

Habitat-specific distinctions in estuarine denitrification affect both ecosystem function and services

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Abstract. Resource limitation controls the base of food webs in many aquatic ecosystems. In coastal ecosystems, nitrogen (N) has been found to be the predominant limiting factor for primary producers. Due to the important role nitrogen plays in determining ecosystem function, understanding the processes that modulate its availability is critical. Shallow-water estuarine systems are highly heterogeneous. In temperate estuaries, multiple habitat types can exist in close proximity to one another, their distribution controlled primarily by physical energy, tidal elevation and geomorphology. Distinctions between these habitats such as rates of primary productivity and sediment characteristics likely affect material processing. We used membrane inlet mass spectrometry to measure changes in N₂ flux (referred to here as denitrification) in multiple shallow-water estuarine habitats through an annual cycle. We found significantly higher rates of denitrification (DNF) in structured habitats such as submerged aquatic vegetation, salt marshes and oyster reefs than in intertidal and subtidal flats. Seasonal patterns were also observed, with higher DNF rates occurring in the warmer seasons. Additionally, there was an interaction between habitat type and season that we attributed to the seasonal patterns of enhanced productivity in individual habitat types. There was a strong correlation between denitrification and sediment oxygen demand (SOD) in all habitats and all seasons, suggesting the potential to utilize SOD to predict DNF. Denitrification efficiency was also higher in the structured habitats than in the flats. Nitrogen removal by these habitats was found to be an important contributor to estuarine ecosystem function. The ecosystem service of DNF in each habitat was evaluated in US dollars using rates from a regional nutrient-offset market to determine the cost to replace N through management efforts. Habitat-specific values of N removal ranged from approximately three thousand U.S. dollars per acre per year in the submerged aquatic vegetation to approximately four hundred U.S. dollars per acre per year in the subtidal flat. Because of the link between habitat type and processes such as DNF, changes in habitat area and distribution will have consequences for both ecosystem function and the delivery of ecosystem services.

Key words: denitrification; ecosystem services; estuary; evaluation; intertidal flat; oyster reef; saltmarsh; submerged aquatic vegetation; subtidal flat.

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INTRODUCTION

Nitrogen (N) limits primary production in most coastal waters (Paerl 1997). Humans have dramatically modified the global N cycle (Vitousek et al. 1997), including significantly increasing

the N load to coastal systems (Howarth et al. 1996, Nixon et al. 1996, Carpenter et al. 1998, Howarth and Paerl 2008). Excessive N loading has led to eutrophication in coastal systems throughout the world (Nixon 1995). Negative impacts linked to excessive N delivery to coastal

regions include, but are not limited to, harmful algal blooms (Paerl 1997), shifts in primary producer communities (Hauxwell et al. 2001) and increased hypoxia (Rabalais et al. 2002). Coastal managers seek to temper the effects of N loading to coastal waters through source reductions and increasing N removal capacity. Estuarine habitat restoration has been offered as a potential mechanism to increase N sinks (Byers et al. 2006, Brush 2009).

Nitrogen delivery to coastal waters is modulated by on-land storage of N as well as processes that retain and remove N from aquatic systems. Few mechanisms exist through which N can be removed. Denitrification transforms biologically available N and releases it to the atmosphere as either N₂ or N₂O and has been identified as an important removal mechanism for N being transported to coastal waters (Seitzinger et al. 2006) and N in estuaries (Seitzinger 1987, Seitzinger 1988). Other removal mechanisms include burial in sediments and removal as N₂ through alternate pathways such as anaerobic ammonium oxidation (ANAMOX). Despite its importance to ecosystem function, gaps remain in our understanding of the spatial and temporal controls on rates of DNF and must continue to be filled to facilitate effective management of watersheds and coastal waters. Significant strides have been made in modeling DNF (Fennel et al. 2009) using process-based indicators such as sediment oxygen demand. Despite these advances, the heterogeneous and dynamic nature of estuaries continues to pose challenges to modeling efforts (Valiela and Bowen 2002, Seitzinger 2008). Studies that compare and contrast N cycling among estuarine habitats enhance ongoing efforts to model N dynamics and manage N loading in estuaries.

Shallow estuarine habitats provide disproportionately large contributions to ecosystem function that have been identified as providing ecosystem services (Peterson and Lipcius 2003). Estuarine habitats such as salt marshes provide refuge and foraging habitat for ecologically important, economically valuable finfish and mobile invertebrates; stabilize the shoreline; and process nutrients (Millennium Ecosystem Assessment 2005). They are subject to significant natural and anthropogenic stressors that have the potential to affect both their function and

delivery of ecosystem services (Vitousek et al. 1997, Valiela et al. 2000, Bertness et al. 2002, Laursen et al. 2002, Seitzinger 2008).

Habitats in temperate estuarine intertidal and shallow subtidal areas include structured habitats such as marshes, submerged aquatic vegetation and oyster reefs. Other common shallow-water estuarine habitats such as sand and mud flats have less physical structure. Subtle changes in shallow-water estuarine geomorphology create a complex landscape mosaic with multiple habitat types in a narrow elevation range (Fagherazzi et al. 2006). Physical and biological characteristics of these habitats vary and have been demonstrated to affect sediment properties and biogeochemical cycling (Gutierrez and Jones 2006, Eyre and Maher 2010, Eyre et al. 2010b).

Nutrient processing receives significant attention from scientists and managers because of the influence it has on water quality and the availability of essential elements for the base of the food web. Nutrient processing has been studied in individual estuarine habitats such as salt marshes (Kaplan et al. 1977, Anderson et al. 1997, Merrill and Cornwell 2000, Valiela et al. 2000, Davis et al. 2004, Hamersley and Howes 2005), submerged aquatic vegetation (Caffrey and Kemp 1990, Shieh and Yang 1997, Risgaard-Petersen and Ottosen 2000, Welsh et al. 2001), and mud and sand flats (Koch et al. 1992, Hamersley and Howes 2003, Patel 2008). Few studies have attempted to simultaneously examine the rates of N processing in the full suite of habitats present in shallow estuarine ecosystems (Eyre and Ferguson 2002, Ferguson et al. 2007, Eyre et al. 2010a) and none that we are aware of have comprehensively addressed representative habitats from temperate estuaries. Contemporaneous quantification of N transformations in individual habitats is critical to informing decisions regarding restoration and protection of estuarine habitats.

