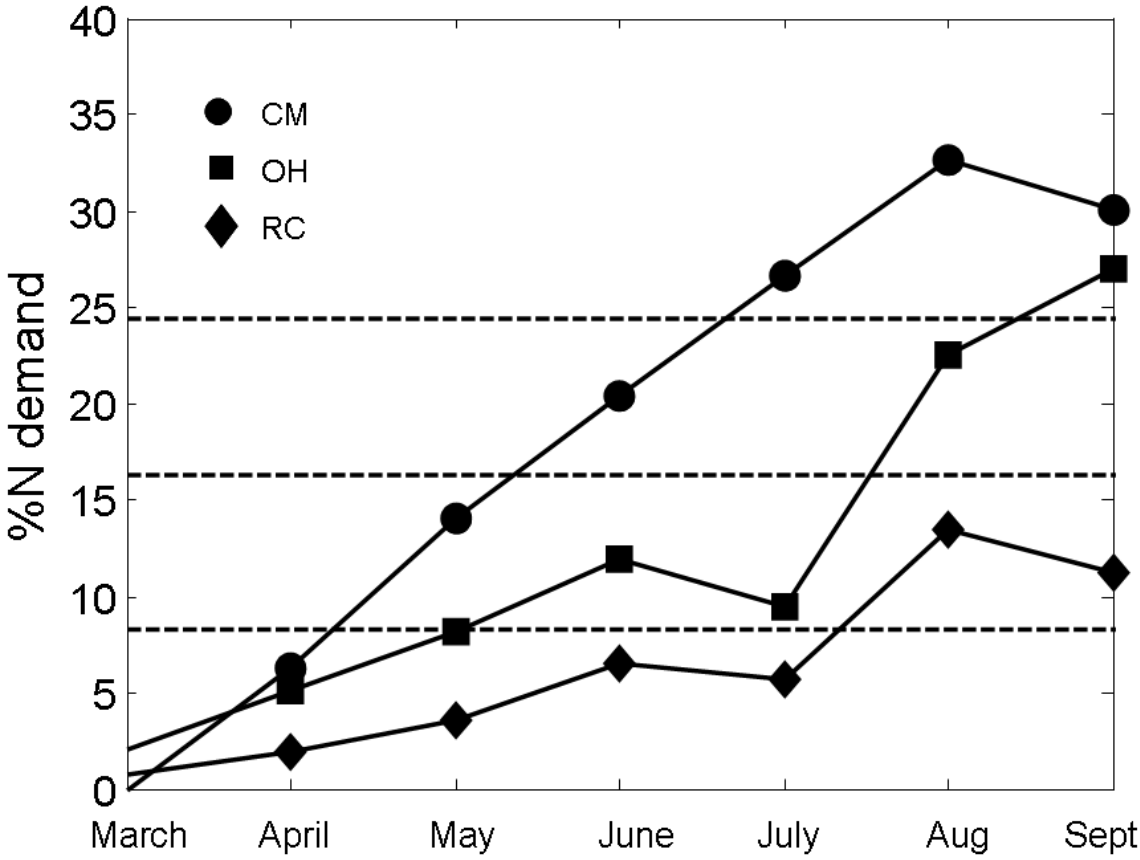

Figure 5-6. Contribution of N demand satisfied by S. alterniflora shoot uptake in salt marsh at the CM, OH, and RC sites at -20 cm MSL. Dashed lines indicate the seasonal N demand that can be attributed to shoot uptake.

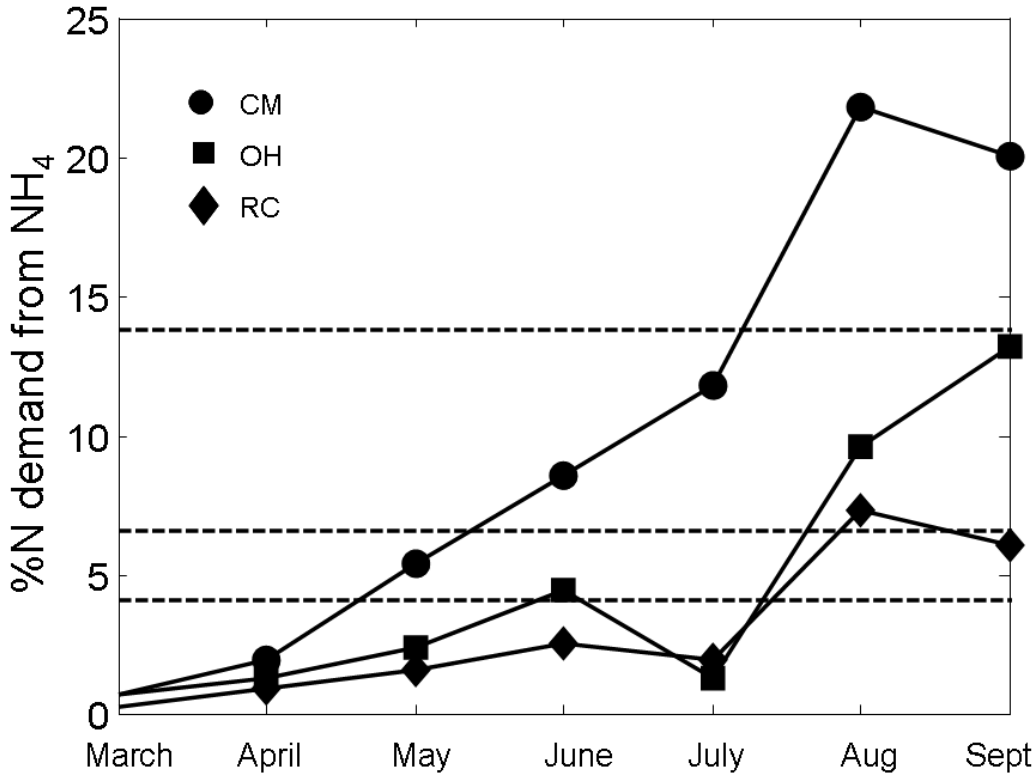

Figure 5-7. Contribution of N demand satisfied by NH4+ shoot uptake in salt marsh at the CM, OH, and RC sites at -20 cm MSL. Dashed lines indicate the seasonal N demand that can be attributed to shoot uptake.

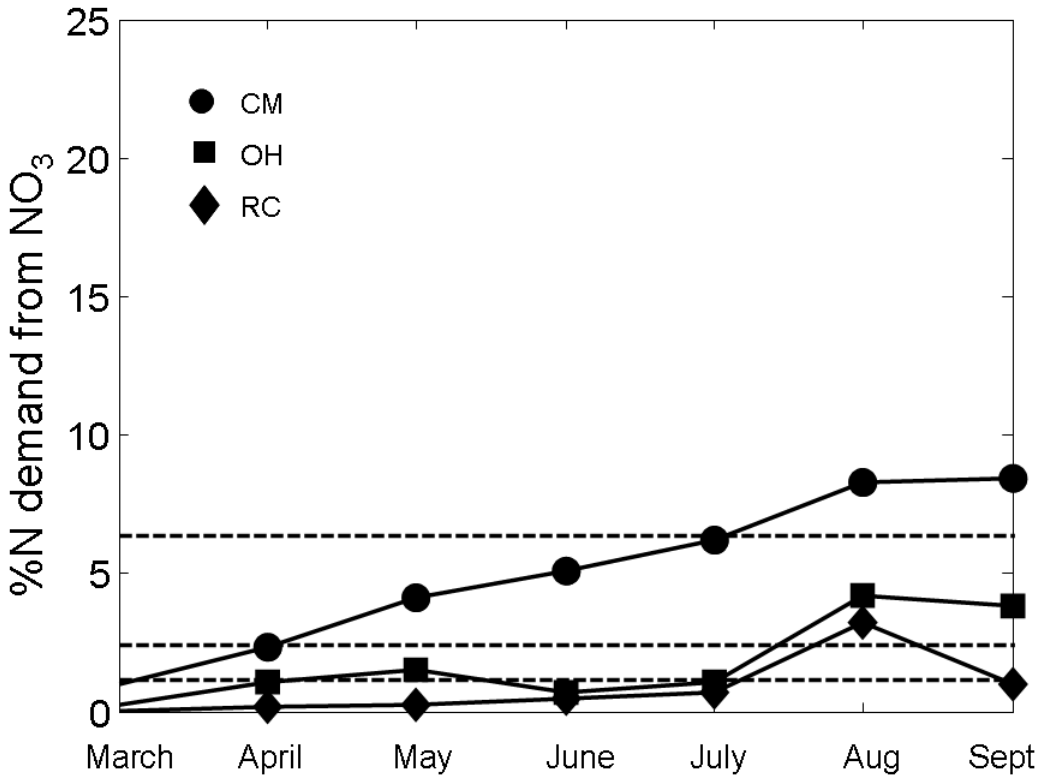

Figure 5-8. Contribution of N demand satisfied by NO3- shoot uptake in salt marsh at the CM, OH, and RC sites at -20 cm MSL. Dashed lines indicate the seasonal N demand that can be attributed to shoot uptake.

