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Habitat context influences nitrogen removal by restored oyster reefs

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Summary

1. Like many ecosystem functions in marine and terrestrial environments, nutrient processing varies dramatically over small spatial scales, making efforts to apply findings within and across ecosystems challenging. In estuaries, information on the influence of habitat context on sediment nutrient cycling is lacking even though this is an important estuarine function with high societal value.

2. We collected triplicate intact sediment cores from restored oyster reefs located in different habitat contexts (adjacent to salt marshes, seagrass beds and mudflats), as well as salt marshes, seagrass beds and mudflats without reefs (controls). Sediment denitrification and fluxes of dissolved inorganic nitrogen were measured under ambient and experimentally elevated water column nitrate levels.

3. Under ambient nitrate, oyster reefs enhanced sediment denitrification by 18–275% over the controls, with highest rates of denitrification in the mudflat context. With experimentally elevated nitrate, the rate of denitrification was higher for oyster reefs compared to the controls in all contexts. This suggests that oyster reefs prime sediments to denitrify nitrate pulses by providing a labile carbon source for denitrifying bacteria.

4. There was a weak positive relationship between oyster density and denitrification under ambient nitrate concentrations and a positive relationship with denitrification that became negative beyond ~ 2400 individuals m^{-2} with elevated nitrate concentrations. The effect of the oyster reef on sediment denitrification was most pronounced in the mudflat context, due to the absence of other structured habitats and higher oyster density, compared to the other two habitat contexts investigated.

5. The consistency of denitrification efficiency across the habitats and lack of difference between habitats with reefs and those without (controls) suggest oyster-mediated denitrification is an effective sink for nitrogen in coastal systems.

6. *Synthesis and applications.* Our study indicates that oyster-mediated denitrification is dependent on the habitat context of the oyster reef, and variation in oyster density and the relative functional redundancy of oyster reefs where other structured habitats exist (e.g. seagrass and salt marshes) may explain this pattern. Efforts to model and predict ecosystem services provided through oyster reef restoration such as the removal of anthropogenically derived nitrogen should incorporate how habitat context influences ecosystem functions.

Key-words: denitrification, habitat context, nutrient cycling, oyster reefs, restoration, water quality

Introduction

Determining how ecological processes and functions scale temporally and spatially is a central theme of environmental science with broad applications for restoration and conservation (Levin 1992). Furthermore, investigations that examine processes occurring not only within one

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habitat but across habitats will help develop a more general understanding of ecological systems. The composition and configuration of habitats in the landscape influences the distribution and abundance of sessile and motile species, which may result in the context of a particular habitat having profoundly different effects on ecological processes (Micheli & Peterson 1999; Grabowski *et al.* 2005; Hosack *et al.* 2006; Smyth *et al.* 2013). Efforts to extrapolate experimental findings from individual habitats to explain processes operating at large spatial and temporal scales are limited by lack of information about the influence of spatial patterns on ecological processes. This is particularly challenging in marine systems because of spatial heterogeneity and connectivity between habitats (Barbier *et al.* 2011; Boström *et al.* 2011).

One of the overarching consequences of anthropogenic activities in many ecosystems is the degradation of structured habitats such as forests, coral reefs and oyster reefs, resulting in a fragmented and simplified landscape (Sala *et al.* 2000; Thrush *et al.* 2008). Yet the design of conservation and restoration strategies, including protected areas and natural parks, has often emphasized conserving individual habitats rather than landscapes or networks of habitats (Margules & Pressey 2000). This approach ignores the importance of habitat context, which affects key ecosystem functions and may result in restoration of ecosystem structure but not function (Simenstad, Reed & Ford 2006).

Excessive nutrient inputs from multiple sources including agricultural runoff and atmospheric deposition have led to eutrophication in many aquatic systems (Vitousek *et al.* 1997; Galloway *et al.* 2003; Diaz & Rosenberg 2008). As nutrient enrichment has accelerated, the balance between nutrient inputs and exports has shifted, affecting growth, composition and biomass of primary producers, and consequently impacting water quality (Conley 2000). In addition, excessive nitrogen loading can impact entire food webs by shifting ecosystems from supporting higher taxa to microbe-dominated communities (Diaz & Rosenberg 2008), resulting in the loss of important ecosystem services such as providing recreationally and commercially valuable fish (Byers & Grabowski 2013). To prevent or mitigate these negative effects, management efforts often focus on reducing nitrogen inputs to coastal waters and converting reactive nitrogen to unreactive N₂ gas (Seitzinger *et al.* 2006). Nitrogen can be removed through physical transport, burial or denitrification – the microbial-mediated conversion of bioavailable nitrogen to N₂ gas (Vitousek *et al.* 1997). Bacteria capable of denitrification are ubiquitous, and denitrification can occur when there is low oxygen concentration, sufficient nitrate levels and a high quantity of labile carbon. Unfortunately, habitat modification and loss have reduced the denitrification capacity of many coastal ecosystems (Brush 2009). To recover this lost service (denitrification) and help reverse eutrophication, it is often necessary to restore and enhance habitats within a context that promotes the

highest rates of denitrification (Fulweiler, Rabalais & Heiskanen 2012).

