Vol. 560: 173–183, 2016
doi: 10.3354/meps11922

MARINE ECOLOGY PROGRESS SERIES
Mar Ecol Prog Ser

Published November 24

Biological activity exceeds biogenic structure in influencing sediment nitrogen cycling in experimental oyster reefs

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ABSTRACT: Oysters are estuarine ecosystem engineers, in that their physical structure and biological function affect ecosystem processes such as organic matter and nutrient cycling. Oysters deliver material to the sediments through biodeposition and sedimentation caused by modification of flow around the reef. We conducted an experiment to distinguish between biotic effects and physical structure of oyster reefs on sediment nitrogen cycling. Experimental reefs consisting of live oysters, oyster shells alone and mudflats (controls) were sampled for a period of 4 wk for sediment organic matter, C and N content and fluxes of nitrogen (NH_4^+, NO_X and N_2) and oxygen (O_2). We hypothesized that the biological activity of the oyster would deposit more, higher quality organic matter compared to deposition from flow modification alone, thus facilitating denitrification and having a larger impact on sediment nitrogen cycling. Compared to the controls, the live oyster experimental reefs increased sediment denitrification by 61% and the shell experimental reefs showed a 24% increase. The live oyster experimental reef also had the largest O_2 demand and NH_4^+ production. Reef structure likely increased organic matter deposition, but the higher quality and larger quantity of organic matter associated with live oysters increased denitrification and microbial respiration. This experiment shows that the ecosystem service of nitrogen removal provided by oysters is primarily driven by the biological function of the oysters and secondarily from the physical structure of the reef. Our increased understanding of how oysters engineer ecosystems and modify nutrient cycling can help guide future oyster restoration efforts.

KEY WORDS: *Crassostrea virginica* · Nitrogen · Denitrification · Oyster reefs · Biogenic habitat · Ecosystem engineers

INTRODUCTION

Many processes in ecosystems are driven by interactions between organisms and their environment. Ecosystem engineers directly and indirectly modify, create or maintain habitat (Jones et al. 1994). These organisms can also influence biogeochemical processes through biotic production or abiotic modification of resources to the microbial community (Gutierrez & Jones 2006, Jones et al. 2006, Mermillod-Blondin 2011). Such engineering in the environment can have a profound influence on ecosystem functioning.

The eastern oyster *Crassostrea virginica* is one example of an ecosystem engineer in estuaries that provides valuable ecosystem services (Coen et al. 2007, Grabowski et al. 2012). Oysters create biogenic reefs, which serve as habitat and shelter from predation for other organisms. Additionally, the reef structure in-

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creases transport of material and energy from the water column to the benthos (Lenihan 1999). Oysters also provide a water quality benefit through filtration (Grizzle et al. 2008, Zu Ermgassen et al. 2013), consuming seston and other particulate material in the water column as they feed. A portion of the nitrogen (N) and carbon (C) in this material is assimilated into biomass, while the undigested and unassimilated fraction is egested and transferred to the sediment surface as feces and pseudofeces, collectively biodeposits (Haven & Morales-Alamo 1966, Dame et al. 1984, Newell 2004). This action cleans the water and also has the potential to modify the benthic nitrogen cycle (Newell et al. 2002, Kellogg et al. 2014).

Oyster reef ecosystems have high rates of N_2 production from denitrification relative to areas without oyster reefs (Piehler & Smyth 2011, Kellogg et al. 2013, Hoellein et al. 2015, Smyth et al. 2015). Enhanced denitrification has been attributed to oyster-mediated benthic–pelagic coupling (Newell et al. 2005, Smyth et al. 2013). Denitrification primarily occurs when O_2 concentration is low, NO_3^-, the electron acceptor, is available, and there is sufficient supply of labile carbon or another electron donor (Seitzinger et al. 2006). The deposition of biodeposits on the sediment surface can modify the availability of oxygen, NO_3^- and carbon and create conditions favorable for denitrification (Newell et al. 2005, Kellogg et al. 2014). Additionally, excretion from oysters of dissolved organic and inorganic nitrogen into the water column can increase NH_4^+ concentration, which can be oxidized to NO_2^- and NO_3^- via nitrification; subsequently, NO_3^- can be reduced to N_2 gas via denitrification or anaerobic NH_4 oxidation to N_2 (anammox). Biodeposition may also fuel microbial metabolism to the extent that increased O_2 demand for mineralization decreases nitrification and enhances NH_4 production.