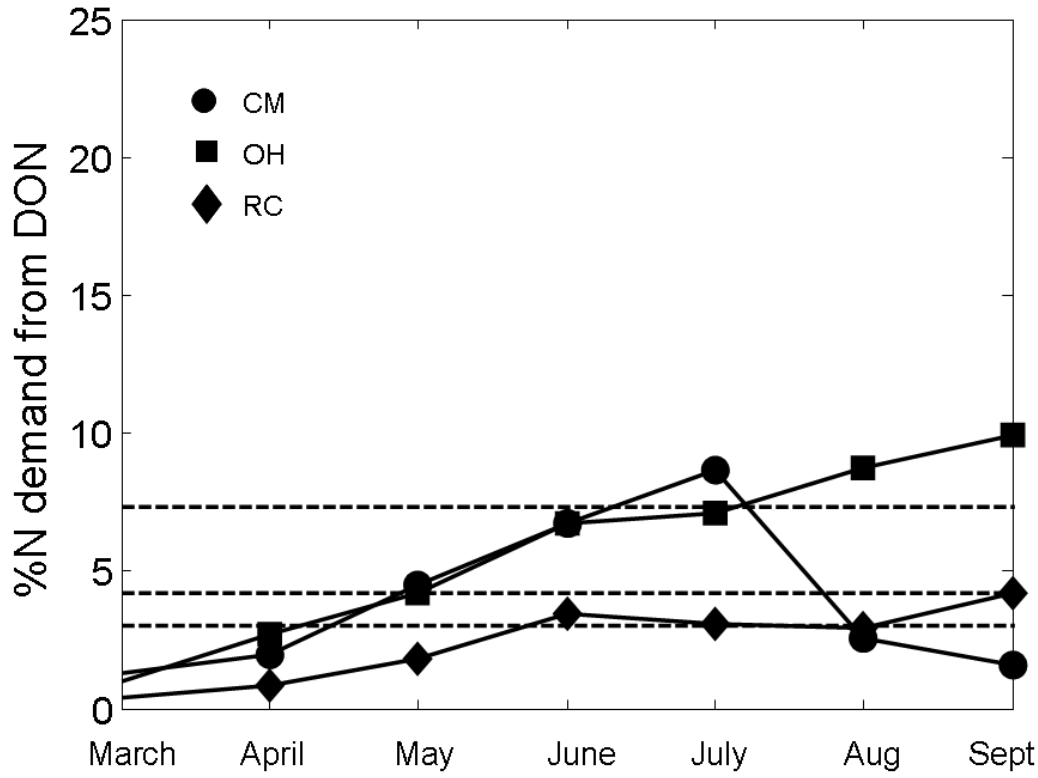

Figure 5-9. Contribution of N demand satisfied by DON shoot uptake in salt marsh at the CM, OH, and RC sites at -20 cm MSL. Dashed lines indicate the seasonal N demand that can be attributed to shoot uptake.

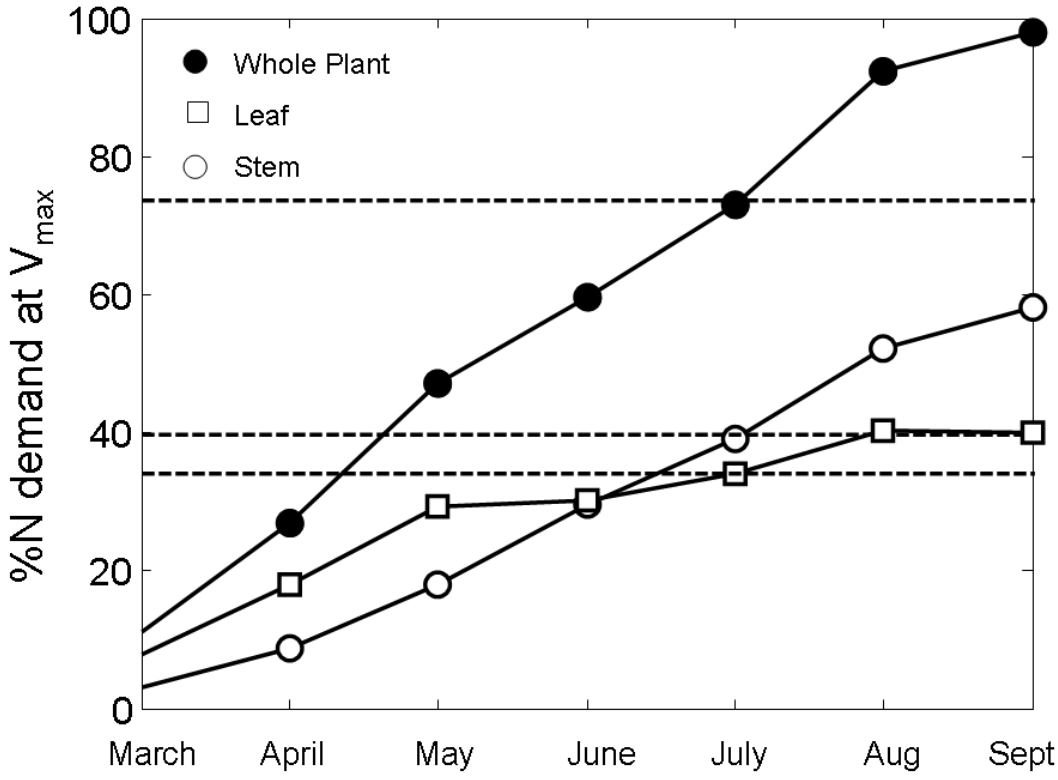

Figure 5-10. Potential contribution of N demand satisfied by N uptake in a eutrophied estuary with N uptake rates at V_{max} at -20 cm MSL. Empty circles represent the contribution from leaf tissue, and empty squares represent stem tissue. Dashed lines indicate the seasonal N demand that can be attributed to shoot, leaf, or stem uptake.

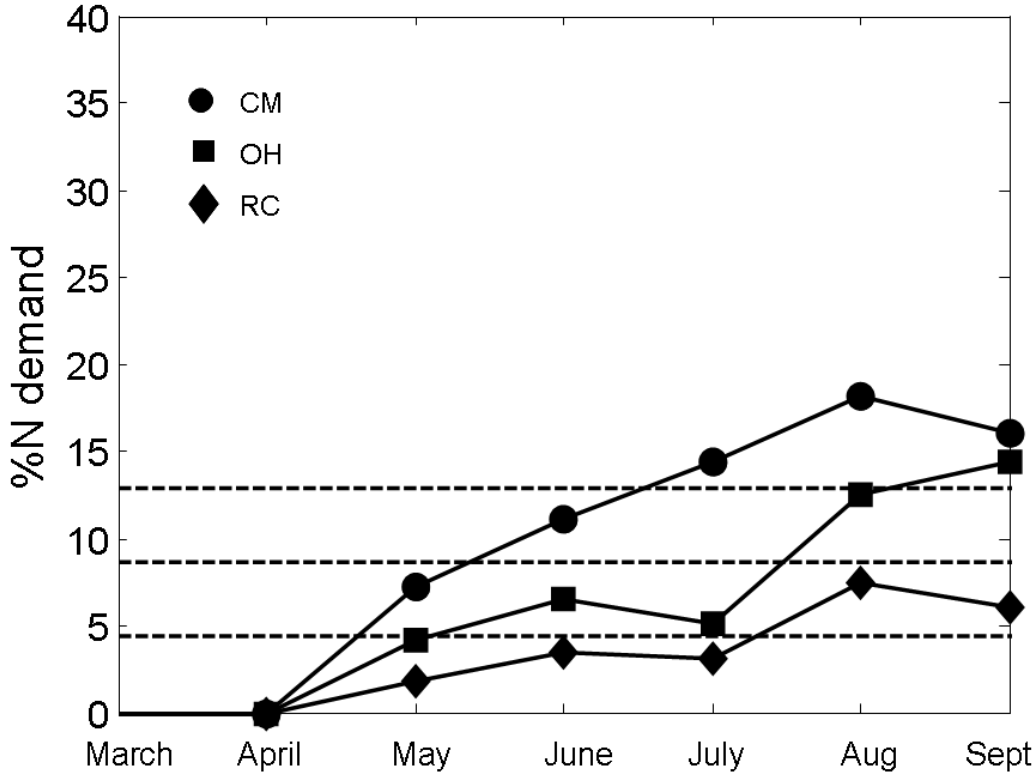

Figure 5-11. Contribution of N demand satisfied by *S. alterniflora* shoot uptake in the CM, OH, and RC field sites at + 20cm MSL. Dashed lines indicate the seasonal N demand that can be attributed to shoot uptake.

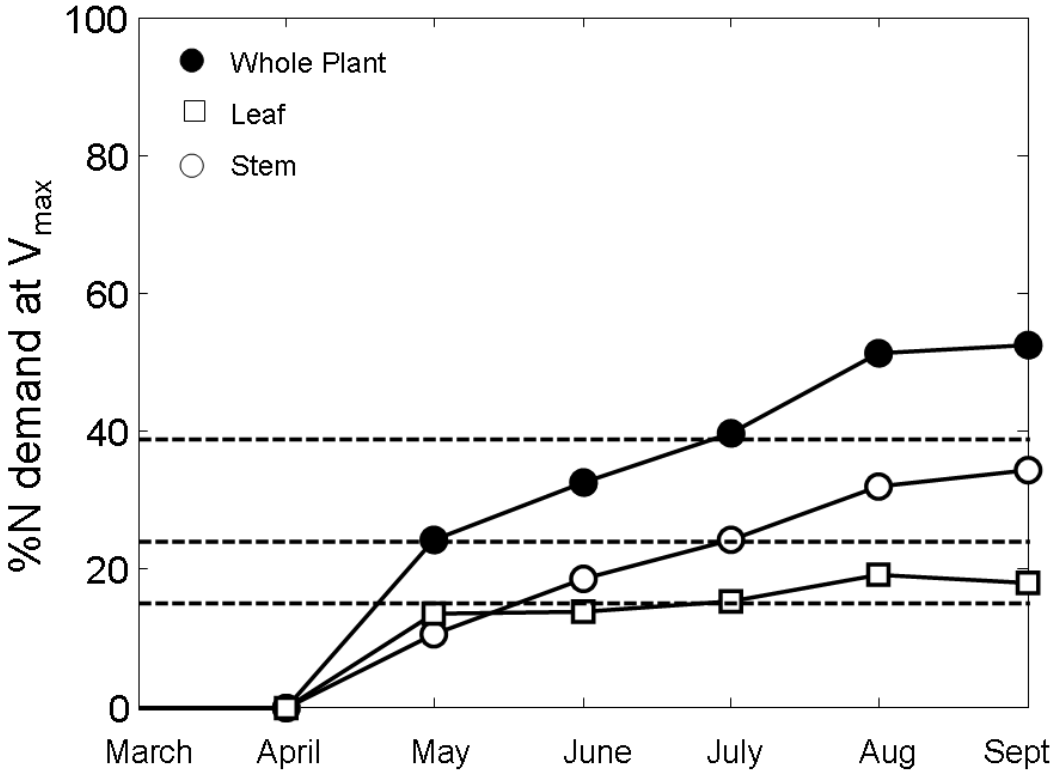

Figure 5-12. Potential contribution of N demand satisfied by N uptake in a eutrophied estuary with N uptake rates at V_{max} at +20 cm MSL. Empty circles represent the contribution from leaf tissue, and empty squares represent stem tissue. Dashed lines indicate the seasonal N demand that can be attributed to shoot, leaf, or stem uptake.

tissue is responsible for 60% of shoot N uptake under both elevation scenarios. This result is counter intuitive, but it is largely dependant upon the part of the plant that that receives the greatest inundation (Figures 3 and 4, Table 5-2). Leaves have a greater contribution to shoot uptake early in the growing season, when plants are shorter and more of the biomass is found in leaf tissue (Table 5-2). As the height of the plant increases, more biomass is allocated to the stem, which also receives greater inundation time than the leaves higher in the plant canopy

Discussion

Our results indicate that shoot uptake can contribute a significant amount of N to support *S. alterniflora* growth. In our model, shoot uptake at ambient field concentrations has the potential to provide up to 25% of a plant's seasonal N demand. These estimates are 2.5× greater than previously reported for *S. anglica* from a eutrophied marsh (Bouma et al. 2002), and are conservative since they were modeled in a mesotrophic estuary. Even at our most N-limited site, shoot uptake by *S. alterniflora* supplied approximately 10% of the seasonal N demand, which was similar to the estimate of Bouma et al (2002) for their most eutrophic site. For *S. alterniflora* in Virginia, the importance of this process is dependant on both elevation within the marsh, which affects the time of inundation and available N concentrations. Assuming marshes of similar elevation and tidal range, our model results suggest that shoot uptake has the potential to supply ~ 75% of plant N demand in eutrophic estuaries. The estimates provided in our

model prediction are representative of net effects since plant epiphytes were removed by wiping the plant biomass clean with paper towels post experiment before analysis. Plant epiphytes have been demonstrated to remove >50% of incoming nutrients in Florida Everglades shallow-water ecosystems (Dodds 2003), suggesting that the overall importance of shoot N removal from the water column may be even greater than our estimates suggest.

The conclusion that shoot uptake by *S. anglica* can at most supply 10% of N demand in a eutrophic system was based upon the assumptions that flood-water N concentrations were low when biomass was great enough to sustain significant N uptake rates, and that inundation time was less than 2.4 hrs a day at this time (Bouma et al. 2002). We came to different conclusions even though *S. alterniflora* N uptake rates are about half of those reported for *S. anglica*. These differences can be largely attributed to differences in inundation time which may vary from 12 hrs a day in April to ~3.3 hrs a day of complete submergence in September. Modeling N uptake on partially submerged plants is critical, since *S. alterniflora* is only submerged ~1.7 hrs per high tide event late in the growing season when plants grow to heights of ~100 cm. However, at this height, more than 88% of *S. alterniflora* shoot has the potential to take up N for ~8 hours a day.

Both water column N concentrations and the form of the N available determine the potential significance of the process. Shoot NH_4^+ uptake is the most important process in satisfying shoot N demand due to high uptake rates when compared to either NO_3^- or DON. Overall, the relative importance of shoot N uptake is largely dependant upon NH_4^+ and DON concentrations in the water column (>80% of N taken up), similar

to results in seagrass dominated ecosystems (Stapel et al. 1996). Although water column NO_3^- concentration may be an order of magnitude greater than the concentration to saturate the uptake kinetics, NO_3^- uptake rates are the lowest of the three treatments. This may be attributed the 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. Low uptake rate of NO_3^- may explain why Drake et al. (2008) concluded that foliar uptake is not an important process in a $^{15}NO_3^-$ enrichment experiment in a New England salt marsh. Our study, and Bouma et al (2002) report that NO_3^- contributes only a small portion of shoot N uptake and we suggest Drake et al (2008) would have come to a dramatically different conclusion for the importance of shoot uptake if they had chosen $^{15}NH_4^+$ as their N source.

Our results suggest that DON uptake has the potential to account for almost 30% of shoot N uptake at ambient concentrations. This finding is similar to estimates that root DON uptake can account for 24% of plant N demand in *S. alterniflora* (Mozdzer et al. in review-b). Other studies in estuarine systems have shown the importance of DON uptake for macroalgae (Tyler et al. 2005, Tyler and McGlathery 2006), and seagrasses (Vonk et al. 2008). DON is more ecologically important than NO_3.due to greater N uptake rates, energetically less costly for two reasons. Overall plant carbon cost of N acquisition are much lower for DFAA vs. NO_3^- (Clarkson 1986), and NO_3^- must be first be reduced to NH_4^+ by the short-lived, and energetically expensive nitrate reductase enzyme (Li and Oaks 1993) before being attached to a carbon skeleton.

The relatively high primary production of salt marshes at low elevations is often attributed to the efficient flushing of salts and sulfides from the rhizosphere, which have been shown to reduce *S. alterniflora* N uptake (Bradley and Morris, 1991; Chambers et al., 1998). Since our results suggest that foliar uptake is a significant process and increases with inundation frequency, we suggest that decreased productivity with increasing elevation on the marsh platform may be one factor potentially attributed to a lower contribution of shoot N uptake, resulting in reduced vegetation growth. Although physio-chemistry plays an important role, we also suggest that the increased productivity of both tall and intermediate growth form *S. alterniflora* may be partially attributed to shoot uptake of nutrients from the water column.

The potential importance of shoot uptake in low-elevation marshes is supported by different strategies in root: shoot biomass partitioning among the different *S. alterniflora* growth forms. Plants from lower potions of the marsh tend to have lower root: shoot ratios (Gallagher 1974, Dame and Kenny 1986). Since shoot uptake may constitute a significant source of N for low elevation *S. alterniflora*, one interpretation may be a greater investment in shoot biomass where N can be easily taken up from an aerobic environment. Maintaining roots in an anaerobic environment is energetically costly and influences estuarine species zonation (Maricle et al. 2006). Shoot N uptake may represent an alternate hypothesis to differences in root: shoot ratios in *S. alterniflora* growth forms.

Marsh elevation and plant height are factors that determine the importance of shoot uptake. Morris et al (2002) suggested that biomass increases with decreasing elevation up to a threshold. We suggest that the increased productivity in low-elevation

marshes (more frequently inundated) may also be partly attributed to shoot uptake of N from the water column. Beyond the elevation threshold, there is most likely a trade off between the potential for shoot N uptake, and reduced photosynthetic capacity attributed to tidal inundation (Kathilankal et al. 2008). With increases in elevation on the marsh platform, the relative importance of this process decreases by almost 50% suggesting that root uptake, controlled by physio-chemistry in the rhizosphere will be the predominant source of N with plants higher in elevation. This transition at the higher elevation also corresponds to a change in plant phenotype from intermediate to short growth form *S. alterniflora* in our study

Differences in relative nutrient ability may dramatically affect the importance of this process. In our model simulations, we have assumed that *S. alterniflora* N uptake rates remain constant in locations within an estuary with different N supplies. However, Bouma et al (2002) observed a 400% increase in leaf NH_4^+ uptake rates in eutrophic salt marshes in *S. anglica* in Denmark suggesting that our rates of N uptake are conservative since they were determined in a mesotrophic estuary. Assuming a similar 400% increase in leaf NH_4^+ uptake rates alone, and uptake at V_{max}, our results indicate that shoot uptake of N can supply between 130 and 240% of seasonal plant demand depending on marsh elevation (Figure 5-13). While these estimates would need to be verified experimentally, the ability of plants to adapt to their environment suggest that rates of shoot N uptake can be enhanced significantly in eutrophic environments to satisfy nearly all *S. alterniflora* N-demand, resulting in an even greater potential for the importance of foliar uptake.

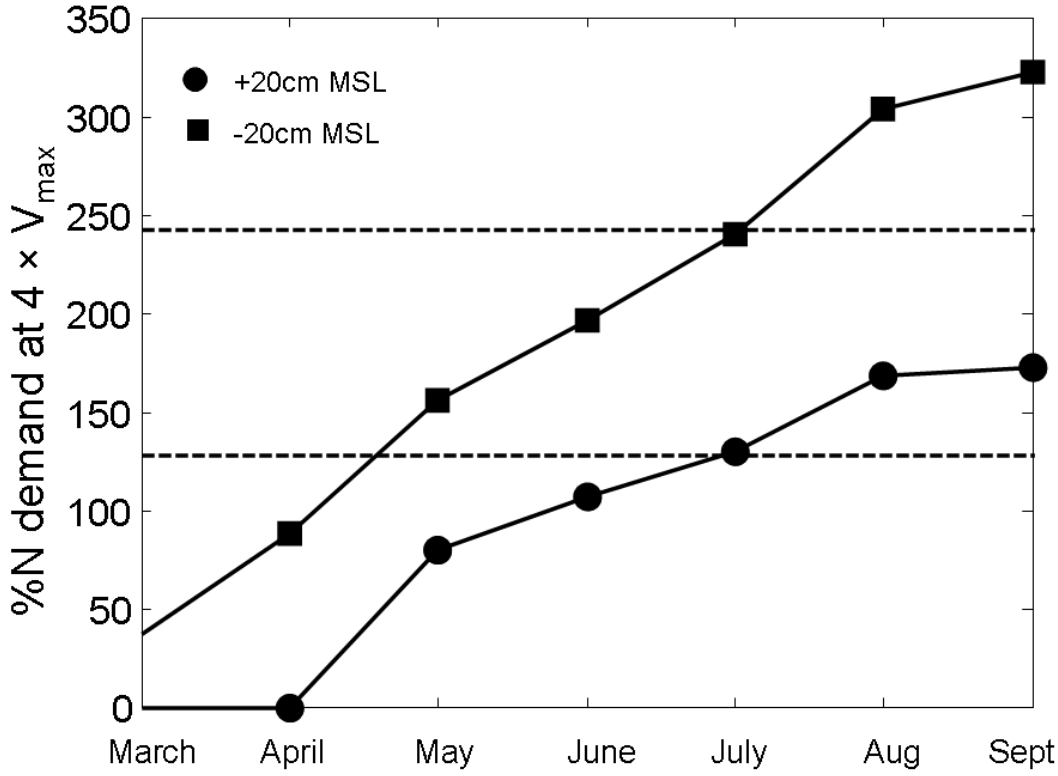

Figure 5-13. Potential contribution of N demand satisfied by N uptake in a eutrophied estuary at 4 times V_{max}. Squares indicate plants at -20 cm MSL, and circles represent plants at +20 cm MSL. Dashed lines indicate the seasonal N demand that can be attributed to shoot uptake.

Conclusions

Shoot uptake of nitrogen from the water column is a significant source of plant N for *S. alterniflora*. Annual estimates for *S. alterniflora,* 6-25% in mesotrophic estuaries, fall within the range of terrestrial plants (<10%) (Wilson 1992, Boyce et al. 1996, Tomaszewski et al. 2003) and seagrasses (50-100%) (Stapel et al. 1996, Terrados and Williams 1997, Peuke et al. 1998, Vonk et al. 2008). However, in eutrophic systems, shoot uptake has the potential to sustain anywhere from 75 to 240% of plant N demand. The importance of this process is directly proportional to the time of inundation (a function of marsh elevation and plant height) and to available N concentrations, which may vary upon the location of the marsh in an estuary. In Virginia salt marshes, NH_4^+ availability drives shoot N uptake since NH_4^+ uptake rates are at least 5 times greater than either DON or NO_3^- uptake rates. After NH_4^+, DON makes the next greatest contribution to overall N demand. Although water column NO_3^- concentrations may be quite high, *S. alterniflora* apparently does not use NO_3^- due to the high energetic cost of using this N source. Our results suggest that shoot uptake by *S. alterniflora* has the potential to effectively remove nutrients from the water column, and the aboveground canopy may be an effective filter for removing nutrients from the water column.

Chapter 6

Conclusions and Synthesis

Dissolved organic nitrogen (DON) has been demonstrated to be an important source of plant N in many ecosystems (Jones and Darrah 1992, Chapin et al. 1993, Kielland 1994, Raab et al. 1996, Nasholm et al. 1998, Raab et al. 1999, Schmidt and Stewart 1999, Lipson and Nasholm 2001, McFarland et al. 2002, Mozdzer et al. 2004, Tyler et al. 2005, Vonk et al. 2008). Contrary to our previous understanding of nitrogen (N) utilization in *Spartina alterniflora* salt marshes, my research shows that dissolved organic nitrogen (DON) may be an important supplemental source in N-limited salt marsh ecosystems. *S. alterniflora* use DON through its roots, and this process varies latitudinally (Chapter 2). Additionally, at ambient concentrations of DON in the water column, shoot uptake of DON alone may provide up to 10% of plant N demand, which is approximately 30% of total shoot N uptake in salt marshes mid-Atlantic salt marshes (Chapter 5). In both these environments, both above- and belowground, the importance of this process will be largely dependent upon the available concentration.

I suggest that *S. alterniflora* ecotypes that grow in the three biogeographic provinces along the North American Atlantic coast have adapted to local N availability attributed to differences in heterotrophic activity (Chapter 2). In high-latitude salt marshes, relative heterotrophic activity is lower, resulting in greater pools of DON, which high latitude *S. alterniflora* may depend more on as a N source. With decreases in latitude, microbial activity increases resulting in lower available pools of DON. In low-latitude salt marshes, *S. alterniflora* ecotypes have a high N demand, greater N uptake rates, and will use either DON or NH_4^+ as a N source depending upon availability.

While pools of DON vary latitudinally based upon relative microbial activity (Chapter 3), in mid-Atlantic *S. alterniflora* sediments, DON availability did not vary

significantly among sites. However due to great spatial heterogeneity in NH_4^+ availability, the amount of DON that may contribute to plant N demand may vary significantly within a single marsh, as well as among *S. alterniflora* growth form zones (Chapter 3). Such differences in the availably of this labile pool of nutrients needs more research, especially at the rhizosphere scale.

One of the most interesting results from this dissertation it the potential importance of shoot N uptake. While previous research on *S. anglica* suggested that shoot uptake may supply ~10% of plant N demand (Bouma et al. 2002), I came to a very different conclusion using *in situ* experiments and numerical models. While NH_4^+ availability accounts for a majority of shoot uptake, shoot DON uptake is ecologically more important than shoot NO_3^- uptake, even though NO_3^- concentrations may be an order of magnitude greater. Overall, shoot uptake by *S. alterniflora* can supply up to 25% of seasonal plant N demand in relatively pristine salt marshes within the VCR-LTER based upon their relative elevation on the marsh platform and water column nutrient concentrations. Eutrophication has the potential to dramatically change the relative importance of the process, and has the potential to account for anywhere from 75% to 240% of plant N demand given similar tidal amplitudes and elevations.

My research shows clearly that *S. alterniflora* will use DON as an N source in both the rhizosphere and the water column given sufficient pools. However, more research is needed to create better estimates of both DON and DIN concentrations in the rhizosphere. Differences in sampling technique may yield dramatically different results (Long et al. 2008), which further complicates quantification of this labile pool of nutrients. Porewater equilibrators used in my study are indicative of long-term pools,

since the equilibration time of the sample volume takes approximately 30 days. Suction lysimeters provide an alternative method for collecting bulk porewater (Chambers and Odum 1990), however, this sampling method gives a static estimate of standing stock nutrients at one point in time. Centrifuged sediment samples (Gardner and Hanson 1979), sever roots in sampling which release both organic and inorganic nutrients potentially skewing the results. These aforementioned sampling techniques present three estimates for collecting bulk porewater, which may not be indicative of nutrient concentrations in the rhizosphere. This suggests the need to more accurately quantify plant available nutrients in the rhizosphere at fine scales. Unfortunately, high resolution water samplers (Berg and McGlathery 2001) designed for use in sandy sediments do not work well in salt marsh sediments due to clogging of the sampling pores (personal observation). Additionally, standard colorimetric methods of porewater analysis require at least 20ml of volume to quantify the standard suite of inorganic nutrients. This volume of water may not be representative of the concentrations of nutrients in the rhizosphere. Long et al. (Long et al. 2008) found that bulk concentrations did not accurately reflect concentrations near the roots where uptake occurs. To truly understand nutrient fluxes in the rhizosphere, small sample volumes representative of the rhizosphere should be taken, leaving only analysis by HPLC or IC which is both more costly and time consuming than standard techniques. Understanding the limitations of the methods, the results presented in the study are conservative estimates of DON availability.

Salt marsh sediments and marine sediments are very different than terrestrial environments where DON uptake has been studied previously (Chapin et al. 1993, Raab et al. 1996, Nasholm et al. 1998, Schmidt and Stewart 1999, Lipson and Nasholm 2001)

porewater estimates from saturated soils present actual estimates of plant available nutrients. Additionally, pools of DFAA accumulate since they are poor carbon substrates for the dominant heterotrophs, sulfate reducing bacteria (Hansen 1993, Hansen and Blackburn 1995), and N concentrations are available in excess to microbial needs since the microbial community is phosphorus limited (Sundareshwar et al. 2003). In terrestrial sediments, heterotrophic decomposition of organic matter is aerobic, and DFAA are short lived due to the high demand for both organic carbon and N by microorganisms.

DFAA fertilization increased both tissue N concentration and productivity in both short and intermediate growth forms of *S. alterniflora* (Chapter 4). However, the increase in productivity and N content created small patches of N rich plants, which herbivorous insects selectively grazed. Although Chapter 4 was not meant to be an herbivory experiment, it presents the case that salt marshes are spatially heterogeneous, and mobile grazers, *e.g.* grasshoppers, graze preferentially on the N-rich intermediate growth form of *S. alterniflora* in mid-Atlantic salt marshes. Under eutrophication scenarios, Bertness et al. (2008) suggested the potential for consumer control *S. alterniflora* salt marshes. While my data indicate the potential for considerable top-down pressure, larger scale experiments are needed to make such claims in mid-Atlantic salt marshes since episodic events may not be indicative of salt marsh ecosystem processes in general.

In conclusion, DON uptake is an important supplemental source of S. alterniflora N in N-limited systems and may supply ~25 % of plant N demand both above- and belowground. However, eutrophication may alter the importance of this process both above- and belowground. In sediments, N loading will likely increase inorganic NH_4^+

concentrations, making plants less dependant upon root DON uptake. On the other hand, increases in water column N have been associated with increases in DFAA concentrations (Verity 2002). Assuming the same trajectory, aboveground, shoot DON uptake may become more important in tidally influences salt marshes low in elevation. More research is suggested to understand competition for DON in the rhizosphere, since bulk estimates presented in this study may not be representative of rhizosphere conditions. Due to ecotypic differences in N-uptake and N-demand, more research in needed to understand the role of genetics in controlling latitudinal trends in productivity in *S. alterniflora* along the North American Atlantic coast. Finally, as coastal eutrophication continues to impact salt marsh ecosystem processes, DON use by *S. alterniflora* is likely to become a more important process. More studies are needed to understand the impacts increased productivity attributed to N loading, which may create greater pools of slowly mineralizing organic matter, resulting in greater DON availability to plants changing the relative importance of this process.

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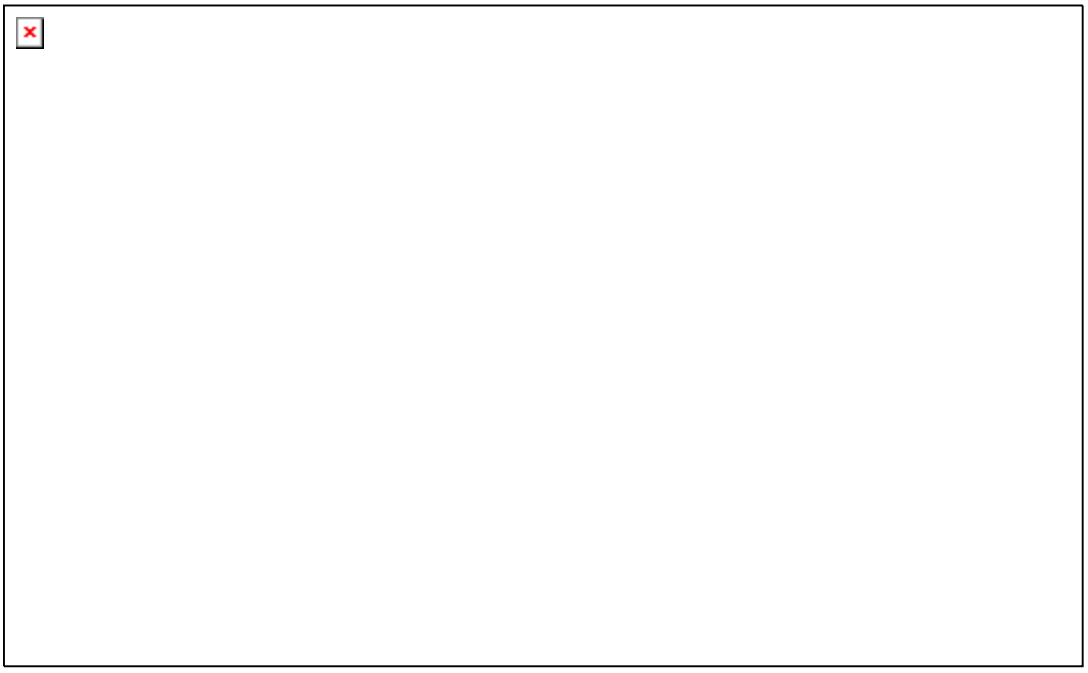
Appendix A: Mean ± SE porewater nutrient data at 20cm depth in August 2006.



Appendix B: Mean ± SE porewater nutrient data from the VCR in May 2007

Site	S. alterniflora Zone	Depth (cm)	Month	Variable µM	n	mean	variance	std. err.
FP	Short	10	May	NH4	3	2.7	1.8	0.8
				PO4	3	40.4	4869.5	40.3
				TDN	3	95.7	183.3	7.8
				DON	3	93.1	150.9	7.1
				Urea	3	2.3	0.4	0.4
				DFAA	3	3.2	0.3	0.3
				H2S	3	211.4	107542.1	189.3
				BDON	3	5.5	0.4	0.4
FP	Short	20	May	NH4	3	21.4	6.9	1.5
				PO4	3	4.6	0.9	0.5
				TDN	3	130.7	349.2	10.8
				DON	3	109.3	391.3	11.4
				Urea	3	3.1	0.7	0.5
				DFAA	3	2.7	1.7	0.8
				H2S	3	144.6	11812.6	62.7
				BDON	3	5.8	2.4	0.9
FP	Short	30	May	NH4	3	104.5	878.3	17.1
				PO4	3	33.8	26.4	3.0
				TDN	3	221.2	3028.5	31.8
				DON	3	116.7	1036.3	18.6
				Urea	3	2.7	0.4	0.4
				DFAA	3	5.6	13.6	
				H2S	3	487.6	129131.8	207.5
				BDON	3	8.3	18.6	2.5
FP	Tall	10	May	NH4	3	25.6	133.6	6.7
				PO4	3	41.5	601.5	14.2
				TDN	3	76.7	218.6	8.5
				DON	3	51.1	12.9	2.1
				Urea	3	1.0	0.0	0.1
				DFAA	3	3.1	0.1	0.2
				H2S	3	475.9	62251.0	144.0
				BDON	3	4.1	0.2	0.3
FP	Tall	20	May	NH4	3	85.8	4470.5	38.6
				PO4	3	53.5	324.2	10.4
				TDN	3	144.4	7828.0	51.1
				DON	3	58.6	478.9	12.6
				Urea	3	1.3	0.2	0.2
				DFAA	3	8.0	21.2	2.7
				H2S	3	1920.1	2873353.5	978.7
				BDON	3	9.3	23.7	2.8
FP	Tall	30	May	NH4	3	184.0	1036.0	18.6
				PO4	3	56.6	305.5	10.1
				TDN	3	227.7	2443.1	28.5
				DON	3	43.7	410.3	11.7
				Urea	3	1.7	0.1	0.2
				DFAA	3	4.1	19.8	2.6
				H2S	3	2004.4	1108498.1	607.9
				BDON	3	5.8	22.7	2.7
FP	Intermediate	10	May	NH4	3	21.4	174.6	7.6
				PO4	3	67.3	796.0	16.3
				TDN	3	178.5	5970.3	44.6
				DON	3	157.1	7435.4	49.8
				Urea	3	1.4	0.0	0.1
				DFAA	3	5.1	5.5	1.4
				H2S	3	902.5	354882.9	343.9
				BDON	3	6.5	6.5	1.5
FP	Intermediate	20	May	NH4	3	260.0	28396.0	97.3
				PO4	3	148.1	2651.5	29.7
				TDN	3	395.7	32762.6	104.5
				DON	3	135.7	6319.4	45.9
				Urea	3	1.3	0.1	0.1
				DFAA	3	4.5	1.4	0.7
				H2S	3	3294.6	961222.3	566.0
				BDON	3	5.8	1.7	0.7
FP	Intermediate	30	May	NH4	3	256.7	6917.3	48.0
				PO4	3	128.7	506.3	13.0
				TDN	3	375.6	6797.3	47.6
				DON	3	118.9	4521.0	38.8
				Urea	3	1.8	0.0	0.0
				DFAA	3	5.2	11.3	1.9
				H2S	3	4079.2	207444.9	263.0
				BDON	3	7.0	10.9	1.9