Once ubiquitous in estuaries, oyster reefs have declined by an estimated 85% world-wide in the last century, with estimates in the USA equally as severe (Rothschild *et al.* 1994; Beck *et al.* 2011; Zu Ermgassen *et al.* 2012). Although oysters have been exploited as a fishery for many years, they are now also recognized for their ecological services (Peterson & Lipcius 2003; Grabowski *et al.* 2012). Oyster reefs provide many valuable ecosystem services such as habitat for fishes, shoreline protection and maintenance of water quality (Grabowski *et al.* 2012). Oyster-mediated benthic–pelagic coupling improves water quality through filtration and biodeposition. Deposition of organic-rich biodeposits (faeces and pseudofaeces) and enhanced settlement of suspended materials on the sediment surface promotes conditions favourable for denitrification by supplying a carbon source for denitrification and stimulating NO₃⁻ production through nitrification (Newell, Cornwell & Owens 2002; Kellogg *et al.* 2013; Smyth, Gherardi & Piehler 2013). While recent studies have shown oyster reefs increase sediment denitrification, questions remain about factors that control denitrification in these habitats and how to include oysters into nutrient management plans (Kellogg *et al.* 2014).

Because of their value as a fishery and for the services they deliver, global oyster reef restoration efforts are currently underway. Successful restoration of oyster reef habitat involves recovering not only the oyster population but also the associated ecosystem services (Coen & Luckenbach 2000; Peterson & Lipcius 2003; Simenstad, Reed & Ford 2006; Boström *et al.* 2011). Determining how the habitat context of a restored oyster reef modifies important functions and consequently impacts the delivery of ecosystem services will improve the ability of managers to increase the return on their investment from oyster reef restoration efforts. We examined whether the habitat context of a restored oyster (*Crassostrea virginica*) reef influences oyster-mediated sediment denitrification by conducting experiments on sediments surrounded by different types of biogenic structures (salt marshes, seagrass beds) or isolated on mudflats. Additionally, we assessed whether the habitat context of a restored oyster reef affects removal of nitrogen in response to anthropogenic nitrate loading.

Materials and methods

STUDY SITE

Habitats used in this study were located in Middle Marsh between Beaufort and Shackleford Banks on the central North Carolina coast in Back Sound (Fig. 1). This area contains seagrass beds, salt marshes, oyster reefs and intertidal mudflats. Oyster reefs used in this study were restored in three distinct habitat contexts in summer 1997: on isolated mudflats (mudflat), adjacent to salt marsh (marsh), or on the edge of salt marsh and

surrounded by seagrass beds (seagrass). We sampled sediments from three distinct areas within Middle Marsh that had a reef and control pair for each context. Reefs were separated from controls by at least 50 m in the 1.3-km² study area (Grabowski *et al.* 2005).

SAMPLE COLLECTION

Sediment cores (contained in 6.4-cm-diameter by 17-cm-long polycarbonate tubes, 10 cm depth) were collected adjacent to each reef habitat ($n = 3$) and control habitat (without reefs, $n = 3$) in each context at low tide on 28 June 2010. Cores collected from oyster reefs did not contain live oysters. Additionally, ~100 L of water was collected for use in the laboratory incubations. Following collection, sediment cores and water were transported to an environmental chamber (Bally, Inc., Morehead City, NC, USA) at The University of North Carolina Institute of Marine Sciences in Morehead City, NC. Surface water measurements of dissolved O₂, salinity and water temperature (YSI 600 Series Sonde and Model 650 data logger; Yellow Springs Instruments, Yellow Springs, OH, USA) were also collected. Oyster density in the reef was determined by placing a 0.25-m² quadrat on each reef (one quadrat per reef) and counting all the oysters present with a shell length >25 mm (Powers *et al.* 2009).

SEDIMENT CORE INCUBATIONS

Within 4 h of collection, sediment cores were set up in a continuous flow core incubation system to measure steady-state nutrient and dissolved gas fluxes, described in Piehler & Smyth (2011). Briefly, cores were sealed with gas-tight lids, which had an inflow and outflow port. Water from a reservoir was pulled over the cores at a flow rate of 1 mL min⁻¹. Triplicate dissolved gases and duplicate dissolved inorganic nitrogen samples were collected from the outflow and inflow periodically over the next 24 h. To examine how sediments from different habitat contexts responded

to nitrate pulses, nitrate concentration in the reservoir water was elevated with NaNO₃ (~800 μM) after 48 h of sampling. Dissolved gas and inorganic nitrogen samples were then collected for an additional 48 h. Incubations were conducted in the dark and at ambient temperature (30 °C).

