

Changes in $\delta^{15}\text{N}$ in salt marsh sediments in a long-term fertilization study

Erin L. Kinney^{1,*}, Ivan Valiela²

¹Department of Marine Biology, Texas A&M University at Galveston, 200 Seawolf Parkway, Galveston, Texas 77553, USA

²The Ecosystems Center, Marine Biological Laboratory, 7 MBL Street, Woods Hole, Massachusetts 02543, USA

ABSTRACT: Nitrogen retention by salt marshes has been suggested as a means of mitigating the delivery of land-derived nitrogen loads to coastal waters. As land-derived nitrogen loads increase, it is unclear whether there is an upper limit to the amount of nitrogen retained by salt marshes. A long-term fertilization study in the Great Sippewissett Marsh on Cape Cod, USA, has been examining the changes to salt marsh vegetation and sediment processes as a result of increased nitrogen loading. To determine whether decadal-scale changes in nitrogen loading and sources are recorded in salt marsh sediments, we examined sediment $\delta^{15}\text{N}$ and %N profiles from below low and high marsh vegetation in control and fertilized plots in Great Sippewissett Marsh. As expected, we found little change in $\delta^{15}\text{N}$ values in control plots. Nitrogen burial, calculated using %N values in bulk sediments, was higher in fertilized plots, but did not increase over time. However, $\delta^{15}\text{N}$ values in fertilized plots were higher than in control plots and increased over time, becoming heavier than the source fertilizer and continuing to increase linearly. The continuous increase in sediment $\delta^{15}\text{N}$ values in fertilized plots over the fertilizer $\delta^{15}\text{N}$ value suggests that denitrifying bacteria are responding to the increased nitrogen load and fractionating the available nitrogen. The nitrogen that remained unaccounted for by burial led us to conclude that 47 to 80% of the fertilizer nitrogen was denitrified.

KEY WORDS: Great Sippewissett Marsh · Nitrogen uptake · Nitrogen burial · $\delta^{15}\text{N}$ · Denitrification · Fractionation · Vegetation

Resale or republication not permitted without written consent of the publisher

INTRODUCTION

Salt marshes provide important ecological services in coastal settings by intercepting land-derived nitrogen and processing that nitrogen, either retaining it within the marsh, releasing it as nitrogen gas, or exporting it to coastal waters (Valiela et al. 2000). Urbanization and atmospheric deposition on coastal watersheds have increased the amount of nitrogen reaching salt marshes (Fry et al. 2000, Bowen & Valiela 2001a), but salt marshes still seem to be sinks for much of the external nitrogen (Valiela & Cole 2002, Wigand et al. 2004, Brin et al. 2010).

Changes in external delivery of nitrogen may be evident in estuarine $\delta^{15}\text{N}$ sediment profiles, which may also reveal changes (Voss et al. 2000) as well as spatial differences (Struck et al. 2000) in nitrogen sources. Zimmerman & Canuel (2002) found an increase in $\delta^{15}\text{N}$ over time in sediment profiles from Chesapeake Bay, likely as a result of increased nitrogen loading, as well as enhancement of microbial processes within sediments as a result of increased nitrogen availability. Church et al. (2006) reported that salt marsh $\delta^{15}\text{N}$ sediment profiles in Delaware reflected the increase in nitrogen inputs from wastewater and fertilizer sources.

*Email: kinneye@tamug.edu

Increased nitrogen loads have, however, changed salt marsh ecosystems, including vegetation growth (Valiela & Teal 1974, Valiela et al. 1978) and type (Fox 2007, Fox et al. 2012), microbial decomposition (Valiela et al. 1985b), and denitrification (Kaplan et al. 1979, White & Howes 1994, Hamersley & Howes 2005). These results have been confirmed by enrichment studies in Georgia (Chalmers 1979), Delaware (Gallagher 1975, Sullivan & Daiber 1974), Rhode Island (Levine et al. 1998, Caffrey et al. 2007), and North Carolina (Broome et al. 1975).

To examine the effect of nutrient enrichment on salt marsh ecosystems, Valiela et al. (1973) established long-term experimental plots in the Great Sippewissett Marsh, on Cape Cod, Massachusetts, USA, which have been maintained for the past 38 yr (Fig. 1). Previous studies in these plots showed that the vegetation growth and composition changed in

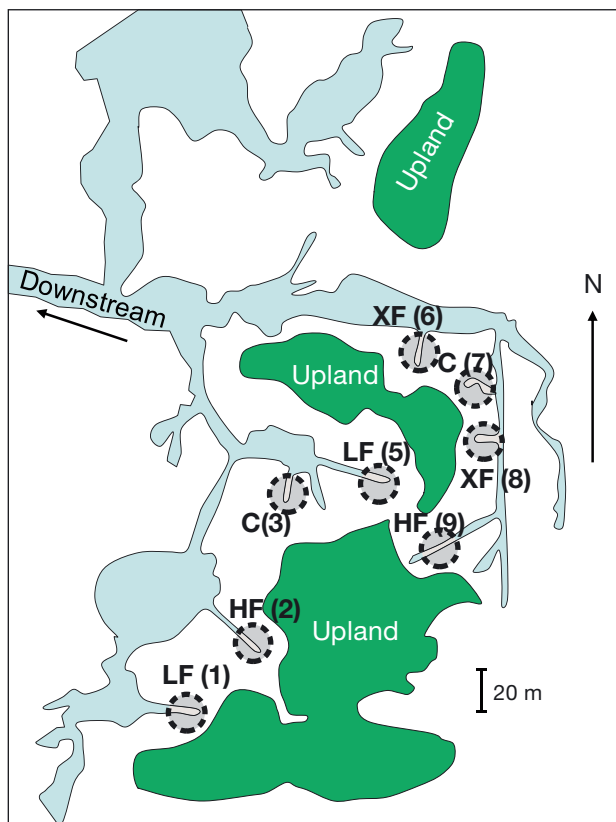


Fig. 1. Experimental plots within the Great Sippewissett Marsh. Plots marked C are controls, the rest are fertilized with mixed fertilizer at 3 dosages (0.85 , 2.52 , and 7.56 $\text{g N m}^{-2} \text{wk}^{-1}$ for low fertilization [LF], high [HF] and extra-high [XF] fertilization, respectively). Dark green areas indicate upland hummocks, blue areas indicate tidal streams. Sediment cores for this study were collected from C, HF and XF plots. Numbers in parentheses are plot numbers

response to fertilization (Valiela & Teal 1974, Fox et al. 2012), but there has been little loss of nitrogen to tidal water (Valiela et al. 1973, Brin et al. 2010). These studies suggest that the bulk of the nitrogen added to the experimental plots (beyond N_2 fixation) must be retained in the vegetation, buried in the sediment, or released as N_2 via denitrification or anammox.

In this paper we first investigate the fate of the nitrogen retained in sediment during the long-term fertilization by examining $\delta^{15}\text{N}$ and %N of salt marsh sediment cores from high and low marsh vegetation habitats within the experimental plots. Second, we use data from the vertical profiles obtained from the cores to estimate the fate of externally derived nitrogen by assessing the amount of nitrogen buried in the sediment, we use fractionation of $\delta^{15}\text{N}$ to evaluate microbial denitrification and/or anammox and we compare these burial and gas losses with previously obtained estimates of denitrification and tidal export (Hamersley & Howes 2005, Brin et al. 2010). There is some evidence that denitrification and nitrogen fixation may co-occur in water columns (De Wit et al. 2001, Welsh et al. 2001), but increased supply of ammonium has been found to strongly inhibit fixation of nitrogen in salt marsh sediments (Van Raalte et al. 1974, Carpenter et al. 1978, Teal et al. 1979).

