

US EPA ARCHIVE DOCUMENT

Denitrification Enzyme Activity of Fringe Salt Marshes in New England (USA)

Cathleen Wigand,* Richard A. McKinney, Marnita M. Chintala, Michael A. Charpentier, and Peter M. Groffman

ABSTRACT

Coastal salt marshes are a buffer between the uplands and adjacent coastal waters in New England (USA). With increasing N loads from developed watersheds, salt marshes could play an important role in the water quality maintenance of coastal waters. In this study we examined seasonal relationships between denitrification enzyme activity (DEA) in salt marshes of Narragansett Bay, Rhode Island, and watershed N loadings, land use, and terrestrial hydric soils. In a manipulative experiment, the effect of nutrient enrichment on DEA was examined in a saltmeadow cordgrass [*Spartina patens* (Aiton) Muhl.] marsh. In the high marsh, DEA significantly ($p < 0.05$) increased with watershed N loadings and decreased with the percent of hydric soils in a 200-m terrestrial buffer. In the low marsh, we found no significant relationships between DEA and watershed N loadings, residential land development, or terrestrial hydric soils. In the manipulation experiment, we measured increased DEA in N-amended treatments, but no effect in the P-amended treatments. The positive relationships between N loading and high marsh DEA support the hypothesis that salt marshes may be important buffers between the terrestrial landscape and estuaries, preventing the movement of land-derived N into coastal waters. The negative relationships between marsh DEA and the percent of hydric soils in the adjacent watershed illustrate the importance of natural buffers within the terrestrial landscape. Denitrification enzyme activity appears to be a useful index for comparing relative N exposure and the potential denitrification activity of coastal salt marshes.

SALT MARSHES often provide the important service of water quality maintenance by intercepting land-derived N from adjacent watersheds (Valiela et al., 2000a). Because these wetlands provide sources of organic matter and an anoxic environment (Stefanson, 1972; Seitzinger, 1988; Groffman, 1994; Nowicki et al., 1999), denitrification (the bacterial conversion of nitrate to gaseous nitrogen) is often high in salt marshes with actual denitrification rates reported to range from 3 to 340 kg N ha⁻¹ yr⁻¹ (Valiela et al., 2000a). However, the relationships between human activities in watersheds and coastal salt marsh denitrification, including the potential for denitrification rates in salt marshes to keep up with increasing anthropogenic N loads, are unclear.

In Narragansett Bay (Rhode Island, USA), coastal salt marshes of similar geomorphology and hydrology but varying watershed N loads are currently under study to determine if ecosystem structure and function associated with the salt marshes vary with watershed N loads

and percent residential land use (McKinney et al., 2001; Wigand et al., 2001, 2003). Nitrogen can be limiting in salt marshes (e.g., Valiela and Teal, 1974), but increasing human activities in watersheds adjacent to coastal habitats may result in human-derived N (e.g., wastewater and fertilizer) as a new source of N to salt marshes that could fuel the denitrification process. Denitrification requires anoxic conditions and organic matter that are often associated with hydric soils characteristic of most wetlands (Mitsch and Gosselink, 2000). Consequently, inland wetlands with hydric soils might act as a natural buffer of human-derived N loads, preventing or minimizing N from reaching coastal habitats such as salt marshes. Nitrogen that is not intercepted or processed in the terrestrial landscape enters the salt marsh and is either retained in the coastal habitat, released into the adjacent coastal waters, or lost through denitrification.

In the Narragansett Bay fringe salt marshes, the high marsh is dominated by saltmeadow cordgrass while the low marsh is dominated by smooth cordgrass (*S. alterniflora* Loisel) (Bertness and Ellison, 1987). These plants have different root architecture and rhizosphere characteristics that can influence denitrification (Woldendorp, 1963; Brar, 1972; Stefanson, 1972). The low marsh is bounded at its upper limit by the extent of the mean high tide, and generally, in Narragansett Bay the low marsh is inundated with sea water twice a day. In addition to plant differences, nutrient and organic exchanges that occur with the rise and fall of the tides and upland contributions of nutrients and organics also could differ between the low and high marsh locations. Finally, seasonal differences in denitrification rates may be detectable among the marshes because of temporal variations in plant production and the supply of nutrients and organics (Groffman, 1994). Most denitrification activity in temperate ecosystems occurs in the early spring and fall, during periods of high soil wetness and low plant productivity (Groffman, 1994). Quantification of actual denitrification rates is hindered by high spatial and temporal variation, and therefore in some cases, assays of denitrifier biomass are a good index of denitrification potential since they integrate multiple factors that influence denitrification activity (Groffman, 1987, 1994). In this study we measured denitrification enzyme activity (DEA) using a simple acetylene-block denitrification enzyme assay as an index of denitrifier biomass in salt marsh soils and an integrated measure of the denitrification potential (Smith and Tiedje, 1979).

In a bay-wide survey, we tested the hypothesis that coastal salt marshes are important natural buffers between developed lands and coastal waters and transform land-derived N into gaseous N. We tested whether the marshes showed increasing DEA with increasing N

C. Wigand, R.A. McKinney, and M.M. Chintala, USEPA Office of Research and Development, National Health and Environmental Effects Research Laboratory, Atlantic Ecology Division, 27 Tarzwell Drive, Narragansett, RI 02882. M.A. Charpentier, OAO Corporation, 27 Tarzwell Drive, Narragansett, RI 02882. P.M. Groffman, Institute of Ecosystem Studies, PO Box AB, Millbrook, NY 12545. Received 8 May 2003. *Corresponding author (wigand.cathleen@epa.gov).

Published in J. Environ. Qual. 33:1144–1151 (2004).

© ASA, CSSA, SSSA

677 S. Segoe Rd., Madison, WI 53711 USA

Abbreviations: DEA, denitrification enzyme activity.

loads and percent residential land use, and if there were significant relationships between marsh DEA and the percent of hydric soils in the surrounding terrestrial lands. Second, we examined whether there were significant differences in DEA between the high and low marsh zones or spring and fall seasons. In addition, we used correlation analyses to identify patterns of marsh soil characteristics with N loads, percent residential land use, and the percent of hydric soils in the adjacent uplands. Finally, in a manipulative field experiment in a saltmeadow cordgrass marsh in the Prudence Island (Rhode Island) National Estuarine Research Reserve, we examined the effect of N and P additions on DEA.