This study focused on five of the major habitat types found in temperate estuaries including salt marsh, submerged aquatic vegetation, oyster reef, intertidal flat and subtidal flat. Quantifying the ecosystem services from these habitats is essential for effective decision-making in rapidly changing coastal regions. Based on past work in individual estuarine habitats, we hypothesized that habitats which modify sediment organic

matter pools through enhanced primary productivity and organic matter repackaging would have higher rates of DNF and DNF efficiency. The purpose of this study was to compare rates of DNF in representative estuarine habitats, identify factors that affect habitat-specific rates of DNF and quantify N removal by these habitats as an ecosystem service. Additionally, we assessed potential predictive factors for DNF including sediment organic matter and sediment oxygen demand.

MATERIALS AND METHODS

Site description

Our study sites were on the southern shoreline of Bogue Sound on the central coast of North Carolina, USA. Bogue Sound has semi-diurnal tides of approximately 0.7 meters. Despite significant human development pressure, the sound has maintained good water quality, including low concentrations of dissolved inorganic nutrients and water-column chlorophyll *a* (O'Connor et al. 2009). Intertidal and shallow-subtidal habitats include fringing marsh, submerged aquatic vegetation (SAV), oyster reef, soft-bottom intertidal flat and subtidal flat.

Sediment N fluxes and N transformations

Continuous flow experiments with intact sediment cores were used to determine the fluxes of nutrients and dissolved gases (Lavrentyev et al. 2000, Gardner et al. 2006, McCarthy et al. 2007). Three intact sediment cores (6.4 cm diameter and approx 17 cm high) and overlying water (~400 ml per core) were collected by hand from each habitat two hours prior to low tide in February, May, July, and October of 2007. Due to the physical structure of the oyster reef, oyster reef sediment cores were collected immediately adjacent to the reef or in interstitial sediments. Cores included only sediment; however, roots and rhizomes of emergent vegetation were often contained within the cores. Additionally, 30 l of sound water was collected as a reservoir for the continuous flow incubations.

Sediment cores and reservoir water were transported to The University of North Carolina Institute of Marine Sciences in Morehead City, NC. The continuous flow system was incubated in an environmental chamber (Bally Inc.) at in

situ temperatures under dark conditions. Each core was capped with a plexiglass top equipped with two O-rings to maintain an air- and water-tight seal. Each cap contained two ports plumbed with Tygon tubing, one for inflow and one for outflow to create a well-mixed water column within the continuous flow chamber. Water column volume was maintained at approximately 400ml. Inflow water from the reservoir was aerated and unfiltered water was passed over cores at a flow rate of 1ml per minute (Lavrentyev et al. 2000).

Cores were pre-incubated for a period of no less than 18 hours prior to sampling to allow the system to reach equilibrium (Eyre et al. 2002). Following pre-incubation, 5 ml samples were collected from the inflow and outflow of each core at 18, 24, 36 and 48-hour increments, to ensure that steady-state conditions were present for analysis of dissolved gases by membrane inlet mass spectrometry (MIMS). MIMS was used to measure concentration of dissolved gasses (N₂, O₂ and Ar) in water (Kana et al. 1994, Kana et al. 1998, An et al. 2001). Additionally, 50 ml water samples were collected for nutrient analysis from the reservoir water and each core. Water was filtered through Whatman GF/F filters (25 mm diameter, 0.7 μm nominal pore size) and the filtrate was analyzed with a Lachat Quick-Chem 8000 automated ion analyzer for NO₃, NH₄. Detection limit for nitrate was 0.04 for NO₃ and 0.18 for NH₄. Following each continuous-flow experiment, sediment samples were taken from each core for organic matter percentages determined by loss on ignition (Byers et al. 1978).

Since there were multiple sediment cores per sample site, the inflow concentration of water entering the core was measured from the reservoir water, which bypassed the cores and flowed directly into the sample vials. Measuring inflow concentrations from the bypass line also accounted for any changes in water chemistry resulting from pump or tubing effects.

Methodological constraints

We used continuous-flow core incubations and analyzed concentrations of dissolved gasses (N₂ and O₂) in water using MIMS. This method is state-of-the-art but, like all DNF methods, it is not without compromises, including the uncertainty of extrapolating from cores to a larger

system and the potential for container effects. Two specific issues related to our methods are the fact that the cores are constantly submerged and that incubations are conducted in the dark. Conducting flux experiments from intertidal sediment in which the tides are not replicated likely results in an under-estimation of sediment denitrification by excluding low-tide DNF. Unfortunately, the ability to simulate tides in MIMS experiments remains a challenge. Other investigators have conducted experiments in both the light and dark and analyzed the samples using a MIMS (Ferguson et al. 2007). In this study we were unable to conduct these experiments without the formation of gas bubbles, which significantly and selectively affect gas concentrations in water (Reeburgh 1969). Light incubations would have been valuable to examine the interactions of benthic microalgae and N cycling. However, our DNF data were extrapolated based on 12 hour days to reflect our assumption of very low rates during the day due to both competition with benthic microalgae for N and increased oxygen concentrations (Tobias 2007, Hochard et al. 2010).

Calculations

Flux calculations were based on the assumption of steady-state gradients that match in situ gradients and a homogenous water column. Benthic fluxes were calculated using the equation $(C_{\text{out}} - C_{\text{in}}) \times F/A$, where C represents the concentration of any analyte, C_{in} and C_{out} are the outflow and inflow concentration (μM), respectively, F is the peristaltic pump flow rate (11 hr^{-1}), and A is the surface area of the core (m^2) (Miller-Way and Twilley 1996). Net DNF was calculated as the flux of N_2 and sediment oxygen demand (SOD) was calculated as the flux of O_2 (Kana et al. 1994, Smith et al. 2006). Individual measurements from each core over time were averaged to yield core-specific values. Fluxes per habitat were calculated as the mean of the core-specific values from three replicate cores. Errors presented here include the standard error between the triplicate cores. Denitrification efficiency was calculated as the proportion of $\text{N}_2\text{-N}$ flux into the water compared to the total dissolved inorganic nitrogen (DIN) flux into the water column using the formula: $[\text{N-N}_2 \text{ flux} / (\text{N-DIN Flux} + \text{N-N}_2 \text{ Flux}) \times 100]$ (Seitzinger 1987, Eyre and Ferguson 2002).

