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Coupled nitrification–denitrification measured *in situ* in a *Spartina alterniflora* marsh with a $^{15}NH_4^+$ tracer

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ABSTRACT: Measurements of N losses by denitrification from saltmarsh sediments have proved difficult because of the importance of plant metabolism and tidal cycles to sediment N cycling. *In vitro* approaches often do not measure the dominant coupled nitrification–denitrification pathway and/or alter *in situ* plant growth and redox conditions. We developed an *in situ* $^{15}NH_4^+$ tracer approach to measure coupled nitrification–denitrification fluxes in an undisturbed New England *Spartina alterniflora* saltmarsh. The tracer was line-injected into sediments underlying natural *S. alterniflora* stands and in similar areas receiving long-term N amendment (up to 11.2 mol organic N m^{-2} yr^{-1} for 16 to 23 yr), and ^{15}N retention and loss routes were followed for 1 to 5 d. Denitrification losses in unfertilized grass stands ranged from 0.4 to 11.9 mmol N m^{-2} d^{-1} (0.77 ± 0.18 mol N m^{-2} yr^{-1}). Denitrification in unfertilized sediments remained low until late summer, but underwent a ca. 4-fold increase in August and September, although sediment temperatures and respiration rates were high throughout the summer. Plant N uptake may limit the availability of N to support denitrification during the early summer, and denitrification may be released from competition with plant uptake in late summer, when plant growth slows. Denitrification rates in fertilized areas ranged from 22 to 77 mmol N m^{-2} d^{-1} (10.5 ± 4.9 mol N m^{-2} yr^{-1}), and denitrification was likely controlled by the availability of fertilizer N rather than by competition with plants, since N was added in excess of plant demand. Our results emphasize the importance of *in situ* measurements of denitrification in understanding the dynamics of saltmarsh N cycling.

KEY WORDS: Saltmarsh · Denitrification · Nitrification · ^{15}N · *Spartina alterniflora*

INTRODUCTION

Denitrification, the anaerobic microbial reduction of oxidized inorganic N to N_2O or N_2, is acknowledged as an important sink in the saltmarsh N cycle, but quantifying its contribution has proven difficult. With few allochthonous sources of NO_3^-, denitrification in highly organic vegetated saltmarsh sediments—such as those in Massachusetts—is supported primarily by coupled nitrification–denitrification of remineralized NH_4^+ (Patrick & Reddy 1976, White & Howes 1994a,b, Howes et al. 1996). *In vitro* methods appropriate for submerged aquatic sediments neglect the effects of growing plants and tidal inundation and exposure. These *in situ* biological and physical factors create a complex sediment redox structure by oxidizing the rhizosphere and sediment pores, thereby increasing the availability of oxygen to support nitrification and decreasing the diffusion distance between oxic sites of nitrate production by nitrifying bacteria and anoxic sites of nitrate consumption by denitrifiers (Dacey & Howes 1984, Risgaard-Petersen & Jensen 1997, Sundby et al. 1998).

A still common *in vitro* technique, the acetylene block (e.g. Chabrerie et al. 2001, Tobias et al. 2001b, Sundareshwar et al. 2003, Hefting et al. 2004, Wigand

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et al. 2004, Dollhopf et al. 2005), also blocks nitrification, making it inadequate for measuring coupled nitrification–denitrification (Van Raalte & Patriquin 1979). The acetylene block is also inhibited by the S^{2-} levels present in many saltmarsh sediments (Sørensen et al. 1987). More recent methods have measured nitrification and denitrification rates through ^{15}N isotope dilution and recovery of reaction products, but their application has typically been limited to *in vitro* incubations (Abd Aziz & Nedwell 1986, DeLaune et al. 1989, Stepanauskas et al. 1996).

In vitro techniques are inadequate to measure saltmarsh denitrification, because they isolate sediments from plant growth, tides, light, sediment hydrology, and redox conditions. Plant N uptake competes with nitrification for available NH_4^+ (Buresh et al. 1981, DeLaune et al. 1983, Dean & Biesboer 1985, White & Howes 1994a), while root oxygen leakage aerates the rhizosphere, promoting coupled nitrification–denitrification (Reddy et al. 1989, Mendelssohn & McKee 1992, Arth & Frenzel 2000). Tidal inundation frequency and its control of the sediment oxidation state may also play a role in regulating nitrification–denitrification (Smith & Patrick 1983). Nevertheless, few measures of denitrification maintain *in situ* conditions, and most previous approaches to *in situ* measurements have been flawed. Landscape-level mass balance approaches suffer from coarse spatial and temporal resolution (Valiela & Teal 1979, Anderson et al. 1997, van Wijnen & Bakker 2000). Haines et al. (1977) estimated denitrification rates from sediment N_2 gas profiles, but their measurements did not consider sediment porosity, nor was it clear how they accounted for diffusion. Kaplan et al. (1979) measured *in situ* N_2 evolution into He headspaces in small bell jars inserted into surficial sediments, but their method involved the removal of aboveground biomass, cutting of roots, alteration of sediment hydrology, and short incubations in dark enclosures. Smith et al. (1983) measured wetland N_2O fluxes into dark enclosures, and estimated denitrification rates from N_2/N_2O ratios determined in a swamp forest (Lindau et al. 1988, DeLaune et al. 1989). ^{15}N tracers have been used in undisturbed vegetated marsh to determine tracer fate and denitrification losses, but not to measure denitrification rates (Buresh et al. 1981, DeLaune et al. 1983). Tobias et al. (2001a) used an *in situ* $^{15}NO_3^-$ groundwater tracer, but their method did not measure coupled nitrification–denitrification, and was used in unvegetated sediments.

In the present study, we developed an *in situ* ^{15}N tracer approach to measuring coupled nitrification–denitrification ('denitrification' or D_n) in undisturbed marsh grass stands. Our approach was modified from the *in situ* isotope tracer method of White & Howes (1994a). In a study of long-term saltmarsh N retention, they injected $^{15}NH_4^+$ directly into undisturbed sediments. During their first measurement interval (3 d), ^{15}N loss was rapid, but subsequent loss was slow, since most of the tracer had become fixed into plant biomass pools with low turnover rates (White & Howes 1994b). However, the pattern and rate of denitrification N losses during the initial 3 d after tracer injection remained unclear, as was the seasonal pattern and annual flux through denitrification.

In the present study, we determined the seasonal pattern of short-term (days) inorganic nitrogen loss from saltmarsh grass stands through nitrification–denitrification reactions by injecting $^{15}NH_4^+$ *in situ* into undisturbed saltmarsh sediments, following total ^{15}N retention through a series of destructively sampled sediment cores, measuring all non-gaseous ^{15}N loss routes, and determining denitrification rates by mass balance. Although this approach has the potential to overestimate denitrification losses, with careful quantification of all other potential loss routes, we believe any disadvantages of our approach are outweighed by the advantages of studying denitrification in undisturbed grass stands, under natural conditions of plant and tidal activity. We analyzed the seasonal pattern of denitrification so obtained with respect to the availability of N, regulated by remineralization and plant uptake. We also examined denitrification in grass stands receiving long-term fertilization, where N was not limiting to plant growth and where our denitrification measurements could be compared to mass balance measures.