MATERIALS AND METHODS

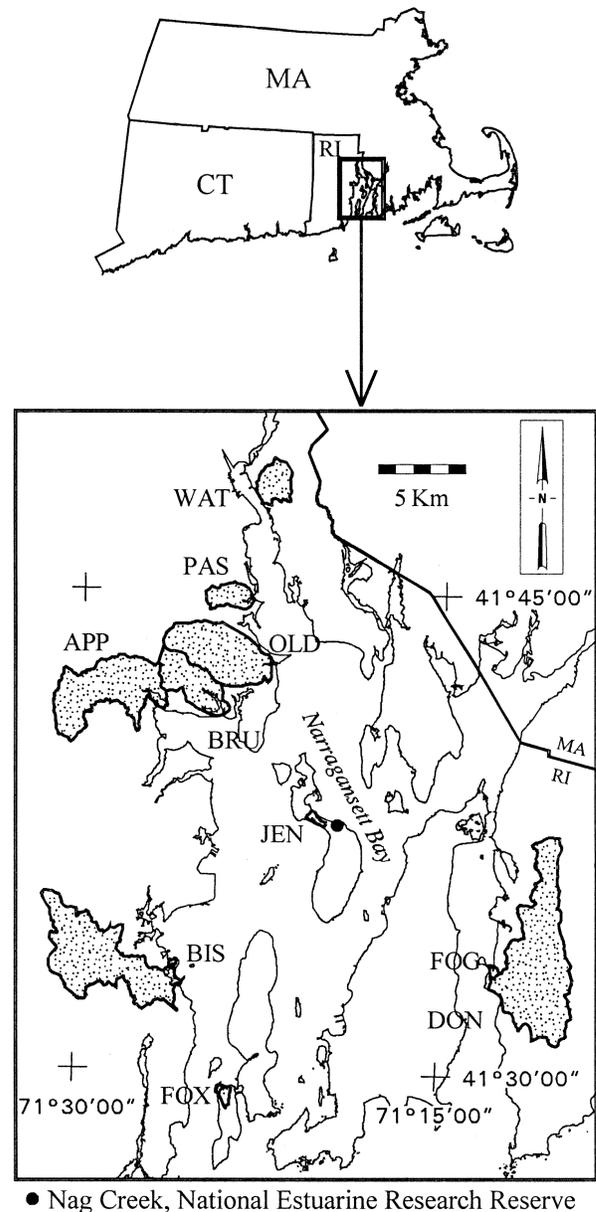
Watershed Analyses and Nitrogen Loads

The Narragansett Bay salt marsh sites and adjoining sub-watersheds have been described previously (McKinney et al., 2001) and consist of 10 sites, which have similar geomorphology and sea exchange (Fig. 1). Tidal ranges estimated from tide charts averaged 1.3 ± 0.1 m (McKinney et al., 2001). Watersheds were delineated using 15-min (1:24 000 scale) topographic maps from the United States Geological Survey (USGS).

Information on watershed characteristics was processed using an ARC/INFO software package (ESRI, 2002). The data layer for land use and land cover was developed from 1995 aerial photography (1:24 000 scale) coded to Anderson modified Level 3 to 0.25-ha minimum polygon resolution (Anderson et al., 1976). In previous reports we described natural land types (i.e., % forested, % brush land, and % inland wetland) and human altered land (i.e., % residential, % agricultural, % industrial and commercial, % recreational) in the subwatersheds adjoining the marshes (McKinney et al., 2001; Wigand et al., 2001). Since the percentage of residential lands dominated the land types altered by humans in this urbanized New England region (Wigand et al., 2001), we used this land type for our correlations with DEA and marsh soil characteristics.

Valiela et al. (1997) suggested that in the New England region, a 200-m buffer zone from the upland edge of salt marshes into the adjacent watershed is most likely the area to influence nutrient exchanges and ecosystem structure. In addition, Rhode Island soils data (1:15 840 scale) have also been shown to be useful for identifying possible sinks (e.g., riparian zones) for ground water N in the terrestrial landscape (Rosenblatt et al., 2001). Therefore, we examined land use patterns and the percent hydric soils in the terrestrial lands in a 200-m buffer zone from the salt marsh upland edge to ascertain whether there were significant relationships of land use or hydric soils in the buffer with DEA.

A 200-m zone in the watershed adjacent to the upland edge of each salt marsh was created using a Geographic Information System (GIS). This buffer was overlaid onto soils drainage class data obtained from the Rhode Island soil survey (Rector, 1981). There are 11 soil drainage classes, ranging from excessively to very poorly drained reported at 1:15 840 scale. We aggregated the three "poorly drained" drainage classes (i.e., somewhat poorly drained, poorly drained, and very poorly drained), which are characteristic of inland wetlands and high denitrification (Simmons et al., 1992; Nelson et al., 1995; Mitsch and Gosselink, 2000), into one "hydric soils" class. The percentage of area of each soil drainage class was calculated by dividing the area of each soil drainage class in the 200-m buffer by the total area of the buffer contained within the



● Nag Creek, National Estuarine Research Reserve
Fig. 1. Location of the salt marshes and adjacent watersheds of the sites in the bay-wide survey (see Table 1 for site descriptions), and Nag Creek, which was the site of the Prudence Island fertilization experiment.

watershed. The same method was used to determine the percentage of residential area in the 200-m buffer for the 10 watersheds (Table 1). Nitrogen loads previously calculated for the same Narragansett Bay marshes in a concurrent study examining plant structure at the sites (Wigand et al., 2003) were used for the present study. To calculate the N loads, we used a nitrogen-loading model (NLM) developed and verified for Cape Cod, Massachusetts by Valiela et al. (1997, 2000b). The NLM estimates coastal N loads from atmospheric deposition, fertilizer, and wastewater (e.g., via septic systems, using values for per capita contributions of N) in watersheds by multiplying the surfaces of various land use types (e.g., natural vegetation, agricultural land, turf, residential land, and impervious surfaces) by an appropriate coefficient and subsequently correcting the loads for N losses in various compartments (e.g., vegetation and soils, vadose zone, aquifer). The estimate

Table 1. Watershed characteristics and calculated N loadings for Narragansett Bay salt marsh sites.