While oyster-mediated benthic–pelagic coupling affects sediment nitrogen cycling, the physical structure of an oyster reef also contributes to organic matter accumulation by reducing water velocity and increasing sedimentation (Lenihan 1999, Lenihan et al. 2001, Falcão et al. 2007). Reef structure also provides habitat for bioturbating organisms (Tolley & Volety 2005, Oakley et al. 2014). Bioturbation can increase the delivery of oxygen to the sediment and enrich sediments with NH_4^+ from excretion by infaunal organisms (Mermillod-Blondin & Rosenberg 2006). Given the links between denitrification, nitrification, oxygen and organic carbon quantity and quality (Eyre & Ferguson 2009, Eyre et al. 2013, Fulweiler et al. 2013), it is likely that the combination of both biological and physical aspects of oyster reefs contribute to elevated rates of denitrification.

The goal of this study was to determine the effects of oyster feeding activity versus physical reef structure on sediment nitrogen dynamics. Based on our understanding of the factors that affect nitrogen regeneration and removal, we hypothesized that the benthic–pelagic coupling facilitated by oyster feeding activity should contribute to a larger portion of sediment net N_2 production than the reef structure alone. To test the effects of reef structure and oyster function on sediment nutrient dynamics, sediment fluxes of N_2, O_2, combined NO_3^- and NO_2^- (NO_x) as well as NH_4^+ were measured within experimental plots containing constructed oyster reef with either live oysters, oyster shell or sediment controls. Sediment organic matter (SOM), C and N content and benthic algal biomass were also quantified.

MATERIALS AND METHODS

Study site

This experiment was conducted at Hoop Hole Creek, in Bogue Sound, NC, USA (34.422° N, 76.455° W), which is located on the sound side of a barrier island. The area contains natural and restored oyster reefs (O'Connor et al. 2008). Water quality in Hoop Hole Creek is generally good and the creek is closed to shellfish harvesting. Our experiment was set up on an intertidal mudflat located approximately 25 m away from the edge of a fringing salt marsh and nearby oyster reefs.

Experimental design

Clusters of live oysters *Crassostrea virginica* were collected from an intertidal reef in Calico Creek, NC (34.728° N, 76.722° W), at low tide on 28 June 2010. Oyster clumps were transported back to The University of North Carolina Institute of Marine Sciences (UNC-IMS). Oyster clumps were haphazardly placed in eight 19 l plastic buckets, and each bucket was assigned to 1 of 2 groups. Oysters in 4 of the buckets were kept alive in continuously flowing tanks with unfiltered water from Bogue Sound and exposed to natural light irradiation. The remaining 4 buckets of oysters were left out of water and exposed to the sun and terrestrial scavengers (primarily ants). This resulted in the removal of the oyster meat and the majority of all organic material while maintaining the

structure of the oyster clumps. Prior to setting up the field experiment, all oysters (>10 mm) were counted, and 50 from each bucket were measured for size distribution.

Experimental reefs made of live oysters or oyster shell were constructed at Hoop Hole Creek to test the effects of reef structure and oyster biological function on sediment nitrogen dynamics. On 16 July 2010 at low tide an intertidal mudflat was divided into twelve 0.56 m^2 plots (2 rows of six 0.75 × 0.75 m plots), each separated by 3 m, or 4 times the width of each plot. Plots were haphazardly assigned an experimental treatment: live oysters, oyster shell, or sediment control (mudflat without oysters) (see Fig. S1 in the Supplement at www.int-res.com/articles/suppl/m560p173_supp.pdf). Constructed reefs were similar in size to the patchy natural oyster reefs commonly found in this area of North Carolina (Macreadie et al. 2012, Geraldi 2015). Oyster reefs (live or shell) had a density of 123 ± 11 individuals per plot (0.56 m^2; mean shell height 37.1 ± 4.21 mm). Clumps of live oysters or oyster shell were positioned in an upright orientation in the water column to mimic a natural and fully functioning oyster reef. A 10 × 10 cm area was left open near the center of each plot for sediment samplings. Plots were checked every 3 d to ensure structure was maintained and repositioned if necessary. Sediment samples were collected from bare area near the center of each plot to quantify O_2, N_2 and dissolved inorganic nitrogen (DIN) fluxes, SOM and sediment C and N content 2 and 4 wk after construction. Samples for sediment properties such as porewater nutrients (DIN collected with a sipper) and benthic algal biomass (chlorophyll *a* [chl *a*]) were collected at the 2 wk sampling only (details below). A 2 wk period was selected to allow time for the sediments to stabilize prior to sampling for biogeochemical analysis (Porter et al. 2006). The experiment was terminated after 4 wk due to wild oyster recruitment on the treatments, which compromised the shell treatment. Water temperature, dissolved oxygen and salinity were measured during each sampling (YSI 600 Series Sonde and Model 650 data logger).