Site	S. alterniflora Zone	Depth (cm)	Month	Variable μM	n	mean	variance	std. err.
SH Y13	Short	10	May	NH4	3	52.1	2276.0	27.5
				PO4	3	1.1	3.5	1.1
				TDN	3	80.4	2993.6	31.6
				DON	3	28.4	49.1	4.0
				Urea	3	5.1	10.7	1.9
				DFAA	3	4.1	6.0	1.4
				H2S	3	8.0	92.0	5.5
				BDON	3	9.2	3.2	1.0
SH Y13	Short	20	May	NH4	3	95.7	16720.6	74.7
				PO4	3	2.9	3.0	1.0
				TDN	3	135.1	21580.8	84.8
				DON	3	39.4	328.6	10.5
				Urea	3	2.9	0.1	0.2
				DFAA	3	4.9	7.4	1.6
				H2S	3	4.5	6.2	1.4
				BDON	3	7.9	9.0	1.7
SH Y13	Short	30	May	NH4	3	165.0	9777.3	57.1
				PO4	3	15.0	129.1	6.6
				TDN	3	200.4	10503.5	59.2
				DON	3	35.4	22.2	2.7
				Urea	3	5.2	1.4	0.7
				DFAA	3	5.9	2.3	0.9
				H2S	3	735.5	1225038.9	639.0
				BDON	3	11.1	1.4	0.7
SH Y13	Tall	10	May	NH4	3	70.0	2427.9	28.4
				PO4	3	11.7	23.3	2.8
				TDN	3	98.7	3740.9	35.3
				DON	3	28.7	150.9	7.1
				Urea	3	3.5	1.5	0.7
				DFAA	3	3.1	1.6	0.7
				H2S	3	8.2	70.4	4.8
				BDON	3	6.6	5.0	1.3
SH Y13	Tall	20	May	NH4	3	174.0	7764.3	50.9
				PO4	3	30.1	530.8	13.3
				TDN	3	202.9	6715.9	47.3
				DON	3	28.9	61.9	4.5
				Urea	3	5.5	9.5	1.8
				DFAA	3	6.6	41.9	3.7
				H2S	3	163.4	11364.0	61.5
				BDON	3	12.1	85.0	5.3
SH Y13	Tall	30	May	NH4	3	117.2	2000.1	25.8
				PO4	3	22.1	19.3	2.5
				TDN	3	139.5	2729.8	30.2
				DON	3	22.3	150.4	7.1
				Urea	3	6.6	3.3	1.1
				DFAA	3	5.5	2.2	0.9
				H2S	3	504.8	462071.3	392.5
				BDON	3	12.1	0.5	0.4
SH Y13	Intermediate	10	May	NH4	3	18.5	370.7	11.1
				PO4	3	0.3	0.0	0.1
				TDN	3	126.1	6535.4	46.7
				DON	3	107.7	7447.9	49.8
				Urea	3	1.0	2.4	0.9
				DFAA	2	10.8	129.9	8.1
				H2S	1	27.1	.	.
				BDON	2	12.4	91.8	6.8
SH Y13	Intermediate	20	May	NH4	3	45.2	1278.3	20.6
				PO4	3	4.7	15.2	2.3
				TDN	3	120.3	4683.1	39.5
				DON	3	75.1	1735.9	24.1
				Urea	3	0.9	1.0	0.6
				DFAA	3	6.8	14.5	2.2
				H2S	3	5.6	25.2	2.9
				BDON	3	7.7	9.2	1.8
SH Y13	Intermediate	30	May	NH4	3	149.2	16333.1	73.8
				PO4	3	11.6	20.4	2.6
				TDN	3	249.9	32930.6	104.8
				DON	3	100.7	4784.8	39.9
				Urea	3	1.6	0.5	0.4
				DFAA	3	2.9	3.2	1.0
				H2S	3	188.1	30416.3	100.7
				BDON	3	4.5	1.3	0.6

Site	S. alterniflora Zone	Depth (cm)	Month	Variable μM	n	mean	variance	std. err.
SH Y150	Short	10	May	NH4	3	174.3	44.3	3.8
				PO4	3	19.0	21.7	2.7
				TDN	2	237.2	54.1	5.2
				DON	2	59.2	20.5	3.2
				Urea	3	3.8	7.0	1.5
				DFAA	3	3.9	2.2	0.9
				H2S	3	2143.7	41596.0	117.8
				BDON	3	7.7	16.4	2.3
SH Y150	Short	20	May	NH4	3	379.0	11809.0	62.7
				PO4	3	22.6	91.6	5.5
				TDN	3	465.6	9450.9	56.1
				DON	3	86.6	134.3	6.7
				Urea	3	2.7	0.1	0.2
				DFAA	3	8.6	109.8	6.1
				H2S	3	2217.8	417207.7	372.9
				BDON	3	11.3	111.1	6.1
SH Y150	Short	30	May	NH4	3	521.7	87376.3	170.7
				PO4	3	18.9	41.6	3.7
				TDN	3	636.0	113392.0	194.4
				DON	3	114.3	1704.3	23.8
				Urea	3	3.1	1.2	0.6
				DFAA	3	4.7	1.7	0.8
				H2S	3	3028.1	1034365.5	587.2
				BDON	3	7.8	1.8	0.8
SH Y150	Tall	10	May	NH4	3	18.8	58.6	4.4
				PO4	3	4.4	7.7	1.6
				TDN	3	63.6	82.6	5.2
				DON	3	44.8	60.8	4.5
				Urea	3	2.5	1.7	0.8
				DFAA	3	3.2	0.5	0.4
				H2S	3	71.1	25.5	2.9
				BDON	3	5.7	4.0	1.2
SH Y150	Tall	20	May	NH4	3	9.8	70.6	4.9
				PO4	3	2.4	0.0	0.1
				TDN	3	46.4	165.2	7.4
				DON	3	36.6	90.0	5.5
				Urea	3	3.4	1.6	0.7
				DFAA	3	3.5	5.0	1.3
				H2S	3	170.5	26992.3	94.9
				BDON	3	6.9	11.8	2.0
SH Y150	Tall	30	May	NH4	3	34.2	29.0	3.1
				PO4	3	5.0	1.3	0.7
				TDN	3	72.1	7.1	1.5
				DON	3	38.0	19.8	2.6
				Urea	3	3.9	0.3	0.3
				DFAA	3	2.4	0.2	0.2
				H2S	3	148.5	4298.8	37.9
				BDON	3	6.3	0.9	0.6
SH Y150	Intermediate	10	May	NH4	3	86.5	71.6	4.9
				PO4	3	11.9	27.3	3.0
				TDN	3	248.0	3258.9	33.0
				DON	3	161.5	2645.8	29.7
				Urea	3	2.9	3.7	1.1
				DFAA	3	3.4	1.3	0.7
				H2S	3	825.5	144438.3	219.4
				BDON	3	6.3	2.7	1.0
SH Y150	Intermediate	20	May	NH4	3	177.0	4269.0	37.7
				PO4	3	16.7	46.7	3.9
				TDN	3	302.8	964.0	17.9
				DON	3	125.8	6037.0	44.9
				Urea	3	2.4	1.6	0.7
				DFAA	3	2.0	1.0	0.6
				H2S	3	2205.7	866350.2	537.4
				BDON	3	4.4	1.0	0.6
SH Y150	Intermediate	30	May	NH4	3	366.7	8025.3	51.7
				PO4	3	16.7	8.0	1.6
				TDN	3	533.2	46167.5	124.1
				DON	3	166.5	20225.7	82.1
				Urea	3	1.6	0.1	0.2
				DFAA	3	3.3	3.0	1.0
				H2S	3	2524.6	92666.7	175.8
				BDON	3	4.8	2.4	0.9

Site	S. alterniflora Zone	Depth (cm)	Month	Variable μM	n	mean	variance	std. err.
UPC	Short	10	May	NH4	4	26.9	89.9	4.7
				PO4	4	5.5	3.0	0.9
				TDN	4	72.8	986.9	15.7
				DON	4	45.9	491.7	11.1
				Urea	4	1.9	1.7	0.7
				DFAA	4	9.5	11.3	1.7
				H2S	4	496.1	138359.6	186.0
				BDON	4	11.4	19.4	2.2
UPC	Short	20	May	NH4	4	200.0	1634.5	20.2
				PO4	4	28.6	59.2	3.8
				TDN	4	260.0	2143.2	23.1
				DON	4	60.0	81.1	4.5
				Urea	4	0.6	0.9	0.5
				DFAA	4	2.8	0.6	0.4
				H2S	4	3033.0	480422.3	346.6
				BDON	4	3.4	0.6	0.4
UPC	Short	30	May	NH4	4	210.4	10122.1	50.3
				PO4	4	25.3	22.6	2.4
				TDN	4	299.2	29826.9	86.4
				DON	4	88.8	7058.5	42.0
				Urea	4	0.2	0.0	0.1
				DFAA	4	3.0	0.1	0.2
				H2S	4	3324.0	1011157.7	502.8
				BDON	4	3.2	0.3	0.3
UPC	Intermediate	10	May	NH4	4	105.1	19222.3	69.3
				PO4	4	21.4	69.4	4.2
				TDN	4	148.1	20560.5	71.7
				DON	4	43.0	87.6	4.7
				Urea	4	0.1	0.0	0.1
				DFAA	4	2.1	0.0	0.1
				H2S	4	1087.2	931094.0	482.5
				BDON	4	2.3	0.1	0.1
UPC	Intermediate	20	May	NH4	4	106.8	3144.5	28.0
				PO4	4	11.8	17.5	2.1
				TDN	4	184.1	5260.6	36.3
				DON	4	77.3	299.3	8.7
				Urea	4	1.9	0.8	0.4
				DFAA	3	18.2	5.0	1.3
				H2S	4	1080.6	403176.3	317.5
				BDON	3	20.4	6.5	1.5
UPC	Intermediate	30	May	NH4	4	397.0	226.0	7.5
				PO4	4	22.7	67.9	4.1
				TDN	4	508.0	1589.3	19.9
				DON	4	111.0	1228.7	17.5
				Urea	4	2.5	1.4	0.6
				DFAA	1	9.8	.	.
				H2S	4	3160.7	331588.3	287.9
				BDON	1	11.0	.	.