Site [†]	Watershed characteristic			Calculated N load		
	Watershed area	Hydric soils (200-m buffer)	Residential (200-m buffer)	Marsh area	Total N load	Marsh area normalized N load
	ha	%		ha	kg N yr ⁻¹	kg N ha ⁻¹ yr ⁻¹
JEN	41	33.4	4.4	11.85	29	2
FOX	62	49.3	0	10.04	103	10
FOG	30	34.7	8.3	4.43	280	63
DON	2 975	29.4	0	29.00	11 593	400
PAS	314	18.1	44.0	4.10	9 917	2 418
BRU	781	23.8	12.3	9.16	22 344	2 440
BIS	2 296	17.1	0	3.85	11 235	2 922
OLD	1 505	22.4	100	9.63	31 587	3 282
WAT	402	22.8	56.7	1.97	11 920	6 037
APP	1 738	0.2	86.6	3.17	32 472	10 253

[†] JEN, Jenny Pond; FOX, Fox Hill Salt Marsh; FOG, Fogland Marsh; DON, Mary Donovan Marsh; PAS, Passeonkquis Cove; BRU, Brush Neck Cove; BIS, Bissel Cove; OLD, Old Mill Creek; WAT, Watchemoket Cove; APP, Apponaug Cove.

of the N load to the marsh was then calculated as the sum of the loads from atmospheric deposition, fertilizer, and wastewater (corrected for losses) and divided by the marsh area in hectares (Table 1). The calculated N loads were examined for significant relationships with DEA and the marsh soil characteristics.

Sewer information for the 10 watersheds was obtained from a GIS data layer, which was compiled in 1995 from available regional and municipal sewer authority maps. According to this information, in three of the 10 Narragansett Bay marsh watersheds studied, less than twenty percent of wastes were sewered. At the time of the study, the balance of residential waste in these three watersheds and all of the waste from the other seven watersheds was primarily treated in domestic septic systems. Rhode Island statewide precipitation data were obtained from the United States National Climate Data Center for the fall and spring in 1998, 1999, and 2000 (National Climatic Data Center, 2004).

Denitrification Enzyme Activity Measurements

For the bay-wide DEA survey, sediment samples from each site were collected at low tide in fall (October, November, December) of 1998 and 2000 and in spring (April, May, June) of 1999 and 2000 for a total of four sampling events at each site. On each sampling date, we collected three replicate cores (4.6-cm diameter) of vegetated soil to a 10-cm depth and about 1 m apart in both the high and low marsh zones. The area sampled in the high marsh zone was characterized by the presence of the dominant plant saltmeadow cordgrass and the low marsh zone by the presence of the tall form of smooth cordgrass. Before sampling the soil with a hand-held hammer corer, the aboveground plant material was removed with clippers in the field. The soil cores were placed in plastic bags, stored in a cooler with ice in the field, and refrigerated at 4°C in the laboratory until processed and analyzed within three days. For the field manipulative experiment, one core per treatment plot was collected as described above.

The top 3 cm of each core was used for the DEA analysis. For each core, two replicates of 6 g of homogenized soil were placed in 70-mL dark serum bottles. The DEA was measured using the acetylene-based anaerobic assay as described by Smith and Tiedje (1979) and Groffman et al. (1996) in which conditions for denitrification are made nonlimiting by the addition of excess nitrate at a rate of 1.4 mg KNO₃ g⁻¹ sediment wet weight and at a rate of 1 mg glucose g⁻¹ sediment wet weight and by removal of oxygen and diffusion limitations in soil slurries. Chloramphenicol was added to the soil samples to inhibit the growth of the bacteria at a rate of 0.25 mg g⁻¹ sediment wet weight. The combined nitrate-glucose-chloramphenicol media was prepared in ultrapure water and 12 mL

was added to each 6 g of soil. Before adding acetylene to the serum bottles, each was stoppered and made anaerobic by a series of evacuations and flushes with N gas. Headspace samples were taken at 30 and 90 min, stored in evacuated glass tubes, and analyzed for N₂O with electron capture gas chromatography (Series II, 5890; Hewlett-Packard, Palo Alto, CA). This short-term anaerobic assay has been employed and described in several other wetland studies (Groffman et al., 1992, 1996; Duncan and Groffman, 1994; Otto et al., 1999).

For determining the characteristics of the marsh sediments, we collected sediment cores from the dominant *Spartina* spp. plant belts in the high and low marsh zones in each marsh in 1998 and 1999. The width of each marsh at the upland edge was approximated by pacing the edge by foot, and three transects were set approximately equidistant from the upland edge beginning at the Jesuit's bark (*Iva frutescens* L.) zone or wooded edge to the cove sea edge. Within each distinct *Spartina* spp. plant belt of at least 1 m in length along each transect, a sediment core 4.6 cm in diameter was taken to estimate the percent moisture, percent organic matter, and bulk density. The top 2 cm of each core was weighed before and after drying at 60°C to calculate the percent moisture and bulk density. The percent organic matter was then calculated by the difference in weight after combustion for 6 h at 450°C. Reported soil data are two-year averages reported on a dry weight basis for the saltmeadow cordgrass sediment in the high marsh and the tall form of smooth cordgrass in the low marsh zone.

In addition, on separate cores from each site, sediment grain size for saltmeadow cordgrass sediment in the high marsh zone and smooth cordgrass sediment in the low marsh zone was determined using a gravimetric method to separate out sand, clay, and silt particles. Three 4.6-cm-diameter cores from the high and low marsh of each site were collected, and the top 3 cm of each core was homogenized for the grain size analysis. Carbonate and other organic fragments of a 5-g homogenized soil sample were dissolved with 1 M acetic acid and subsequently with 30% hydrogen peroxide. The sand fraction was separated from the clay and silt fraction using a 63- μ m sieve, with the clay and silt fraction collected on a preweighed filter with a 1.2- μ m pore size.