MATERIALS AND METHODS

Experimental plots

The circular experimental plots in the Great Sippewissett Marsh are 10 m in radius, are bisected by a small creek, and originally contained creek bed, creek banks, low marsh and high marsh habitats. Tall-form *Spartina alterniflora* grows on creek banks, and medium- and short-form *S. alterniflora* grow in low marsh areas (Valiela et al. 1985a, Rogers et al. 1998).

The ongoing fertilizer treatments include mixed fertilizer applications in different doses (extra-high fertilization [XF]: 7.56 ; high fertilization [HF]: 2.52 $\text{g N m}^{-2} \text{wk}^{-1}$; Table 1), applied by hand broadcasting every 2 wk during the growing season (20 wk yr^{-1}) to replicate plots. There are 2 XF (plots 6 and 8), 2 HF (plots 2 and 9), and 2 control (C; plots 3 and 7) plots (Fig. 1). Control and HF plots were established in 1971 and XF plots were established in 1974 (Giblin et al. 1980). The fertilizer used is a mixed slow-release fertilizer, with a ratio of 6:4 N:P (Valiela et al. 1973).

Table 1. Mass balance of external nitrogen added in experimental plots in the Great Sippewissett Marsh. NA: data not available; -: data not applicable. C: control; HF: high fertilization; XF: extra-high fertilization; LM: low marsh; HM: high marsh; PN: particulate nitrogen

Treatment:	C		HF		XF	
Experimental fertilization dose ($\text{g N m}^{-2} \text{yr}^{-1}$)	0		52.2		156.7	
PN exported by tide ($\text{g N m}^{-2} \text{yr}^{-1}$) ^a	5		5		5	
% of N added that was exported by tide ($\text{g N m}^{-2} \text{yr}^{-1}$) ^a	-		9		3	
N retention ($\text{g N m}^{-2} \text{yr}^{-1}$) ^b	-		43-50		123-148	
% of N added retained ^b	-		85.7-95.2		83.2-94.2	
Vegetation type:	LM	HM	LM	HM	LM	HM
Measured denitrification rate ($\text{g N m}^{-2} \text{yr}^{-1}$) ^c	10.9	NA	52.2	NA	148.1	NA
Burial ($\text{g N m}^{-2} \text{yr}^{-1} \pm \text{SE}$) ^d	7 \pm 0.2	6 \pm 0.2	15 \pm 0.7	14 \pm 0.4	18 \pm 0.8	24 \pm 1.2
% of fertilizer N buried ^d	-	-	28	27	11	15
Estimated denitrification by difference (N added - tidal export - burial) ($\text{g N m}^{-2} \text{yr}^{-1}$)	-	-	22-30	24-31	100-125	94-118
Estimated % N in fertilizer that was denitrified ^e	-	-	47-58	48-59	68-80	63-76

^aCalculated using data on PN export and vegetated area in the Great Sippewissett Marsh from Valiela & Teal (1979)
^bBrin et al. (2010); ^cHamersley & Howes (2005); ^dPresent study, see Fig. 8
^eEstimated as the nitrogen remaining in the retention term after the losses from PN export and burial

Collection of sediment cores

To examine the impact of long-term fertilization on bulk sediments, one sediment core was collected from each of the 2 C, HF, and XF plots from areas with low marsh vegetation habitat (*Spartina alterniflora* of intermediate height), and a second core was collected from the same 6 plots from areas supporting high marsh vegetation (monoculture of *Spartina patens* or *Distichlis spicata*) during the summer of 2008. Cores were collected using a 6.6 cm diameter, 50 cm long plexiglass cylinder sharpened at the bottom. Cores were extracted by pushing gently on the bottom of the core to minimize compaction. Cores were wrapped in foil after removal from the tube, and were put on ice until they could be transported to the laboratory and frozen. Frozen cores were cut in half longitudinally and one half was kept frozen. The other half was cut into 1-cm sections using a band saw. Sections were weighed, dried at 60°C, reweighed and homogenized using a Retsch Mixer Mill. $\delta^{15}\text{N}$, %N and %C analysis was performed at the Stable Isotope Laboratory at the Marine Biological Laboratory.

Sediment dating

Sediment sections were dated using ^{137}Cs radioisotopic methods. There has been some debate as to

whether ^{137}Cs or ^{210}Pb dating is the most appropriate for organic sediments. Mudd et al. (2009) suggested that the ^{137}Cs method is more appropriate for young, highly organic sediments. This method has been used successfully in Great Sippewissett Marsh (Turner et al. 2009). Accretion rates were estimated as the column of sediment found above the peak in ^{137}Cs activity, which was taken as corresponding to 1963, the year of maximum atmospheric fallout from testing of nuclear devices (DeLaune et al. 1978, Milan et al. 1995).

To examine the possible effects of organic matter decomposition and compaction, we looked for trends in vertical profiles of bulk density. Differences in compaction of sediments over time could result in sediment depth not being directly related to accretion rate. Differences were small and did not follow any pattern that would suggest differential compaction over time. There was no evidence of compaction over time, so we calculated accretion using sediment depth and ^{137}Cs dates to re-cast the vertical profiles in terms of years instead of absolute depth of sediment.

To define vertical profiles of $\delta^{15}\text{N}$ and %N, we used a spline smoothing method (with $\lambda = 10$), removing 2 values whose residuals were greater than 1. Because accretion rates in each core were slightly different, we interpolated values from the spline fit per year to calculate average values and performed ANOVA for data grouped by decadal sections that were analyzed

as nested within treatment. Tukey-Kramer HSD post hoc tests were used to reveal significant differences within and between treatments and decades for all pairs.

Relationship of $\delta^{15}\text{N}$ between shoots and below-ground peat

To examine the relationship between sediment and above-ground plant nitrogen contents, 5 to 10 stalks of the dominant salt marsh vegetation were collected from above and surrounding each coring site. Low marsh vegetation was medium-form *Spartina alterniflora* in all plots. High marsh vegetation was *Spartina patens* in control plots and *Distichlis spicata* in HF and XF plots. Samples of above-ground vegetation were dried at 60°C and ground for stable isotope analysis at the Stable Isotope Laboratory at the Marine Biological Laboratory.

Fate of added nitrogen

Mass balance estimates

The second aim in this work was to use the information from the vertical profiles, plus previously available data on the 2 major mechanisms (tidal export and denitrification) that remove nitrogen from sediments in the experimental plots, to estimate annual nitrogen burial within plot sediments.

Estimates of nitrogen burial

We calculated net nitrogen burial in sediment core sections by multiplying the percent nitrogen in the core sections by the bulk density of each section and the accretion rate of the core, which were estimated from the ^{137}Cs data. As the ages for each core segment were slightly different, spline fits (with $\lambda = 1$) were used to estimate average burial for each core at specific time points.

Export by tidal water

Exports of dissolved inorganic and organic nitrogen from the plots were reported in Brin et al. (2010). Brin et al. (2010) did not measure particulate exchanges from the plots, so we roughly estimated potential loss of particulate nitrogen from the experi-

mental plots using estimates of export of particulate nitrogen from the Great Sippewissett Marsh (Valiela & Teal 1979), adjusted to area of the plots. These particulate exports are most appropriate for the control plots; since they purposely ignore the effects of fertilization, they probably underestimate particulate exports from fertilized plots. We note, however, that Brin et al. (2010) did not find clear evidence that increased fertilization led to increased tidal export of dissolved nitrogen, and that Valiela & Teal (1979) showed that particulate tidal losses were smaller than other terms, so the underestimation in particulate nitrogen loss via export might be a minor factor.