MATERIALS AND METHODS

We measured nitrification–denitrification ('denitrification' or D_n) by injecting $^{15}NH_4^+$ *in situ* into undisturbed vegetated saltmarsh sediments and following total ^{15}N retention over 1 to 5 d in a series of destructively sampled 6.5 cm diameter × 10 cm deep sediment cores and their associated aboveground biomass. Our measure of total ^{15}N retention included dissolved inorganic as well as organic (plant and microbial) fractions. From the decrease in ^{15}N recovery over time, we calculated a rate constant for all ^{15}N losses. We identified and measured all non-gaseous ^{15}N loss routes out of the sampled core volume and associated aboveground biomass, and adjusted the ^{15}N loss rate constant to calculate a denitrification rate constant (Fig. 1). This rate constant was applied to the *in situ* NH_4^+ pool to determine the overall rate of coupled nitrification–denitrification. We further compared these *in situ* measures to measurements in greenhouse lysimeters, where denitrification was the only loss route, and under *in vitro* conditions without normal plant function or tidal inundation.

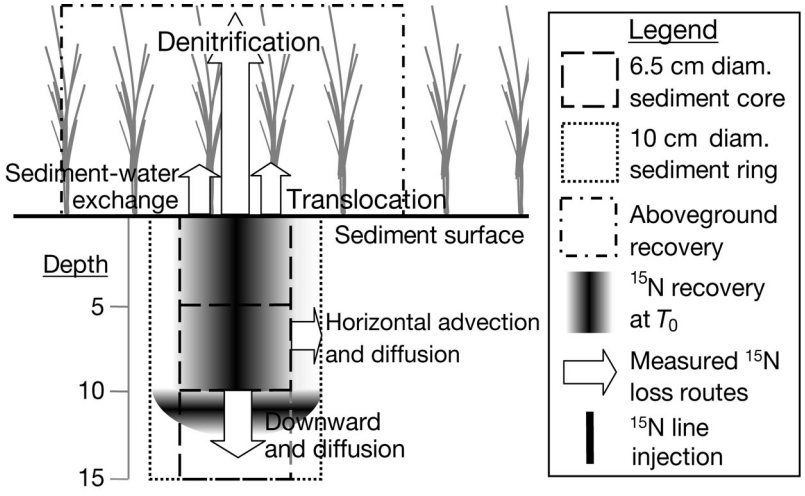

Fig. 1. Measured loss routes of injected ^{15}N. $^{15}NH_4^+$ was line-injected to 10 cm depth in undisturbed vegetated saltmarsh sediments. Total ^{15}N recovery (during a time course of 1 to 5 d) was measured in a series of destructively sampled, 6.5 cm diameter sediment cores sectioned into 5 cm intervals (– – –). T_0 recovery of ^{15}N within the core averaged 93% (see Table 2; approximate distribution of tracer shown by shading). Changes in ^{15}N recovery in the 10 to 15 cm depth interval (no denitrification losses; White & Howes 1994a) were used to determine downward advection and diffusion. Changes in ^{15}N recovery in a 10 cm diameter sediment ring surrounding the core (·····) (corrected for denitrification losses) were used to measure horizontal advection and diffusion. Sediment–water exchanges were determined from the differences in ^{15}N loss rates between conditions of tidal inundation and exposure. Translocation losses were measured in aboveground biomass harvested in a 20 cm diameter area (– · – · –). ^{15}N losses are summarized in Table 3

Study site. Great Sippewissett Marsh (Cape Cod, MA, USA) is a typical New England saltmarsh dominated by the short form of *Spartina alterniflora* (Loisel.) growing in near-monospecific stands on the marsh plain (Teal & Howes 1996). The sediments consist of a highly organic peat (21 to 35% C) of low hydraulic conductivity (Redfield 1972, Howes & Goehringer 1994). Nitrification and denitrification are tightly coupled in these carbon-rich sediments (Valiela & Teal 1979, Howes & Goehringer 1994). We performed our experiments in undisturbed stands of short-form *S. alterniflora* similar to those previously investigated in this saltmarsh (e.g. Valiela & Teal 1979, Howes et al. 1984, 1985, 1986, White & Howes 1994a,b, Teal & Howes 1996), and in plots originally identical to control areas that have received high N and P fertilization for 16 to 23 yr (Valiela et al. 1973, 1975). Fertilized plots were treated with 3.7 or 11.2 mol organic N m^{-2} yr^{-1} (71% as pelletized sewage sludge [6.1% N] and 29% as urea) and 2.2 mol P m^{-2} yr^{-1} (as pelletized sewage sludge and triple phosphate), applied 10 times in each growing season (May to September).

Field work. We measured ^{15}N retention and loss rates in all seasons, with measurements concentrated in the summer (unfertilized grass stands: n = 9; fertilized areas: n = 6; see dates in Table 6). For each denitrification rate measurement, we first made up to 14 line injections (0 to 10 cm deep, ca. 0.5 m apart) of $^{15}NH_4Cl$ solution into the marsh sediments, performing all work from boardwalks to avoid compaction. We followed total ^{15}N retention for 1 to 5 d, with measurements concentrated in the first 24 h, by destructively sampling duplicate sediment cores for each time point (total time points n = 3 to 7). Immediately after injection and at each subsequent sampling time, we harvested aboveground biomass and extracted duplicate piston cores (6.5 cm diameter, 15 cm deep) centered on randomly selected injection sites. We immediately (in the field) sectioned the sediment cores into three 5 cm thick layers, and packed them in dry ice to halt biological activity. This treatment lowered the temperature of sediments by 10°C within 6 min. Sections were completely frozen within 20 min of collection and were kept at –20°C until preparation for analysis (see below). Additional uninjected cores were collected for measurements of KCl-extractable NH_4^+ (n = 3), porosity (n = 3), and natural abundance of ^{15}N (n = 2), made at 5 cm depth intervals (methods below). We immediately filled all core holes with sediment from an adjacent area to prevent water table disturbance and air entry into nearby injection sites.

Method verification. The line-injected tracer solution (0.82 ml cm^{-1}) increased the sediment water content by <3%. The tracer (4.1 to 5.6 µmol $^{15}NH_4^+$ cm^{-1}) increased the total sediment N mass by only ca. 0.1%, but increased the ^{15}N content of the recovered sediment from 0.37 at.% to ca. 0.46 at.%. The tracer increased sediment NH_4^+ pools by an average of 68 and 27% in natural and fertilized plots, respectively. In order to examine the effect of these short-term increases in NH_4^+ availability on D_n rates, an experiment was performed comparing ^{15}N losses in unfertilized areas at injections of 33, 66, and 100% of the normal tracer addition level, representing increases in sediment NH_4^+ of 43, 82, and 122%, respectively.

We measured tracer retention (including all nongaseous ^{15}N forms) in the upper 10 cm of the 6.5 cm diameter sediment cores, the site of 93% of CO_2 production and 97% of the live belowground plant biomass (Fig. 1) (Howes et al. 1985, White & Howes 1994a). We measured aboveground translocation from

temporal changes in ^{15}N recovery in a 20 cm diameter area of aboveground biomass centered on each injection site. We compared ^{15}N losses during intervals between core samplings when the marsh surface was inundated with losses occurring during tidal exposure, in order to determine if ^{15}N losses differed under these 2 conditions and whether sediment–water exchanges were a significant loss route. Downward tracer losses (translocation, advection, or diffusion) were determined from temporal changes in ^{15}N recovery in the metabolically inactive 10 to 15 cm depth horizon. Horizontal tracer losses were determined from changes in ^{15}N recovery (corrected for D_n losses) in a 10 cm diameter sediment ring collected around the 6.5 cm diameter sampling area (Fig. 1). Although our method for determining D_n did not depend on knowing the fate of ^{15}N retained within the 6.5 cm × 10 cm core sections, we measured the partitioning of injected ^{15}N between aboveground biomass, live roots and rhizomes, remaining sediment, and unreacted $^{15}NH_4^+$ on 1 occasion in May after 3 and 7 d on duplicate cores by the methods of White & Howes (1994a).