A manipulative field experiment was conducted in the high marsh at Nag Creek (41°37' N, 71°19' W) in the National Estuarine Research Reserve on Prudence Island, RI (Fig. 1) from May 2000 to June 2002 to test the effect of nutrient enrichment on DEA. A 2 × 2 factorial design was used, with N and P as the treatments and DEA as the response. The four treatment groups were N and P, P alone, N alone, and a control with no added N or P. For each treatment, there were four replicate 1-m² plots that were semi-randomly located

in vegetated patches at least 3 m apart in the high marsh. When random selection of experimental plot coordinates resulted in a plot located in a bare patch, the plot was moved to the nearest vegetated patch. Dissolved nutrient additions of $\text{Ca}(\text{NO}_3)_2$ and P_2O_5 (i.e., 2 g N m^{-2} and 0.2 g P m^{-2}) were sprinkled with a watering pot on the sediments by moving the plant shoots aside once every two weeks in the spring, summer, and fall; in winter (December, January, and February), the nutrient additions were only applied once per month. The total amount of dissolved N and P for the extent of the DEA experiment was 72 g N m^{-2} and 7.2 g P m^{-2} . The DEA was measured in each plot ($n = 16$) in June 2002 using the methods described above for the bay-wide survey. The DEA results for one N-amended replicate were lost due to mechanical problems with the gas chromatograph during the laboratory analyses.

Statistical Analyses

Pearson correlation analyses were used to examine relationships of DEA with the N loads, percent residential development, percent hydric soils, and marsh sediment characteristics. Paired, two-tailed, student t tests were used to examine spatial (low vs. high marsh zone) and temporal (seasons) differences in mean DEA. The DEA values from the fertilization experiment were transformed using natural logarithms to accommodate homogeneity of variance assumptions before running a two-way ANOVA to examine for the main effects of N or P and for an $\text{N} \times \text{P}$ interaction. The probability for significance is reported at $p \leq 0.05$ for all the statistical analyses.

RESULTS

In the fertilization experiment, the N-amended treatments had significantly (two-way ANOVA, log-transformed data, $p < 0.05$) greater DEA than the controls or the P-amended treatments (Fig. 2). Although there was a significant main effect of N (f value = 8.36, $p < 0.05$), there was no significant effect of P and no significant $\text{N} \times \text{P}$ interaction (Fig. 3). In the bay-wide survey, the fall (1998 and 2000) and spring 1999 DEA in the high marsh significantly ($p \leq 0.05$) increased with increasing N loads (Fig. 4; Table 2). In contrast, the fall 1998 and spring 1999 DEA significantly ($p < 0.05$) decreased with increasing hydric lands in the surrounding terrestrial buffer (Fig. 5; Table 2). In the fall of 2000 and spring of 1999, the significant relationships of DEA with N loads and hydric soils were primarily driven by rates from one marsh (APP site; see Fig. 4b, 5b), and removal of this site from the statistical analyses results in

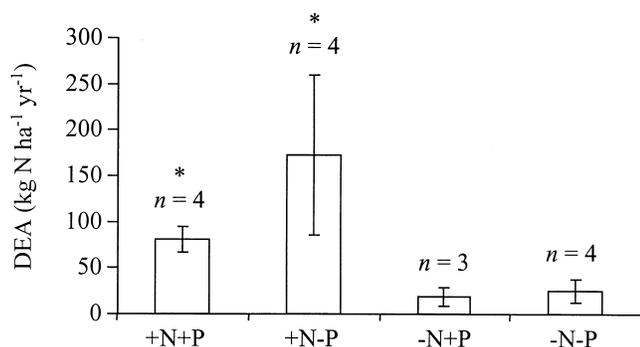


Fig. 2. Denitrification enzyme activity (DEA) values (\pm SE) of the controls and N- and P-amended plots from the Prudence Island fertilization experiment. * Significant at the 0.05 probability level.

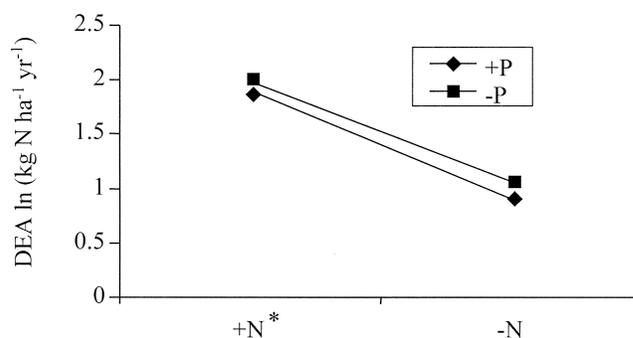


Fig. 3. Response (mean denitrification enzyme activity, log-transformed $\text{kg N ha}^{-1} \text{ yr}^{-1}$) to N and P treatments. There was a significant ($p < 0.05$) main effect of N, but no significant effect of P and no significant $\text{N} \times \text{P}$ interaction. * Significant at the 0.05 probability level.

nonsignificant relationships. The relationships of DEA with N load, hydric lands, and percent residential development were strongest in the fall of 1998 (Table 2), which, of the periods we sampled, was the season with the least precipitation (Table 3). We found no significant correlations of DEA in the low marsh with N load, percent residential development, or percent hydric soils in either the spring or fall. Finally, we found no significant relationships of DEA with percent sand or organic matter for the high or low marsh for either season.