Appendix C: Mean ± SE porewater nutrient data from the VCR in September 2007

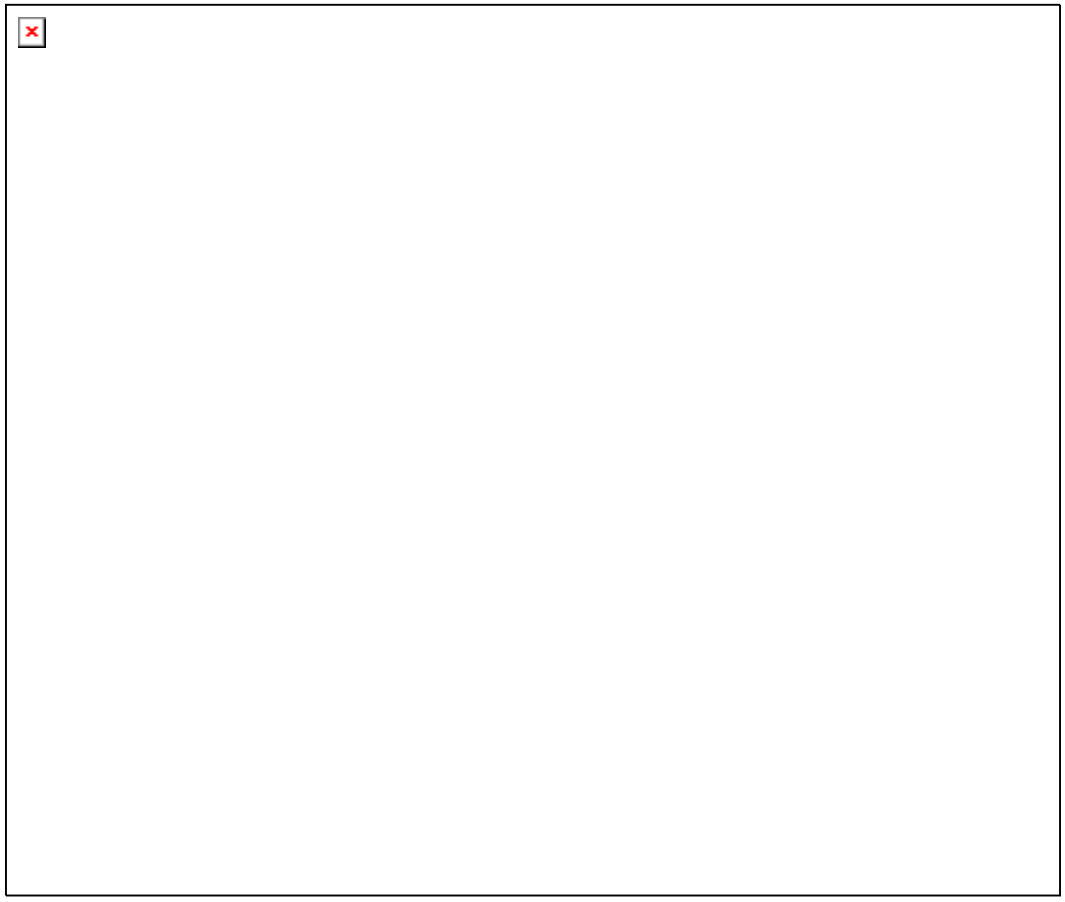
Site	S. alterniflora Zone	Depth (cm)	Month	Variable μM	n	mean	variance	std. err.
FP	Short	10	Sept	NH4	3	6.2	96.8	5.7
				PO4	3	14.9	60.1	4.5
				TDN	3	97.3	1796.9	24.5
				DON	3	91.1	1112.9	19.3
				Urea	3	1.9	0.5	0.4
				DFAA	3	1.9	1.1	0.6
				H2S	3	137.1	33861.5	106.2
				BDON	3	3.8	1.0	0.6
FP	Short	20	Sept	NH4	3	27.5	557.2	13.6
				PO4	3	21.6	54.1	4.2
				TDN	3	123.7	1964.9	25.6
				DON	3	96.2	440.7	12.1
				Urea	3	1.5	0.2	0.3
				DFAA	3	2.0	0.4	0.3
				H2S	3	163.3	3133.5	32.3
				BDON	3	3.5	1.2	0.6
FP	Short	30	Sept	NH4	3	104.3	3670.3	35.0
				PO4	3	35.6	79.8	5.2
				TDN	3	222.0	2859.5	30.9
				DON	3	117.7	158.7	7.3
				Urea	3	3.9	1.3	0.7
				DFAA	3	1.7	0.4	0.4
				H2S	3	1253.3	24332.4	90.1
				BDON	3	5.7	2.0	0.8
FP	Tall	10	Sept	NH4	3	251.2	20970.1	83.6
				PO4	3	79.5	742.8	15.7
				TDN	3	364.9	28000.7	96.6
				DON	3	113.8	612.2	14.3
				Urea	3	3.3	0.4	0.3
				DFAA	3	1.9	0.2	0.3
				H2S	3	6497.5	514117.1	414.0
				BDON	3	5.2	0.4	0.4
FP	Tall	20	Sept	NH4	3	190.7	3218.6	32.8
				PO4	3	55.8	316.4	10.3
				TDN	3	284.9	6114.8	45.1
				DON	3	94.3	575.6	13.9
				Urea	3	1.7	0.1	0.2
				DFAA	3	2.0	1.0	0.6
				H2S	3	5974.5	64364.3	146.5
				BDON	3	3.6	1.6	0.7
FP	Tall	30	Sept	NH4	3	257.3	2076.1	26.3
				PO4	3	52.7	6.3	1.5
				TDN	3	342.7	3113.7	32.2
				DON	3	85.3	276.6	9.6
				Urea	3	4.5	10.9	1.9
				DFAA	3	2.2	0.3	0.3
				H2S	3	5566.3	1509312.3	709.3
				BDON	3	6.7	14.8	2.2
FP	Intermediate	10	Sept	NH4	3	314.7	5302.3	42.0
				PO4	3	121.5	2084.3	26.4
				TDN	3	478.5	8036.9	51.8
				DON	3	163.9	295.9	9.9
				Urea	3	2.7	6.3	1.4
				DFAA	3	1.2	0.1	0.2
				H2S	3	6343.3	918482.8	553.3
				BDON	3	3.9	5.2	1.3
FP	Intermediate	20	Sept	NH4	3	402.0	39109.0	114.2
				PO4	3	89.5	1876.0	25.0
				TDN	3	566.7	52837.3	132.7
				DON	3	164.7	1334.3	21.1
				Urea	3	2.8	0.0	0.1
				DFAA	3	1.9	1.1	0.6
				H2S	2	6556.5	387050.4	439.9
				BDON	3	4.7	1.3	0.7
FP	Intermediate	30	Sept	NH4	2	390.0	1058.0	23.0
				PO4	2	69.0	288.0	12.0
				TDN	2	526.0	968.0	22.0
				DON	2	136.0	2.0	1.0
				Urea	2	2.0	0.9	0.7
				DFAA	2	1.4	0.0	0.1
				H2S	2	6202.7	2082514.0	1020.4
				BDON	2	3.4	0.6	0.6