There were other nitrogen inputs that we left out of the calculations. Nitrogen fixation occurs in the plot sediments, and there is some evidence that denitrification and nitrogen fixation may co-occur in water columns; however, in the fertilized salt marsh sediments the concentrations of ammonium are so high as to strongly inhibit nitrogen fixation (Van Raalte et al. 1974, Carpenter et al. 1978, Teal et al. 1979), so given the uncertainty in the various terms, in the calculations, we opted to ignore fixation in the fertilization plots. Anammox in these salt marsh habitats is of minor importance; Koop-Jakobsen & Giblin (2009) showed that anammox amounted to only 3% of nitrogen gas generation by denitrification. Atmospheric deposition was smaller than other terms (Valiela & Teal 1979), so we also omitted this input.

Estimates of denitrification

We first approximately estimated rates of denitrification by difference, based on known inputs to sediments by fertilization, minus losses via tidal export and by burial. The estimates of denitrification rates by this approximate mass-balance approach could then be compared with previous direct measurements of denitrification (Kaplan et al. 1979, Hamersley & Howes 2005), to see whether we had reasonably constrained nitrogen burial estimates for the plots.

RESULTS

Vertical profiles in sediments

$\delta^{15}\text{N}$ with depth

The vertical profiles of $\delta^{15}\text{N}$ in the plots all showed lighter values at depth, and heavier values nearer the

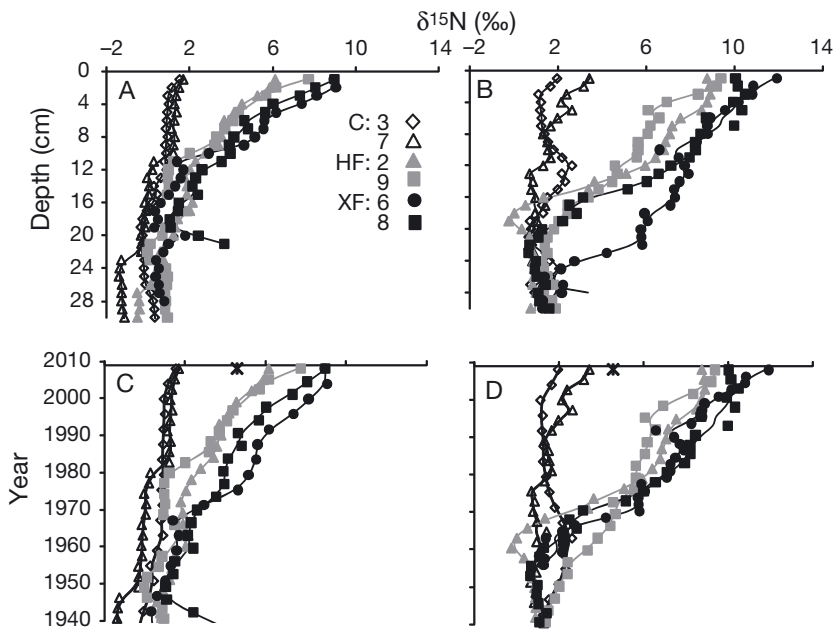


Fig. 2. (A,B) Depth and (C,D) age profiles of $\delta^{15}\text{N}$ of bulk sediments from low marsh (left) and high marsh (right) cores from six experimental plots in the Great Sippewissett Marsh. Accretion rates were calculated based on ^{137}Cs peaks for each core. See Fig. 1 for plot descriptions. Fertilizer $\delta^{15}\text{N}$ value = 4.6‰ (asterisk). Splines ($\lambda = 10$) were fit to the points to smooth the profile and identify outliers

surface (Fig. 2A,B). Values measured at the lower end of the cores were similar in all treatments, but changes became evident at shallower depths. The shift toward heavier values was more prominent in HF plots, and even more so in XF plots. Replicate profiles per treatment were reasonably similar. Below-ground growth of vegetation (roots and rhizomes) occurs mainly between 0 and 20 cm (Valiela et al. 1976). Given previous information that showed different accretion rates in the treatments (Turner et al. 2009), we adjusted the vertical profiles for sediment age before performing statistical analyses.

Accretion rates

To account for differential accumulation rates between sites, we used the ^{137}Cs -calculated accretion rates to compare sediment sections by age. Accretion rates can be calculated by depth, assuming constant accretion and little compaction, or by bulk density, to account for compaction of deeper sediments. Bulk density of our core sections ranged between 0.1 and 0.4 g cm^{-3} and did not change greatly with depth in any of the cores (Fig. 3). There were a few excep-

tional sections within some cores (e.g. Fig. 3D, 18 cm depth) that contained sand, probably the result of over-wash during a storm event, which resulted in much higher bulk densities (1.1 g cm^{-3}). We ignored the anomalous sandy layers and calculated the accretion rate of the peaty layers as relative elevation above peak ^{137}Cs activity, assumed to be circa 1963 (DeLaune et al. 1978, Milan et al. 1995).

^{137}Cs profile dated cores showed that there were indeed differences in accretion associated with different sites and with different treatments. Our high marsh accretion rates were similar to those reported by Turner et al. (2009) for areas '2 m inside the plot', which seems most likely to be taken from areas of high marsh vegetation. In our measurements, low and high marsh accretion rates had similar accretion rates (Table 2).

Vertical profiles of $\delta^{15}\text{N}$

In all plots, $\delta^{15}\text{N}$ became heavier in more recently accreted layers (Fig. 2C,D). The increases in sediments under different treatments differed after treatments were established in 1970 (Fig. 2C,D). The increases in $\delta^{15}\text{N}$ were especially marked for fertilized plots, and more so for the XF plots. For both low marsh (Fig. 2C) and high marsh (Fig. 2D), the 2 replicate cores subject to HF doses were heavier than the 2 replicate control plots, and $\delta^{15}\text{N}$ values in XF cores were, in turn, heavier than those in HF cores. Even though there was some (1 to 2 cm) apparent downward mixing of the $\delta^{15}\text{N}$ below the 1970 sediments (Fig. 2), the increases largely occurred after treatments were established. Increases in $\delta^{15}\text{N}$ were evident, albeit to a much lesser degree, in control plots. This minor increase may reflect regional effects of human activities (McClelland & Valiela 1998).

We tested the effect of treatment and age on the $\delta^{15}\text{N}$ data after 1970. Fertilized high marsh sediments had significantly heavier $\delta^{15}\text{N}$ values than low marsh sediments at the same dose after the start date of 1970 (ANOVA, $F = 25.26$, $p < 0.0001$, Tukey-Kramer HSD post hoc tests showed significant differences; Table S1a-f in the supplement at www.int-res.com/articles/suppl/m477p041_supp.pdf), indicating that a