Rhode Island statewide precipitation data from the

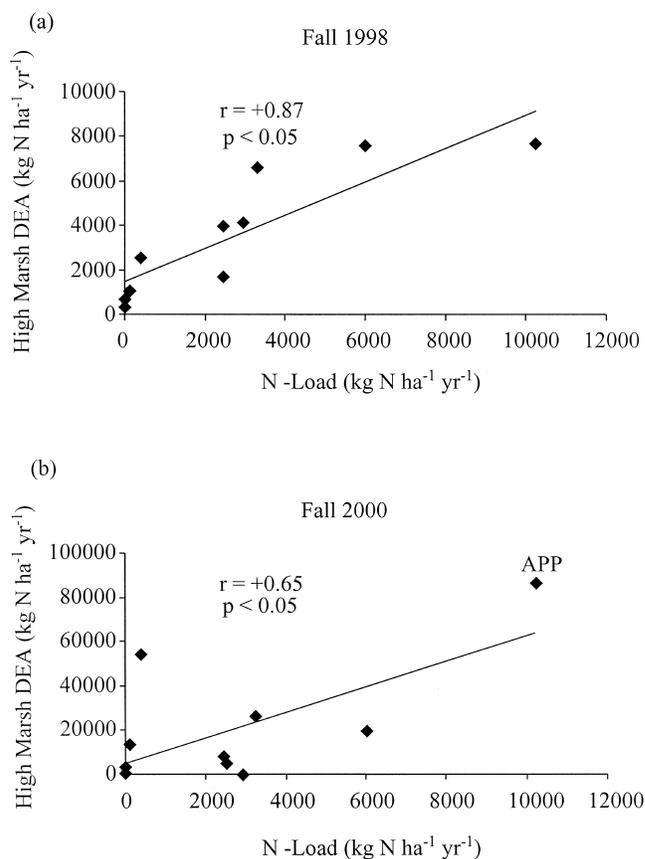


Fig. 4. The relationship from the bay-wide survey between denitrification enzyme activity (DEA) in the high marshes and watershed N loads in fall (a) 1998 and (b) 2000.

Table 2. Relationships of seasonal denitrification enzyme activity (DEA) in the high marsh with N loads, percent residential development, and percent hydric soils in the surrounding watershed.

Time	N load†		% Residential‡		% Hydric soils§	
	<i>r</i>	<i>p</i>	<i>r</i>	<i>p</i>	<i>r</i>	<i>p</i>
Fall 1998	+0.87	0.001	+0.85	0.002	-0.77	0.009
Fall 2000	+0.65	0.04	+0.51	NS¶	-0.57	NS
Average, fall	+0.70	0.02	+0.56	NS	-0.61	NS
Spring 1999	+0.62	0.05	+0.24	NS	-0.64	0.04
Spring 2000	+0.20	NS	+0.05	NS	-0.005	NS
Average, spring	+0.37	NS	+0.11	NS	-0.15	NS

† Estimated total N load from the watershed to the salt marsh measured as kg N ha⁻¹ yr⁻¹. (Values for the estimated N loads are found in Table 1 and details on the model used to estimate the loads are described in the text.)

‡ Percent residential land use in a 200-m buffer zone from the upland marsh edge into the adjacent watershed.

§ Percent hydric soils in a 200-m buffer zone from the upland marsh edge into the adjacent watershed.

¶ Not significant.

United States National Climate Data Center reported seasonal average precipitation for the 10-yr period (1990–2000) as 310.1 mm for the fall and 318.4 mm for the spring (Table 3). The fall and spring precipitation in 2000 was greater than the season averages in fall 1998 and spring 1999 (Table 3). Average DEA for the fall and spring of 2000 were greater than the average DEA in fall 1998 or spring 1999 (Table 3). In the low marsh, the fall DEA was significantly ($p = 0.05$) greater for

2000 than 1998. There were trends ($p = 0.07$), albeit not statistically significant, of greater DEA in the high marsh in fall 2000 compared with fall 1998 and of greater DEA in the low marsh in spring 2000 compared with spring 1999. There was no significant difference of average DEA between seasons or between high and low marsh.

The bay-wide average values for the percent sand, percent moisture, percent organic matter, and bulk density for the high and low marsh sediments are reported in Table 4. At sites where there were high N loads, high percent residential development, and low percent hydric soils in the terrestrial buffer, the salt marsh sediments had high percent sand (Table 5). We did not detect any significant relationships between percent moisture, percent organic matter, or bulk density with the N loads associated with the 10 sites (Table 5). However, for the low marsh only, the percent moisture and bulk density did correlate significantly ($p < 0.05$) with the percent residential development in the 200-m buffer (Table 5).

DISCUSSION

The high DEA that was measured in the 10 coastal fringe salt marshes supports the hypothesis that these habitats are important natural buffers between the land and the sea and may provide the important service of intercepting land-derived N and transforming it into N gas. The findings that the DEA in the high marsh increased with watershed N loadings, and likewise, that DEA increased in the N-amended treatments in the fertilization experiment, suggests that marsh denitrification could represent an important sink that regulates N loading to the estuary. Although the low marsh DEA did not correlate with the watershed N loads, the denitrification potential of these sediments is high. The denitrification activity in the low marsh might better correlate with the nutrient load exchanged from the sea with the rise and fall of the tides, which was not measured in this study. The DEA values of the coastal salt marshes that we studied were similar to the DEA values reported for tidally influenced, freshwater marshes in the Hudson River (New York) (Otto et al., 1999; Findlay et al., 2002).

The areal extent and location of salt marshes are important when evaluating their potential to transform anthropogenic N loads in a particular watershed. In this study, marshes with lower N loads (less than 500 kg N ha⁻¹ yr⁻¹) had the highest marsh area for N exchange, which might allow for a high percentage or complete processing of anthropogenic N loads. On the other hand, the greatest DEA values were associated with marshes having the highest land-based N inputs, but reduced areal extent. Generally, the potential denitrification activity in the high marsh (fall and spring averages: 12 218 and 5732 kg N ha⁻¹ yr⁻¹, respectively) was similar or greater than the estimated N loading to the marshes (average load: 2783 kg N ha⁻¹ yr⁻¹). The potential denitrification activity of the low marsh was often greater than the high marsh, and therefore, the low marsh could be an additional buffer preventing land-derived N from entering coastal waters. However, it is important to note