Laboratory incubations. We also assessed our method of measuring ^{15}N retention and loss in greenhouse lysimeters, where only gaseous ^{15}N losses were possible. Sediment cores from unfertilized grass stands (20.3 cm diameter, n = 4) with actively growing *Spartina alterniflora* were maintained in lysimeters in a greenhouse with water table levels manipulated to simulate field conditions (Arenovski & Howes 1992, White & Howes 1994a). Incubations were conducted in late August, as for the field incubations.

We further measured ^{15}N-N_2 production from sediments held *in vitro* without normal plant function or tidal inundation. Vegetation was cut to 1 cm, and sediment cores (8.8 cm diameter × 14 cm deep, collected on 2 October 2003) were line-injected with $^{15}NH_4^+$ (3.2 mmol cm^{-1}) and incubated (20°C) in sealed glass-walled chambers. D_n was measured by sediment ^{15}N-N_2 efflux (Finnegan 251 mass spectrometer) and as N_2 flux (O_2 and N_2, Shimadzu GC-14A gas chromatograph). Sediments from both control and fertilized sites were incubated in replicate chambers (4 each with 80% He/20% O_2 [N_2-free to facilitate N_2 detection] and 2 each with He only [to control for diffusive N_2 flux from dissolved porewater N_2]; method described in Hamersley & Howes 2003). Gas samples were analyzed daily for 5 d, after which the total sediment ^{15}N content was determined as with the *in situ* experiments.

Calculations. ^{15}N retention was calculated as the excess above natural abundance (0.3663 at.%, CV < 8 × 10^{-6}%) recovered in the 6.5 cm × 10 cm sediment cores and associated aboveground biomass. The time series of destructively sampled core sections was used to determine k_T, the ^{15}N decay constant, using either an exponential or linear model. We adjusted k_T arithmetically for other measured tracer losses (k_L) (Fig. 1) to obtain the ^{15}N decay constant for nitrification–denitrification (k_D, expressed as % d^{-1}):

$$k_D = -100(k_T - k_L) \quad (1)$$

In the *in vitro* experiment, k_D was calculated from the ratio of the production rate of ^{15}N-N_2 and the mass of tracer ^{15}N injected into the core. Since coupled nitrification–denitrification rates of $^{14}NH_4^+$ and $^{15}NH_4^+$ do not significantly differ (*in situ* isotopic fractionation [α] of NH_4^+ by nitrification–denitrification in sediments = 0.993; Brandes & Devol 1997), D_n was calculated by applying the decay constant k_D to the *in situ* sediment NH_4^+ pool (KCl-extractable) (White & Howes 1994a) such that:

$$D_n = k_D [NH_4^+] \quad (2)$$

All correlation coefficients (R) are Pearson product moment correlations, and errors are standard errors (SE).

Analytical approach. For our total ^{15}N retention measurements, the pH of previously frozen core sections was reduced to ca. 2 by shaking with 0.2 N H_2SO_4 for 24 h at 4°C. This was the best method found to inhibit biological activity during wet sample processing and prevent $^{15}NH_4^+$ volatilization during subsequent drying (to constant weight: ca. 48 h at 60°C). ^{15}N recovery from sediment sections injected with tracer in the laboratory averaged 104 ± 1% (n = 12). Sediments and dried aboveground biomass were milled (Cyclotec) to <0.5 mm, and total N and ^{15}N content was determined by mass spectrometry (Carlo Erba T1500, Stable Isotope Facility, University of California, Davis). This analysis measured all non-gaseous forms of ^{15}N (including belowground plant and microbial biomass) within the core sections. KCl-extractable NH_4^+ ('sediment NH_4^+') was determined by extraction of known sediment volumes with acidified (pH = 2) 2 N KCl at 4°C for 24 h (White & Howes 1994a), followed by sterile filtration and analysis by a colorimetric indophenol method (Scheiner 1976). The longer extraction times required for these peats are preferable to homogenization and do not result in NH_4^+ mineralization (authors' unpubl. data). Known volumes of sediment were weighed wet and dry (to constant weight at 60°C) to determine porosity and density. Organic C and N contents were determined by elemental analysis (Perkin-Elmer 2400). Porewaters were extracted with sippers and analyzed for salinity (conductivity) and sulfide (Cline 1969).

Table 1. Characteristics of unfertilized and XF fertilized (11.2 mol organic N m^{-2} yr^{-1} for 16 to 23 yr) marsh plots. Standard errors in parentheses

	Unit	Unfertilized	XF fertilized
Sediments (top 10 cm)[a]			
Porosity	ml cm^{-3}	0.86 (0.01)	0.80 (0.01)
Density	g cm^{-3}	0.16 (0.01)	0.21 (0.01)
Organic N	mmol cm^{-3}	0.16 (0.01)	0.34 (0.02)
Organic C	mmol cm^{-3}	3.1 (0.1)	3.9 (0.2)
KCl-extractable NH_4^+	µmol cm^{-3}		
0–5 cm		0.13 (0.02)	0.84 (0.33)
5–10 cm		0.15 (0.04)	0.70 (0.25)
10–15 cm		0.12 (0.04)	0.55 (0.16)
Porewater salinity		26.2 (2.9)	28.4 (2.9)
Porewater S^{2-}	mM	1.66 (0.50)	0.42 (0.15)
Spartina alterniflora [b]			
Stem height	cm	30	102
Peak biomass	g m^{-2}	270 (66)	1100 (90)

[a]n > 24 (unfertilized) and n > 18 (fertilized)
[b]Howes et al. (1986)

RESULTS

Fertilization resulted in a >3-fold increase in the aboveground biomass and height of *Spartina alterniflora* plants over control areas (Table 1). Sediment characteristics also differed; fertilized sediments were denser and contained nearly twice the organic N and >4 times the NH_4^+. Although sediment salinity levels were similar, aeration of the sediments by increased evapotranspiration reduced porewater S^{2-} concentrations nearly 4-fold.

Evaluation of the *in situ* method

Line-injections of $^{15}NH_4^+$ into undisturbed *Spartina alterniflora* saltmarsh sediments resulted in a reproducible distribution of label through the sediment, and most of the label was recovered at T_0 within the 6.5 cm diameter sediment core. The ^{15}N recovery at T_0 averaged 99% in unfertilized and 98% in fertilized sediments within a 5 cm radius (10 cm diameter) of the injection site (Table 2, Fig. 1). Most (ca. 93%) of the injected ^{15}N was recovered within a 6.5 cm diameter area in both sediment types, and only 6.2 ± 1.8 and 5.4 ± 1.8% was recovered outside of the 6.5 cm diameter sample core in unfertilized and fertilized sediments, respectively. The distribution of recovered ^{15}N at each depth interval was consistent within sediment type (unfertilized versus fertilized). Some ^{15}N was recovered in the 10 to 15 cm depth layer since the line injection was begun with the syringe needle tip at 10 cm.

Non-denitrification ^{15}N loss routes were small relative to total losses (Table 3). Measurements made on each sampling date were used to calculate k_L (Eq. 1). Overall, downward losses of ^{15}N were not significant (unfertilized sediments: mean = –2.0 ± 5.4% d^{-1}, t = 1.2, df = 8, p = 0.27; fertilized sediments: mean = 4.1 ± 9.8% d^{-1}, t = 0.55, df = 5, p = 0.6). Horizontal losses of ^{15}N through the sediments averaged only 1.8 ± 0.7% d^{-1} (n = 8) in unfertilized sediments and 1.7 ± 0.5% d^{-1} (n = 6) in fertilized sediments. Sediment–water exchanges during tidal inundation were not significant either (0.5 ± 13% d^{-1}, t = 0.005, df = 6, p = 0.5). The large error associated with the sediment–water exchanges results exists because they were determined from the differences between 2 ^{15}N recovery measurements typically only hours apart (Fig. 2). Translocation of ^{15}N into aboveground biomass during the first 24 h was small, averaging 0.9 ± 1.4% d^{-1} (n = 9) in unfertilized and 2.0 ± 0.8% d^{-1} (n = 6) in fertilized sediments (December excluded [no translocation]). Since changes in the aboveground biomass pool were included in k_T and sediment–water exchanges were not significant, k_L was calculated as the sum of the measured horizontal and vertical losses. The overall mean rate of horizontal movement of ^{15}N was used to calculate k_L when actual measurements during 3 sampling dates were not made.

Enhancement of sediment NH_4^+ pools by the $^{15}NH_4^+$ tracer had no significant effect on the decay constant k_D or, therefore, on D_n rates (Fig. 2). Measurements at tracer injection levels (33, 66, and 100% of normal tracer addition levels) representing sediment NH_4^+ pool enhancements of 43, 82, and 122%, respectively, resulted in k_D values of 71 ± 5, 72 ± 23, and 73 ± 10% d^{-1},

Table 2. Mean ^{15}N recovery (SE) in sediments sampled from unfertilized and XF fertilized (11.2 mol organic N m^{-2} yr^{-1}) saltmarsh grass stands ca. 30 min after line-injecting $^{15}NH_4^+$ solution into undisturbed sediments (0 to 10 cm)

	^{15}N recovered at T_0 (% of total injected)	
Depth interval (6.5 cm diameter core)[a]	Unfertilized	XF fertilized
0–5 cm	38.3 (3.8)	37.7 (4.0)
5–10 cm	40.6 (3.3)	49.2 (3.8)
10–15 cm	13.8 (2.5)	5.8 (1.8)
Subtotal	92.8 (4.5)	92.7 (1.4)
Outside 6.5 cm diameter core[b]	6.2 (1.8)	5.4 (1.8)
Total	99.0	98.0

[a]n = 18 (unfertilized) and n = 12 (fertilized)
[b]10 cm diameter outer core taken surrounding 6.5 cm diameter inner core, n = 8 (unfertilized) and n = 6 (fertilized)

Table 3. Mean ^{15}N losses (SE) from vegetated saltmarsh sediment volumes (6.5 cm diameter × 10 cm) injected *in situ* with $^{15}NH_4^+$. Losses are expressed as a percentage of injected ^{15}N lost in the first 24 h and can be compared with denitrification loss rates (k_D) (see Table 6). Results from an earlier study (White & Howes 1994a) are included, with the time span of measurements within square brackets. Positive values indicate a loss

^{15}N loss route (Fig. 1)	Method of measurement	^{15}N loss rate	
		Unfertilized	XF fertilized
Downward advection and diffusion	Change in ^{15}N recovery below injection depth (10 cm)	–2.0 (5.4)% d^{-1}	4.1 (9.8)% d^{-1}
	Change in ^{15}N recovery below injection depth (15 cm) in undisturbed field sites[a]	0 (1.4)% [3 d]	
Horizontal advection and diffusion	Change in ^{15}N recovery in 10 cm diameter outer sediment ring	1.8 (0.7)% d^{-1}	1.7 (0.5)% d^{-1}
	Difference in ^{15}N recovery between undisturbed field sites and open-bottomed *in situ* lysimeters[a]	–6.2% [100 d]	
Sediment–water exchanges	Difference in ^{15}N loss rates between inundated and exposed conditions	0.5 (13)% d^{-1}	0.5 (13)% d^{-1}
	Difference in ^{15}N recovery between *in situ* and laboratory lysimeters[a]	–3.1% [30 d]	
Translocation	^{15}N recovery in aboveground biomass	0.9 (1.4)% d^{-1}	2.0 (0.8)% d^{-1}

[a]White & Howes (1994a)

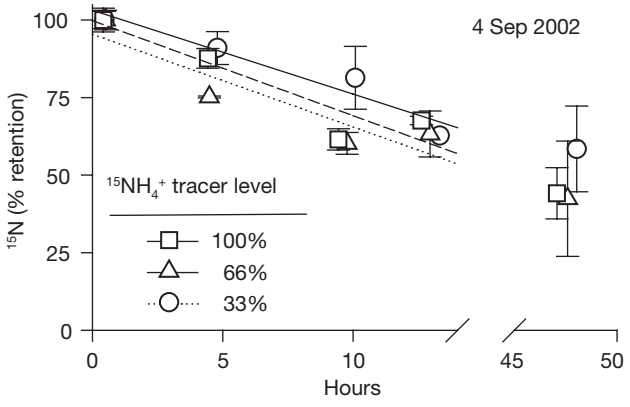

Fig. 2. ^{15}N decay constant in *in situ* incubations is independent of initial $^{15}NH_4^+$ tracer concentration. Short-term *in situ* ^{15}N recovery from unfertilized vegetated saltmarsh sediments with $^{15}NH_4^+$ added at 33, 66, and 100% of standard injection levels (4.6 mmol $^{15}NH_4^+$ cm^{-1}). ^{15}N recovery includes aboveground biomass. The ^{15}N decay constants (k_D, Eq. 1) were independent of the tracer addition level (71 ± 5% d^{-1}, 72 ± 8% d^{-1}, and 73 ± 10% d^{-1}, respectively). Error bars are SE (n = 2)

respectively (1-way ANCOVA test for homogeneity of regressions, df = 2, 6, p = 0.99).

The pattern of loss and retention of the ^{15}N label was similar to that observed previously (White & Howes 1994a), with initial rapid losses slowing as the $^{15}NH_4^+$ label became incorporated into plant biomass (Fig. 3). Although most of the retained ^{15}N was bound up in plant biomass after 3 d, aboveground translocation was relatively small, varying from 1 to 6% during the growing season (Fig. 4), similar to earlier observations (White & Howes 1994a). Of the recovered label, 81% was found in live roots and rhizomes (Fig. 3). More ^{15}N was translocated to aboveground *Spartina alterniflora* biomass in fertilized grass stands than was translocated in unfertilized control stands.

Late summer comparisons of D_n measured *in situ* and in greenhouse lysimeters (with growing plants and simulated tidal cycles in which denitrification was the only possible ^{15}N loss route) were similar (6.9 ± 1.9 versus 8.3 ± 2.1 mmol N m^{-2} d^{-1}; Table 4), confirming that no significant loss terms were unaccounted for by our method. However, when sediments were incubated *in vitro* with disrupted plant activity and no tidal cycles, D_n became insignificant in unfertilized and very low in fertilized sediments, although oxygen uptake rates were similar to those reported earlier for this same marsh (Table 5; Howes et al. 1984).