Statistical analysis

Mean values of DNF were compared using one- and two-way analysis of variance (ANOVA) and post-hoc Student Newman-Keul tests (SigmaStat 3.0, SPSS, 2003). Linear regressions were used to assess the relationship between SOD and DNF (Fennel et al. 2009). Analysis of covariance (ANCOVA) was used to compare the slopes of the lines describing SOD and DNF relationships within seasons and among different habitats (Matlab 7.4, Mathworks 2007).

Economic evaluation

The dollar value of N removal via DNF was estimated using the rates from the North Carolina nutrient offset program. The North Carolina nutrient offset payment value was a regionally derived number that had significant stakeholder input in its determination. The current trading price of the North Carolina Nutrient Offset Credit Program is \$13 per kilogram of N removed (15A NCAC 02B .0240). To best estimate the annual value of habitat specific N removal, mean annual rates of DNF and the standard error of these means were multiplied by \$13. North Carolina's program is similar to many others; the Chesapeake Bay in particular has offset programs with rates that are comparable to North Carolina's. Breetz et al. (2004) described approximately 70 programs using nutrient trading or offsets.

RESULTS

All habitats were located in full to near-full salinity waters (27–36, Table 1), with generally low levels of water column DIN (Table 1). Temperature of the overlying water varied from 11.32°C in the winter to 29.45°C in the summer (Table 1). Sediment organic matter was generally quite low in all habitats ($<2\%$); most of the values higher than 2% organic matter were in the marsh (Table 2).

Both habitat type and season affected the rate of DNF (ANOVA, $p < 0.05$) (Fig. 1). There was also a significant interaction between habitat type and season (Fig. 1). Post hoc tests revealed that summer rates were the highest, followed by spring and fall, with lowest rates in the winter (Student-Newman Keuls, $p < 0.05$, Fig. 1). Annual mean DNF rates were higher in struc-

Table 1. Physical and chemical characteristics of the overlying water during each of the sampling events.

Date	Temp (°C)	Sal.	NO _x (μmol N l ⁻¹)	NH ₄ ⁺ (μmol N l ⁻¹)
Feb. 2007	11.32	27.34	0.15	1.58
May 2007	14.95	30.16	0.0	1.87
July 2007	29.45	36.36	0.49	0.76
Oct. 2007	24.02	32.54	0.32	0.98
2007 Average	19.93	31.60	0.24	1.13

Table 2. Physical and chemical characteristics of five shallow water estuarine habitats. Positive values indicate flux out of the sediment and negative values indicates flux into the sediment (n = 3).

Month	Site	Sediment Organic Matter (%)	DIN Flux (μmol N m ⁻² hr ⁻¹)
Feb.	Marsh	6.65 ± 0.58	16.47 ± 5.68
	Oyster reef	0.92 ± 0.42	-7.70 ± 1.58
	SAV	0.47 ± 0.20	2.31 ± 3.00
	Intertidal Flat	0.37 ± 0.03	-4.37 ± 2.44
May	Subtidal Flat	0.45 ± 0.21	-5.65 ± 1.19
	Marsh	3.20 ± 1.63	6.71 ± 13.54
	Oyster reef	1.33 ± 0.09	-20.10 ± 5.59
	SAV	0.71 ± 0.14	49.60 ± 57.08
July	Intertidal Flat	1.30 ± 0.75	-13.14 ± 2.03
	Subtidal Flat	0.60 ± 0.06	-15.68 ± 1.42
	Marsh	11.92 ± 2.70	2.78 ± 5.28
	Oyster reef	2.28 ± 1.19	35.05 ± 11.04
Oct.	SAV	0.79 ± 0.10	-10.64 ± 0.77
	Intertidal Flat	0.93 ± 0.26	-1.41 ± 4.11
	Subtidal Flat	0.62 ± 0.03	-1.17 ± 2.64
	Marsh	2.12 ± 0.39	-12.11 ± 0.69
	Oyster reef	2.59 ± 0.35	-8.50 ± 2.54
	SAV	1.13 ± 0.18	11.56 ± 16.68
	Intertidal Flat	0.78 ± 0.06	1.08 ± 7.79
	Subtidal Flat	0.60 ± 0.03	-10.83 ± 0.60

tured habitats (oyster reef, marsh, SAV) than in unstructured habitats (intertidal flat, subtidal flat) (Fig. 1). Intertidal flat DNF rates were significantly higher than subtidal flat rates (Student-Newman Keuls, $p < 0.05$) (Fig. 1).

Comparisons of the relationship of DNF and SOD in individual habitats revealed significant relationships in every habitat ($p < 0.001$, Table 3, Fig. 2). Values of r^2 spanned from 0.73 in the subtidal flat to 0.88 in the marsh. There were no significant differences in the slopes of the lines for each habitat (ANCOVA, $p > 0.05$, Table 3). Regression analyses found significant relationships between rates of DNF and SOD during every season ($p < 0.001$, Fig. 3, Table 3). The range of r^2 was from 0.673 in the winter to 0.975 in the spring. We plotted all of our DNF and SOD data with a single regression line; additionally, the lines generated by Seitzinger and Giblin (1996) and Fennel et al. (2009) are plotted in Fig. 4 for context.