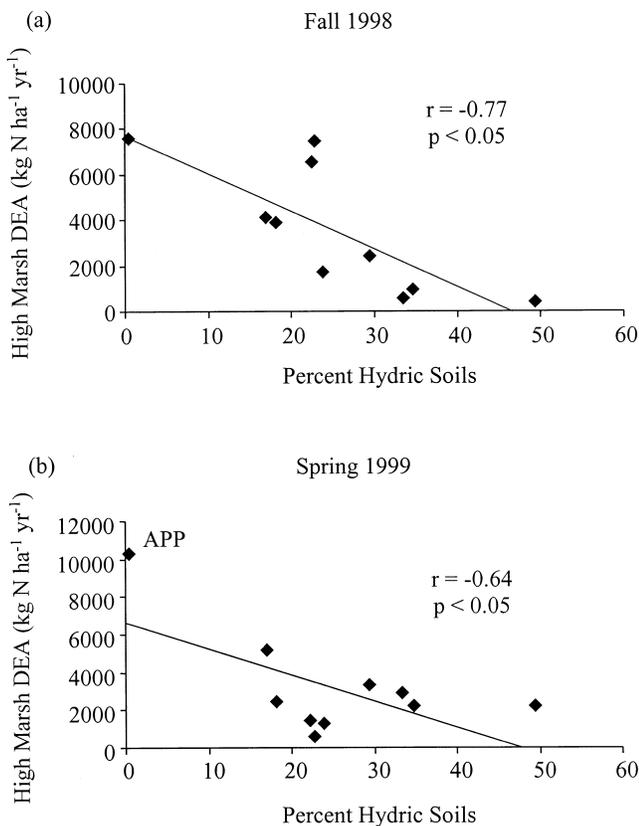


Fig. 5. The relationship from the bay-wide survey between denitrification enzyme activity (DEA) in the high marshes and percent hydric soils in an adjacent terrestrial buffer in (a) fall 1998 and (b) spring 1999.

Table 3. Rhode Island statewide seasonal precipitation and high and low marsh denitrification enzyme activity (DEA) among the 10 marshes.

Time	Rhode Island statewide precipitation	High marsh DEA	SE	Low marsh DEA	SE
	mm	kg N ha ⁻¹ yr ⁻¹			
Fall 1998	219.3	3 592	892	11 951	9 382
Fall 2000	255.3	20 844	8 986	55 674	20 824
Average†, fall	310.1	12 218	4 748	33 812	12 868
Spring 1999	273.5	3 186	881	4 216	1 689
Spring 2000	390.3	8 278	3 993	47 197	22 047
Average†, spring	318.4	5 732	1 850	25 706	11 682

† Rhode Island 10-yr (1990–2000) statewide seasonal average precipitation.

that DEA is frequently one to two orders of magnitude higher than actual measured denitrification rates in tidal and nontidal wetlands (Seitzinger, 1988; Otto et al., 1999; Lowrance et al., 1995; Valiela et al., 2000a). Therefore, the DEA represents a denitrification potential that a marsh system might provide under optimal environmental conditions. Ultimately, the ability of marsh systems to process land-based N loads will depend on marsh area, plant productivity, availability of labile organic matter, hydrologic flowpath, and a suitable geochemical environment (e.g., oxidation–reduction status of the sediments) to facilitate biological processes and transformations.

Furthermore, other microbial processes such as N fixation (the conversion of N gas to organic N) could act as an N source in a salt marsh and offset the benefit of the denitrification process acting as an N sink (Kaplan et al., 1979; Valiela and Teal, 1979; Currin et al., 1996). For example, in some disturbed salt marshes, loss of emergent plant cover could result in bare spots with extensive cyanobacterial mats capable of high rates of N fixation. At an impacted Narragansett Bay salt marsh with low macrophyte cover, sandy sediment, and high N loads, N fixation rates exceeded actual denitrification rates (J. Davis, personal communication, 2002). In some newly created salt marshes with low emergent macrophyte cover and coarse sediments in North Carolina, Currin et al. (1996) measured N fixation rates that were three orders of magnitude greater than denitrification rates. Therefore, researchers and managers need to assess the net N flux from these fringe salt marshes to determine the N buffering capacity of these habitats.

Microbes may be limited by P in some coastal ecosystems, and in a South Carolina salt marsh study, P additions resulted in lower potential denitrification rates (Sundareshwar et al., 2003). Phosphorus fertilization appears to enhance heterotrophic activity and carbon mineralization at the expense of denitrifiers in some salt marshes (Sundareshwar et al., 2003). Our results do not show a significant P effect on the denitrification enzyme activity in the high marsh on Prudence Island, RI, but the DEA results were highly variable within each treatment. Therefore, it is possible that P limitation may alter N cycling in some New England salt marshes, as was reported in South Carolina.

In earlier studies at these same Narragansett Bay coastal salt marshes we demonstrated that the stable N isotope ratio of the marsh biota [i.e., ribbed mussels (*Geukensia demissa*); smooth cordgrass] increased with

increasing watershed N loadings and percent residential land development, suggesting that anthropogenic, land-based N was affecting marsh processes (McKinney et al., 2001; Wigand et al., 2001). Furthermore, at the same sites where DEA and the N loads were greatest, we observed a decrease in high marsh plant species richness and the extent of saltmeadow cordgrass (Wigand et al., 2003). These concomitant changes in the plant structure may have a negative effect on the ability of the salt marsh to process N. The roots of saltmeadow cordgrass form a tightly woven turf matrix that binds the soil and helps resist erosional processes such as those that occur during storm events. For example, the loss of saltmeadow cordgrass might make the marsh more susceptible to scouring action by ice floes. As a result, the marsh would erode and the area available to process N would be reduced. The loss of some salt marsh plants (e.g., smooth cordgrass) would also reduce the oxic–anoxic interfaces in the rhizosphere that occur from the channeling of oxygen from the leaves to the roots (Teal and Kanwisher, 1966). These oxic–anoxic interfaces are important for the coupling of nitrification and denitrification processes in marsh sediments (Mitsch and Gosselink, 2000). In addition, water uptake by *Spartina* spp. roots results in increased air entry into the sediment and increased sediment oxidation, which could also promote the coupling of the nitrification and denitrification processes (Dacey and Howes, 1984). Finally, a reduction in the root density and the organic exudates that roots release could limit the availability of labile organic carbon for the denitrification process (Sherr and Payne, 1978).

Salt marshes with a high percentage of lands with hydric soils in the 200-m buffer zone surrounding the sites had lower DEA than salt marshes with a lower percentage of hydric soils in their adjacent watersheds. Poorly drained soils in landscapes are often associated with inland wetlands, nitrate sinks, and high denitrification rates (e.g., Groffman and Tiedje, 1989; Mitsch and Gosselink, 2000; Rosenblatt et al., 2001), which can filter

Table 4. Salt marsh sediment characteristics averaged across 10 Narragansett Bay sites.†

Sediment characteristic	High marsh	Low marsh
Sand, %	60.8 ± 5.9	54.1 ± 9.0
Moisture, %	60.4 ± 6.6	63.8 ± 2.8
Organic matter, %	29.7 ± 5.8	20.8 ± 2.2
Bulk density, g cm ⁻³	0.56 ± 0.11	0.44 ± 5.1

† Values are means ± standard errors.