Nitrification–denitrification

We measured D_n rates in both unfertilized and fertilized grass stands. We made late-summer measurements in each year from 1997 to 2000, and collected seasonal data over 2 yr (see dates in Table 6). The short-term time course of ^{15}N retention in the *Spartina alterniflora* stands within Great Sippewissett Marsh yielded a temporal pattern consistent with coupled nitrification–denitrification losses (Fig. 5). After an initial period of rapid ^{15}N loss, short-term nitrification–denitrification losses of ^{15}N slowed, as the $^{15}NH_4^+$ tracer became incorporated into biomass and was less available to support D_n (Figs. 3 & 4). In unfertilized *S. alterniflora* stands, the ^{15}N decay constant due to

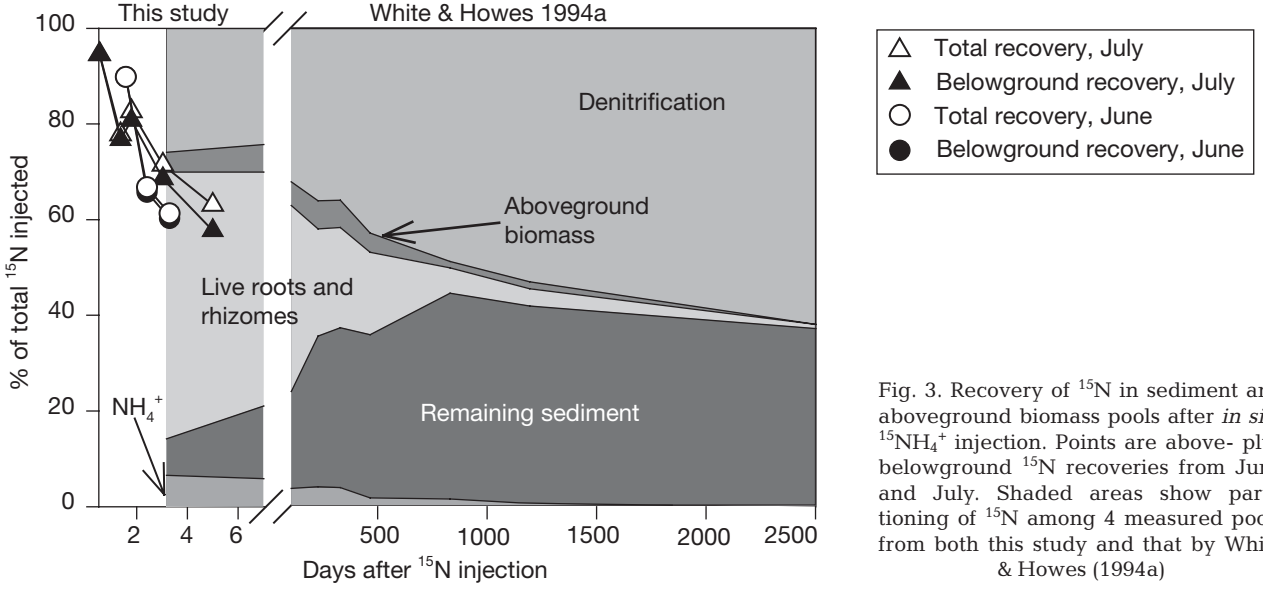

Fig. 3. Recovery of ^{15}N in sediment and aboveground biomass pools after *in situ* $^{15}NH_4^+$ injection. Points are above- plus belowground ^{15}N recoveries from June and July. Shaded areas show partitioning of ^{15}N among 4 measured pools from both this study and that by White & Howes (1994a)

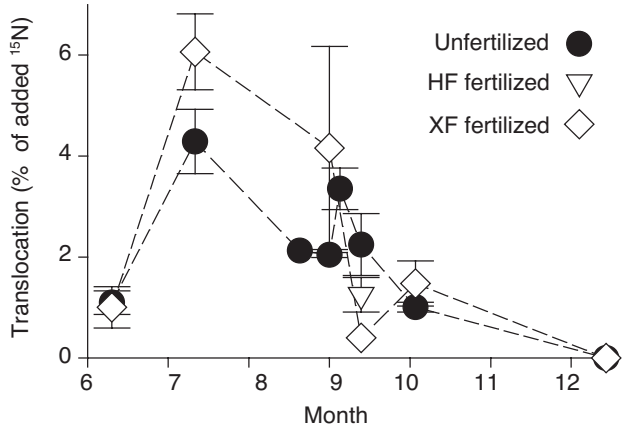

Fig. 4. Translocation of $^{15}NH_4^+$ injected into sediments in aboveground biomass in natural unfertilized and fertilized (XF: 11.2 mol organic N m^{-2} yr^{-1}; HF: 3.7 mol organic N m^{-2} yr^{-1}) saltmarsh grass stands. Points are from different years; sampling dates in Table 6. Error bars are SE (n = 2)

coupled denitrification (k_D) ranged from lows of 10 to 16 % d^{-1} in fall and winter up to 148 % d^{-1} in late summer (Table 6). In fertilized areas, k_D ranged between 44 and 134 % d^{-1}, with the peak occurring in late June. Sediment NH_4^+ (KCl-extractable) concentrations in unfertilized sediments ranged from 0.064 to 0.25 µmol N cm^{-3}, with no apparent seasonality (Table 6). In contrast, in fertilized sediments, sediment NH_4^+ had a summer maximum associated with the timing of fertilizer application, and NH_4^+ concentrations ranged from 0.21 to 1.6 µmol cm^{-3}, up to 10 times the unfertilized concentrations.

Summer D_n maxima were observed in both natural and fertilized grass stands (Fig. 6A,B). D_n rates in unfertilized areas were low from October through July (0.4 to 2.2 mmol N m^{-2} d^{-1}), but late summer measurements in all years were high, up to 11.9 mmol N m^{-2} d^{-1}. In fertilized areas, high summer rates began in July and were more than an order of magnitude higher

Table 4. Mean denitrification (SE) measured under field and laboratory conditions. Lysimeters containing vegetated sediments treated with simulated tidal inundation cycles in which denitrification was the only possible loss pathway were used to check the validity of *in situ* assays. For the *in vitro* experiment in which denitrification was measured by ^{15}N-N_2 production, *in situ* conditions could not be maintained; aboveground plant biomass was removed and sediments were kept flooded. n.a., not applicable

Experiment	k_D (% d^{-1})	n	Sediment NH_4^+[a] (mmol cm^{-3})	Translocation[b] (% d^{-1})	Denitrification rate (mmol N m^{-2} d^{-1})
In situ (19 Aug)	30.6 (4.0)	2	0.23 (0.05)	1.0 (0.5)	6.9 (1.9)
Greenhouse lysimeter (6–19 Aug)[c]	20.1 (1.7)	4	0.41 (0.12)	1.9 (0.6)	8.3 (2.1)
In vitro[d]	0.02 (0.01)	4	0.094 (0.027)	n.a.	−0.002 (0.001)

[a]KCl-extractable
[b]Translocation into aboveground biomass. This biomass was removed for *in vitro* experiment due to headspace limitations
[c]Lysimeters contain undisturbed sediment with live vegetation collected from the same site as the *in situ* experiments and were hydrologically controlled to simulate tidal cycles (see 'Materials and methods')
[d]Sediments collected 2 October, but incubated at August temperatures (20°C)

Table 5. Mean metabolism (SE) of saltmarsh sediments incubated *in vitro* (n = 4). Sediments were incubated with aboveground biomass removed and were kept flooded due to methodological constraints. Positive values are fluxes out of sediments