Exchanges at the sediment–water interface

At each plot, 1 sediment core (6.4 cm diameter by 17 cm depth) was collected from bare area near the center of the plot for use in continuous-flow core incubations designed to measure exchanges across the sediment–water interface (Lavrentyev et al. 2000, McCarthy & Gardner 2003). The same plot was sampled during both the 2 and 4 wk sampling events. Sediment cores were extracted by hand and did not contain live oysters or oyster shells. Site water (75 l) for use in the continuous-flow incubation and cores were immediately (<1 h) transported to an environmental chamber at UNC-IMS, set to *in situ* (30°C) temperature. Dark conditions were maintained throughout the course of the incubation to reduce the effects of photosynthetic algae (An & Joye 2001) and to prevent the formation of bubbles that would affect gas concentrations (Reeburgh 1969). Sediment cores were submerged in a water bath and sealed with gastight lids equipped with inflow and outflow ports and connected to a peristaltic pump. Unfiltered site water (37 ppt) was continuously pulled over the cores at a flow rate of 1 ml min^{-1} (Piehler & Smyth 2011). Site water was aerated to maintain oxygenated conditions in the water entering the cores.

Following a 24 h pre-incubation period, water samples were collected for dissolved gas and nutrient analysis from the outflow port of each core and from a bypass that flowed directly into a sample vial to measure the inflow concentration. Dissolved gases were analyzed immediately after collection 3 times over a 48 h period (24, 30 and 48 h) to ensure that steady-state conditions were achieved. Average outflow oxygen concentration was 68.9 ± 5.34 µM at the end of 48 h. Samples for dissolved nutrient analysis were collected approximately 24 h after the incubation began, filtered through Whatman GF/F glass microfiber filters (25 mm diameter, 0.7 µm nominal pore size) and frozen until analysis.

Concentrations of dissolved gases were measured using a Balzers Prisma QME 200 quadrupole mass spectrometer (MIMS; Pfeiffer Vacuum, Nashua, NH, USA). Concentrations of O_2 and N_2 were determined using the ratio with Ar (Kana et al. 1994). Nutrient samples were analyzed with a Lachat Quick-Chem 8000 automated ion analyzer for NO_x (combined NO_3^- + NO_2^-) and NH_4^+ concentrations using standard protocols (Lachat Instruments: NO_3^-/NO_2^- [collectively NO_x^-] Method 31-107-04-1-A; NH_4^+ Method 31-107-06-1-A; detection limits: 0.02 µM NO_x, 0.36 µM NH_4^+).

Flux 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 (measured from the bypass line), f is the flow rate (0.06 l h^{-1}), and a is the sediment surface area

(0.0032 m^2). A positive flux represents production from the sediment, while a negative flux is uptake by the sediment. Fluxes of oxygen directed into the sediment were considered rates of sediment oxygen demand (SOD). This technique determines a net N_2 flux, which is calculated as the difference between gross denitrification and gross nitrogen fixation, and does not discern whether N_2 is produced through denitrification or anammox. We consider net positive N_2 fluxes to be denitrification.

Successive measurements from each core were averaged to give core specific values. Denitrification efficiency (%), the relative amount of mineralized nitrogen removed via denitrification, was calculated as follows:

$$\text{Denitrification efficiency} = \frac{N - N_2}{\sum NO_X + NH_4^+ + N - N_2} \times 100 \quad (1)$$

Sediment properties

Additional samples were collected at the 2 wk sampling only for analysis of porewater nutrients and benthic algal biomass (sediment chl *a*). Samples for sediment C and N content and SOM were collected at the 2 and 4 wk samplings. Porewater was collected using sipper samplers (McGlathery et al. 2001), positioned to collect water in the pore space from 1 to 6 cm depth. Porewater samples were filtered through Whatman GF/F glass microfiber filters (25 mm diameter, 0.7 µm nominal pore size) and frozen until analysis for DIN (NO_x and NH_4^+) as described above. Samples for sediment chl *a* (0.5 cm^2 surface area, 1cm depth) were placed in polypropylene centrifuge tubes with 10 ml of 45:45:10% methanol:acetone:water solvent, sonicated over ice for 30 s and extracted at –18°C for 18 h. Chl *a* (a proxy for benthic microalgal biomass) in the upper 1 cm of sediment was determined using spectrophotometry (Lorenzen 1967, modified by Pinckney et al. 1994). This analysis did not account for phaeopigments. Sediment C and N content was assessed in the upper 5 cm. Sediments for C and N measurements were dried at 70°C, ground with a mortar and pestle, fumed for 48 h with 1 N HCl to remove inorganic C and dried again. Fumed sediment samples were analyzed for organic C and N content with a Perkin Elmer CHN analyzer (Model 2400 Series II) standardized with acetanilide. SOM was quantified in the upper 2 cm of sediment. Sediments were dried for 24 h at 60°C and then combusted at 525°C for 4 h. The difference between dried and combusted samples constituted SOM and is expressed as a percentage of the total.

Statistical analysis

Statistical analyses were performed using R 2.13.1 (R Development Core Team 2011). Linear mixed effects models (*lme* in the R *nlme* package) were used to investigate the effects of treatment on fluxes of N_2, NO_x, NH_4^+, denitrification efficiency, SOD, SOM and C:N. Because sampling time may be a source of variation, time was nested within experimental plot as a random variable. A mixed-effects model where plot number was included as a random variable was used to examine the effects of treatment on benthic algal biomass and porewater nutrients, which were only assessed at the 2 wk sampling. When effects were significant, Tukey's HSD post-hoc test was used to determine differences between the treatments. The relationships between fluxes and sediment properties were analyzed using Spearman rank correlations and linear regression (*corrplot* in *R corrplot* package). Assumptions of homogeneity were tested using Levene's tests. Normality was assessed by visually inspecting the Q-Q plots. Results were considered statistically significant at $\alpha = 0.05$.

RESULTS

Flux experiment

Water temperature (30°C) and salinity (37 ppt) were the same for both sampling events. Dissolved oxygen was 4.5 mg l^{-1} at the 2 wk sampling and 3.8 mg l^{-1} at the 4 wk sampling. NO_x concentration in the site water used for the continuous flow incubations was 0.05 µM at the 2 wk and 1.16 µM at the 4 wk samplings. NH_4^+ concentration increased from 0.79 µM at the 2 wk sampling to 1.10 µM at the 4 wk sampling.

All treatments had net positive N_2 fluxes, suggesting denitrification occurred in excess of nitrogen fixation. Denitrification (net positive N_2 flux) was significantly different between treatments (Fig. 1). Sediments from the live oyster treatment had higher denitrification compared to the shell and control treatments. Denitrification was lowest for the control treatment (167 ± 17.5 µmol $N\text{-}N_2$ m^{-2} h^{-1}) and highest for the live oyster treatment (269 ± 29.4 µmol $N\text{-}N_2$ m^{-2} h^{-1}). The live oyster treatment increased denitrification over the control by 61%. The shell treatment had a 24% increase in denitrification compared to the control, but this increase was not significant. SOD was also significantly different between treatments (Fig. 2). The control treatment had the lowest SOD (1240 ± 272 µmol O_2 m^{-2} h^{-1}), and the live oyster treatment had the

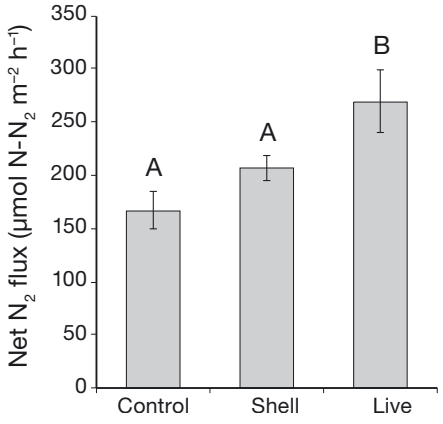

Fig. 1. Net N_2 flux for each treatment. A positive flux indicates denitrification in excess of nitrogen fixation. Significant treatment differences are denoted with different letters ($p < 0.05$). Data are mean ± SE (n = 8)

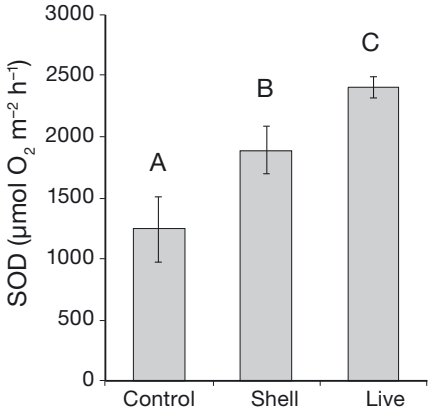

Fig. 2. Sediment oxygen demand (SOD) for each treatment. Significant treatment differences are denoted with different letters ($p < 0.05$). Data are mean ± SE (n = 8)

highest SOD (2400 ± 60.8 µmol O_2 m^{-2} h^{-1}), with the shell treatment having an intermediate rate of SOD (1890 ± 138 µmol O_2 m^{-2} h^{-1}).

There was an effect of treatment on NH_4^+ flux but not on NO_x flux. Sediments from all experimental treatments consumed NO_X during both sampling events (Table 1), while NH_4^+ efflux was evident from all treatments (Table 1). The live oyster treatment had the highest rate of NH_4^+ production but was only significantly greater than the control. On average, there was more NH_4^+ production for the shell treatment compared to the control, but this difference was not significant. Overall, NO_x demand and NH_4^+ production were higher for the live oyster treatment than the shell and control treatments. However, NO_x fluxes were not different from zero for any of the treatments.

Average denitrification efficiency based on efficiency from each core ranged from 53.9% for the live oyster treatment to 89.8% for the control (Table 1). The control treatment was more efficient at denitrification compared to the live oyster treatment. The shell treatment, which had an intermediate denitrification efficiency, was statistically similar to both the control and live oyster treatments.

Denitrification was enhanced in the live oyster treatment relative to the sediment control. The contribution of reef structure and biological activity of the oysters to this enhancement was calculated based on absolute differences in denitrification rates between our treatments. The contribution of structure was determined by subtracting the mean denitrification at control from the denitrification at shell treatment. The effect of oyster biological activity was calculated by subtracting the calculated contribution of structure from the enhanced denitrification at the live oyster treatment. Based on these calculations, the enhancement in denitrification at our experimental oyster reefs was 39% attributed to the 3-dimensional structure of the reef and 61% to the biological function of the oyster.

Table 1. Experimental mean fluxes of NO_x and NH_4^+ across the sediment–water interface and denitrification efficiency for each treatment. A positive flux for NO_x and NH_4^+ indicates a flux from the sediment to the water column, and a negative value indicates a flux from the water column to the sediment. Data are mean ± SE (n = 8). Significant treatment differences are denoted with different letters ($p < 0.05$)

	NOx flux (µmol N m^{-2} h^{-1})	NH_4^+ flux (µmol N m^{-2} h^{-1})	Denitrification efficiency (%)
Control	−1.670 ± 3.79	22.13^{A} ± 18.11	89.79^{A} ± 6.78
Shell	−7.500 ± 4.73	101.4^{AB} ± 70.76	76.72^{AB} ± 14.03
Live	−10.53 ± 4.87	499.9^{B} ± 294.7	53.90^{B} ± 14.67

Sediment properties

Sediment C:N was similar between treatments; however, there was a trend for lower C:N in the live oyster treatment compared to the control and shell treatments. C:N ranged from 9.58 ± 0.26 in the live oyster treatment to 10.9 ±0.36 in the shell treatment (Fig. 3). While there was no difference in the sediment C:N ratio between the treatments, there was a significant effect of treatment on both C and N content. Sediments from the live oyster treatment had significantly more C and N compared to the

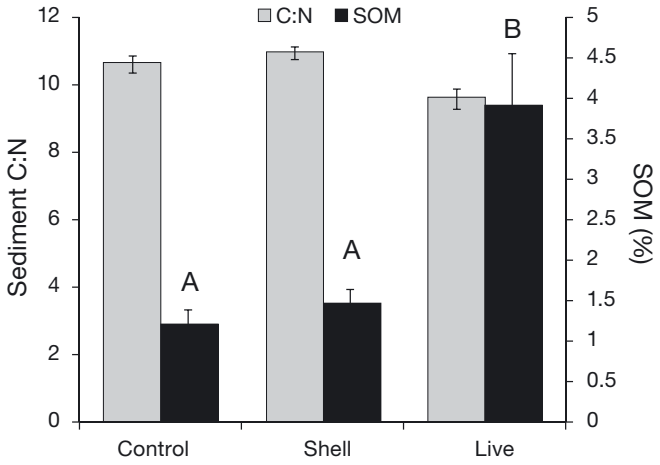

Fig. 3. Sediment carbon:nitrogen ratios and sediment organic matter (SOM) for each treatment. Treatments with different letters are significantly different ($p < 0.05$). Data are mean ± SE (n = 4)

shell and control treatments, which were similar to each other. SOM was also significantly higher for the live oyster treatment relative to the control and shell treatments, which had similar SOM contents (Fig. 3).

Benthic microalgal biomass (chl *a*) and porewater nutrient samples were collected after 2 wk only (Table 2). The level of DIN in porewater was highest for the live oyster treatment, lowest for the control and intermediate for the shell treatment, although the treatment differences were not significant. NH_4^+ constituted 98.9, 96.1 and 99.6% of the porewater DIN pool for the control, shell and live oyster treatments, respectively. Benthic microalgal biomass was also higher for the live oyster treatment compared to the shell and control treatments, although this difference was not significant.

Denitrification was significantly and positively correlated with SOD (Fig. 4a) and SOM (Fig. 4b). The relationships between SOD and SOM explained 35 and 48% of the variation in N_2 fluxes, respectively. The observed positive relationship between denitrification and SOM were primarily driven by 2 samples from the live oyster treatments. Denitrification was also correlated with C and N content of the sediment (Table 3). NH_4^+ fluxes were correlated with SOM and SOD, as well as with sediment C and N (Table 3), suggesting mineralization of organic material in the sediment was the source. NO_x fluxes were not correlated with other fluxes or sediment characteristics.

Table 2. Mean porewater DIN ($NO_x + NH_4^+$) concentration and benthic chlorophyll biomass for each plot. NH_4^+ constituted >96% of the porewater DIN pool. Data are mean ± SE (n = 4). There was no difference between the treatments for either of these parameters

	Porewater DIN (µM)	Benthic chlorophyll (mg m^{-2})
Control	46.19 ± 15.28	65.18 ± 6.80
Shell	76.97 ± 14.51	51.17 ± 17.13
Live	130.18 ± 43.60	88.96 ± 17.14

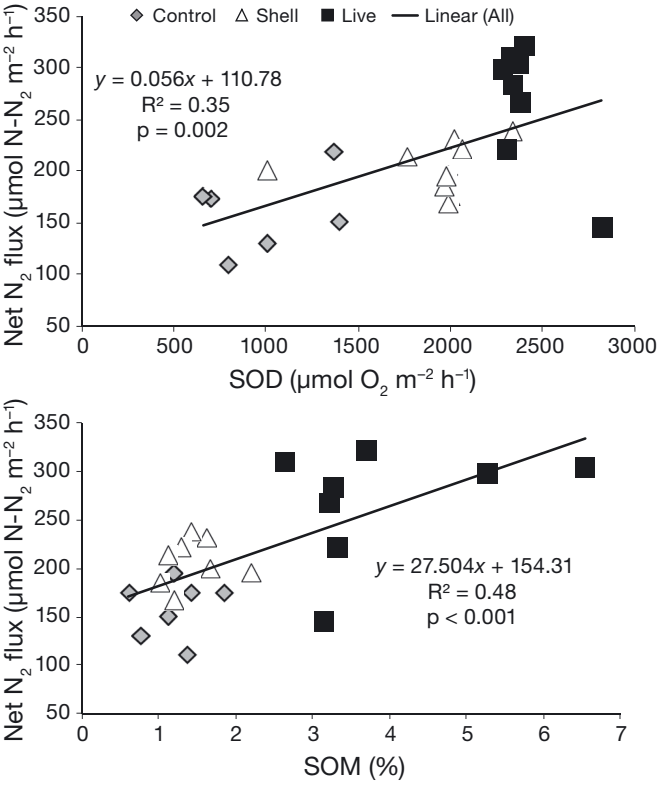

Fig. 4. Net sediment N_2 flux as a function of (a) sediment oxygen demand (SOD) and (b) sediment organic matter (SOM) for each treatment during each sampling period

Table 3. Spearmann correlation coefficient matrix of sediment C, N, C:N, sediment organic matter (SOM) and fluxes of N_2, sediment oxygen demand (SOD), NO_x and NH_4^+ for all treatments. