*Ecology*, 95(12), 2014, pp. 3293–3303


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

Thomas J. Mozdzer,[1,2,3] Karen J. McGlathery,[2] Aaron L. Mills,[2] and Joseph C. Zieman[2]

[1]*Bryn Mawr College, Department of Biology, 101 N. Merion Avenue, Bryn Mawr, Pennsylvania 19010 USA*
[2]*University of Virginia, Department of Environmental Sciences, 291 McCormick Road, Charlottesville, Virginia 22903 USA*

*Abstract*. North American Atlantic salt marshes are generally considered to be nitrogen (N) limited systems, and plants within these marshes were historically thought to use only inorganic forms of N, such as $NH_4^+$. Recent research has suggested that *Spartina alterniflora* may take up some organic nitrogen compounds directly. To determine the availability of dissolved organic nitrogen (DON) to *S. alterniflora* in marshes along the North American Atlantic coast, porewater was sampled at eight field sites along the North American Atlantic Coast, ranging from Maine (44° N) to Florida (30° N). To determine if a latitudinal gradient existed in the assimilation of either DON or $NH_4^+$, replicated mesocosm experiments were conducted with *S. alterniflora* plants at three field sites (Massachusetts, Virginia, South Carolina), which represented three distinct ecotypes in the latitudinal range. To determine how microbial activity may influence DON availability, we also assessed rates of microbial activity. We found that porewater DON availability increased linearly with latitude. While *S. alterniflora* assimilated DON at all sites, plants from the southernmost site had 50% greater $NH_4^+$ assimilation rates than the other two sites, suggesting that low-latitude *S. alterniflora* may rely more strongly on $NH_4^+$ as an N source. In contrast, high-latitude salt marshes contain greater pools of available DON, and high-latitude *S. alterniflora* plants exhibit similar uptake rates of DON and $NH_4^+$, suggesting a greater reliance upon DON as an N source. We also found that microbial activity decreased by an order of magnitude with increasing latitude along our latitudinal gradient. This suggests that DON uptake is more important at high-latitude locations, where temperature constrains microbial mineralization of organic N. Our analysis also suggests that N limitation of *S. alterniflora* increases with decreasing latitude. We suggest that other detritus-based ecosystems may exhibit similar latitudinal patterns regarding DON availability and use.

*Key words:* *amino acid; DON; latitude; mineralization; N; $NH_4$; salt marsh;* Spartina; *temperature*.

## Introduction

A central hypothesis in the field of biogeography is 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 of the North American Atlantic Coast are an ideal ecosystem in which to explore central questions in biogeography because they are dominated by monocultures of the foundation species, *Spartina alterniflora* (Mitsch and Gosselink 1993, Pennings and Bertness 2001). Temperature and length of the growing season have been correlated to observed latitudinal trends in salt marsh ecosystem productivity (Kirwan et al. 2009), with nitrogen (N) availability generally limiting the productivity of ecosystems (Valiela and Teal 1974). *S. alterniflora* from mid-Atlantic salt marshes uses dissolved organic nitrogen (DON) to supplement inorganic N uptake (Mozdzer et al. 2010). However, it is not known how the use of DON by *S. alterniflora* varies across its range along the North American Atlantic coast. The dominance of a single foundation species along a large latitudinal range provides a unique opportunity to study how biogeography, biogeochemistry, and ecophysiology interact to influence ecosystem-level productivity.

Few studies have investigated how ecotypic or genetic variation correlate with the observed productivity gradients along the North American Atlantic coast. High-latitude salt marshes experience a colder, shorter growing season, resulting in slower organic matter decomposition, and greater accumulation of refractory materials such as peat (Craft 2007, McCall and Pennings 2012). Additionally, high-latitude salt marshes can be severely impacted by winter-time ice scour, further limiting primary production. Conversely, low-latitude salt marshes experience a longer, warmer growing season, greater salinity due to increased evaporation, 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).

Manuscript received 26 September 2013; revised 24 February 2014; accepted 27 February 2014; final version received 30 May 2014. Corresponding Editor: S. C. Pennings.
[3] E-mail: tmozdzer@brynmawr.edu

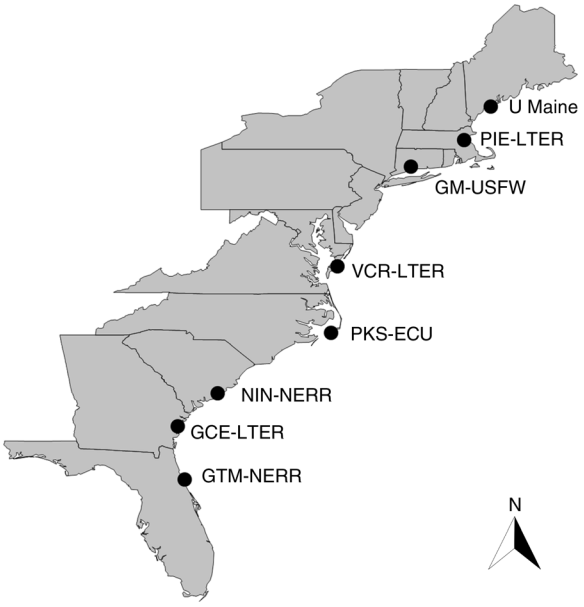


FIG. 1. Location of the field sites along the North American Atlantic coast forming our latitudinal gradient.

Geographic barriers to gene flow and environmental variation along the North American Atlantic coast have resulted in a continuum of genetic and phenotypic variation in *S. alterniflora* (Blum et al. 2007), with high-latitude ecotypes exhibiting greater shoot N concentrations, lower stem density, and smaller canopy size than those of southern marshes (Seliskar et al. 2002). High-, mid-, and low-latitude salt marshes correlate well with the recently identified genetic lineages (chloroplast DNA haplotypes) of *S. alterniflora* (Blum et al. 2007); with distinct northern, mid-, and southern Atlantic coast groups. At this time, it is unclear if these ecotypic/genetic differences are associated with traits that could affect productivity.

Productivity along this latitudinal gradient is primarily attributed to bottom-up physio-chemical forcing. For example, the addition of N results in increased salt marsh productivity regardless of the latitudinal context (Valiela and Teal 1974, Gallagher 1975, Chalmers 1979, Mendelssohn 1979, Dai and Wiegert 1997). 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) also can affect *S. alterniflora* productivity.

While dissolved inorganic nitrogen (DIN) is assumed to support plant N demand, there is recent evidence suggesting that dissolved organic nitrogen (DON) is also important. Direct uptake of DON in salt marsh ecosystems may help explain the high productivity of oligotrophic marshes. We previously demonstrated that *S. alterniflora* can use DON directly via roots (Mozdzer et al. 2010) as well as through foliar uptake (Mozdzer et al. 2011), and that DON can provide up to 24% of plant N demand (Mozdzer et al. 2010). Similarly, DON is an important N source in a variety of detritus-based ecosystems including arctic tundra (Chapin et al. 1993, Kielland 1994, Schimel and Chapin 1996), arctic salt marshes (Henry and Jefferies 2003), boreal forests (Nasholm et al. 1998), grasslands (Streeter et al. 2000, Weigelt et al. 2003), and temperate coastal lagoons (Tyler et al. 2001). However, few have studied the potentially important pools of dissolved free amino acids (DFAA) and urea-N in temperate salt marshes. Previous studies reported DFAA-N concentrations up to 9 μmol/L N in short-form *S. alterniflora* sediments in Georgia, USA (Gardner and Hanson 1979) and up to 12.8 μmol/L DFAA-N in a mid-Atlantic *S. alterniflora* marsh in Virginia, USA (Mozdzer et al. 2010). At the northern extreme, DFAA in arctic salt marshes dominated by *Pucinellia phrygranodes* were up to 80 μmol/L (Henry and Jefferies 2002). These values suggest a possible gradient in the availability of DON with latitude.

Due to differences in *S. alterniflora* productivity with latitude at the continental scale, 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- vs. low-latitude salt marshes due to slow organic matter mineralization, and that (2) high-latitude *S. alterniflora* ecotypes would rely more heavily upon this N source. We present here the results of in situ short-term mesocosm experiments designed to assess the relative magnitudes of DON and DIN utilization, and a survey to quantify N pools along a latitudinal gradient. These studies were combined with estimates of heterotrophic microbial activity to determine how N availability and ecotypic variation interact to influence DON uptake over a latitudinal range from 30° to 44°. Our data suggest that DON uptake is more important at high-latitude locations, where temperature constrains microbial decomposition of organic N.

## METHODS

### *Plant N assimilation rates*

Three field sites were selected that spanned the biogeographic provinces of the North American Atlantic coast (Hayden and Dolan 1973, 1976, Seliskar et al. 2002; see Plate 1). The sites included Plum Island Ecosystems (PIE) Long Term Ecological Research (LTER) site, Virginia Coast Reserve (VCR) LTER site, and North Inlet (NIN) National Estuarine Research Reserve (NERR) (Fig. 1). Earlier studies have found that *S. alterniflora* originating from these biogeographic provinces also correspond to distinct ecotypes (Seliskar et al. 2002) and *cp*DNA genotypes (Blum et al. 2007). Our high-latitude site, PIE-LTER, is at the northernmost extent of the gradient, and located within the

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

To quantify in situ plant N uptake, $^{15}N$ was used as a tracer in short-term mesocosm experiments. Using a 10-cm internal diameter × 35 cm long section of PVC pipe, 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 collected to a depth of at least 30 cm, which is beyond the active root zone of *S. alterniflora*. Root depth profiles were not quantified in this study, but observations indicated that roots were almost exclusively restricted to the upper 20 cm at all sites. Each open-ended core was placed into an individual 5-L container that was filled to a depth of 10 cm with creek water from the site. This flooded the bottom of the core and maintained a constant hydroperiod and anoxic conditions. The core mesocosms were placed in an open area near the salt marsh, and thus were exposed to ambient light and temperature conditions. The experiments were conducted early in the growing season when plants were actively growing. We began with the low-latitude site, NIN (30 May–1 June 2006), followed by VCR (5–7 June 2006), and finally PIE (19–21 June 2006).

Each mesocosm was amended with 14 mL of DI water containing $^{15}N$-$NH_4$, $^{13}C^{15}N$ glycine, or only DI water as a control. Five replicate mesocosms at each site were randomly assigned to each of the three treatments. Each N treatment received a total of 30 μmol $^{15}N$ dissolved in 14 mL of DI water, which is <1% of available porewater, given the porosity of the marsh soils. N treatments were added through a series of seven equally spaced injections throughout the core; 2 mL were added per injection to equally distribute the $^{15}N$.

The experiment was terminated after 48 hours by cutting off the aboveground biomass at the sediment surface. The stems and leaves were washed carefully under tap water, wiped dry, promptly frozen, and stored until they could be lyophilized. Sediments were rinsed from the belowground biomass with tap water, and subsamples of roots and rhizomes were selected from the mesocosm core. Only live, turgid roots were selected to measure assimilation of N by active plant uptake. Additionally, dead root and rhizome material was collected and analyzed as a control for abiotic sorption + adherent microbial assimilation. The root materials were blotted dry, frozen, and subsequently freeze-dried. 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 sent to the University of California Davis Stable Isotope Facility for analysis of $^{15}N$ and $^{13}C$ isotope ratios, and also for elemental C and N analysis. Assimilation of stable isotopes was determined by comparing stable isotope ratios in control plants, which received only DI water, to N-treated enriched plants, using the following equations:

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

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

where $N_{total}$ = the total amount of N (in grams) in the total mass of each tissue fraction; %N is the elemental N content in the analyzed sample; DM is the dry mass (in grams) of the representative biomass; $^{15}N_{asm}$ is the mass of $^{15}N$ (in micrograms) assimilated into the representative biomass in the analyzed sample; $\%^{15}N_{trt}$ is the $^{15}N$ concentration of the sample from the N-treated mesocosm at the end of the 48-hour experiment; $^{15}N_{control}$ is the concentration of $^{15}N$ in the unlabeled-control plant material. Dead belowground roots and rhizomes were used as the control for root and rhizome material with respect to sorption onto the plant material or uptake by adherent microbes. To correct for abiotic sorption and adherent microbial assimilation in roots and rhizomes, the $^{15}N$ content of the dead material was subtracted from the $^{15}N_{trt}$ for each component. These calculations allowed for the determination of $^{15}N$ assimilated throughout the plant. Root mass specific N uptake rates were determined using the equation

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

where $N_{uptake}$ is the total plant N uptake rate normalized to the dry mass of the roots expressed as micrograms $^{15}N$ per gram dry mass of root; $^{15}N$ with subscripts "root," "rhizome," "stem," "leaves," is the amount (in micrograms) of N recovered in each component of the plant, $DM_{root}$ is the dry mass (in grams) of total root biomass, and $t_{exp}$ is the duration of the uptake experiment. Since only a subsample of both roots and rhizomes was 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 equal proportions of roots and rhizomes.

Analysis of variance (ANOVA) was used to test for effects of N treatment and location (biogeographic province) using an $\alpha$ value of 0.05 to determine significant differences. Our experimental design was factorial, with three latitudinal locations and two nitrogen treatments. When significant effects were found by ANOVA, post hoc tests (Tukey's honestly significant

difference tests) were performed to identify pairwise differences among means. All statistical analyses were performed in SAS version 9.2 (SAS Institute 2008).

### *N availability*

Eight field sites were selected along the North American Atlantic coast to determine how the concentration of bioavailable DON varied over the latitudinal range extending from Maine to Florida (Fig. 1; Appendix A). Of these eight, six have been described and used in an earlier latitudinal study (Blum et al. 2004). These sites included the three sites where the plant assimilation rates were determined (PIE-LTER, VCR-LTER, and NIN), as well as the Georgia Coastal Ecosystems (GCE-LTER), the Great Meadows salt marsh in the Stewart B. McKinney National Wildlife Refuge, in Connecticut (previously identified as Fairfield University [FU]), and a salt marsh in Wiscasset, Maine, previously identified as the University of Maine. We also included a second National Estuarine Research Reserve (Guana-Tolomato-Matanzas [GTM]), Florida, and the Pine Knoll Shores (PKS) site established by East Carolina University (Fig. 1). These sites were approximately equally distributed throughout the three biogeographic provinces along our latitudinal gradient. At each site, porewater was sampled in August 2006 using porewater equilibrators situated 10 cm below the soil surface. This depth was within the active root zone of both the low and high marsh, which corresponded to intermediate and short growth form *S. alterniflora*, respectively, and represented increased levels of abiotic stress from higher flooding frequency and duration. We did not measure porewater N in creek bank, tall-form *S. alterniflora*. We assumed that if nutrients crossed the equilibrator membrane, they were available for plant uptake and were representative of long-term pools. We focused our study on porewater N pools because this represents plant available N in the soil matrix, whereas extractable N, e.g., via KCl extraction, can overestimate "plant available" cations, such as $NH_4^+$ and amino acids, that are tightly bound to soil particles. Collected porewater was filter sterilized (GHP, 0.2 mm, Pall Microfilters, Cortland, New York, USA) and frozen for subsequent analyses. Water from the PIE and NIN sites was collected as part of a long-term experiment conducted by J. T. Morris, and was also filter sterilized and frozen. $NH_4^+$ was analyzed using a Lachat QuickChem 8500 (Lachat, Loveland, Colorado, USA) 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, California, USA) using the AccQ-Tag chemistry package from Waters Corporation (Milford, Massachusetts, USA). We report total DFAA-N, and adjusted N content accordingly for amino acids containing more than one N atom (e.g., arginine, histidine, and lysine). Regression analysis was performed in SAS (proc REG) to investigate how N availability varied among salt marshes in each marsh zone along the latitudinal gradient.

### *Estimation of microbial activity*

To estimate rates of microbial mineralization, four 7.6 cm internal diameter × 40 cm deep cores were taken from each field site on 4 August 2006. Plant shoots were cut at the sediment surface, and intact sediment cores were sealed immediately to maintain anaerobic conditions, and shipped overnight on ice to the laboratory in Charlottesville, Virginia, USA. The following morning, sub-cores were taken at depths of 2, 5, 10, 20, 30 cm 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 sharpened de-tipped 10-$cm^3$ plastic syringe as a piston corer (Herlihy and Mills 1985). The sub-core was immediately capped with a serum stopper to minimize exposure to the atmosphere.

Each sub-core was injected with 100 μCi of 2-$^{14}$C-acetate in 25 μL of sterile DI water, and recapped. Acetate was chosen since it is the preferred C substrate of sulfate-reducing bacteria, which are the main heterotrophs in salt marsh sediments (Hansen 1993). The sub-cores were incubated for two hours at room temperature (22° ± 1°C). The contents of the sealed sediment core were transferred into 50-mL Erlenmeyer flasks and slurried with 20 mL of deionized water. The Erlenmeyer flask was capped with a rubber septum, and microorganisms were killed by the addition of 2 mL of 4 mol/L $H_2SO_4$ to the slurry. The $^{14}CO_2$ resulting from $^{14}$C-acetate mineralization was trapped in 0.1 mL phenethylamine. The phenethylamine was placed into liquid scintillation cocktail, and mineralized $^{14}CO_2$ was measured on a Beckman LS 6500 Multi Purpose Scintillation Counter (Beckman Instruments, Palo Alto, California, USA). The results were corrected for quench, and the relative microbial activity is reported as disintegrations per minute (dpm) $^{14}CO_2$ per cubic centimeter of sediment. Statistical analysis was conducted in SAS (V9.2, SAS Institute 2008); we used ANOVA to investigate differences among provinces in relative microbial activity by site and depth, and through the interaction between variables.

## Results

### *Plant N assimilation*

Plant N assimilation rates varied significantly by latitudinal location (site) ($F_{2,29} = 15.54$, $P < 0.0001$) and N treatment ($F_{1,29} = 4.26$, $P = 0.0500$) (Fig. 2A) with greater mass-specific N uptake rates of DIN vs. DON. There was a nonsignificant trend indicating a site × N-treatment interaction, suggesting that DON and DIN uptake rates were similar in the higher-latitude sites (PIE and VCR) ($F_{2,29} = 2.95$, $P = 0.07$). Because high-latitude S. *alterniflora* had significantly less biomass than either the mid- or low-latitude plants ($F_{2,44} = 45.66$, $P < 0.001$)

(Appendix B), plant uptake rates were normalized to the gram dry mass (gdm) of roots. The amount of $^{15}N$ incorporated into the plant biomass varied by site ($F_{2,29} = 25.69$, $P < 0.0001$) (Fig. 2B), although there was no significant effect of N treatment on $^{15}N$ assimilation ($F_{1,29} = 2.31$, $P = 0.14$). $NH_4^+$ uptake rates varied significantly by site ($F_{1,14} = 13.35$, $P = 0.0009$), and post hoc tests indicated that rates were greatest at the southernmost site, NIN (Fig. 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% (mean ± SE) of the amount taken up recovered in the plant biomass (Fig. 2B). Significantly less $^{15}NH_4^+$ was taken up at sites with increasing latitude, i.e., lower $NH_4^+$ uptake rates were observed with high- and mid-latitude plants (Fig. 2B).

DON uptake did not vary significantly among the three latitudinal sites ($F_{2,14} = 2.735$, $P = 0.11$) (Fig. 2A). However, rates from high- and mid-latitude plants tended to be greater than those observed at PIE. More than 49% of the amended $^{15}N$ DON was recovered in the plant biomass in the mid- and low-latitude plants, whereas only 18% was recovered in high-latitude plants ($F_{2,14} = 11.48$, $P = 0.0016$) (Fig. 2B). We used estimates of microbial activity described below to estimate the amount of mineralization that may have occurred prior to assimilation.

Our estimates of N uptake are conservative, since the processes of $^{15}N$ sorption onto root and rhizome surfaces was corrected for based on the $^{15}N$ content of dead biomass being used for controls. At all sites, mean dead belowground material was enriched by 12% and 18% above natural $^{15}N$ abundance for glycine and $NH_4^+$, respectively. By using dead roots and rhizome material, our estimates remove both the process of sorption of the $^{15}N$ onto the plant material and an undefined amount of $^{15}N$ processed by rhizoplane microbes. As such, our estimates are strictly indicative of plant uptake.

N translocation of assimilated N differed among the three sites. While the majority (~85%) of assimilated N was recovered in roots and rhizomes with no significant difference between sites (Fig. 3), N translocation to leaf tissue varied by site ($F_{2,29} = 15.38$, $P < 0.0001$). Post hoc tests indicated that *S. alterniflora* plants at our high-latitude location, PIE, had significantly more recoverable N in leaf tissues than did plants from either VCR or NIN (Fig. 3). The greater amount of N assimilated into the leaf tissue may be attributed to the greater leaf-to-stem ratio in *S. alterniflora* at our high-latitude site when compared to plants at either mid- or low-latitude locations (Appendix B). Even though the high-latitude plants (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 latitude (Appendix B). Belowground, only rhizome translocation varied by site ($F_{2,29} = 3.97$, $P < 0.0313$), with greater $^{15}N$ found at the VCR and PIE sites than at NIN (Fig. 4).

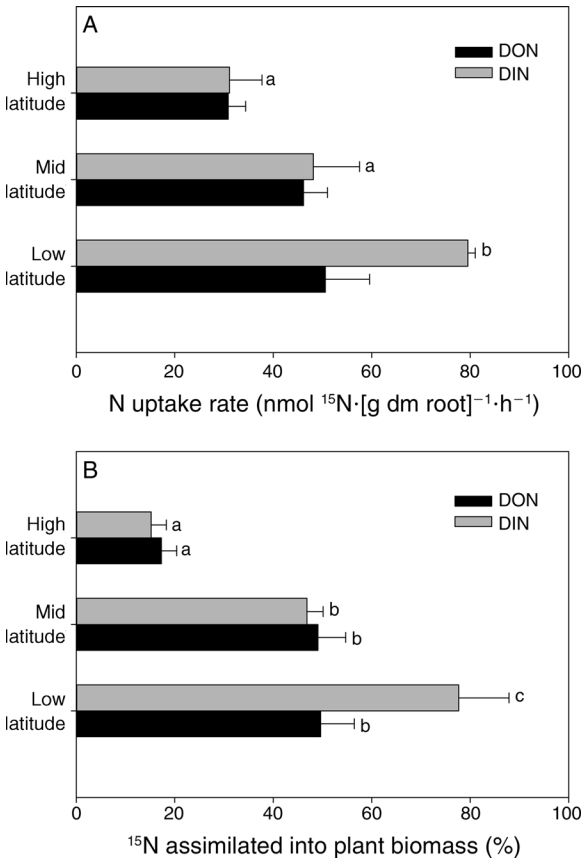


FIG. 2. (A) Effects of N treatment and site on $^{15}N$ assimilation rates (mean + SE). These included the Plum Island Ecosystems (PIE) LTER, the Virginia Coast Reserve (VCR) LTER, and the North Inlet (NIN) National Estuarine Research Reserve, corresponding to high-, mid-, and low-latitude locations, respectively. Data were normalized to g dry mass of root per hour, $n = 5$ per N treatment. Significant pairwise differences (±) are indicated by different letters. N uptake rate varied significantly by site ($P < 0.0001$) and by available N form ($P = 0.050$) (DON, dissolved organic N; DIN, dissolved inorganic N). (B) Mean (±SE) percentage of the $^{15}N$ tracer assimilated by *S. alterniflora* at each site during the 48-h experiment. The amount assimilated varied significantly by site ($P < 0.0001$), but N form had no significant effect ($P = 0.142$). Significant differences among pairs of means ($\alpha = 0.05$) are indicated by different letters. $^{15}N$ content of dead root and rhizome material were subtracted from each $^{15}N$ treatment to correct for abiotic sorption and/or adherent microbial assimilation (see *Methods*).

### *N availability*

Plant-available N varied by N species across the broad latitudinal range. Individual amino acid concentrations ranged from nanomolar to micromolar concentrations, and are presented as total DFAA-N. Combined plant-available DON concentrations (DFAA-N + urea-N), hereafter DON, and urea-N, were log transformed to satisfy homogeneity of variance assumptions.

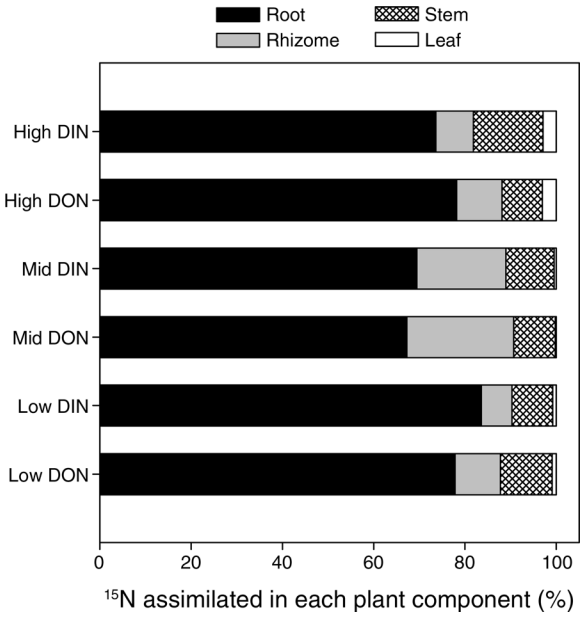


FIG. 3. The percentage of assimilated $^{15}N$ DON and DIN in roots, rhizomes, stems, and leaves of *S. alterniflora* at the conclusion of the 48-h experiment in plants from high-, mid-, and low-latitude locations (PIE, VCR, and NIN, respectively; see *Methods*). Assimilation of $^{15}N$ into each plant component only varied significantly by leaves ($P < 0.0001$), with *S. alterniflora* from the high-latitude location assimilating significantly more N than plants at the mid- and low-latitude locations.

In the low-marsh, intermediate-form *S. alterniflora* zone, porewater DFAA ($P = 0.0004$, $r^2 = 0.36$) and urea-N ($P = 0.016$, $r^2 = 0.23$) increased significantly with increasing latitude (Fig. 4B). No effect of latitude was observed for $NH_4^+$ ($P = 0.79$) (Fig. 4A). Similarly, in the high-marsh, short-form *S. alterniflora* zone, DFAA also increased significantly with increasing latitude ($P < 0.0001$, $r^2 = 0.52$), but exhibited a marginally nonsignificant increase in urea-N ($P = 0.081$, $r^2 = 0.13$). In contrast to the low-marsh zone, $NH_4^+$ increased significantly with increasing latitude ($P = 0.038$, $r^2 = 0.15$) in the high-marsh zone (Fig. 4C).

To estimate how strongly DON varied along the Atlantic Coast latitudinal gradient, DON data were pooled for both marsh zones. We found significant increases in DON availability with increasing latitude ($P < 0.0001$, $r^2 = 0.46$) along the latitudinal gradient (Fig. 5).

### *Microbial activity and DON uptake*

We observed a significant shift in relative microbial activity by latitude (Fig. 6). Two-way ANOVA indicated that microbial activity increased with decreasing latitude ($F_{8,59} = 23.77$, $P < 0.0001$) and decreased by depth ($F_{4,59} = 4.88$, $P < 0.0023$). We also identified an interaction between site and depth where relative microbial activity decreased more by depth in both the low- and mid-latitude sites ($F_{2,59} = 2.47$, $P < 0.0258$). Post hoc tests indicated that rates differed between all pairs of sites, with NIN (at low latitude) exhibiting the greatest rates of microbial activity, VCR (at mid-latitude) exhibiting intermediate rates, and PIE (at high latitude) exhibiting the lowest rates. Post hoc tests investigating differences in microbial activity by depth indicated significantly decreasing rates with depth. All sites had similar microbial activity at the 30-cm depth, which is below the active root zone in *S. alterniflora* marshes. Elemental C:N ratios of soil samples collected from the same sites 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 alanine in vegetated salt marsh sediments were 8.32 $pmol \cdot cm^{-3} \cdot h^{-1}$ at 10 cm depth in short-form *S. alterniflora* sediments, and 23.4 $pmol \cdot cm^{-3} \cdot h^{-1}$ at 20 cm depth in tall-form sediments (Hanson and Gardner 1978). In this case, if we assume that these rates are constant throughout the mesocosm, we can expect 0.97 to 2.72 μmol of the initial 30 μmol $^{15}N$ glycine to have been mineralized to $NH_4^+$ during the 48-hour experiment ((8.32 to 23.4 $pmol \cdot cm^{-3} \cdot h^{-1}$) × 48 h × (2430 $cm^3$ sediment)/$10^6$ = 0.970 to 2.72 μmol mineralized glycine N). This estimate assumes that $^{15}N$ glycine was the sole source of heterotrophic metabolism, and therefore gives a theoretical maximum. While some of the $^{15}DON$ was likely mineralized prior to assimilation, under the described circumstances, this would account for at most 9% of the starting material. This estimate is conservative, since it does not account for differences in heterotrophic activity that may change with depth, potentially by an order of magnitude (Fig. 5, and Hanson and Gardner 1978). Even given 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 site (2.72 μmol mineralized out of 18.11 ± 1.78 μmol recovered from plants) and VCR site (2.72 μmol mineralized out of 19.21 ± 1.08 μmol recovered in the plant). Due to lower total assimilation at PIE, microbial mineralization may potentially account for 35% of the DON assimilated (2.72 μmol mineralized out of 7.62 ± 1.31 μmol recovered from plants) (Fig. 2B). Since microbial activity at PIE is an order of magnitude lower than NIN (Fig. 5), we assume that nearly all DON uptake at PIE can be attributed to plant processes alone and was not mineralized prior to assimilation.