	Unit	Sediment type	
		Unfertilized	Fertilized[a]
O_2 flux	(mmol O_2 m^{-2} d^{-1})	–104 (10)	–163 (28)
N_2 flux[b]	(mmol N m^{-2} d^{-1})	1.7 (1.2)	2.3 (6.7)
^{15}N-N_2 flux	(mmol ^{15}N m^{-2} d^{-1})	–0.0012 (0.0004)	0.039 (0.004)
KCl-extractable NH_4^+	(mmol cm^{-3})	0.094 (0.027)	0.33 (0.01)
Denitrification (from ^{15}N-N_2 flux)	(mmol N m^{-2} d^{-1})	–0.002 (0.001)	0.26 (0.03)
^{15}N recovered from sediments after incubation	(%)	99.2 (3.7)	98.6 (1.6)

[a]11.2 mol organic N m^{-2} yr^{-1}
[b]N_2 production in excess of N_2 flux in chambers where coupled nitrification–denitrification was inhibited by anoxia (Hamersley & Howes 2003)

Table 6. Denitrification in saltmarsh grass stands measured by ^{15}N recovery in *in situ* incubations with $^{15}NH_4^+$. k_D is the ^{15}N decay constant (adjusted for simultaneously measured non-denitrification tracer losses, see Fig. 1, Table 3, Eq. 1). Sediment NH_4^+ is KCl-extractable NH_4^+. Denitrification was calculated by applying k_D to the sediment NH_4^+ pool (Eq. 2). Standard errors in parentheses. n.a., not applicable

Date (mm/dd/yy)	k_D ^{15}N (% d^{-1})	n[a]	r^2	Sediment NH_4^+ (mmol cm^{-3})	Denitrification (mmol N m^{-2} d^{-1})
Unfertilized					
08/19/97	31 (4)	7	0.95[b]	0.23 (0.05)	6.9 (1.9)
10/02/97	10 (5)	3	0.54[b]	0.18 (0.03)	1.8 (1.0)
04/13/98	11	2	n.a.	0.20 (0.03)	2.2 (0.3)
06/09/99	30 (6)	3	0.98[c]	0.05 (0.01)	1.6 (0.4)
09/01/99	148 (1)	3	1.00[c]	0.081 (0.003)	11.9 (0.5)
12/13/99	16 (1)	4	0.97[b]	0.11 (0.02)	1.7 (0.4)
07/10/00	5.9 (1.9)	6	0.86[b]	0.06 (0.00)	0.4 (0.1)
09/12/00	22 (2)	6	0.96[c]	0.25 (0.04)	5.6 (1.0)
09/04/02	69 (11)	12	0.81[b]	0.11 (0.02)	7.8 (1.7)
Annual[d]					0.77 (0.18) mol N m^{-2} yr^{-1}
Fertilized (HF)					
09/12/00	26 (4)	6	0.96[c]	0.50 (0.13)	13 (4)
Fertilized (XF)					
10/02/97	61 (3)	3	1.00[ab]	0.48 (0.07)	29 (4)
06/09/99	134 (25)	4	0.96[c]	0.21 (0.01)	29 (6)
09/01/99	43 (17)	4	0.96[b]	1.6 (1.5)	69 (71)
12/13/99	78 (19)	3	1.00[c]	0.28 (0.11)	22 (10)
07/10/00	54 (17)	5	0.96[c]	1.4 (0.5)	48 (5)
09/12/02	71 (6)	6		0.68 (0.03)	
Annual[d]					10.5 (4.9) mol N m^{-2} yr^{-1}

[a]Number of time points per rate determination (n = 2 sediment cores per time point)
[b]Linear model
[c]Exponential model
[d]Annually integrated rate. Assumptions: low interannual variability in denitrification rates (Teal & Howes 1996); no denitrification from 15 December to 28 February, when sediments are typically frozen; denitrification on 15 September equal to that measured on 2 October, since regional temperatures fall rapidly in mid-September (Fig. 6C)

than those in unfertilized areas, or 48 to 69 mmol N m^{-2} d^{-1}. Non-peak denitrification rates in fertilized grass stands also exceeded those of unfertilized areas, with a range of 22 to 29.2 mmol N m^{-2} d^{-1}. In both treatments, the period of high D_n rates was narrower than the sediment temperature curve. Although sediment temperatures remained constant from June until mid-September (ca. 19°C) (Fig. 6C), peak D_n rates were only measured from late August to early September in unfertilized grass stands and from July to September in fertilized areas. The D_n rate in unfertilized stands covaried with k_D rather than sediment NH_4^+ (coefficient of variation [CV] = 120 versus 52%), whereas in fertilized stands, the variation in the D_n rate was primarily a result of variation in sediment NH_4^+ rather than k_D (CV = 76 versus 44%). There was a 13-fold difference in the annual rate of D_n (obtained by integrating the seasonal denitrification curve formed by the measurement taken in different years) between natural grass stands (0.77 mol N m^{-2} yr^{-1}) and fertilized areas (10.5 mol N m^{-2} yr^{-1}) (Table 6).

DISCUSSION

The short-term retention of ^{15}N in undisturbed *Spartina alterniflora* grass stands labeled with $^{15}NH_4^+$ showed a pattern consistent with that suggested by long-term experiments (White & Howes 1994a,b). Initially rapid denitrification losses slowed as ^{15}N became bound up in plant biomass pools (Fig. 3). Non-denitrification losses were quantified, and errors were small enough to enable measurement of a large late-summer D_n peak in natural grass stands. The low rates of coupled nitrification–denitrification during mid-summer suggested that plant uptake might be competing with nitrifiers for regenerated NH_4^+. Such a pattern has not been observed in saltmarshes with methods that exclude plant growth or isolate sediments from *in situ* conditions (Table 7 and references therein). In contrast, in fertilized areas, where N was

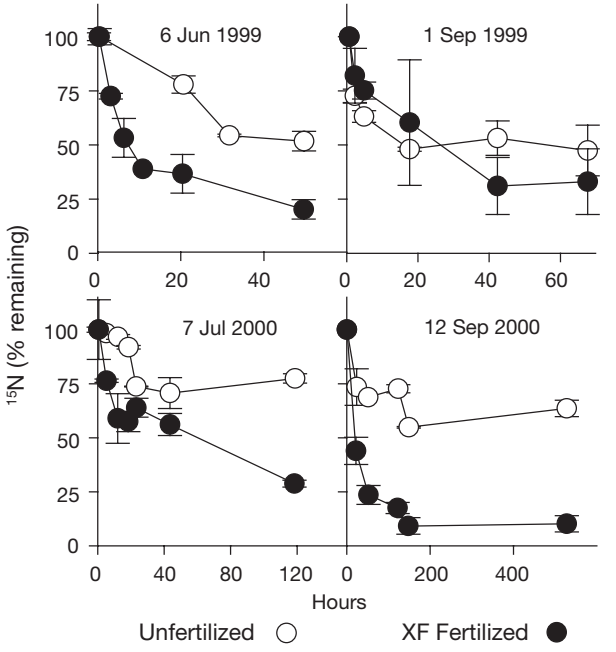

Fig. 5. Examples of ^{15}N recovery time courses (within 6.5 cm diameter × 10 cm sediment volume plus aboveground biomass) in incubations with $^{15}NH_4^+$ in undisturbed natural (unfertilized) and fertilized (11.2 mol organic N m^{-2} yr^{-1}) saltmarsh grass stands. The initial rapid loss of ^{15}N due to denitrification slows after $^{15}NH_4^+$ becomes fixed into biomass pools with slow turnover (Fig. 3). Error bars are SE (n = 2)