SEDIMENT AND WATER ANALYSIS

Water samples from laboratory experiments were analysed immediately upon collection for dissolved gasses (N₂, O₂ and Ar) with membrane inlet mass spectrometry (MIMS). Concentrations of dissolved N₂ and O₂ were determined using the ratio with Ar (Kana *et al.* 1994). Coefficients of variation for N₂/Ar were 0.05% and 0.04% for O₂/Ar. Water samples from laboratory experiments for dissolved nutrient determination were filtered through Whatman GF/F glass fibre filters (25 mm diameter, 0.7 μm nominal pore size) and frozen until analysis. Dissolved inorganic nutrients were analysed with a Lachat Quick-Chem 8000 automated ion analyser for NO₃⁻+NO₂⁻ (reported as NO_x) and NH₄⁺ concentrations using standard protocols (Lachat Instruments, Milwaukee, WI, USA: NO₃⁻/NO₂⁻ method 31-107-04-1-A, NH₄⁺ method 31-107-06-1-A; detection limits: 0.04 μM NO_x, 0.18 μM NH₄⁺; CV(%): 0.9% NO_x and 2.6% NH₄⁺).

Upon completion of the incubations, the upper 2 cm of sediment in each core was sampled for organic matter content by mass difference from dried sediments before ignition (105 °C for 6 h) and after ignition (525 °C for 3 h).

CALCULATIONS

Fluxes across the sediment–water interface were calculated as $(C_o - C_i) \times f/a$, where C_o is the outflow concentration (μmol L⁻¹), C_i is the inflow concentration, f is the flow rate (0.06 L h⁻¹), and a is the sediment surface area (0.0032 m²). Successive measurements from each core (triplicates for dissolved gas and duplicates for dissolved inorganic nutrients) were averaged to give

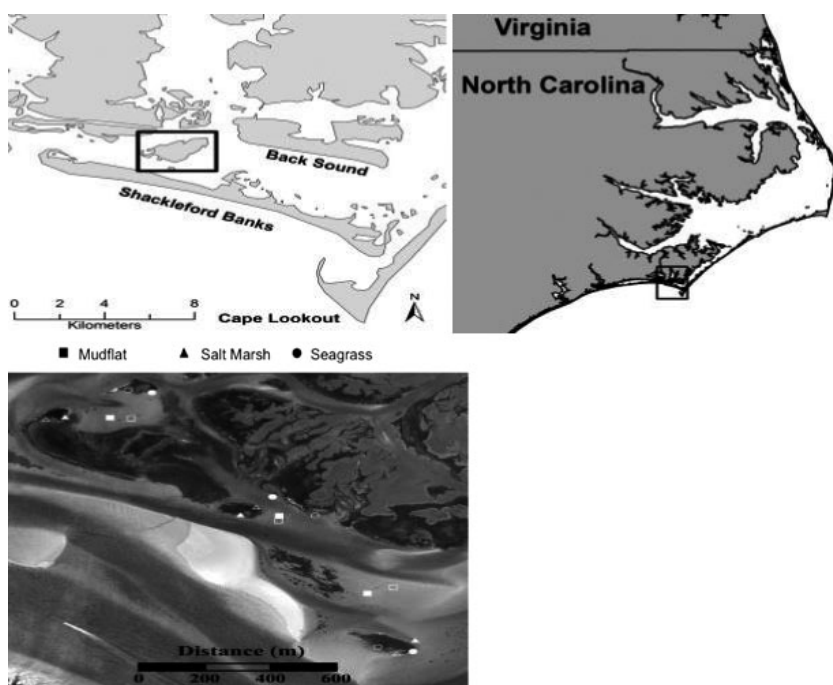


Fig. 1. Reef and control habitats in Middle Marsh, NC. Control habitats are open symbols and reefs are solid symbols.

core-specific values. This results in a net N_2 flux (gross denitrification – gross nitrogen fixation) and does not distinguish between the sources of N_2 . Consequently, denitrification refers to net N_2 production. Oxygen fluxes were calculated using the concentrations of O_2 obtained from the MIMS, presented as sediment oxygen demand (SOD), and serve as an indicator of organic matter quality, such that more labile organic matter is associated with higher SOD (Ferguson, Eyre & Gay 2003). To determine the influence of oyster reefs on sediment N_2 fluxes, the change in denitrification between the control and reef habitat pair in each zone was calculated (Kellogg *et al.* 2014). Denitrification efficiency was computed as the percentage of the dissolved inorganic nitrogen efflux that was N_2 (Piehler & Smyth 2011).

STATISTICAL ANALYSES

Statistical analyses were performed using R 2.13.1 (R Foundation for Statistical Computing 2011). Linear mixed-effects models (*lme* in R *nlme* package), where habitat nested in sampling location was included as a random effect for the intercept, were used to investigate the effects of oyster reef presence, habitat context, nitrate concentration (ambient vs. elevated) and the interaction between these factors on response variables. Fluxes of N_2 , NO_x ($NO_3^- + NO_2^-$) NH_4^+ , denitrification efficiency and SOD were analysed using all three fixed effects. For sediment organic matter, only habitat context and reef presence were included as fixed effects. The effects of ambient vs. elevated nitrate concentration and habitat context on oyster reef-mediated changes in denitrification were also analysed with a mixed-effects model (fixed effects: nitrate concentration \times habitat context; random effects: habitat nested in location). Relationships between oyster density and habitat context were made using a mixed-effects model (fixed effects: habitat context; random effects: habitat nested in location). Comparisons were conducted using linear contrasts and judged against an alpha level of 0.05. Interactions were assessed using Tukey's HSD (*lsmeans* in R *lsmeans* package). Assumptions of homogeneity were tested using Levene's tests. Regression analyses were used to investigate the effect of oyster density on denitrification. Models with the lowest Akaike's information criterion corrected for small sample sizes (AIC_c) were chosen.

Results

SEDIMENT DENITRIFICATION RATES

Patterns in denitrification (net N_2 production) were affected by reef presence, habitat context and water column nitrate (linear contrasts $P = 0.0381$, Fig. 2a,b), indicating the effect of the reef and water column nitrate was different for each habitat context. For ambient nitrate concentrations, reefs had higher rates of denitrification compared to the controls, increasing denitrification by 275%, 18% and 70%, in the mudflat, marsh and seagrass context, respectively (Fig. 2a). However, the difference in sediment denitrification between the control and reef was only significant for oyster reefs in the mudflat habitat context (Tukey, $P < 0.0001$). Rates of denitrification in oyster reefs were similar for all habitat contexts as were rates of denitrification in control habitats. When water column

nitrate was elevated, sediment denitrification increased for both reef and control habitats. This increase was significant for all habitats except the seagrass-reef and mudflat-control habitats (Fig. 2b). Within each habitat context, the reef habitats significantly increased sediment denitrification compared to the control habitats with elevated nitrate (Fig. 2b). Overall, mudflat-reefs had the highest rate of denitrification, about $10 \mu\text{mol N m}^{-2} \text{h}^{-1}$ higher compared to the other reefs for ambient nitrate and about $200 \mu\text{mol N m}^{-2} \text{h}^{-1}$ higher for elevated nitrate. Reefs increased denitrification with the addition of nitrate to the water column in all habitat contexts.

In general, oyster reefs enhanced sediment denitrification (positive change in denitrification) over the controls, but the magnitude of the oyster reef's effect on sediment denitrification was dependent on habitat context and water column nitrate (Fig. 3; linear contrasts, $P = 0.0365$). The effect of oyster reefs on sediment denitrification was similar between all contexts under ambient nutrients and for the marsh and seagrass context with elevated nitrate. Oyster reefs had the largest effect on sediment denitrification in the mudflat context after water column nitrate was elevated, increasing denitrification by about $500 \mu\text{mol N m}^{-2} \text{h}^{-1}$ (Tukey, $P < 0.0001$).

SEDIMENT NITRATE AND AMMONIUM FLUXES

Water column nitrate, habitat context and oyster reef presence affected sediment NO_x fluxes (linear contrasts, $F_{2,18} = 4.95$, $P = 0.02$). Under ambient levels of nitrate, nitrate fluxes varied between uptake (negative flux) in the mudflat habitat and seagrass-control, to production in the seagrass-reef and marsh-control (Fig. 2c). However, NO_x fluxes were not different between the controls and reefs in any habitat context.

Sediment NO_x fluxes were significantly affected by water column nitrate and nitrate uptake increased for most habitats (Fig. 2d). The mudflat-control was the only habitat to produce nitrate after water column nitrate was elevated ($488.48 \pm 1024.25 \mu\text{mol N m}^{-2} \text{h}^{-1}$), and was significantly different from the other habitats (Tukey, $P < 0.0001$) including the mudflat-reef (Tukey, $P = 0.0065$). Nitrate uptake was greater in the seagrass-control than the seagrass-reef habitat (Tukey, $P < 0.0004$).

There was production of ammonium from all sediments with both ambient (Fig. 2e) and elevated concentrations of nitrate (Fig. 2f). Under ambient nitrate, the largest production of ammonium occurred in the seagrass habitat, where both the control and reef had higher levels of ammonium production compared to the other habitats. With elevated nitrate, ammonium production increased for all habitats. The only difference between the control and reef occurred in the mudflat context, where the mudflat-reef produced significantly more ammonium than the mudflat-control with elevated nitrate (Tukey, $P = 0.01$).

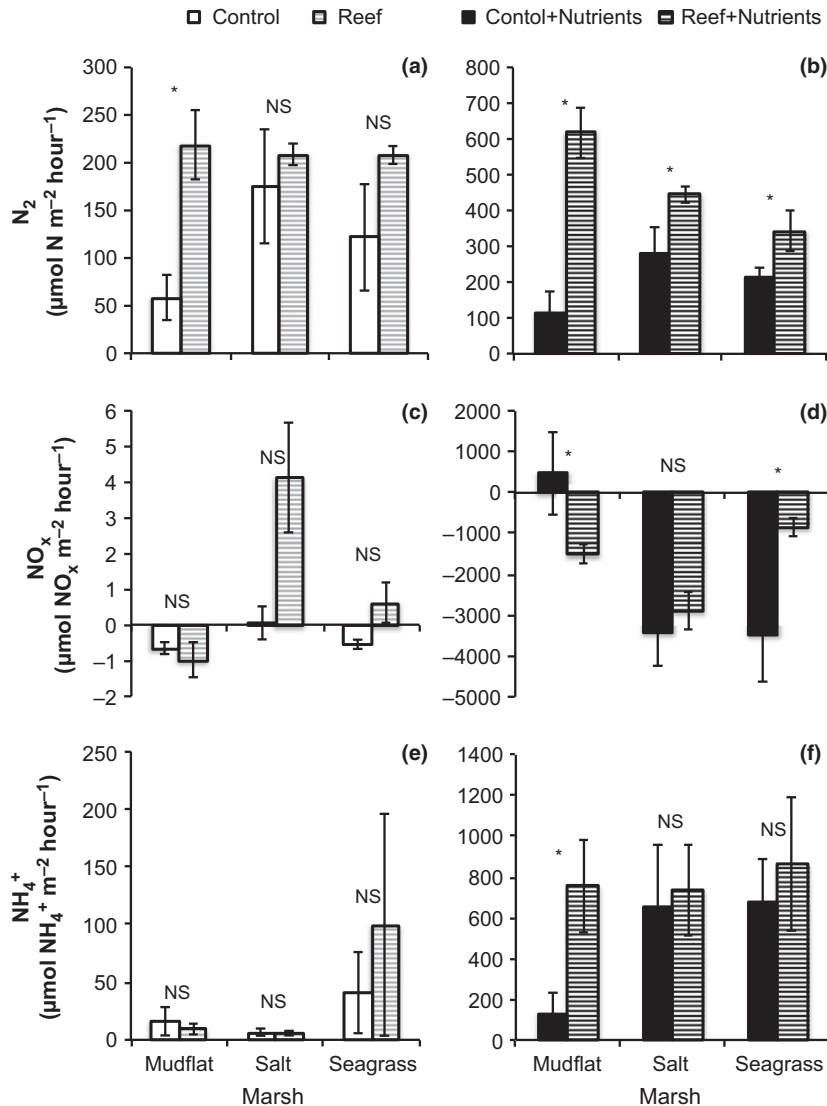


Fig. 2. Mean net fluxes of N_2 , NO_x and NH_4^+ before (a, c, e) and after (b, d, f) experimental nitrogen additions for reef and control areas in each habitat ($n = 3$). A positive value indicates flux out of the sediment, and a negative value indicates flux from the water column to the sediment. Significant differences ($P < 0.05$) between control and reefs for each landscape are indicated with asterisks. NS indicates no significant difference. Error bars are one standard error of the mean.

DENITRIFICATION EFFICIENCY

Denitrification efficiency with ambient nitrate ranged from 78% in the mudflat-control to 95% in the mudflat-reef and marsh-reef (Fig. 4). Although reef presence did not significantly affect denitrification efficiency, there was a trend of higher efficiencies for reefs compared to controls. Denitrification efficiency significantly decreased (Tukey, $P < 0.0001$) when nitrate concentrations were experimentally elevated. Ammonium production increased for all control and reef habitats with experimentally elevated nitrate concentrations. There was no difference in denitrification efficiency between the reef and control in any habitat context.

SEDIMENT OXYGEN DEMAND AND ORGANIC MATTER

There was no effect of water column nitrate on SOD, indicating that O_2 dynamics did not change throughout the experiment, and SOD measured during ambient and

nitrate addition experiments was pooled for each habitat (Table 1). SOD was similar for all reefs and in every case higher than controls. This difference was significant in the seagrass and mudflat context, where the seagrass-control was 30% lower than the seagrass-reef and the mudflat-control was 125% lower than the mudflat-reef (Tukey, $P = 0.04$, $P < 0.0001$, respectively).

Sediment organic matter (SOM) was higher in habitats with reefs than in the controls (Table 1). Among control habitats, the mudflat had the lowest SOM content. The seagrass-reef had about 1.3% higher SOM content compared to the other reef habitats and significantly more compared to the seagrass-control (Tukey, $P < 0.0001$).

DENSITY AND SIZE OF OYSTERS

Oyster densities were greatest on the mudflat-reef (Table 1, $F_{2,42} = 28.21$, $P = 0.004$). Oyster length was similar between all habitats. The mudflat-reef had the longest mean shell length but was also the most variable. Before

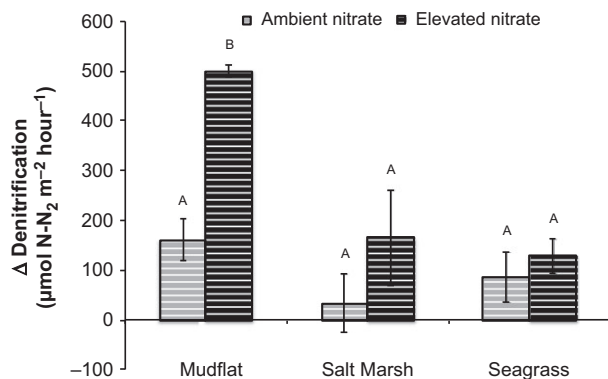


Fig. 3. Patterns of oyster-mediated change in denitrification (Δ Denitrification) associated with oyster reef compared to the control in each habitat context. Errors are one standard error of the mean. Histograms not sharing letters are significantly different from each other ($P < 0.05$).

the addition of nitrate, a linear regression model best explained the relationship between sediment denitrification and oyster density, but this relationship was not significant (Fig. 5a, $R^2 = 0.19$, $P = 0.07$). The relationship between denitrification and oyster density after the addition of nitrate to the water column was best explained by a second-order polynomial relationship (Fig. 5b, $R^2 = 0.65$, $P = 0.0004$).

Discussion

Oyster density is often considered an indicator of restoration success (Luckenbach *et al.* 2005; Powers *et al.* 2009; Zu Ermgassen *et al.* 2012). Several services such as water filtration, habitat complexity and invertebrate refugia positively correlate with oyster density (Peterson, Grabowski & Powers 2003; Soniat, Finelli & Ruiz 2004; Rodney & Paynter 2006; Zu Ermgassen *et al.* 2013). However, relationships between oyster density and ecosystem processes such as nitrogen assimilation are not always linear (Dame *et al.* 2002; Carmichael *et al.* 2012). Our model indicates that under ambient nitrate concentrations, denitrification is weakly related to oyster density. With elevated nitrate, the relationship between oyster density and denitrification at low to intermediate oyster densities was positively correlated, whereas at high oyster densities, there was an apparent threshold resulting in decreased sediment denitrification at the highest densities. The eastern oyster produces 1.33–16.8 mg C as biodeposits per g of oyster tissue per day (Haven & Morales-Alamo 1966; Higgins *et al.* 2013). Our model suggests a threshold of ~ 2400 individuals m^{-2} , underscoring the importance of high density for oyster augmentation of denitrification and the potential for very high densities to lead to reductions in sediment denitrification. The volume of biodeposits produced at high oyster densities may cause sediments to become anoxic, resulting in sulphide accumulation and nutrient

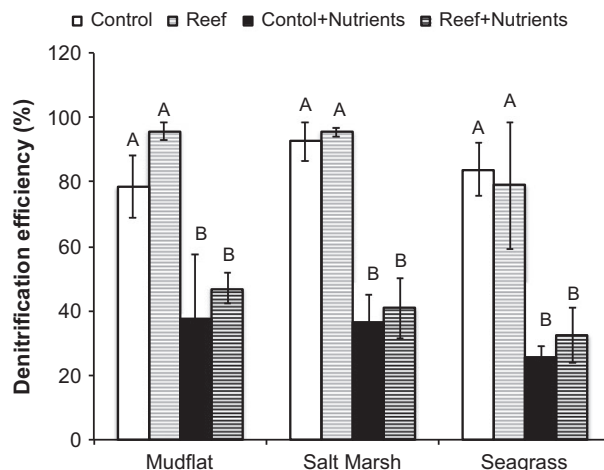


Fig. 4. Denitrification efficiency from controls and oyster reefs in different habitat contexts under both ambient and experimental nitrate levels. Errors are one standard error of the mean. Histograms not sharing letters are significantly different from each other ($P < 0.05$).

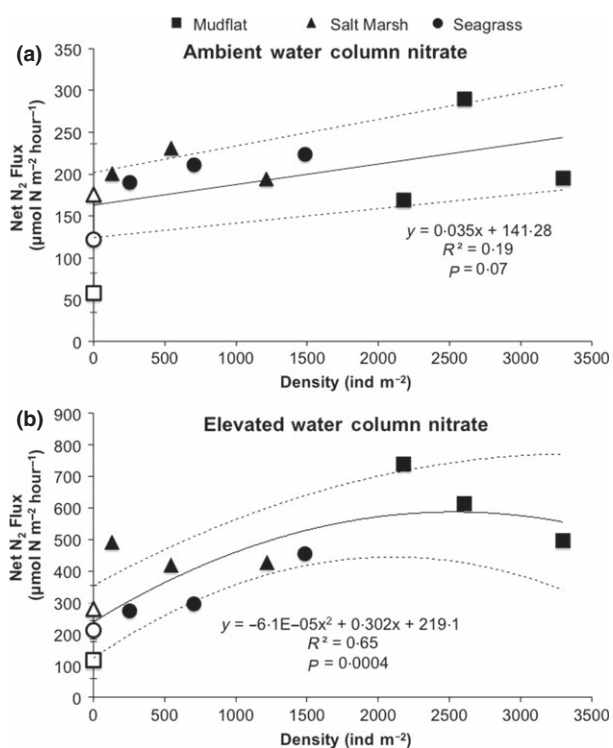
regeneration rather than removal through denitrification (Tenore & Dunstan 1973; Kemp *et al.* 1990).

This relationship between oyster density and denitrification was driven by the high density and high rate of denitrification at the mudflat-reef. Oyster density on the mudflat-reef was comprised of a range of sizes, as indicated by the broad oyster size distributions on those reefs. The patterns of oyster density and habitat context were similar to the patterns found in Grabowski *et al.* (2005) and more recent estimates of oyster density on the same reefs (F.J. Fodrie, unpublished data). The larger oysters on the mudflat-reef had higher filtration rates and subsequently likely produce and accumulate more biodeposits (Zu Ermgassen *et al.* 2013). Other characteristics of the mudflat-reef, such as the relative isolation from other habitats, lack of predation (Micheli & Peterson 1999), sediment type and elevation (Fodrie *et al.* 2014; Rodriguez *et al.* 2014), may also contribute to the higher oyster density and higher denitrification found for reefs in this context. Additional research on the interactions between oyster density and habitat context would improve our understanding of this relationship. Our data also indicate that in oligotrophic systems with low nitrate levels, oyster reefs increase denitrification regardless of oyster density. Further investigation of the relationships between oyster density and ecosystem functioning will increase the accuracy of models aimed at predicting the delivery of ecosystem services derived from conservation and restoration activities by helping identify target restoration densities and sustainable levels of oyster harvesting.

Structured habitats, such as seagrass beds, salt marshes and oyster reefs, tend to have higher rates of denitrification compared to unstructured habitats because of production and trapping of organic matter (Piehler & Smyth 2011). Enhanced sediment denitrification by oyster reefs

Table 1. Mean sediment oxygen demand (SOD), sediment organic matter (SOM), oyster density and oyster shell length for controls and oyster reefs in each context

Variable	Landscape	Control (SE)	Reef (SE)
SOD ($\mu\text{mol O}_2 \text{ m}^{-2} \text{ h}^{-1}$)	Mudflat	480.73 (206.53)	1322.44 (110.79)
	Salt marsh	1085.77 (422.40)	1359.39 (58.75)
	Seagrass	1052.07 (336.27)	1374.23 (47.66)
SOM (%)	Mudflat	0.91 (0.13)	1.26 (0.06)
	Salt marsh	1.51 (0.19)	1.45 (0.21)
	Seagrass	1.40 (0.15)	2.63 (0.32)
Oyster density (individuals m^{-2})	Mudflat		2693.33 (326.12)
	Salt marsh		629.33 (316.96)
	Seagrass		816.00 (360.03)
Oyster shell length (mm)	Mudflat		67.24 (45.70)
	Salt marsh		42.00 (19.44)
	Seagrass		49.035 (25.83)

**Fig. 5.** Relationship between denitrification and oyster density before (a) and after (b) nitrogen addition. Solid line is the model and dashed lines are 95% prediction intervals. Regression includes all reefs (solid). The mean value of the controls in each habitat (open) was not included in the regression.

results from the oyster producing biodeposits that supply organic nitrogen and carbon to the sediment microbial community (Newell, Cornwell & Owens 2002). Given that salt marshes and seagrass beds produce new organic matter through photosynthesis, the reef's functional role in enhancing denitrification may become redundant when other biogenic habitats are present. This functional redundancy has been demonstrated for the delivery of other ecosystem services by reefs, such as use by juvenile fish (Heck, Hays & Orth 2003; Grabowski *et al.* 2005; Gerald

et al. 2009). The mudflat-control, which lacked three-dimensional biogenic structure and was relatively isolated, had a lower quality and quantity of organic matter, as indicated by a lower SOD and SOM, compared to the other habitats. Furthermore, the high rates of NO_x production with elevated nitrate indicate that nitrification is occurring in this habitat. Therefore, organic matter rather than nitrate likely regulates denitrification in the mudflat-control. This limitation was alleviated with the addition of the oyster reef on the mudflat. Our results suggest that targeted oyster reef restoration and conservation efforts stand to have the largest impact on nutrient dynamics and the removal of anthropogenic nitrogen in the mudflat habitat context.

Denitrification in the marsh-reef increased relative to the control with the addition of nitrate to the water column. The effect of the reef was subtler in the seagrass habitat context, which consisted of oyster reef, marsh and seagrass habitats. The additional structure and organic matter delivery from the oyster reef to the seagrass sediments had little effect on denitrification because organic matter was not limiting in this setting. Similar results have been found in eutrophic systems where the effect of oysters is less evident since the production of labile organic matter such as pseudofaeces by oysters does not limit denitrification under these conditions (Hoellein & Zarnoch 2014). In our study system, with low dissolved nutrients and high water quality, nitrate in the water column increased the reef-enhanced denitrification in all habitat contexts.

Organic matter loading can hinder coupled nitrification–denitrification, but enhance direct denitrification when nitrate is available in the overlying water (Caffrey *et al.* 1993; Cornwell, Kemp & Kana 1999). The increase in denitrification detected with elevated nitrate was greater in reef sediments than in control sediments. Oyster reefs in eutrophic waters, with higher nitrate levels, tend to have higher denitrification than sediments without reefs (Hoellein, Zarnoch & Grizzle 2014). Sediment denitrification in salt marshes, seagrass beds and mudflats was limited by carbon availability, which was alleviated with the

addition of the oyster reef because it supplies organic matter to the sediment through benthic–pelagic coupling and primes the sediments for denitrification when nitrate is available. Oyster reefs have a larger impact on sediment nitrogen removal than habitats without reefs, enhancing removal of bioavailable nitrogen. Restoring oyster reefs to enhance denitrification may be used as a tool for water quality management to combat anthropogenically derived eutrophication because this process is a sink for reactive nitrogen in the coastal zone. However, the habitat context of the reef affects denitrification, and mudflat-reefs will remove more anthropogenic nitrogen than reefs set in other contexts. Comparing how factors such as reef properties (e.g. oyster density, elevation) and habitat context impact the delivery of ecosystem services relative to reference areas without reef habitat aids in identifying restoration criteria that can maximize the delivery of societal benefits (Palik *et al.* 2000).