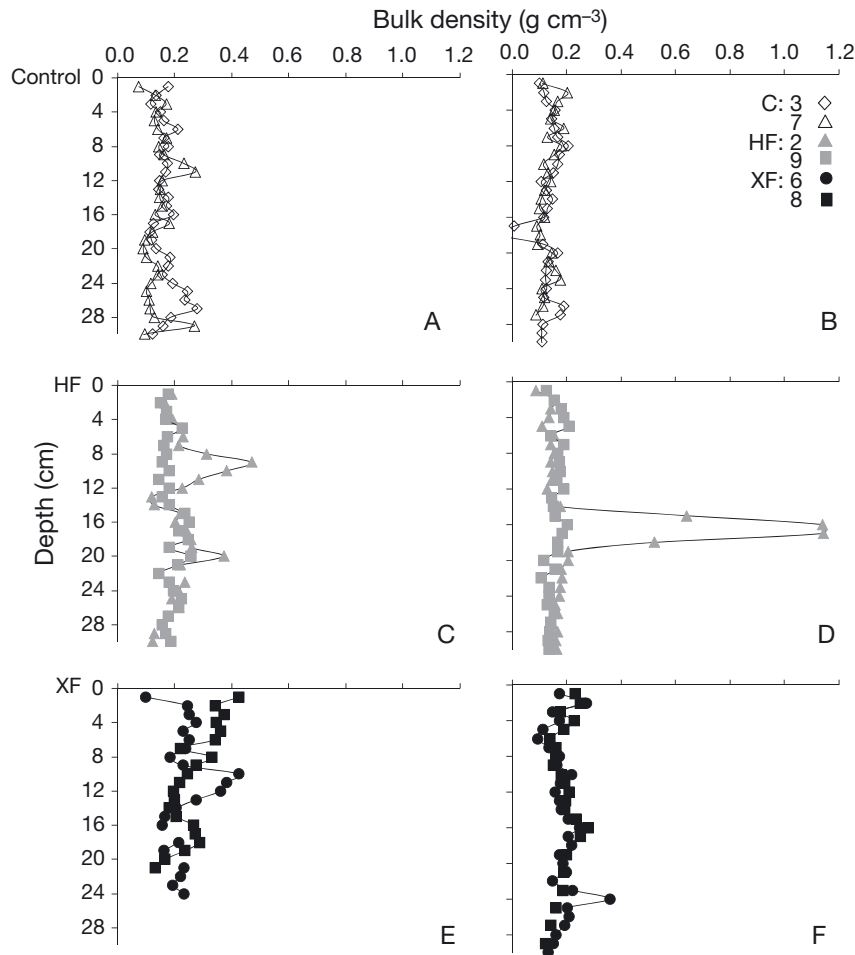


Fig. 3. Depth profiles of bulk density of sediments from low marsh (left) and high marsh (right) cores from 6 experimental plots in the Great Sippewissett Marsh. See Fig. 1 for plot descriptions. Plot 2 bulk density values between 14 and 18 cm (not shown) fell between 0.6 and 1.2 g cm⁻³. These values exceptionally were high due to a high proportion of sand in those sections, likely the result of over-wash during a storm event

Table 2. Accretion rates (cm yr⁻¹) for low and high marsh cores calculated as cm of sediment above the depth of ¹³⁷Cs peak for this study and from Turner et al. (2009). Control plot accretion rates shown for Turner et al. (2009) are averages for unfertilized sediments near the appropriate plots. ANOVA, low marsh: df = 2, F = 1.5, p = 0.35; high marsh: df = 2, F = 4.58, p = 0.12. See Fig. 1 for details

Treatment	Plot number	Low marsh	High marsh	Turner et al. (2009)
C	3	0.24	0.28	0.30
	7	0.35	0.17	0.37
HF	2	0.33	0.38	0.35
	9	0.36	0.31	0.37
XF	6	0.24	0.56	0.54
	8	0.28	0.40	0.63

fractionating process was more active in high marsh sediments than in low marsh sediments (Fig. 2), perhaps because of more anoxic conditions in high marsh sediments (Howes et al. 1991).

The post-treatment profiles for high marsh showed a more rapid shift to heavier values than sediments in low marsh. Linear regressions of δ¹⁵N sediment values from each treatment between 1970 and 2008 showed that the slopes of the fertilized treatments (HF and XF) were higher than the slopes of the control sediments in both low and high marsh vegetation (Fig. 4, Table 3). In particular, high marsh sediments exceeded the δ¹⁵N value of the added fertilizer (Fig. 4, dashed line), which suggested that fractionation was more pronounced in high than in low marsh, a notion we revisit below when discussing denitrification rates.

%N values with depth

In all plots, %N values increased slightly toward the surface, most likely due to higher density of live plant roots and rhizomes close to the surface (Valiela et al. 1976). Most %N values ranged between 1 and 3% nitrogen (Fig. 5), with a few outliers. One exception was the high marsh core from HF plot 2, which had a several sections containing a high proportion of sand between 14 and 18 cm. This layer is

also visible in the bulk density profile (Fig. 3). In both the low and high marsh cores, the HF and XF treatments had higher %N than control plots closer to the surface. In the low marsh vegetation, the control and fertilized sediment values diverged above 12 cm (Fig. 5A). In the high marsh cores, the control and XF sediment diverged at 16 cm depth and the HF sediment diverged from the control values at 12 cm. Below we present a more detailed examination of the profiles, using the ¹³⁷Cs dates.

%N with age

The vertical profiles of %N were also adjusted for different accretion rates (Fig. 5C,D). The %N dif-

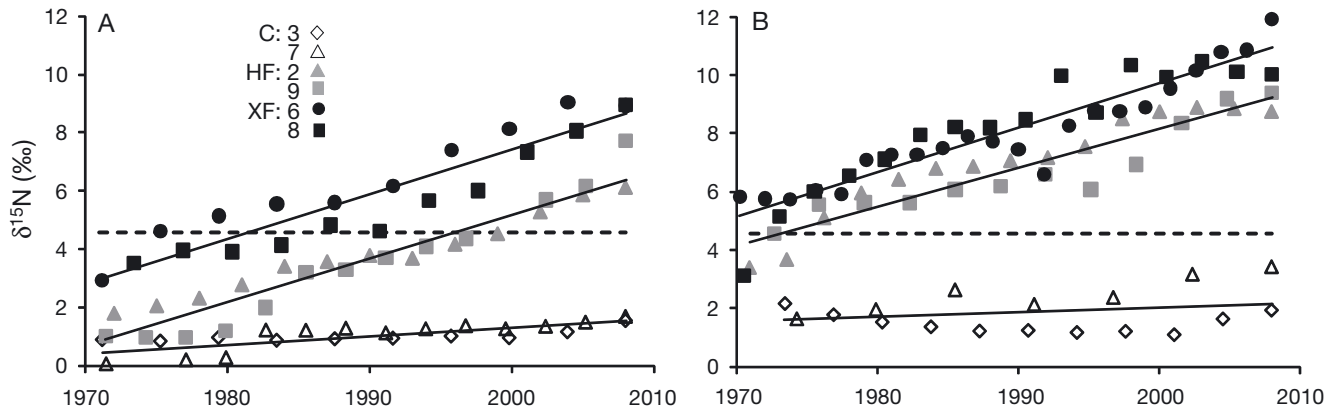


Fig. 4. Linear regressions of control, high fertilization and extra-high fertilization $\delta^{15}\text{N}$ values of bulk sediments versus age between 1970 and 2008 for (A) low marsh (LM) and (B) high marsh (HM) habitats (see Table 3 for individual regression equations). ANCOVA results indicated that y -intercepts are significantly different (LM: $F = 15.38$, $p = 0.008$; HM: $F = 8.81$, $p = 0.05$). Slopes of the control and HF regression lines were significantly different (LM: $T = 11.97$, $p < 0.0005$; HM: $T = 7.10$, $p < 0.0005$). Slopes of the HF and XF regression lines were not significantly different (LM: $T = 0.33$; ns; HM: $T = 1.53$, ns). Dashed line represents the $\delta^{15}\text{N}$ value of fertilizer (4.6‰)

Table 3. Regression results for $\delta^{15}\text{N}$ values of bulk sediments versus age between 1970 and 2008 for low marsh and high marsh habitats (data shown in Fig. 4). C: control; HF: high fertilization; XF: extra-high fertilization

Vegetation type	Treatment	Regression equation	R^2	F	p
Low marsh	C	$y = 0.03x - 59.08$	0.60	33.18	<0.0001
	HF	$y = 0.15x - 292.49$	0.92	300.69	<0.0001
	XF	$y = 0.15x - 300.16$	0.87	130.06	<0.0001
High marsh	C	$y = 0.01x - 27.45$	0.06	1.05	0.32
	HF	$y = 0.13x - 259.64$	0.87	171.46	<0.0001
	XF	$y = 0.15x - 297.34$	0.87	242.66	<0.0001

ferred significantly between high marsh and low marsh within fertilizer treatments (ANOVA, $F = 35.94$, $p < 0.0001$). Tukey-Kramer HSD comparisons revealed that vertical profiles of %N from control treatments were not different between low and high marsh vegetation (Table S2a,b in the supplement). In low marsh vegetation habitats, scatter within the core profiles made determining divergence of control and fertilized %N values difficult to date. One HF plot (plot 2) did not diverge from control values until 1990, and low marsh control treatments were not significantly different in %N from low marsh HF treatments, which in turn, were not significantly different from low marsh XF treatments. Low marsh XF treatment %N values were significantly higher than control values. In the high marsh vegetation habitats, %N values for fertilized treatments became significantly higher than control values around 1970. The high marsh HF and XF %N values were not significantly different (Table S2c–f in the supplement).