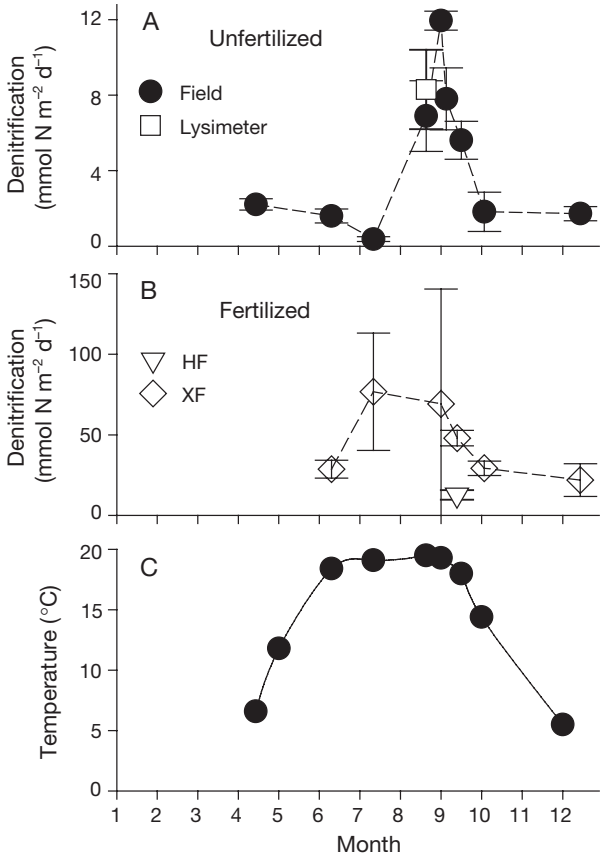

Fig. 6. Denitrification rates in *Spartina alterniflora* stands in the Great Sippewissett saltmarsh. Rates were measured in different years; see Table 6 for sampling dates. Note differing *y*-axis scales in Panels A and B. (A) Natural unfertilized areas. (B) Fertilized areas (XF: 11.2 mol organic N m^{-2} yr^{-1}; HF: 3.7 mol organic N m^{-2} yr^{-1}). Error bars are SE (n = 2). (C) Seasonal sediment temperature in natural unfertilized grass stands

added in excess of plant growth (and competitive interactions would not be expected to dominate), nitrification–denitrification losses reflected seasonal patterns of N addition and/or temperature (Fig. 6B).

Non-denitrification ^{15}N losses

^{15}N measures of nitrogen loss from saltmarsh sediments would ideally include recovery of ^{15}N-N_2 for a complete mass balance. However, complete ^{15}N recovery is not possible without experimental conditions that disturb plant growth, evapotranspiration, and tidal cycles. In order to constrain uncertainties in the fate of missing ^{15}N, we performed experiments (White & Howes 1994a, this paper) to measure the magnitude and significance of non-D_n loss routes (Fig. 1, Table 3). D_n rates measured *in situ* and in greenhouse lysimeters where D_n was the only possible ^{15}N loss route were similar (Table 4), as were their patterns of long-term ^{15}N retention (in experiments by White & Howes 1994a). In contrast, in plant-free sediments incubated *in vitro*, D_n was nearly zero, possibly because of the reduction in oxygen transport into the sediments when evapotranspiration and leakage of O_2 from roots were suppressed under *in vitro* conditions (Table 4) (Dacey & Howes 1984, Howes & Teal 1994). Although sediment NH_4^+ levels differed among the 3 incubation conditions that we compared (Table 4), we do not believe that these differences explain the D_n rates we measured. Sediment NH_4^+ levels in both the *in situ* and *in vitro* treatments were within the range of sediment NH_4^+ levels measured during the growing season in the field (Table 6), and there was little correlation of sediment NH_4^+ with D_n ($R = -0.05$). That no D_n was measured in the *in vitro* treatment was more likely a result of the experimental conditions required for *In vitro* measurement than the sediment NH_4^+ level. In the *in vitro* measurement, it was necessary to remove the aboveground biomass (typically 30 to 40 cm tall) in order to have a small headspace to increase the sensitivity of ^{15}N-N_2 production. In this, we followed the practice of all previous saltmarsh investigators (see Table 7). In addition, in the water-saturated atmosphere of an *in vitro* chamber, the absence of evapotranspiration prevents sediment aeration, likely inhibiting nitrification (Dacey & Howes 1984).

Table 7. Denitrification rates determined in saltmarsh sediments

Denitrification (mmol N m^{-2} d^{-1})	Denitrification Annually integrated (mol N m^{-2} yr^{-1})	Method	Vegetation	Experimental conditions	Source
0.4–11.9	0.77	$^{15}NH_4^+$ retention	Short *Spartina alterniflora*	*In situ*, plants	Present study
1.8	0.29 – 0.40	$^{15}NH_4^+$ retention mass balance	Short *S. alterniflora*	*In situ*, plants	White & Howes (1994a)
2.3	–	Sediment N_2 profiles	*S. alterniflora*	*In situ*	Haines et al. (1977)
0.4–2.1	0.40	N_2 production	Short *S. alterniflora*	*In situ*, no plants	Kaplan et al. (1979)
0.21	–	$^{15}N_2$ generated from $^{15}NO_3^-$	P*uccinellia maritima* *S. townsendii* *Halimione portulacoides*	*In vitro*, no plants	Abd Aziz & Nedwell (1986)
–	0.29	N_2O, corrected for N_2/N_2O ratio	*S. alterniflora*	*In situ*	DeLaune et al. (1989)
0.06–1.4	–	Acetylene block	*H. portulacoides*	*In vitro*, no plants	Koch et al. (1992)
0–0.61	–	Acetylene block	*S. alterniflora*	*In vitro*, no plants	Thompson et al. (1995)
–	0.043	$^{15}N_2O$ isotope dilution	Short *S. alterniflora*	*In vitro*, no plants	Anderson et al. (1997)
423	–	Acetylene block short *S. alterniflora*	*S. cynosuroides*,	*In vitro*, no plants	Tobias et al. (2001b)
0–2.2		Isotope pairing	*Limonium serotinum*	*In vitro*, no plants	Eriksson et al. (2003)
–9.0 to 10.1		N_2 changes in overlying water exposed to atmosphere	*S. patens*, *Schoenplectus pungens*	*In vitro*, plants	Davis et al. (2004)
5.6–13.8		Acetylene block	Short *S. alterniflora*	*In vitro*, no plants	Dollhopf et al. (2005)

Specific measurements of potential ^{15}N loss routes confirmed that they were small relative to denitrification losses, supporting earlier work (Table 3; White & Howes 1994a). Losses through diffusion and advection are small because the $^{15}NH_4^+$ label rapidly exchanges with 'bound' porewater and sorbed NH_4^+ pools, and the specific yield of porewater (the pool for which movement is possible) is only 1 to 3% of the total water content (Dacey & Howes 1984, White & Howes 1994a). Further, the hydraulic conductivity of these marsh peats is low, making significant tracer movement unlikely (Howes & Goehringer 1994). Any physical processes responsible for ^{15}N loss from sediments (horizontal and vertical losses through the sediments and losses during tidal inundation) would likely be similar, not only across seasons, but between fertilized and unfertilized sediments (Table 3). Nevertheless, in our experiments, strong differences in D_n (>3-fold) were seen between seasons, and annual losses in fertilized sediments were 13-fold greater than in unfertilized sediments. Any unaccounted for physical processes leading to loss were therefore small enough to allow this seasonal and experimental variation to be measured. However, non-denitrification ^{15}N loss routes and the errors associated with them (Table 3) were significant relative to the D_n losses in unfertilized grass stands from October to July (Table 6), and the variation in D_n rates during this period (Fig. 6A) is likely not significant.