Mean annual DNF efficiency (percent benthic

efflux as N₂) ranged from approximately 74% for the subtidal flat to approximately 80% for the intertidal flat and 80–100% for the structured habitats (Fig. 5A). Structured habitats had higher efficiency than the subtidal flat but did not differ from the intertidal flat (Student-Newman Keuls, $p < 0.05$). Efficiency in the winter was significantly lower than in the other seasons (Student-Newman Keuls, $p < 0.05$). There was no consistent seasonal trend for the other structured habitats, while efficiency in the intertidal and subtidal flat varied significantly between winter and other seasons (Student-Newman Keuls, $p < 0.05$). SAV did not follow this pattern and instead had highest mean efficiency in the winter, spring and summer, and lowest in fall. Oyster reef sediments had significantly lower efficiencies in the summer compared to other seasons (Student-Newman Keuls, $p < 0.05$) (Fig. 5B). The relationship between SOD and DNF efficiency revealed high DNF efficiency values even at the lowest SOD values (Fig. 6). High efficiency

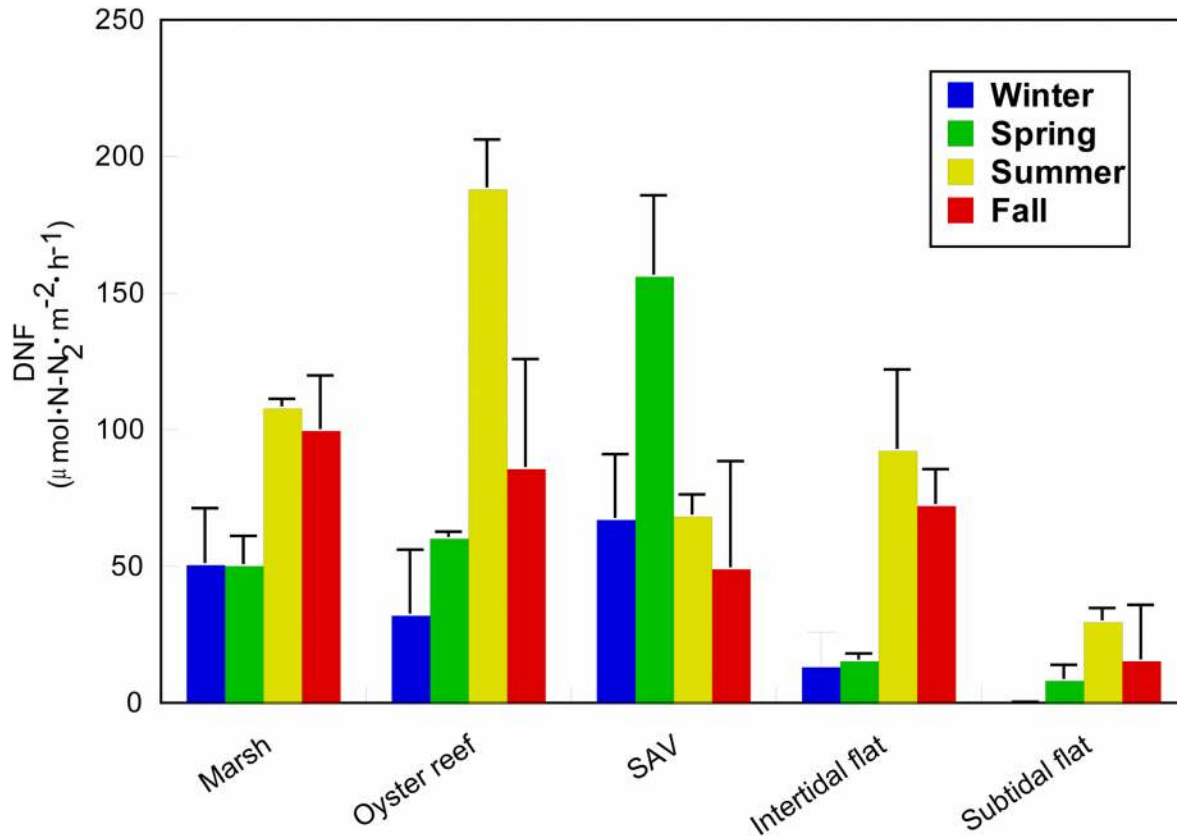


Fig. 1. Mean rates of denitrification by habitat for each season. Error bars are one standard error.

Table 3. The slopes of the regression analyses comparing fall and spring were significantly different from summer, but not from each other. None of the habitat slopes were significantly different from each other.

Season	Habitat	Slope	R ²	P < 0.001	ANCOVA
Winter		0.1070	0.673	*	AB
Spring		0.0615	0.975	*	A
Summer		0.0994	0.785	*	B
Fall		0.0700	0.943	*	A
	Marsh	0.0563	0.789	*	A
	Oyster reef	0.0708	0.732	*	A
	SAV	0.0569	0.830	*	A
	Intertidal Flat	0.0687	0.880	*	A
	Subtidal Flat	0.0359	0.795	*	A

continued through the highest SOD in this study, but showed a slight decline above SOD of 2000 $\mu\text{mol O}_2 \text{ m}^{-2} \text{ hr}^{-1}$ (Fig. 6).

We estimated that the annual cost to replace the removal of N through habitat-specific DNF ranged from nearly \$3,000 per acre per year for the oyster reefs and SAV to \$414 per acre per year in the subtidal flat. The marsh DNF was valued at nearly \$2,500 per acre per year, and the

intertidal flat was approximately \$1,500 per acre per year (Table 4).

DISCUSSION

Knowledge of habitat-specific attributes is critical to predicting changes in ecosystem function that may result from changes in the amount or distribution of particular habitats