Fertilization increased %N in core sediments (Fig. 5). Generally, %N values in fertilized plots were higher than %N values found in salt marsh sediment profiles from other sites (Table 4). There was large variation in %N over time, more than was reported in Delaware salt marsh and Baltic Sea sediment cores, but similar to the variation found elsewhere on Cape Cod (Waquoit Bay, Table 4). Despite high nitrogen loads, the %N values in the fertilized plots (Fig. 5)

did not continue to increase over time like the $\delta^{15}\text{N}$ values (Fig. 2). After the initial increase in %N around the start of the fertilization experiment in 1971, the %N values were fairly consistent through 2008. The %N values in XF cores were higher than the %N values in HF cores, but not in proportion to the dose of fertilizer applied to each treatment. It appears that %N in the sediments was limited by some process that removed more nitrogen from XF

Table 4. Reported ranges of percent nitrogen values of salt marsh and estuarine sediment cores

Location	%N	Source
Delaware Estuary salt marsh	0.15–0.32	Church et al. (2006)
Baltic Sea	0.2–1.3	Struck et al. (2000)
Waquoit Bay salt marsh	1.5–2.5	Kinney (2010)
Great Sippewissett Marsh	1.1–3.8	Present study

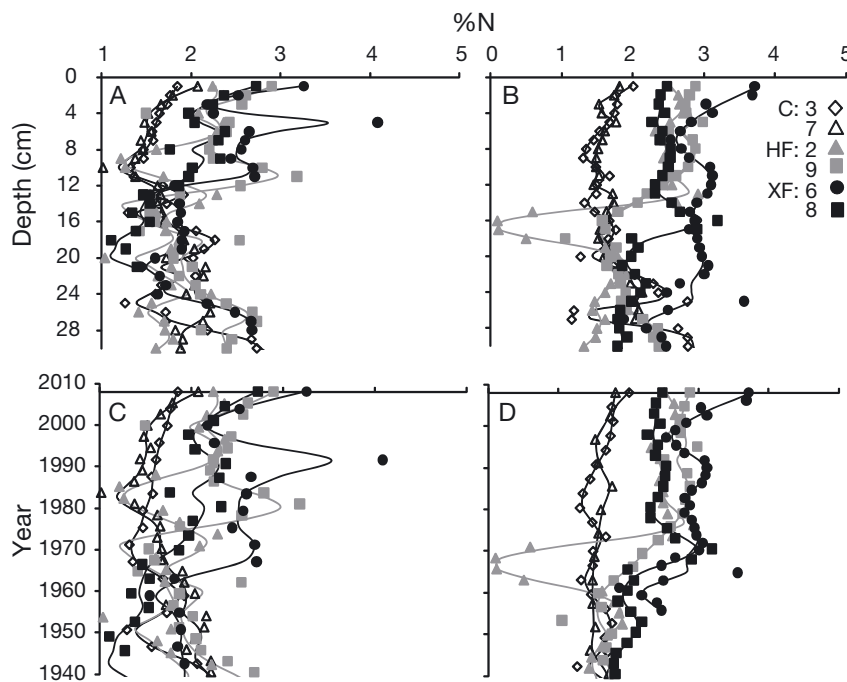


Fig. 5. (A,B) Depth and (C,D) age profiles of %N of bulk sediments from low marsh (left) and high marsh (right) cores from 6 experimental plots in the Great Sippewissett Marsh (see Fig. 1). Accretion rates were calculated based on ^{137}Cs peaks for each core. Splines ($\lambda = 10$) were fit to the points to smooth the profile and identify outliers. Note: Plot 2 %N values were affected by the high proportion of sand found in sections between 14 and 18 cm depth (Fig. 3)

plots than from HF plots, which led us to assess whether denitrification was a feasible mechanism.

Influence of live plant roots and rhizomes

To determine whether $\delta^{15}\text{N}$ values of the sediments were mainly a result of the presence of roots and rhizomes, we compared $\delta^{15}\text{N}$ of sediment within the upper 1cm of sediment with $\delta^{15}\text{N}$ of shoots of above-ground vegetation (Fig. 6). In 9 out of 12 measurements, vegetation values were similar to those of surface sediment $\delta^{15}\text{N}$ and fell near the 1:1 line (Fig. 6, dashed line). These results suggest that vegetation took up nitrogen that had already been fractionated by the microbial community in the sediments, and that there was higher fractionation in XF plots (Fig. 2). The 3 apparent outliers in Fig. 6 might represent the scatter inherent in $\delta^{15}\text{N}$ uptake in different salt marsh species, particularly *Distichlis spicata*, which contributed the 2 XF high marsh points. Moreover, $\delta^{15}\text{N}$ values for above-ground vegetation growing on the core locations increased with increasing

nitrogen load (Fig. 7). All XF and most HF above-ground shoots had heavier $\delta^{15}\text{N}$ values than the fertilizer (4.57‰, mean of samples collected at different times across the treatment period; Fig. 7, dashed line).

Mass balance estimates

From the cores data, plus previously published evidence, we could assemble values that quantify inputs and outputs of nitrogen in the plots (Table 1). We knew the amount of nitrogen added by fertilization, and we knew from Brin et al. (2010) the amount of dissolved inorganic nitrogen exported by tidal exchange (Table 1). From these data, we calculated that only a small percentage (2 to 6%) of the nitrogen inputs was removed by tides.

In contrast, the burial of externally derived nitrogen in sediments was larger than the amount of nitrogen lost by tidal export (Fig. 8, Table 1). Burial increased rapidly once fertilization began, but there was no significant increase in burial over time after the initial increase (Fig. 8, Table S3a–f in the supplement). Burial was significantly higher in fertilized

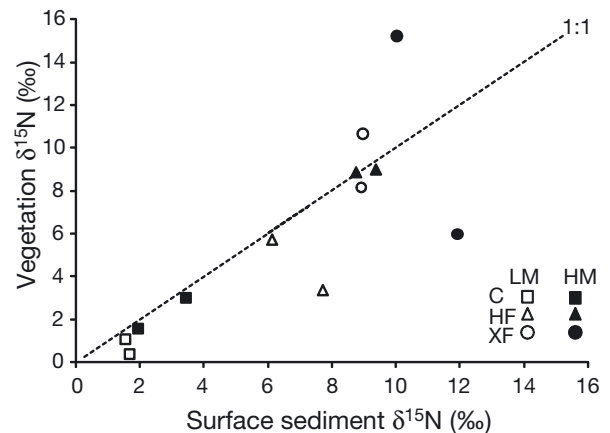


Fig. 6. Surface sediment $\delta^{15}\text{N}$ values versus above-ground vegetation $\delta^{15}\text{N}$ values for all cores. Low marsh vegetation (LM) is *Spartina alterniflora*. High marsh vegetation (HM) is *Spartina patens* in C plots, *Distichlis spicata* in HF and XF plots (see Fig. 1)