Plant uptake

Translocation of ^{15}N into aboveground biomass peaked in July (Fig. 4). However, recovery of ^{15}N in aboveground biomass never exceeded 6%. Nonetheless, plant uptake accounted for a significant proportion of the ^{15}N pool retained within the 6.5 cm × 10 cm core section after 3 d in May, with 81% of the retained label found in plant biomass (Fig. 3). The ratio between the recovery of ^{15}N in above- and belowground plant biomass after 3 d (about 10%) was similar to the ratio of annual plant production measured previously in the same marsh (Valiela et al. 1976). Translocation from the injection site into a 10 cm diameter ring around the sampled 6.5 cm diameter core was <1.8% d^{-1}. Translocation of ^{15}N outside of this 10 cm diameter sampled sediment area was likely even lower, and not likely an important loss route, since although translocation and belowground biomass growth were highest in July and June, losses of ^{15}N in unfertilized grass stands at this time were very low (Fig. 4; Valiela et al. 1976).

Nitrification–denitrification

The pattern of D_n in natural unfertilized *Spartina alterniflora* grass stands did not appear to be forced primarily by sediment temperature (Fig. 6A,C). Although sediment temperature reached its summer plateau by June, D_n rates in June and July were low,

similar to the rates measured at other times of the year. D_n in these saltmarsh grass stands is likely controlled by $^{15}NH_4^+$ availability (Hamersley & Howes 2003), which itself is regulated by the turnover of belowground plant biomass. Although KCl-extractable NH_4^+ pools in the sediment did not show any seasonal trend (Table 6), the availability of NH_4^+ may have changed in late summer as a result of changes in organic matter degradation or of competition for regenerated NH_4^+ between plants and nitrifiers. The large and rapid incorporation of label into plant biomass in May (Fig. 3) suggests that plant N uptake competes with nitrification–denitrification for remineralized NH_4^+ early in the growing season, reducing the availability of N for denitrification (Valiela et al. 1975, DeLaune et al. 1983, Anderson et al. 1997). Measures of above- and belowground plant biomass indicate that plant growth stops in late summer and belowground biomass begins to decrease (Valiela et al. 1976). Sediment organic matter degradation (and NH_4^+ regeneration) peaks sharply during August and falls rapidly during September (Teal & Howes 1996). Therefore, the late summer peak in D_n is likely fueled by this increase in NH_4^+ availability. The importance of *S. alterniflora* N uptake in limiting nitrification–denitrification losses has also been shown in experiments with $^{15}NH_4^+$ where ^{15}N retention in the presence of living marsh plants was higher than that in sediments without plants (Buresh et al. 1981, Dean & Biesboer 1985). Competition between plants and nitrifiers for uptake of limiting quantities of N is also known from a variety of other systems, including seagrass meadows and hardwood forests (Zak et al. 1990, Welsh et al. 2000), as well as in monospecific pot experiments (Verhagen et al. 1995, Norton & Firestone 1996, Bodelier et al. 1998).

The range of denitrification rates measured in unfertilized grass stands in the present study is within the range reported with older methods (Table 7). Most *in vitro* measurements were lower than the *in situ* measurements, probably due to anoxic sediment conditions in *in vitro* studies, which inhibited nitrification. Denitrification rates determined by the acetylene block method were usually much lower than those determined by other methods, as expected from the known limitations of this technique and the inhibition of nitrification by acetylene. Our annual rate (0.77 mol N m^{-2} yr^{-1}) represents a significant increase from the annual denitrification flux (0.29 to 0.40 mol N m^{-2} yr^{-1}) used by White & Howes (1994a) in their N budget of the short *Spartina alterniflora* marsh, which assumed no denitrification during the coldest 6 mo of the year. In contrast, our study showed denitrification losses from April to December (the entire period we measured). We assumed no denitrification only for the 2 mo during which the marsh sediments are typically frozen. In addition, previous measurements did not capture the denitrification peak in late August and early September, which accounted for nearly half of our measured annual flux. Although our integrated annual denitrification rate in unfertilized grass stands is higher than suggested by mass balance measures, changing our assumption of linearity between measurements and rejecting the highest point yields an annual rate of 0.59 mol N m^{-2} yr^{-1}, a value more closely in line with mass balance estimates.

In fertilized marsh areas, however, D_n rates appeared to be controlled primarily by the high N availability resulting from fertilization. Competition with plant N uptake was less important, since the added N was >10 times the plant demand (White & Howes 1994b). In July and early September, sediment NH_4^+ concentration was >10 times higher than in unfertilized sediments (Table 6), and D_n rates were similarly enhanced (Fig. 6B). However, although sediment temperature was high in June, both sediment NH_4^+ and D_n remained low. Little fertilizer (3.1 mol N m^{-2}) had been applied at this point, and there may not have been enough time for the fertilizer N to penetrate into actively cycling sediment zones. Additionally, high plant uptake early in the growing season could have accounted for as much as one-third of the applied fertilizer, further reducing its influence on sediment NH_4^+ availability (based on measured aboveground biomass N and a belowground:aboveground N ratio of 2.4; Valiela et al. 1976, White & Howes 1994b).

D_n rates calculated by our ^{15}N tracer method were verified by a mass balance of N in the fertilized plots calculated after 16 to 23 yr of N amendments (authors' unpubl. data). Annual denitrification losses were assessed relative to the known addition versus retention of fertilizer N within the sediments, since N losses to tidal waters are known to be small (Valiela et al. 1973, Wolaver et al. 1983, White & Howes 1994b). In 4 plots receiving 11.2 mol N m^{-2} yr^{-1}, only 6.2% was retained in sediment pools (in excess of controls) (n = 8), indicating a maximum denitrification loss of 10.5 ± 0.2 mol N m^{-2} yr^{-1}, similar to the 10.5 ± 4.9 mol N m^{-2} yr^{-1} loss by denitrification determined from the $^{15}NH_4^+$ tracer measurements of the present study. Although the D_n rates measured in our fertilized saltmarsh grass stands are high, they are within the range of previously published rates for the vegetated saltmarsh, and rates up to 273 mmol N m^{-2} d^{-1} (determined by isotope-pairing) have been reported in estuarine sediments receiving similarly high inputs of anthropogenic N (Trimmer et al. 2000).

The faster turnover of ^{15}N (k_D) in fertilized sediments probably results from a combination of factors (Table 6). Organic N concentration and belowground biomass in fertilized sediments are more than twice those in unfertilized sediments (Table 1; Valiela et al.

1976), and ^{15}N turnover in fertilized sediments may have been stimulated by elevated microbial populations and the resulting rapid remineralization of plant biomass and organic fertilizer. In addition, high *Spartina alterniflora* growth rates under fertilization lead to increased evapotranspiration, deeper intertidal water table excursions, and increased sediment oxidation, which may stimulate nitrification rates (Dacey & Howes 1984, Howes et al. 1986).

Our measurements of denitrification fluxes from saltmarshes are the first we know of to show a sharp increase in D_n during a brief period in late summer, rather than exhibiting the broad sinusoidal distribution that might be expected were denitrification rates primarily regulated by temperature. Our results emphasize the importance of *in situ* measures of denitrification in understanding the seasonal cycle of denitrification in saltmarsh grass stands. *In vitro* measures may overemphasize temperature effects, while neglecting the important structuring effects of sediment oxidation and plant N uptake on the availability of NH_4^+ to support coupled nitrification–denitrification.

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