Although rates of denitrification were higher in the reefs compared to the controls, denitrification efficiency measured in this study was similar for all habitat contexts. Denitrification efficiency does not indicate high rates of denitrification, but rather the percentage of nitrogen removed as N_2 gas relative to the total amount of nitrogen delivered back to the water column. Efficiencies from reefs were similar to those of sediments from natural oyster reefs in North Carolina (Piehler & Smyth 2011), but 60–80% higher than the efficiencies associated with restored oyster reefs in Maryland (Kellogg *et al.* 2013). In this study, controls and reefs were equally as efficient at removing nitrogen, despite differences in NO_x and NH_4^+ fluxes. All habitats had an increase in ammonium production after the addition of nitrate to the water column, which led to a decrease in denitrification efficiency. The consistency of denitrification efficiency across the habitats and lack of difference between reefs and controls in any context suggest that even though oyster reefs are recycling nutrients, oyster reefs are denitrifying at least as efficiently as the controls.

Alteration of temperate coastal ecosystems as a result of increasing urbanization of coastal watersheds is accelerating (Diaz & Rosenberg 2008). Two drivers of estuarine ecosystem degradation are nitrogen enrichment and loss of biogenic habitats (Diaz & Rosenberg 1995, 2008; Paerl *et al.* 1998). Oyster reefs are among the most threatened habitats in marine ecosystems due to a variety of factors including over-harvesting, disease and degraded water quality (Lenihan & Peterson 1998; Jackson *et al.* 2001; Beck *et al.* 2011; Zu Ermgassen *et al.* 2012). Increased nutrient loading and the reduction in the natural grazing pressure by suspension-feeding bivalves have dramatically changed estuarine ecosystem functioning. Increasing oyster biomass in a system is often suggested as a management strategy to improve water quality (Bricker, Rice & Bricker 2014). However, we demonstrated that environmental factors associated with location (i.e. habitat context, tidal height, region) and reef properties (i.e. the density of adult oysters) will affect the recovery of

reef-associated services such as removal of anthropogenically derived nitrogen in estuaries by promoting denitrification, especially during periods when nitrate loading is high. Our results suggest that reefs restored on tidal mudflats will achieve the greatest nitrogen removal benefit during ambient and elevated nitrate conditions because these reefs have higher adult oyster densities. Meanwhile, the nitrogen removal benefits derived from oyster reef restoration efforts in salt marsh or seagrass habitat contexts were 4% lower than those of mudflat-reefs under ambient nitrate and 27–44% lower under elevated nitrate levels. This reduced benefit is likely a result of lower oyster densities and because oyster reefs in the two vegetated habitats are proximal and potentially functionally redundant to marsh and seagrass habitats that also enhance denitrification (Piehler & Smyth 2011; Smyth *et al.* 2013). While recent studies have demonstrated that oysters are able to remove nutrients either through assimilation (Carmichael *et al.* 2012) or enhanced denitrification through production and accumulation of biodeposits (Kellogg *et al.* 2013; Smyth, Geraldi & Piehler 2013; Smyth *et al.* 2013), the magnitude of this effect is dependent on habitat context. Increased nitrogen removal via denitrification during periods of elevated nitrate loading coupled with the accumulation of organic matter associated with oyster reefs provides further evidence that oyster reefs can reduce anthropogenic nitrogen from estuarine systems.

Efforts to measure the delivery of ecosystem services from habitats such as oyster reefs will benefit from studies that investigate how habitat context and ecosystem processes influence reef properties and functioning. We found that many aspects of reef-mediated sediment fluxes are influenced by the habitat context of an oyster reef. Modification of nitrogen cycling is just one of many reef functions that are affected by habitat context. For instance, similar to the effects of reefs on denitrification, oyster reef augmentation of finfish and crustacean production is attenuated when oyster reefs are adjacent to structured habitats (Grabowski *et al.* 2005; Geraldi *et al.* 2009). We found that oyster density is strongly and positively correlated with denitrification rates except at extremely high densities. Studies have demonstrated that habitat context and reef elevation, which is positively correlated with oyster density, are important determinants of overall reef growth and persistence, with reefs located on flats that are isolated from vegetated structure achieving high growth rates and easily outpacing rises in sea level (Rodriguez *et al.* 2014). Collectively, these results suggest that habitat context affects ecosystem processes and should be considered when siting future intertidal oyster reef restoration projects. Moreover, developing a more comprehensive framework of how ecosystem functions are influenced by landscape- and ecosystem-scale characteristics and processes will enhance the efforts of resource managers to maximize service delivery and consequently the return on investment from restoration activities.

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Data accessibility

Data used in this manuscript are available through Dryad: doi: <http://dx.doi.org/10.5061/dryad.040mt> (Smyth, Piehler & Grabowski 2015)

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Supporting Information

Additional Supporting Information may be found in the online version of this article.

Data S1. R code for statistical analysis.