## DISCUSSION

Our data indicate that DON is an available N source for *S. alterniflora* in temperate salt marshes, and that the utilization of this DON pool varies with latitude. High-latitude salt marshes had the greatest pools of available DON, and because plants had similar rates of DON and

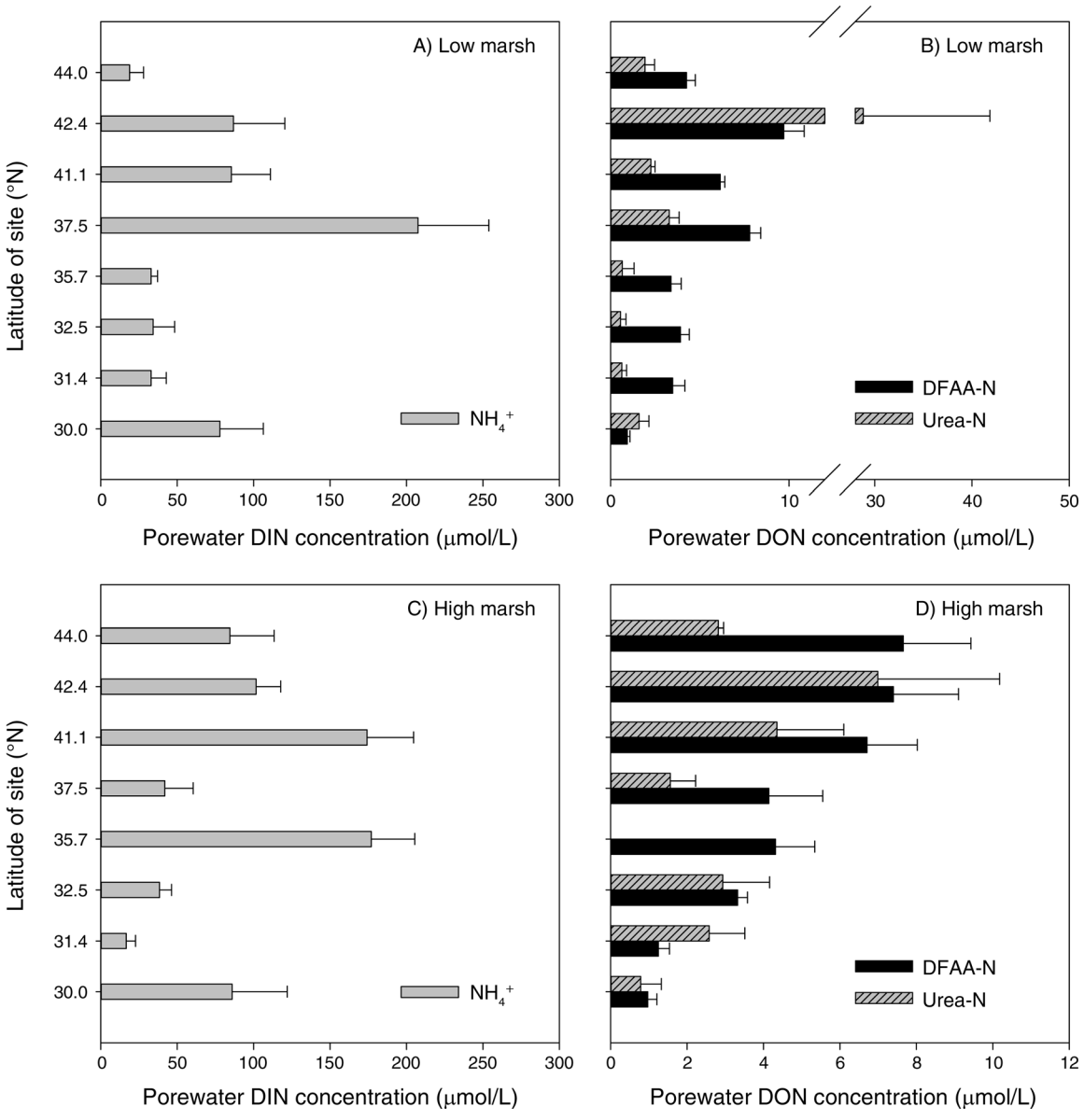


FIG. 4. Mean (+SE) porewater N availability of (A) $NH_4^+$ in low-marsh, intermediate growth form *S. alterniflora*, (B) dissolved free amino acids (DFAA) and urea-N in low-marsh, intermediate-form *S. alterniflora*, (C) $NH_4^+$ in high-marsh, short growth form *S. alterniflora*, and (D) DFAA and urea-N in high-marsh, short growth form *S. alterniflora* at the 10-cm depth in eight field sites forming a latitudinal gradient (see *Methods*). Linear regression of porewater-N over latitude indicate: (A) no significant relationship ($P = 0.79$) of $NH_4^+$ in the low marsh; (B) increases of both DFAA ($P = 0.0004$, $r^2 = 0.36$) and log-transformed urea-N ($P = 0.0156$, $r^2 = 0.23$) with increasing latitude; (C) increases of log-transformed $NH_4^+$ with latitude ($P = 0.0376$, $r^2 = 0.15$); and (D) increases in both DFAA ($P < 0.0001$, $r^2 = 0.52$) and a nonsignificant increase in log-transformed urea-N with latitude ($P = 0.0808$, $r^2 = 0.13$).