Site	S. alterniflora Zone	Depth (cm)	Month	Variable μM	n	mean	variance	std. err.
SH Y13	Short	10	Sept	NH4	2	110.0	242.0	11.0
				PO4	2	12.6	61.1	5.5
				TDN	2	391.8	73881.7	192.2
				DON	2	281.8	65666.9	181.2
				Urea	2	1.7	0.1	0.2
				DFAA	2	2.7	8.2	2.0
				H2S	2	651.7	670169.4	578.9
				BDON	2	4.4	10.3	2.3
SH Y13	Short	20	Sept	NH4	3	56.5	934.8	17.7
				PO4	3	15.6	4.9	1.3
				TDN	3	146.9	973.8	18.0
				DON	3	90.4	36.6	3.5
				Urea	3	2.3	6.3	1.4
				DFAA	1	0.5	.	.
				H2S	3	1056.8	73475.1	156.5
				BDON	1	1.5	.	.
SH Y13	Short	30	Sept	NH4	2	330.8	41328.1	143.8
				PO4	2	20.6	13.0	2.6
				TDN	2	482.4	57528.3	169.6
				DON	2	151.7	1336.5	25.9
				Urea	2	0.3	0.2	0.3
				DFAA	0	.	.	.
				H2S	3	2524.6	2169036.3	850.3
				BDON	0	.	.	.
SH Y13	Tall	Low 10	Sept	NH4	3	64.4	1347.7	21.2
				PO4	3	26.4	438.8	12.1
				TDN	3	116.5	271.4	9.5
				DON	3	52.2	432.7	12.0
				Urea	3	0.3	0.3	0.3
				DFAA	3	2.0	1.9	0.8
				H2S	3	2240.4	2563731.5	924.4
				BDON	3	2.3	0.8	0.5
SH Y13	Tall	20	Sept	NH4	3	314.8	53520.1	133.6
				PO4	3	68.4	3553.0	34.4
				TDN	3	416.5	45876.1	123.7
				DON	3	101.7	2542.3	29.1
				Urea	3	1.5	0.6	0.4
				DFAA	3	5.6	26.7	3.0
				H2S	3	3563.2	1086074.4	601.7
				BDON	3	7.1	29.8	3.2
SH Y13	Tall	30	Sept	NH4	3	206.8	19598.6	80.8
				PO4	3	24.3	67.8	4.8
				TDN	3	277.5	18896.4	79.4
				DON	3	70.6	7.0	1.5
				Urea	3	1.8	0.4	0.4
				DFAA	3	1.8	0.2	0.2
				H2S	3	3248.8	380245.2	356.0
				BDON	3	3.6	0.9	0.6
SH Y13	Intermediate	10	Sept	NH4	3	39.1	209.6	8.4
				PO4	3	14.4	474.8	12.6
				TDN	3	55.8	1343.6	21.2
				DON	2	45.0	14.6	2.7
				Urea	3	0.9	1.2	0.6
				DFAA	3	3.5	2.3	0.9
				H2S	3	550.5	568404.3	435.3
				BDON	3	4.5	2.7	0.9
SH Y13	Intermediate	20	Sept	NH4	3	37.8	676.5	15.0
				PO4	3	30.5	308.4	10.1
				TDN	3	99.1	724.2	15.5
				DON	3	61.3	57.3	4.4
				Urea	3	0.1	0.0	0.1
				DFAA	3	4.5	5.3	1.3
				H2S	3	847.7	318268.2	325.7
				BDON	3	4.6	4.8	1.3
SH Y13	Intermediate	30	Sept	NH4	3	72.1	2196.1	27.1
				PO4	3	46.5	250.9	9.1
				TDN	3	142.4	2650.1	29.7
				DON	3	70.3	338.4	10.6
				Urea	3	0.0	0.0	0.0
				DFAA	3	3.2	0.5	0.4
				H2S	3	784.6	240731.6	283.3
				BDON	3	3.3	0.5	0.4

Site	S. alterniflora Zone	Depth (cm)	Month	Variable μM	n	mean	variance	std. err.
SH Y150	Short	10	Sept	NH4	3	215.7	1501.6	22.4
				PO4	3	45.7	59.3	4.4
				TDN	3	378.7	750.3	15.8
				DON	3	163.0	1269.6	20.6
				Urea	3	2.2	1.0	0.6
				DFAA	3	3.3	6.8	1.5
				H2S	3	5490.7	2440107.3	901.9
				BDON	3	5.6	6.8	1.5
SH Y150	Short	20	Sept	NH4	3	310.5	7575.3	50.3
				PO4	3	46.8	583.2	13.9
				TDN	3	450.5	14422.5	69.3
				DON	3	140.0	1266.7	20.5
				Urea	3	1.7	0.0	0.1
				DFAA	3	0.8	0.1	0.2
				H2S	3	5629.8	528547.4	419.7
				BDON	3	2.4	0.1	0.2
SH Y150	Short	30	Sept	NH4	3	320.3	10803.1	60.0
				PO4	3	51.2	1443.1	21.9
				TDN	3	458.4	21105.3	83.9
				DON	3	138.1	1957.6	25.5
				Urea	3	1.8	0.4	0.4
				DFAA	3	1.0	0.2	0.2
				H2S	3	4562.5	543352.2	425.6
				BDON	3	2.8	1.1	0.6
SH Y150	Tall	10	Sept	NH4	3	30.8	429.6	12.0
				PO4	3	8.6	34.6	3.4
				TDN	3	85.6	247.8	9.1
				DON	3	54.8	25.1	2.9
				Urea	3	0.3	0.1	0.2
				DFAA	3	0.4	0.0	0.1
				H2S	3	1771.8	2235423.8	863.2
				BDON	3	0.7	0.0	0.1
SH Y150	Tall	20	Sept	NH4	3	45.7	1034.7	18.6
				PO4	3	11.7	7.4	1.6
				TDN	3	93.6	1573.9	22.9
				DON	3	47.9	60.3	4.5
				Urea	3	0.8	1.4	0.7
				DFAA	3	1.0	0.3	0.3
				H2S	3	1948.7	881465.8	542.1
				BDON	3	1.8	2.0	0.8
SH Y150	Tall	30	Sept	NH4	3	135.8	9933.6	57.5
				PO4	3	13.4	18.4	2.5
				TDN	3	111.1	914.3	17.5
				DON	2	49.9	48.0	4.9
				Urea	3	1.2	2.0	0.8
				DFAA	3	0.4	0.1	0.1
				H2S	3	2453.6	1108669.7	607.9
				BDON	3	1.6	2.2	0.8
SH Y150	Intermediate	10	Sept	NH4	2	302.5	24.5	3.5
				PO4	2	60.3	3.1	1.3
				TDN	2	435.5	1200.5	24.5
				DON	2	133.0	882.0	21.0
				Urea	3	3.2	4.5	1.2
				DFAA	3	5.8	1.7	0.8
				H2S	3	6675.9	275145.1	302.8
				BDON	3	9.1	7.9	1.6
SH Y150	Intermediate	20	Sept	NH4	3	438.5	33522.3	105.7
				PO4	3	74.9	1972.7	25.6
				TDN	3	574.8	27370.6	95.5
				DON	3	136.3	752.3	15.8
				Urea	3	4.5	2.0	0.8
				DFAA	3	3.7	2.6	0.9
				H2S	3	6056.1	235650.9	280.3
				BDON	3	8.2	2.6	0.9
SH Y150	Intermediate	30	Sept	NH4	3	455.0	15825.0	72.6
				PO4	3	61.5	481.2	12.7
				TDN	3	622.7	17966.3	77.4
				DON	3	167.7	76.3	5.0
				Urea	3	7.5	25.6	2.9
				DFAA	3	3.1	0.5	0.4
				H2S	3	6016.8	975418.6	570.2
				BDON	3	10.5	32.3	3.3

Site	S. alterniflora Zone	Depth (cm)	Month	Variable μM	n	mean	variance	std. err.
UPC	Short	10	Sept	NH4	3	62.3	893.9	17.3
				PO4	3	16.7	15.6	2.3
				TDN	2	273.0	3042.0	39.0
				DON	3	132.9	13485.2	67.0
				Urea	3	1.7	2.7	0.9
				DFAA	3	471.0	657797.6	468.3
				H2S	3	1420.3	1758405.4	765.6
				BDON	2	3.4	0.2	0.3
UPC	Short	20	Sept	NH4	4	379.5	13031.2	57.1
				PO4	4	53.8	254.7	8.0
				TDN	4	827.5	72769.0	134.9
				DON	4	448.0	24908.2	78.9
				Urea	4	3.9	1.0	0.5
				DFAA	4	2.6	0.1	0.1
				H2S	4	4994.1	4675220.9	1081.1
				BDON	4	6.4	1.2	0.6
UPC	Short	30	Sept	NH4	4	521.5	171840.3	207.3
				PO4	4	35.7	67.9	4.1
				TDN	4	970.0	435874.7	330.1
				DON	4	448.5	66367.0	128.8
				Urea	4	3.7	1.1	0.5
				DFAA	4	2.7	0.3	0.3
				H2S	4	5121.1	794272.6	445.6
				BDON	4	6.4	1.1	0.5
UPC	Intermediate	10	Sept	NH4	4	250.3	29516.1	85.9
				PO4	4	60.7	190.7	6.9
				TDN	4	604.3	113474.9	168.4
				DON	4	354.0	48653.2	110.3
				Urea	4	4.0	0.4	0.3
				DFAA	4	2.6	0.5	0.4
				H2S	4	3980.5	301528.3	274.6
				BDON	4	6.6	0.9	0.5
UPC	Intermediate	20	Sept	NH4	4	156.7	8533.6	46.2
				PO4	4	15.8	10.7	1.6
				TDN	4	470.4	46546.2	107.9
				DON	4	313.7	18121.6	67.3
				Urea	4	0.5	0.3	0.3
				DFAA	3	3.8	1.2	0.6
				H2S	4	1518.6	1344565.8	579.8
				BDON	3	4.1	0.5	0.4
UPC	Intermediate	30	Sept	NH4	4	844.5	139011.7	186.4
				PO4	4	279.4	239853.3	244.9
				TDN	4	1139.4	719629.7	424.2
				DON	3	730.7	8929.3	54.6
				Urea	4	1059.9	4487364.4	1059.2
				DFAA	3	3.6	4.2	1.2
				H2S	3	3851.9	739238.7	496.4
				BDON	3	4.4	3.6	1.1

Appendix D: Allometric relationship between plant height and dry mass.



Appendix E. *S. alterniflora* height (cm) versus elevation of the marsh surface to mean sea level on July 21, 2008.