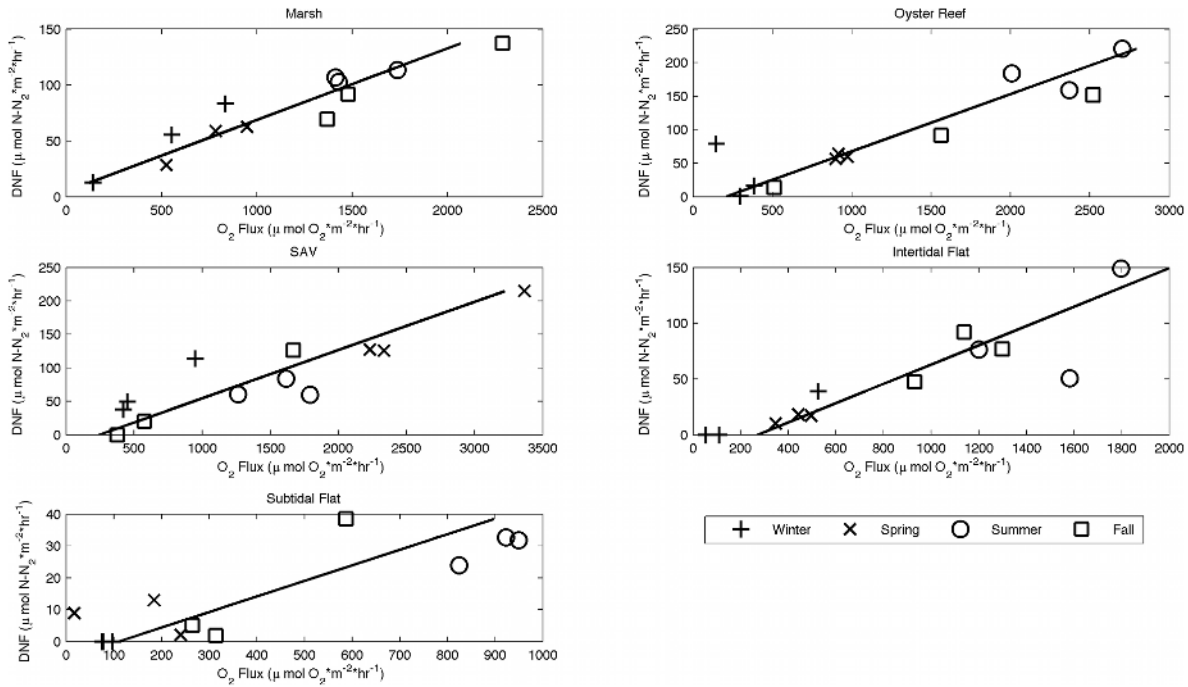


Fig. 2. Habitat specific relationships between SOD and DNF. Statistics on slope and fit are in Table 3.

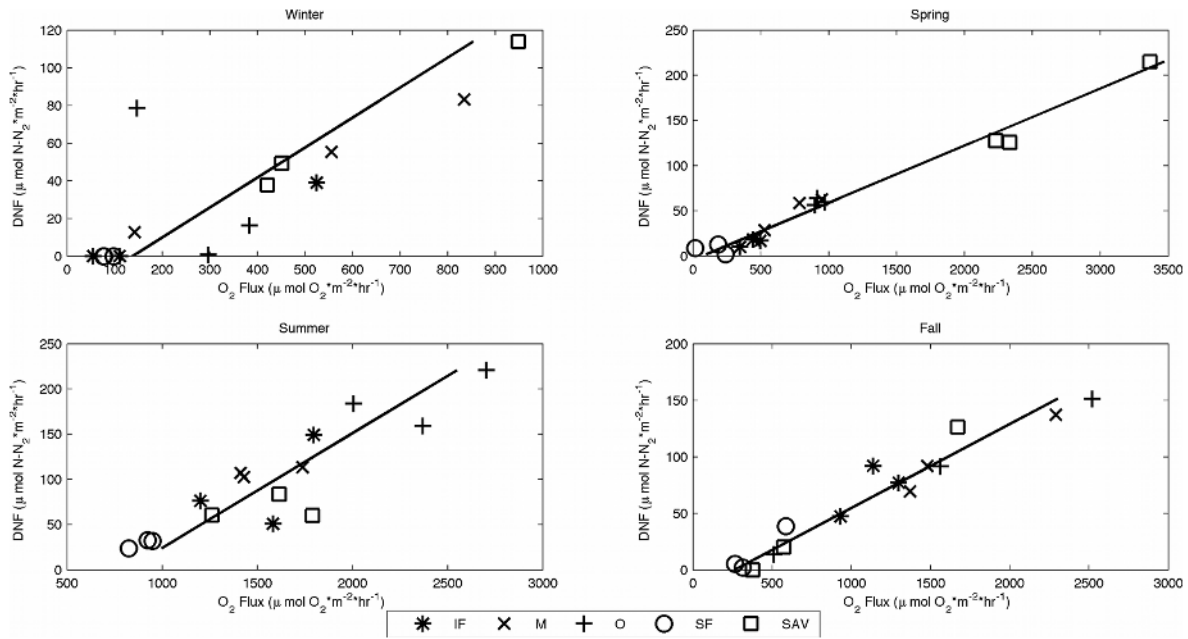


Fig. 3. Seasonal relationships between SOD and DNF. Slopes and regression statistics are in Table 3.

(Craft et al. 2009). In many systems, individual habitats provide disproportionate contributions to both ecosystem function and delivery of

ecosystem services. In estuaries, marshes, SAV and oyster reefs have all experienced tremendous aerial loss (Beck et al. 2009) and are further

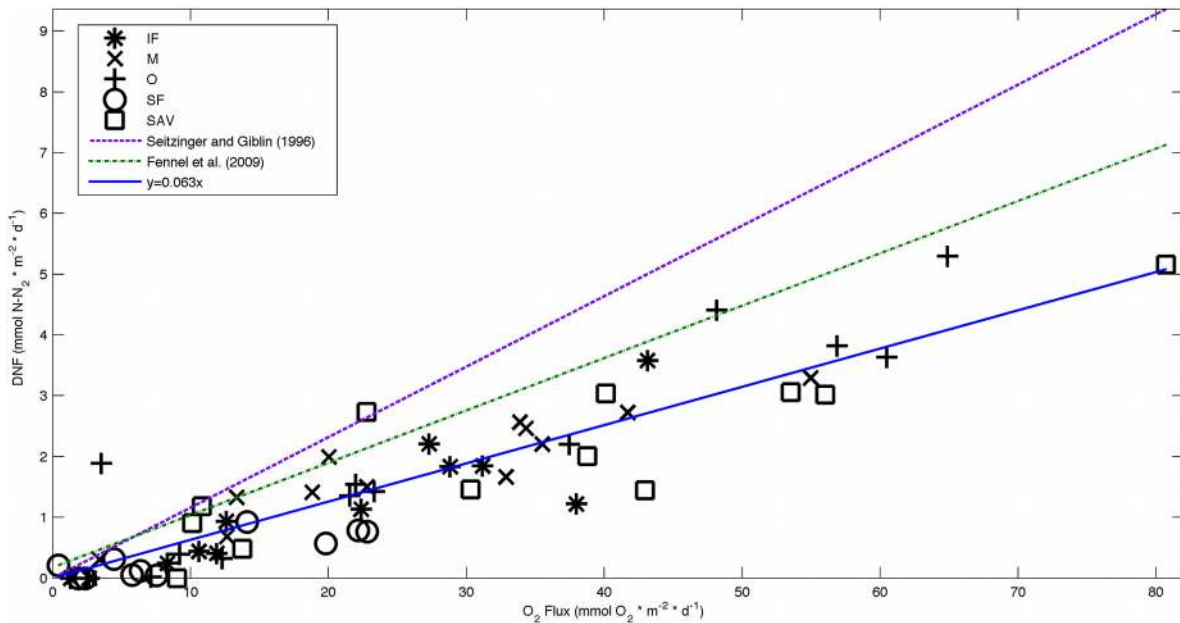


Fig. 4. SOD DNF relationship for all data from this study. The equation of the best-fit line is included in the legend. Also shown are the best-fit lines from Seitzinger and Giblin (1996) and Fennel et al. (2009).

impaired due to threats from a range of stressors. Previous work has examined N cycling in multiple estuarine habitats. Eyre et al. (2010a) measured N cycling in shallow water environments including mangroves, seagrass and mudflats in subtropical coastal systems. Our study provides a comprehensive examination of habitat-specific DNF in a system that is representative of temperate estuaries.