Table 5. Relationships of marsh soil characteristics with N loads, percent residential development, and percent hydric soils among the 10 marsh sites.

Relationship	N load [†]		% Residential [‡]		% Hydric soils [§]	
	r	p	r	p	r	p
High marsh, % sand	+0.73	0.02	+0.34	NS	-0.64	<0.05
Low marsh, % sand	+0.81	0.005	+0.70	0.02	-0.66	0.04
High marsh, % moisture	-0.26	NS	-0.12	NS	+0.43	NS
Low marsh, % moisture	-0.52	NS	-0.71	0.02	+0.60	NS
High marsh, % organic matter	-0.16	NS	-0.12	NS	+0.41	NS
Low marsh, % organic matter	-0.29	NS	-0.48	NS	+0.53	NS
High marsh, bulk density	+0.21	NS	+0.05	NS	-0.40	NS
Low marsh, bulk density	+0.45	NS	+0.70	0.04	-0.47	NS

[†] Estimated total N load from the watershed to the salt marsh measured as kg N ha⁻¹ yr⁻¹. (Values for the estimated N loads are found in Table 1 and details on the model used to estimate the loads are described in the text.)

[‡] Percent residential land use in a 200-m buffer zone from the upland marsh edge into the adjacent watershed.

[§] Percent hydric soils in a 200-m buffer zone from the upland marsh edge into the adjacent watershed.

^{||} Not significant.

out N that might enter the adjacent coastal salt marshes. Given that DEA increases with N loading, it is a reasonable finding that watersheds with high amounts of hydric soils have lower loadings and lower marsh DEA.

The denitrification process and the resulting improvement in water quality provided by salt marshes could be in decline because of the degradation and loss of salt marshes due to ditching, in-filling, and land development (Nixon, 1982; Roman et al., 2000), as well as N overenrichment (Bertness and Pennings, 2000; Wigand et al., 2003). Denitrification enzyme activity appears to be a useful index for comparing the relative N exposure and the potential denitrification of marshes with similar geomorphology and hydrology but varying anthropogenic N loads. Because of the pulsed nutrient regime and seasonal variability associated with salt marshes some microbial N transformations may be more important at some times of the year than others (Odum et al., 1995). Describing the denitrification enzyme activity of coastal salt marshes is only a first step toward understanding the importance of these habitats as critical filters of land-derived N. Measurements of actual denitrification and N fixation rates under various nutrient-loading regimes and for a variety of temporal settings (e.g., seasonal, climatic) are necessary to evaluate the water quality improvement provided by coastal salt marshes.

ACKNOWLEDGMENTS

The authors thank Sibylle Otto, Alan Loreface, and Kelly Addy for providing advice on DEA methods. Dr. Art Gold and Dr. Barbara Nowicki provided thoughtful discussions on nitrogen cycling during the study. Kenny Raposa and Roger Green facilitated the implementation of the manipulative field experiment on the National Estuarine Research Reserve on Prudence Island, RI. Beth Hinchey, Earl Davey, and Jim Latimer provided insightful comments on an early draft of the manuscript. Thanks to Jim Heltshe for his assistance with the statistical analyses. Mention of trade names or commercial products does not constitute endorsement or recommendation for use by the USEPA. This report, contribution no. AED-03-035, has been technically reviewed by the USEPA's Office of Research and Development, National Health and Environmental Effects Research Laboratory, Atlantic Ecology Division, Narragansett, RI, and approved for publication. Approval does not signify that the contents necessarily reflect the views and policies of the Agency.

REFERENCES

- Anderson, J.R., E.E. Hardy, and J.T. Roach. 1976. A land use and land cover classification system for use with remote sensor data. USGS Professional Paper 964. A revision of the land use classification system as presented in USGS Circ. 671. U.S. Gov. Print. Office, Washington, DC. Also available online at <http://www.ncrs.fs.fed.us/4153/deltawest/landcover/LLCoverPub.html> (verified 29 Jan. 2004).
- Bertness, M.D., and A.M. Ellison. 1987. Determinants of pattern in a New England salt marsh plant community. *Ecol. Monogr.* 57: 129-147.
- Bertness, M.D., and S.C. Pennings. 2000. Spatial variation in process and pattern in salt marsh plant communities in eastern North America. p. 39-58. In M.P. Weinstein and D.A. Kreeger (ed.) *Concepts and controversies in tidal marsh ecology*. Kluwer Academic Publ., Dordrecht, the Netherlands.
- Brar, S. 1972. Influence of roots on denitrification. *Plant Soil* 36:713-715.
- Currin, C.A., S.B. Joye, and H.W. Paerl. 1996. Diel rates of N₂ fixation and denitrification in a transplanted *Spartina alterniflora* marsh: Implications for N-flux dynamics. *Estuarine Coastal Shelf Sci.* 42: 597-616.
- Dacey, J.W.H., and B.L. Howes. 1984. Water uptake by roots controls water table movement and sediment oxidation in short *Spartina* marsh. *Science* (Washington, DC) 224:487-489.
- Duncan, S.P., and P.M. Groffman. 1994. Comparing microbial parameters in natural and artificial wetlands. *J. Environ. Qual.* 23:298-305.
- ESRI. 2002. ARC/INFO software package. ESRI, Redlands, CA.
- Findlay, S.E.G., E. Kiviat, W.C. Nieder, and E.A. Blair. 2002. Functional assessment of a reference wetland set as a tool for science, management and restoration. *Aquat. Sci.* 64:107-117.
- Groffman, P.M. 1987. Nitrification and denitrification in soil: A comparison of enzyme assay, incubation and enumeration methods. *Plant Soil* 97:445-450.
- Groffman, P.M. 1994. Denitrification in freshwater wetlands. *Curr. Top. Wetland Biogeochem.* 1:15-35.
- Groffman, P.M., A.J. Gold, and R.C. Simmons. 1992. Nitrate dynamics in riparian forests: Microbial studies. *J. Environ. Qual.* 21:666-671.
- Groffman, P.M., G.C. Hanson, E. Kiviat, and G. Stevens. 1996. Variation in microbial parameters in four different wetland types. *Soil Sci. Soc. Am. J.* 60:622-629.
- Groffman, P.M., and J.M. Tiedje. 1989. Denitrification in north temperate forest soils: Relationships between denitrification and environmental factors at the landscape scale. *Soil Biol. Biochem.* 21: 621-626.
- Kaplan, W., I. Valiela, and J.M. Teal. 1979. Denitrification in a salt marsh ecosystem. *Limnol. Oceanogr.* 24:726-734.
- Lowrance, R., G. Vellidis, and R.K. Hubbard. 1995. Denitrification in a restored riparian forest wetland. *J. Environ. Qual.* 24:808-815.
- McKinney, R.A., W.S. Nelson, M. Charpentier, and C. Wigand. 2001. Ribbed mussel nitrogen isotope signatures reflect nitrogen sources in coastal salt marshes. *Ecol. Appl.* 11:203-214.
- Mitsch, W.J., and J.G. Gosselink. 2000. *Wetlands*. 3rd ed. John Wiley & Sons, New York.
- National Climatic Data Center. 2004. Rhode Island climate summary,