DIN uptake, this suggests a greater dependence on DON as an N source. Ecotypic differences in N uptake rate and the form of N used may be attributed to differences in productivity at the continental scale (Kirwan et al. 2009). Ecotypes originating from high-latitude sites may have adapted to greater organic N availability, and our data suggest that high-latitude *S. alterniflora* relies more heavily on DON as an N source to sustain its N requirements.

The use of DON by *S. alterniflora* in salt marshes is consistent with other detritus-based ecosystems characterized by slow organic matter decomposition that result in pools of available DON. While DON uptake by *S. alterniflora* may not account for as much N as it does in arctic or boreal forest ecosystems (up to 80% of N demand [Chapin et al. 1993, Kielland 1994]), our results are consistent with previous estimates suggesting that DON uptake may account for ~24% of total N demand (Mozdzer et al. 2010) in mid- and high-latitude salt marsh ecosystems. In these systems, DON availability is greater and plants have equal preference for DON and DIN. Due

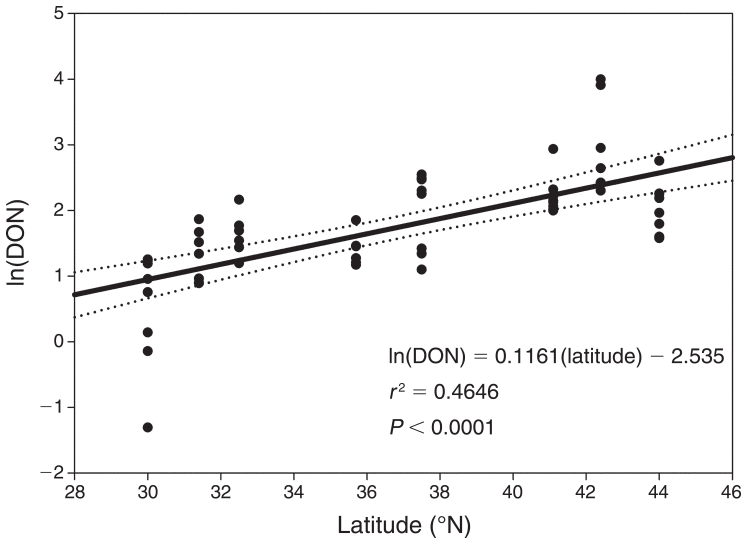


FIG. 5. Relationship between bioavailable dissolved organic nitrogen (DON = DFAA-N + urea-N) and latitude in both high- and low-marsh *S. alterniflora* marshes. DON availability increased significantly ($P < 0.0001$) with increasing latitude. Each point is an independent measurement of DON; dotted lines indicate 95% confidence intervals. Data were log transformed to satisfy homogeneity of variance assumptions.

to temperature effects on mineralization (Gao et al. 2011), the relative importance of DON use will likely vary along a latitudinal/temperature gradient. Thus, DON uptake may be a more ecologically important process in high-latitude salt marshes, due to both greater DON pools and similar uptake rates of DON and DIN.

DON availability, and its relative importance as an N source to salt marsh plants, increased with increasing latitude. Our synoptic data show that porewater DON was strongly correlated with latitude (Figs. 5 and 6). Hence, given declining DON pools with decreasing latitude, the potential contribution of DON as an N source also decreases. For example, in the low-latitude site, DIN uptake rates were approximately twice those at high-latitude sites, and *S. alterniflora* plants relied more strongly on DIN as an N source. Even though DON uptake rates were similar across sites, DON pools were low relative to DIN pools, suggesting that DON does not likely contribute strongly to plant N demand in low-latitude salt marshes.

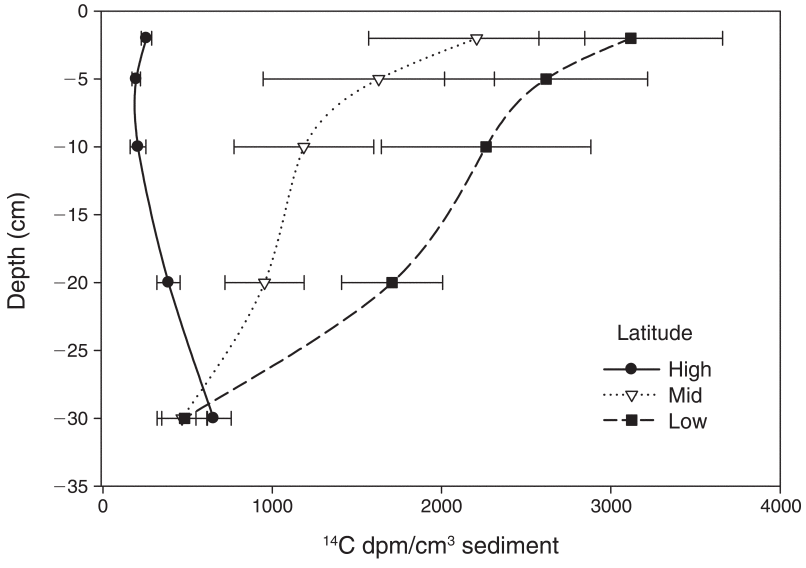


FIG. 6. Relative microbial activity with depth at the high-, mid-, and low-latitude field site, expressed as $^{14}$C disintegrations per minute (dpm) per cubic centimeter of sediment (mean ± SE; $n = 4$ cores per site). Microbial activity varied significantly with depth ($P < 0.0001$) and by site ($P = 0.023$). Post hoc tests indicated that means at all depths were significantly different from each other, except at the 30-cm depth, where no significant differences were observed.