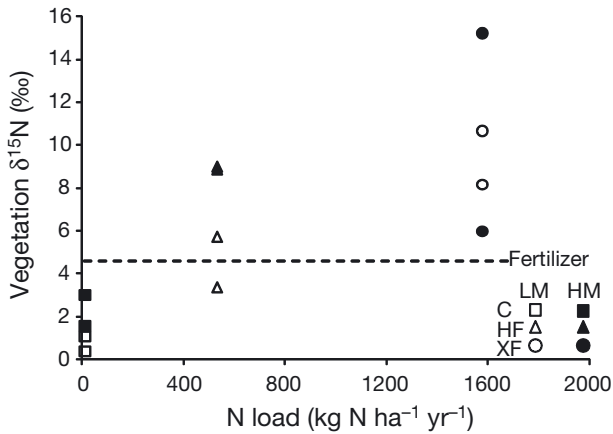


Fig. 7. Nitrogen load of control and experimentally fertilized plots compared with the $\delta^{15}\text{N}$ value of low marsh (LM) and high marsh (HM) vegetation. LM vegetation is *Spartina alterniflora*. HM vegetation is *Spartina patens* in C plots, and *Distichlis spicata* in HF and XF plots (see Fig. 1). Dashed line represents the $\delta^{15}\text{N}$ value of fertilizer (4.6‰)

treatments (14 to 24 $\text{g N m}^{-2} \text{yr}^{-1}$) than in controls (6 to 7 $\text{g N m}^{-2} \text{yr}^{-1}$) in both marsh habitats (see standard errors in Table 1; $F = 12.48$, $p < 0.0001$). Only the XF treatments from high marsh vegetation habitat had higher burial than the other fertilized cores. The annual burial estimates were calculated by multiplying the %N in the cores by the bulk density and the accretion rate. The burial estimates in Table 1 are the average burial rates across the years shown in Fig. 8.

We estimated the amount of nitrogen lost by denitrification as nitrogen added as fertilizer, minus tidal export and minus burial (Table 1). Ranges of estimated denitrification were higher in XF treatments than in HF treatments, corroborating what we inferred from the results of the $\delta^{15}\text{N}$ profiles (Fig. 2), and the differences in %N.

Denitrification estimates derived from the mass balance suggested that denitrification loss was larger than either tidal export or burial, and ranged between 47 and 80% of externally derived nitrogen (Table 1). Our mass balance-derived denitrification estimates (HF: 22 to 31 $\text{g N m}^{-2} \text{yr}^{-1}$; XF: 94 to 125 $\text{g N m}^{-2} \text{yr}^{-1}$) are somewhat lower, but are on the same order of magnitude as the low marsh estimates calculated by Hamersley & Howes (2005) (HF: 52.2 $\text{g N m}^{-2} \text{yr}^{-1}$; XF: 148.1 $\text{g N m}^{-2} \text{yr}^{-1}$; Table 1). This check against measured rates suggests that our approximate estimates were reasonably constrained, and corroborates the importance of denitrification as a loss term, as suggested by Brin et al. (2010) and previous papers.

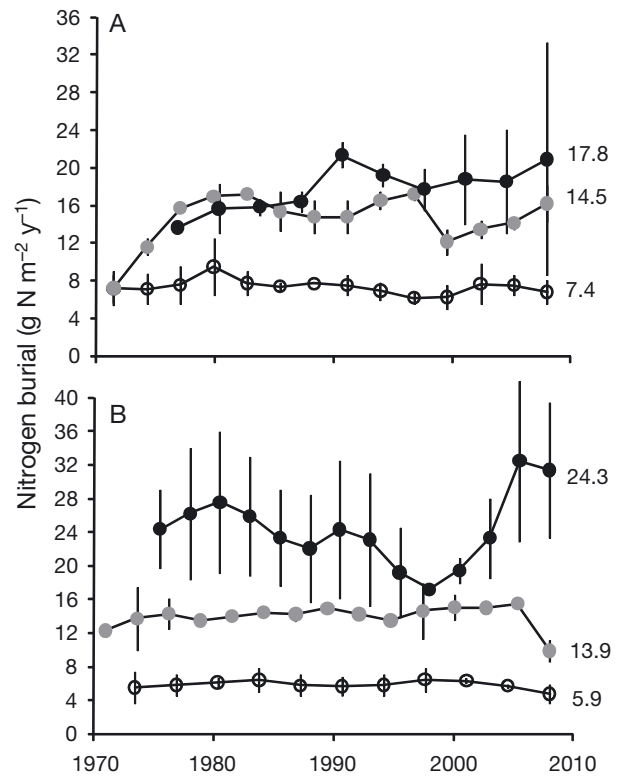


Fig. 8. Low marsh (A) and high marsh (B) nitrogen burial since the beginning of the fertilization experiment in the Great Sippewissett Marsh. Control treatments (open symbols) and high fertilization treatments (grey symbols) were established in 1971. Extra-high fertilization treatments (black symbols) were established in 1974. Splines ($\lambda = 1$) were fit to the points to smooth the profile and provide estimates for corresponding years in order to average two plots per treatment. Mean burial values appear on the right side of the graphs

DISCUSSION

The sediment accretion rates we calculated were similar to those reported for other sites on Cape Cod (Table 5). These are within the range of estimated local sea level rise, indicating that the surface of salt marsh platforms is rising in some proportion to sea level rise, implying that nitrogen burial could be actively taking place. The ^{137}Cs dates were similar to those reported by Turner et al. (2009) and allowed us to establish a time series for the accumulation of nitrogen and sediment in the profiles.

The vertical profiles of $\delta^{15}\text{N}$ showed a record of the past history of conditions relative to nitrogen inputs. There was a small, likely regional-scale increase in $\delta^{15}\text{N}$, prompted by external human influence, as well as a more marked response to the fer-

tilization treatment itself (Fig. 6). From the $\delta^{15}\text{N}$ of the sediment cores, it was clear that fertilizer itself had an effect, as well as microbial denitrification within the sediment. Denitrification seemed the

most important forcing process in establishing the $\delta^{15}\text{N}$ of the sediment. The %N in the profiles increased in response to fertilization, but the increase slowed over time. This slowing may be an expression of the dose-dependent importance of denitrification in these sediments.

Table 5. Reported accretion rates (cm yr^{-1}) from Cape Cod salt marsh sediment cores

Location	Accretion rate	Source
Waquoit Bay	0.28–0.46	Orson & Howes (1992)
Waquoit Bay	0.24–0.33	Kinney (2010)
Great Sippewissett Marsh	0.30–0.63	Turner et al. (2009)
Great Sippewissett Marsh	0.17–0.56	Present study

There has been a lot of discussion as to the source of sediment involved in accretion in salt marshes (Morris et al. 2002, Cahoon et al. 2004, Morris 2006, Mudd et al. 2009). Our finding that the $\delta^{15}\text{N}$ of live shoots resembles that of the surface sediment suggests that the accumulation of organic matter derived from the growth of salt marsh grasses was the principal source of materials underlying accretion of these salt marshes.

From a variety of papers, we can show that in spite of 4 decades of treatments, there was a remarkable

amount of interception of externally provided nitrogen in the experimental plots in the Great Sippewissett Marsh (Table 1, Valiela et al. 1973, Valiela & Teal 1979, Brin et al. 2010). The results of mass-balance calculations suggest that ecosystem-scale interception of nitrogen is, for the most part, related to activity of denitrifying bacteria. In particular, the rates of denitrification in plots receiving larger external nitrogen inputs were higher than those in plots receiving smaller dosages (Fig. 9). Even though the highest dose of nitrogen addition in the XF treatment was quite large, there was no indication that the range of fertilization doses was saturating the ability of denitrifiers to deal with the externally provided nitrogen.