- December 2003 [Online]. Available at www.ncdc.noaa.gov/oa/climate/research/cag3/RI.html (verified 29 Jan. 2004). NCDC, Asheville, NC.
- Nelson, W.M., A.J. Gold, and P.M. Groffman. 1995. Spatial and temporal variation in groundwater nitrate removal in a riparian forest. *J. Environ. Qual.* 24:691–699.
- Nixon, S.W. 1982. The ecology of New England high salt marshes: A community profile. FFWS/OBS-81/55. U.S. Fish and Wildlife Serv., Washington, DC.
- Nowicki, B.L., E. Requentina, D. Van Keuren, and J. Portnoy. 1999. The role of sediment denitrification in reducing groundwater-derived nitrate inputs to Nauset Marsh Estuary, Cape Cod, Massachusetts. *Estuaries* 22:245–259.
- Odum, W.E., E.P. Odum, and H.T. Odum. 1995. Nature's pulsing paradigm. *Estuaries* 18:547–555.
- Otto, S., P.M. Groffman, S.E.G. Findlay, and A.E. Arreola. 1999. Invasive plant species and microbial processes in a tidal freshwater marsh. *J. Environ. Qual.* 28:1252–1257.
- Rector, D.D. 1981. Soil survey of Rhode Island. USDA Soil Conserv. Serv., West Warwick, RI.
- Roman, C.T., N. Jaworski, F.T. Short, S. Findlay, and S. Warren. 2000. Estuaries of the northeastern United States: Habitat and land use signatures. *Estuaries* 23:743–764.
- Rosenblatt, A.E., A.J. Gold, M.H. Stolt, P.M. Groffman, and D.Q. Kellog. 2001. Identifying riparian sinks for watershed nitrate using soil surveys. *J. Environ. Qual.* 30:1596–1604.
- Seitzinger, S.P. 1988. Denitrification in freshwater and coastal marine ecosystems: Ecological and geochemical significance. *Limnol. Oceanogr.* 33:702–724.
- Sherr, B.F., and W.J. Payne. 1978. Effect of the *Spartina alterniflora* root-rhizome system on salt marsh soil denitrifying bacteria. *Appl. Environ. Microbiol.* 35:724–729.
- Simmons, R.C., A.J. Gold, and P.M. Groffman. 1992. Nitrate dynamics in riparian forests: Groundwater studies. *J. Environ. Qual.* 21:659–665.
- Smith, M.S., and J.M. Tiedje. 1979. Phases of denitrification following oxygen depletion in soil. *Soil Biol. Biochem.* 11:262–267.
- Stefanson, R.C. 1972. Soil denitrification in sealed soil-plant systems. I. Effect of plants, soil water content and soil organic matter content. *Plant Soil* 33:113–127.
- Sundareshwar, P.V., J.T. Morris, E.K. Koepfler, and B. Fornwalt. 2003. Phosphorus limitation of coastal ecosystem processes. *Science* (Washington, DC) 299:563–565.
- Teal, J.M., and J.W. Kanwisher. 1966. Gas transport in the marsh grass, *Spartina alterniflora*. *J. Exp. Bot.* 17:355–361.
- Valiela, I., M.L. Cole, J. McClelland, J. Hauxwell, J. Cebrian, and S.B. Joye. 2000. Role of salt marshes as part of coastal landscapes. p. 23–38. *In* M.P. Weinstein and D.A. Kreeger (ed.) *Concepts and controversies in tidal marsh ecology*. Kluwer Academic Publ., Dordrecht, the Netherlands.
- Valiela, I., M. Geist, J. McClelland, and G. Tomasky. 2000b. Nitrogen loading from watersheds to estuaries: Verification of the Waquoit Bay Nitrogen Loading Model. *Biogeochemistry* 49:277–293.
- Valiela, I., G. Collins, J. Kremer, K. Lajtha, M. Geist, B. Seely, J. Brawley, and C.H. Sham. 1997. Nitrogen loading from coastal watersheds to receiving estuaries: New method and application. *Ecol. Appl.* 7:358–380.
- Valiela, I., and J.M. Teal. 1974. Nutrient limitation in salt marsh vegetation. p. 563–574. *In* R.J. Reimold and W.H. Queen (ed.) *Ecology of halophytes*. Academic Press, New York.
- Valiela, I., and J.M. Teal. 1979. The nitrogen budget of a salt marsh ecosystem. *Nature* (London) 280:652–656.
- Wigand, C., R. Comeleo, R. McKinney, G. Thursby, M. Chintala, and M. Charpentier. 2001. Outline of a new approach to evaluate ecological integrity of salt marshes. *Hum. Ecol. Risk Assess.* 7:1541–1554.
- Wigand, C., R. McKinney, M. Chintala, M. Charpentier, and G. Thursby. 2003. Relationships of nitrogen loadings, residential development, and physical characteristics with plant structure in New England salt marshes. *Estuaries* 26:1494–1504.
- Woldendorp, J.W. 1963. The influence of living plants on denitrification. *Meded. Landbouwhoges. Wageningen* 63:1–100.