Historically, habitat degradation in estuaries has selectively affected structured habitats and consequently has shifted estuaries towards higher proportions of unstructured habitats (Beck et al. 2009, Brush 2009). The net result of these anthropogenic activities is that estuaries remove far less N currently than they did centuries ago (Brush 2009). Our data suggest that habitat shifts resulting from climatic and anthropogenic stressors such as sea level rise, coastal urbanization and shoreline stabilization could dramatically alter N cycling on the ecosystem scale and further modify the degree to which estuaries perform valuable ecosystem services such as DNF.

Habitat-specific DNF

Denitrification in individual habitats is well

documented. Salt marsh DNF has been studied for decades, with early work identifying the importance of landscape heterogeneity on rates of DNF (Kaplan et al. 1979). Rates of salt marsh DNF have been linked to organic matter levels (Seitzinger 1994, Starr and Gillham 1993), substrate availability (Seitzinger 1988, Tobias et al. 2001, Hamersley and Howes 2005) and frequency and duration of inundation (Morris 1991, Ensign et al. 2008). Rates of DNF in SAV beds have been found to vary widely. Although we found that DNF rates in SAV beds were higher than both unstructured habitats, other studies have suggested that rates of DNF are actually lower in SAV than in sediments without SAV (Kenworthy et al. 1982, Welsh et al. 2001). Some discrepancy in the evaluation of the importance of DNF in SAV is attributable to the characteristics of the reference sites. Our reference subtidal flats had relatively low rates of DNF, whereas reference sites in other studies have had very high rates of DNF.

DNF data have been reported from experiments with simulated oyster biodeposits (Newell et al. 2002) and a conceptual model of the potential for oysters to enhance estuarine DNF has been presented (Newell et al. 2005), but ours

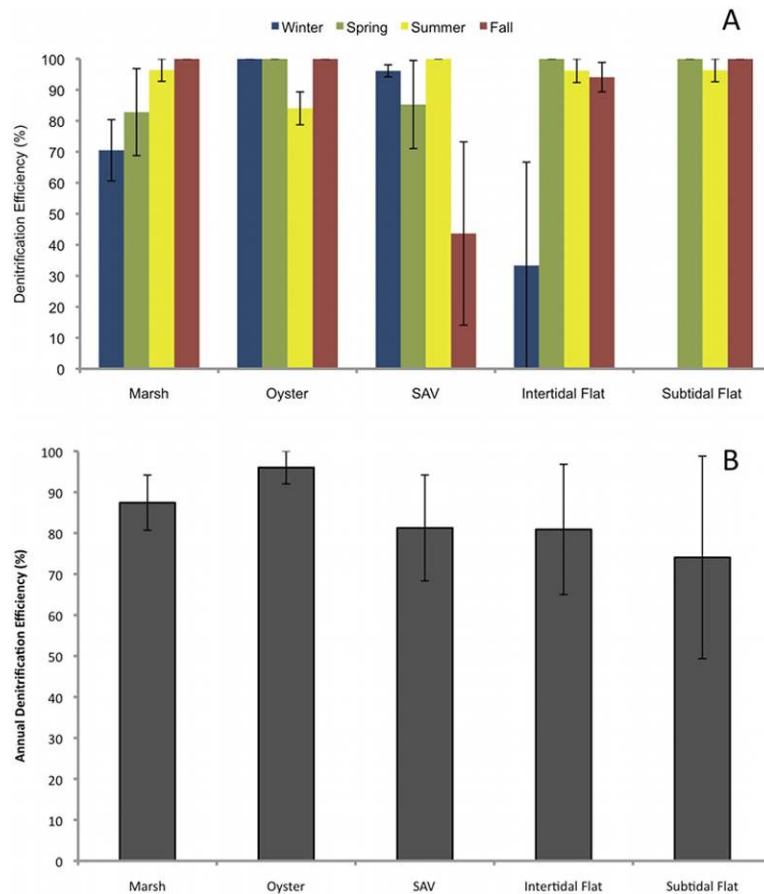


Fig. 5. A: Habitat specific denitrification efficiency expressed as percent of benthic efflux of N that is N_2 . Error bars are one standard error. B: mean annual denitrification efficiency by habitat. Error bars are one standard error.

is the first study to directly measure rates of DNF in oyster reef sediments. Our rates of oyster reef sediment DNF were lower than rates measured with simulated biodeposits and those in the conceptual model presented by Newell and colleagues (2005). Rates from reef sediments were on par with the other structured habitats in our study, and were highest in the summer when oyster filtration is at its peak. Intertidal and subtidal flat DNF rates have generally been found to be lower than those seen in salt marshes and SAV (Anderson et al. 1997, Welsh et al. 2001, Eyre and Ferguson 2002). However, because of their large area within coastal systems, the result of DNF in intertidal and especially subtidal flats can be significant (Eyre et al. 2010a).

Our work suggests that structured habitats are more active sites for nutrient processing than

unstructured habitats. Among the unstructured habitats, the intertidal flat had higher rates of DNF than the subtidal flat. This finding suggests that the position of the intertidal flat in the estuarine landscape (adjacent to the structured habitats, subjected to tidal inundation) may explain the higher rates of DNF. The intertidal flat DNF rates may be elevated relative to subtidal flats due to subsidies of organic matter from adjacent marshes, SAV or oyster reefs. The elevated rates may also be due to the intertidal flats' position higher in the tidal frame and proximity to terrestrial N sources.