The degree of eutrophication by nitrogen enrichment is commonly thought to be increasing in most (Valiela 2006, Bricker et al. 2007), though perhaps not all (Ruhl & Rybicki 2010, Nixon et al. 2009), coastal waters of the world. Where nitrogen loads from land increase, salt marshes will be exposed to increased nitrogen inputs. The results of the present study suggest that the capacity of denitrification in salt marshes may be able to increase in response to interactive effects of increased eutrophication and sea level rise, dampening the effects of increased nitrogen supply.

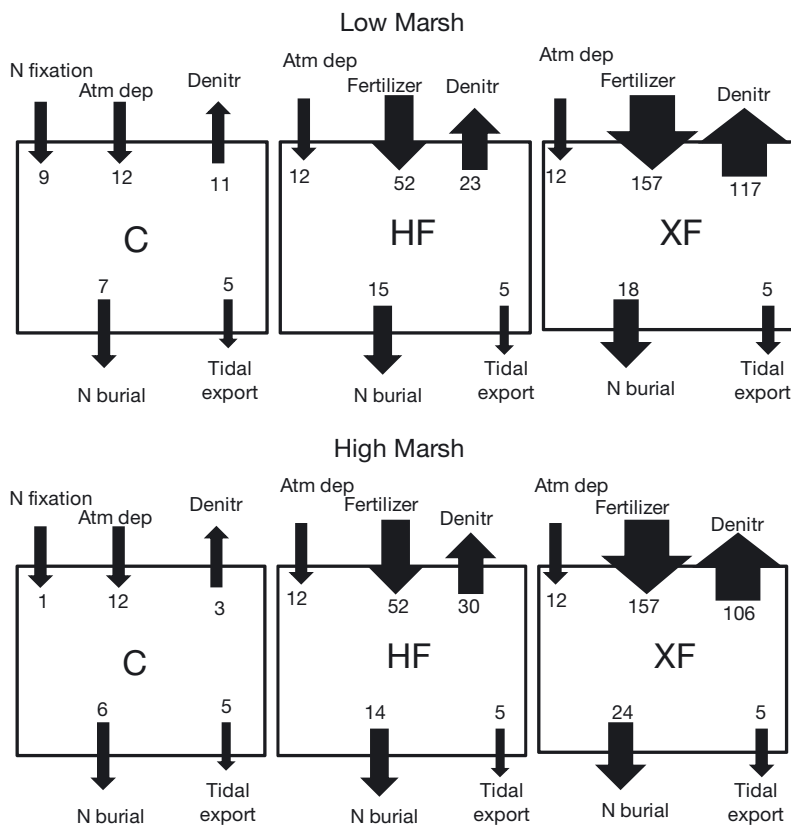


Fig. 9. Box diagrams of nitrogen transformations ($\text{g N m}^{-2} \text{yr}^{-1}$) within C, HF, and XF experimental plots (see Fig. 1) in low marsh and high marsh vegetation habitats. Nitrogen loads, tidal export, nitrogen burial, and potential denitrification ($\text{g N m}^{-2} \text{yr}^{-1}$) are listed in Table 1. Atmospheric deposition of nitrogen through precipitation was obtained from Bowen & Valiela (2001b). Nitrogen fixation data was obtained from Teal et al. (1979). Denitrification values in low marsh control plots were $10.8 \text{ g N m}^{-2} \text{yr}^{-1}$ from Kaplan (1977) and $10.9 \text{ g N m}^{-2} \text{yr}^{-1}$ from Hamersley & Howes (2005). Denitrification values in high marsh control plots were obtained from Hamersley & Howes (2005). Relative size of the arrows approximates the relative quantity of nitrogen transformed by each process

Acknowledgements. We thank Salt Pond Sanctuaries and Dr. E. F. X. Hughes and family for allowing access to the Great Sippewissett Marsh. We are indebted to B. Howes, D. Goehringer and their students at the School of Marine Sciences and Technology, University of Massachusetts, Dartmouth, for their efforts in maintaining the experimental plots. We thank Research Experience for Undergraduates (REU) intern Emily Olesin for her assistance in the field. This work was supported by NSF grants DEB-0516430, DEB-0914795, OCE-0453292, and NOAA grant NA07NOS4200025 to I.V.

LITERATURE CITED

- Bowen JL, Valiela I (2001a) The ecological effects of urbanization of coastal watersheds: historical increases in nitrogen loads and eutrophication of Waquoit Bay estuaries. *Can J Fish Aquat Sci* 58:1489–1500
- Bowen JL, Valiela I (2001b) Historical changes in atmospheric nitrogen deposition to Cape Cod, Massachusetts. *Atmos Environ* 35:1039–1051
- Bricker S, Longstaff B, Dennison W, Jones A, Boicourt K, Wicks C, Woerner J (2007) Effects of nutrient enrichment in the nation's estuaries: a decade of change, Vol 26. National Centers for Coastal Ocean Science, Silver Spring, MD
- Brin LD, Valiela I, Goehringer D, Howes B (2010) Nitrogen interception and export in Great Sippewissett salt marsh plots exposed to chronic experimental nutrient addition. *Mar Ecol Prog Ser* 400:3–17
- Broome SW, Woodhouse WW Jr, Seneca ED (1975) The relationship of mineral nutrient to growth of *Spartina alterniflora* in North Carolina. II. The effects of N, P, and Fe fertilizers. *Soil Sci Soc Am* 39:301–307
- Caffrey JM, Murrell MC, Cathleen W, McKinney R (2007) Effect of nutrient loading on biogeochemical and microbial processes in a New England salt marsh. *Biogeochemistry* 82:251–264
- Cahoon DR, Ford MA, Hensel PF (2004) Ecogeomorphology of *Spartina patens*-dominated tidal marshes: soil organic matter accumulation, marsh elevation dynamics, and disturbance. In: Fagherazzi S, Marani M, Blum LK (eds). The ecogeomorphology of tidal marshes. *Coastal and Estuarine Studies* 59. American Geophysical Union, Washington, DC, p 247–266
- Carpenter EJ, van Raalte CD, Valiela I (1978) Nitrogen fixation by algae in a Massachusetts salt marsh. *Limnol Oceanogr* 23:318–327
- Chalmers AG (1979) The effects of fertilization on nitrogen distribution in a *Spartina alterniflora* salt marsh. *Estuar Coast Mar Sci* 8:327–337
- Church TM, Sommerfield CK, Velinsky DJ, Pont D and others (2006) Marsh sediments as records of sedimentation, eutrophication and metal pollution in the urban Delaware Estuary. *Mar Chem* 102:72–95
- De Wit R, Stal LJ, Lomstein BA, Herbert RA and others (2001) ROBUST: the role of buffering capacities in stabilising coastal lagoon ecosystems. *Cont Shelf Res* 21:2021–2041
- DeLaune RD, Patrick WH Jr, Buresh RJ (1978) Sedimentation rates determined by ^{137}Cs dating in a rapidly accreting salt marsh. *Nature* 275:532–533
- Fox L (2007) The effect of nutrient enrichment and sea level rise on salt marsh vegetation. MA thesis, Boston University
- Fox L, Valiela I, Kinney EL (2012) Vegetation cover and elevation in long-term experimental nutrient enrichment plots in Great Sippewissett salt marsh, Cape Cod, Massachusetts: implications for eutrophication and sea level rise. *Estuar Coast* 35:445–458
- Fry B, Vern AL, Ross MS, Meeder JF (2000) $\delta^{15}\text{N}$ studies of nitrogen use by the red mangrove, *Rhizophora mangle* L. in south Florida. *Estuar Coast Shelf Sci* 50:291–296
- Gallagher JL (1975) Effect of an ammonium nitrate pulse on the growth and elemental composition of natural stands of *Spartina alterniflora* and *Juncus roemerianus*. *Am J Bot* 62:644–648
- Giblin AE, Bourg A, Valiela I, Teal JM (1980) Uptake and cycling of heavy metals in sewage sludge in a New England salt marsh. *Am J Bot* 67:1059–1068
- Hammersley MR, Howes BL (2005) Coupled nitrification–denitrification measured *in situ* in a *Spartina alterniflora* marsh with a $^{15}\text{NH}_4^+$ tracer. *Mar Ecol Prog Ser* 299:123–135
- Howes BL, Howarth RW, Teal JM, Valiela I (1981) Oxidation–reduction potentials in a salt marsh: spatial patterns and interactions with primary production. *Limnol Oceanogr* 26:350–360
- Kaplan W (1977) Denitrification in a Massachusetts salt marsh. PhD thesis, Boston University
- Kaplan W, Valiela I, Teal JM (1979) Denitrification in a salt marsh ecosystem. *Limnol Oceanogr* 24:726–734
- Kinney EL (2010) Experimental and regional studies of sources of nitrogen using models and stable isotopes in salt marshes. PhD dissertation, Boston University
- Koop-Jakobsen K, Giblin AE (2009) Annamox in tidal marsh sediments: the role of salinity, nitrogen loading, and marsh vegetation. *Estuar Coast* 32:238–245
- Levine JM, Hacker SD, Harley CDG, Bertness MD (1998) Nitrogen effects on an interaction chain in a salt marsh community. *Oecologia* 117:266–272
- McClelland JW, Valiela I (1998) Linking nitrogen in estuarine producers to land-derived sources. *Limnol Oceanogr* 43:577–585
- Milan CS, Swenson EM, Turner RE, Lee JM (1995) Assessment of the ^{137}Cs method for estimating sediment accumulation rates: Louisiana salt marshes. *J Coast Res* 11:296–307
- Morris JT (2006) Competition among marsh macrophytes by means of geomorphological displacement in the intertidal zone. *Estuar Coast Shelf Sci* 69:395–402
- Morris JT, Sundareshwar PV, Nietch CT, Kjerfve B, Cahoon DR (2002) Responses of coastal wetlands to rising sea level. *Ecology* 83:2869–2877
- Mudd SM, Howell SM, Morris JT (2009) Impact of dynamic feedbacks between sedimentation, sea-level rise, and biomass production on near-surface marsh stratigraphy and carbon accumulation. *Estuar Coast Shelf Sci* 82:377–389
- Nixon SW, Fulweiler RW, Buckley BA, Granger SL, Nowicki BL, Henry KM (2009) The impact of changing climate on phenology, productivity, and benthic–pelagic coupling in Narragansett Bay. *Estuar Coast Shelf Sci* 82:1–18
- Orson RA, Howes BL (1992) Salt-marsh development studies at Waquoit Bay, Massachusetts: influence of geomorphology on long-term plant community structure. *Estuar Coast Shelf Sci* 35:453–471
- Rogers J, Harris J, Valiela I (1998) Interaction of nitrogen supply, sea level rise, and elevation on species form and composition of salt marsh plants. *Biol Bull* 195:235–237

- Ruhl HA, Rybicki NB (2010) Long-term reductions in anthropogenic nutrients link to improvements in Chesapeake Bay habitat. *Proc Natl Acad Sci USA* 107: 16566–16570
- Struck U, Emeis KC, Voss M, Christiansen C, Kunzendorf H (2000) Records of southern and central Baltic Sea eutrophication in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of sedimentary organic matter. *Mar Geol* 164:157–171
- Sullivan MJ, Daiber FC (1974) Response in production of cord grass, *Spartina alterniflora*, to inorganic nitrogen and phosphorus fertilizer. *Chesap Sci* 15:121–123
- Teal JM, Valiela I, Berlo D (1979) Nitrogen fixation by rhizosphere and free-living bacteria in salt marsh sediments. *Limnol Oceanogr* 24:126–132
- Turner RE, Howes BL, Teal JM, Milan CM, Swenson EM, Goehringer-Toner DD (2009) Salt marsh eutrophication: an unsustainable outcome. *Limnol Oceanogr* 54: 1634–1642
- Valiela I (2006) *Global coastal change*. Blackwell Publishing, Malden, MA
- Valiela I, Cole ML (2002) Comparative evidence that salt marshes and mangroves may protect seagrass meadows from land-derived nitrogen loads. *Ecosystems* 5:92–102
- Valiela I, Teal JM (1974) Nutrient limitation in salt marsh vegetation. In: Reimold RJ, Queen WH (eds) *Ecology of halophytes*. Academic Press, New York, NY, p 547–563
- Valiela I, Teal JM (1979) The nitrogen budget of a salt marsh ecosystem. *Nature* 280:652–656
- Valiela I, Teal JM, Sass W (1973) Nutrient retention in salt marsh plots experimentally fertilized with sewage sludge. *Estuar Coast Mar Sci* 1:261–269
- Valiela I, Teal JM, Persson NY (1976) Production and dynamics of experimentally enriched salt marsh vegetation: belowground biomass. *Limnol Oceanogr* 21:245–252
- Valiela I, Teal JM, Volkmann S, Shafer D, Carpenter EJ (1978) Nutrient and particulate fluxes in a salt marsh ecosystem: tidal exchanges and inputs by precipitation and groundwater. *Limnol Oceanogr* 23:798–812
- Valiela I, Teal JM, Cogswell C, Hartman J, Allen S, Van Etten R, Goehringer D (1985a) Some long-term consequences of sewage contamination in salt marsh ecosystems. In: Godfrey PJ, Kaynor ER, Pelczarski S, Benforado J (eds) *Ecological considerations in wetlands treatment of municipal wastewaters*. Van Nostrand Reinhold, New York, NY, p 301–316
- Valiela I, Teal JM, Volkmann S, Van Etten R, Allen S (1985b) Decomposition in salt marsh ecosystems: the phases and major factors affecting disappearance of above-ground organic matter. *J Exp Mar Biol Ecol* 89:29–54
- Valiela I, Cole ML, McClelland J, Hauxwell J, Cebrian J, Joye S (2000) Role of salt marshes as part of coastal landscapes. In: Weinstein MP, Kreeger DA (eds) *Concepts and controversies in tidal marsh ecology*. Kluwer Academic Publishers, Dordrecht, p 23–38
- Van Raalte CD, Valiela I, Carpenter EJ, Teal JM (1974) Inhibition of nitrogen fixation in salt marshes measured by acetylene reduction. *Estuar Coast Mar Sci* 2:301–305
- Voss M, Larsen B, Leivuori M, Vallius H (2000) Stable isotope signals of eutrophication in Baltic Sea sediments. *J Mar Syst* 25:287–298
- Welsh D, Castadelli G, Bartoli M, Poli D, Careri M, de Wit R, Viaroli P (2001) Denitrification in an intertidal seagrass meadow, a comparison of ^{15}N -isotope and acetylene-block techniques: dissimilatory nitrate reduction to ammonia as a source of N_2O ? *Mar Biol* 139: 1029–1036
- White DS, Howes BL (1994) Long-term ^{15}N -nitrogen retention in the vegetated sediments of a New England salt marsh. *Limnol Oceanogr* 39:1878–1892
- Wigand C, McKinney RA, Chintala MM, Charpentier MA, Groffman PM (2004) Denitrification enzyme activity of fringe salt marshes in New England (USA). *J Environ Qual* 33:1144–1151
- Zimmerman AR, Canuel EA (2002) Sediment geochemical records of eutrophication in the mesohaline Chesapeake Bay. *Limnol Oceanogr* 47:1084–1093

Editorial responsibility: Jana Davis, Annapolis, Maryland, USA

*Submitted: October 15, 2012; Accepted: October 26, 2012
Proofs received from author(s): February 3, 2013*