PLATE 1. (Top) *Spartina alterniflora* salt marsh at the Virginia Coast Reserve LTER and (bottom) a salt marsh on Sapelo Island, part of the Georgia Coastal Ecosystems LTER site, both east coast U.S. locations. Photo credits: T. J. Mozdzer.

Our data clearly demonstrate that DON utilization can vary across latitudinal scales; however, there are also limitations to our approach. Our analysis of DON uptake occurred at only three sites within a 14° latitudinal range; therefore, our results should be interpreted cautiously. Given this caveat, these sites were chosen because they likely contained *S. alterniflora* ecotypes (Seliskar et al. 2002), which are likely representative of *S. alterniflora* genotypes that vary along the latitudinal continuum of the North American Atlantic coast (Blum et al. 2007).

Observed differences in DIN uptake rates may be attributed to ecophysiological adaptations to the form of N available and to differences in N demand at each latitudinal location. The gradient in DIN uptake rate from high- to low-latitude salt marshes corresponds well with documented increases in productivity (Turner 1976, Kirwan et al. 2009), and greater plant N demand with decreasing latitude. To illustrate differences in plant N demand, we estimated the aboveground N demand using the regression of latitudinal origin and biomass from Kirwan et al. (2009) in combination with our aboveground N content data. This calculation suggests that mid- and low-latitude plants require 14–20% more N than high-latitude *S. alterniflora*, 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 that greater growth and N demand occurs in low- and mid-latitude salt marshes. Due to greater N demand and lower shoot N concentrations, our data also suggest that *S. alterniflora* experiences greater N limitation with decreasing latitude.

Patterns in N translocation also suggest that there are 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 leaf tissue than did mid- or low-latitude plants from either VCR or NIN. The rapid translocation of N to leaves may be attributed to faster growth in the shorter growing season at high-latitude sites, where plants allocate proportionally more biomass to leaves (greater leaf : stem), to support the photosynthetically active tissue. More research is needed on differences in N translocation strategies among the three *S. alterniflora* ecotypes, and the ecological implications of translocation differences.

Our data suggest that heterotrophic microbial activity drives the availability of N forms along the Atlantic Coast latitudinal gradient. These results are consistent with observed decreases in denitrification with increasing latitude in coastal watersheds (Schaefer and Alber 2007). Because 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 high-latitude site, PIE. The most parsimonious explanation for our observed patterns in DON availability can be attributed to differences in microbial abundance and sediment temperatures, which also follow the reported increases in both microbial and fungal abundance on standing dead biomass with decreasing latitude (Blum et al. 2004). Therefore, as microbial activity increases with decreasing latitude, less DON is available for plant use, which corresponds to greater reliance upon DIN to satisfy plant N demand in low-latitude salt marshes.

By combining N availability, N use, and microbial activity, we present a more complete picture of the factors controlling primary production in Atlantic coast salt marshes. We suggest that there is potential for DON uptake by *S. alterniflora* in high-latitude salt marshes to account for more than the 24% of plant N demand previously reported for mid-latitude salt marshes (Mozdzer et al. 2010, 2011). Specifically, in high-latitude marshes, where plant available N pools are approximately 2:1 for $NH_4^+$:DON, and given that DON and $NH_4^+$ uptake rates are similar, our data suggest DON may account for up to 50% of plant available N. Conversely, in low-latitude marshes, where pools of bioavailable DON are significantly lower, the contribution of DON to total plant N demand may be even lower than the 24% reported in mid-latitude marshes. Our data suggest that the availability of DON is largely dependent on rates of microbial activity, which we show can change by an order of magnitude in North American Atlantic coast salt marshes. Although the N cycle differs between salt marshes and upland systems, the influence of temperature on the microbial community, rates of N mineralization, and organic N availability is likely shared. Our results raise the possibility that latitudinal patterns of organic N use and availability may be an underappreciated phenomenon of soil N dynamics in general.

## Acknowledgments

Support for this study was provided by the National Science Foundation (NSF) Division of Environmental Biology (DEB) to the Virginia Coast Reserve Long Term Ecological Research Program (DEB-0621014 and DEB-1237733) and a Fred Holmsley Moore Research Award to T. J. Mozdzer. Collaborative support was provided by NSF DEB-1052636 to the University of South Carolina and NSF OCE 1238212 and NSF OCE 0423565 to the Marine Biological Laboratory. We also thank Luke Cole, Rick Gleeson, Kimberly Holzer, Paul Kenny, Gary King, Ron Kneib, Mack Lee, Christina Maki, Margot Miller, Lidia Mozdzer, Karen Sundberg, and Christine Voss for field, laboratory, and coordination assistance. Special thanks to Jim Morris for sharing porewater samples and porewater data. Site access, permits, and/or field support were provided at the Great Meadows Salt Marsh, Guana Tolomato Matanzas National Estuarine Research Reserve, Bell Baruch Marine Laboratory, and the North Inlet National Estuarine Research Reserve, and three LTER sites including Plum Island Ecosystems, Virginia Coast reserve, and the Georgia Coastal Ecosystem. We also thank Joshua Caplan, J. Adam Langley, and two anonymous reviewers for suggestions that greatly improved the manuscript.

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## Supplemental Material

### Ecological Archives

Appendices A and B are available online: http://dx.doi.org/10.1890/13-1823.1.sm