We hypothesized that higher rates of DNF in structured habitats resulted from the production of new organic material through photosynthesis or, in the case of oyster reefs, repackaging organic matter from the water column and

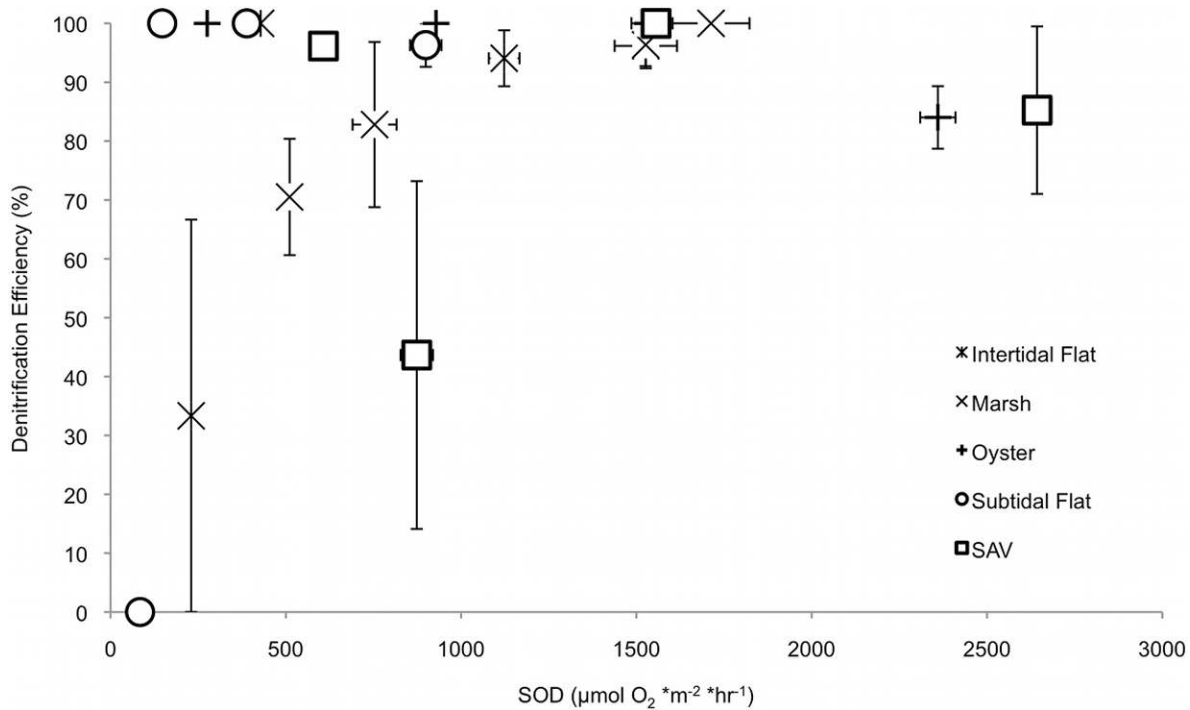


Fig. 6. The relationship between SOD and denitrification efficiency (% benthic efflux as N₂).

Table 4. Cost of replacement of N removal via habitat specific denitrification using nitrogen costs from the NC nutrient offset program. Habitat areas are derived from the North Carolina Coastal Habitat Protection Plan (NC Dept. Environment and Natural Resources).

Habitat	Mean cost to replace N removal (\$ acre ⁻¹ y ⁻¹)	Standard error (\$ acre ⁻¹ y ⁻¹)	Habitat areas in study site (acres)
NC nutrient trading rate (\$13/kg N)			
Marsh	\$2,480	\$487	29,652
Oyster reef	\$2,969	\$1,115	2,718
SAV	\$2,999	\$695	18,285
Intertidal Flat	\$1,552	\$648	1,779
Subtidal Flat	\$414	\$192	135,905

depositing it to the benthos. Data from this study support accepting this hypothesis. Oyster reef, salt marsh and SAV enrichment of sediment organic matter leads to increased supplies of ammonium through mineralization, which can then be nitrified and denitrified. Both mineralization and nitrification demand significant amounts of oxygen, likely explaining the strong correlation between SOD and DNF in this system.

Relationship of DNF and SOD

DNF is a difficult, expensive and time-consuming process to measure. It is often estimated based on correlated parameters that are straightforward to measure, such as residence time, sediment organic matter, redox conditions, or nitrate concentrations (Seitzinger 1987, Nixon et al. 1996, Seitzinger et al. 2006, Quan and Falkowski 2009). For the shallow-water estuarine habitats in our study system, we found a strong correlation between SOD and DNF in all habitats throughout the seasonal cycle. Measuring SOD is

a simple, low-cost alternative to directly measuring DNF (Coffin et al. 1993, APHA 1998, Grenz et al. 2003). This relationship has the potential to be used as a predictive tool for determining spatial and temporal scales of ecosystem services provided by estuarine habitats. The same relationship was found on the continental shelf (Seitzinger and Giblin 1996), but was not as predictive in a recent synthesis of data from a broader suite of habitats (Fennel et al. 2009). The predictive relationship between SOD and DNF is likely applicable to other low nitrate coastal systems. This is due in part to the close coupling between nitrification and DNF in coastal ecosystems, where ambient nitrate concentrations are low (Jenkins and Kemp 1984, Seitzinger 1988). Seitzinger et al. (2006) suggested systems with nitrate concentrations below 10 μM will have primarily coupled nitrification-denitrification, and thus the potential for a predictive relationship between DNF and SOD.

In our study, SOD was a more accurate predictor of DNF than sediment organic matter. SOD is a process-based indicator of the conditions conducive to elevated rates of DNF because it reflects a difference in microbial processing of organic matter, not only pools of organic matter. SOD also reflects the presence of an active microbial community and the changes in redox chemistry that result from organic matter degradation and creation of conditions favorable for DNF. Because lability of organic matter can vary widely in shallow coastal systems, SOD as a proxy for its use is a better indicator of DNF. The tight coupling of nitrification and DNF in our study system drove the strong positive SOD-DNF relationship.

The relationship between SOD and DNF varied in strength among seasons, but not among habitats. Variation in the slope of this relationship is an indicator of changes in the manner by which DNF is occurring; the higher slopes in winter and summer indicate a closer coupling between nitrification and DNF than in the fall and spring. It is reasonable to expect some variation in the slopes of the lines describing the relationship between SOD and DNF because of site-specific variations in critical factors that control DNF, such as the nitrate pool, quantity and quality of organic matter and redox conditions. However, the slopes were not significantly

different for the different habitats, supporting the use of one model of DNF based on the relationship with SOD for all habitats and seasons. The linear relationship between SOD and DNF that Seitzinger and Giblin (1996) found on the continental shelf had a steeper slope than we found in this study, indicating a stronger coupling between nitrification and DNF on the continental shelf than in the shallow water systems in this study. This was anticipated because these shallow water systems are directly connected to fluxes of nitrate and organic matter from adjacent terrestrial land uses (Bertness et al. 2002).

Direct measurement of DNF and DNF efficiency

Directly quantifying rates of DNF in various shallow-water estuarine habitats has significant implications for decision-making in the coastal zone. Nitrogen is often the currency of estuarine eutrophication management (Conley et al. 2009, Paerl 2009), and N offsets are currently traded in several states along the eastern U.S. Therefore, quantifying the relative importance of estuarine habitats as N sinks is a valuable step in effective N management. DNF assessments allow managers to evaluate the important role that shallow estuarine habitats play in removing biologically available N from coastal waters. Despite the recognized importance of DNF in the global N cycle, accurate assessment of rates of DNF remains an enormous challenge due to the large concentration of N_2 in the atmosphere relative to small changes produced from DNF (Groffman et al. 2006). The methodological and logistical challenges related to measurements of DNF likely contribute to the remaining need for direct measurement of N_2 fluxes from shallow-water coastal ecosystems.

Assessing DNF efficiency by determining the proportion of the total N flux out of sediments that is N_2 is another metric to quantify the role of individual habitats in the overall ecosystem level processing of N (Seitzinger 1987, Eyre and Ferguson 2002). We found patterns in mean annual DNF efficiencies that were similar to the patterns in mean annual rates of DNF. Additionally, oyster reef and salt marsh efficiencies were highest in the summer and fall when their productivity/repackaging activities were highest. SAV DNF efficiencies were higher earlier in the

year, likely a result of the distribution of seagrass primary productivity throughout the year due to the presence of two dominant species in the study area. Our study area is at the northern limit of *Halodule* sp., whose peak growth is in winter, and the southern limit of *Zostera* sp., whose peak growth is in summer (Kenworthy et al. 1982). DNF efficiency is an important habitat attribute that does not necessarily accompany high rates of DNF. We found a positive relationship between SOD and DNF, but also assessed how DNF efficiency changed with increasing SOD. Increased SOD can lead not only to increased DNF, but also N regeneration and release that could decrease DNF efficiency. We found high levels of DNF efficiency through the range of SOD with more variability at lower SOD and the suggestion of decreasing DNF efficiency above 2000 $\mu\text{mol O}_2 \text{ m}^{-2} \text{ hr}^{-1}$. This relationship was similar to the pattern observed in Eyre and Ferguson (2009). Very high SOD has been found to correspond with decreased DNF (An and Gardner 2002) and was attributed to sulfide toxicity to nitrifying bacteria and associated decrease in nitrification (Joye and Hollibaugh 1995, An and Gardner 2002).

Monetary value of N removal

Assigning a monetary value is an effective method for conveying the significance of ecosystem services in a form that decision makers can utilize. Since rates of DNF and consequently the economic value of this service were highest for structured habitats, the cost to replace the N that is removed by these habitats using alternative means is also high. Therefore, measures to restore and protect these habitats have additional economic incentives. Conversion of SAV to subtidal flat that could result from physical disruption or degradation of water quality would result in loss of \$2,500 per acre per year worth of N removal. Conversion of an intertidal flat to a subtidal flat that could result from an increase in relative sea level or the installation of a vertical shoreline stabilization structure within the intertidal zone would lead to a loss of more than \$1,000 per acre per year worth of N removal. These examples of the potential application of our data to habitat loss scenarios are a small subset of the possible benefits of this study's multi-habitat assessment approach. Habitat res-

toration and targeted conservation are additional areas that could benefit significantly from improved evaluation of estuarine habitat N removal via DNF. Comprehensive assessments of the global value of ecosystem service have been made (Farber et al. 2002) and detailed habitat-specific ecosystem service evaluations have also been conducted on some systems (Coen et al. 2007, Grabowski and Peterson 2007).

Quantifying ecosystem services is being pursued in many natural systems (Daily et al. 2009), having become more prevalent after the release of the Millennium Ecosystem Assessment (Carpenter et al. 2006). Decisions that involve loss or creation of habitats that provide services can be made on a cost-benefit basis with a common monetary unit. However, there are also tremendous data requirements for accurate quantification and valuation of ecosystem services. Nutrient cycling in estuarine ecosystems has been the topic of research for many decades. Despite the highly developed literature on the subject, more data are required to assure that the ecosystem services quantified are transferable (Plummer 2009). Transferability needs to exist within a system (e.g., are rates of DNF the same across habitat types and landscapes) and between systems (e.g., are habitat-specific DNF rates similar from estuary to estuary) before these processes can be modeled effectively at the ecosystem level and extended to economic evaluations.

Conclusions

We conducted a comprehensive assessment of DNF and an accompanying monetary evaluation of the ecosystem service of shallow water estuarine DNF. Linking habitat structure to ecosystem function is essential for effective decision making regarding management, preservation and restoration of valuable habitats (Strayer and Findlay 2010). Our findings corroborate the focus to date on restoration and preservation of structured coastal habitats such as salt marshes and SAV in temperate estuaries. Our findings also provide additional support for the restoration and preservation of oyster reefs based on their contribution to N removal. Managing habitats to optimize denitrification will also result in enhancement of other ecosystem services such as fish habitat and shoreline

stabilization. Our results provide additional breadth to the understanding of the factors that limit DNF and compliment the recent models for higher nitrate areas (Böhlke et al. 2009) and a broad range of aquatic systems (Fennel et al. 2009). As evaluation of the economic and ecological contributions of processes such as DNF continues, multiple predictive metrics will be required to quantify those values.

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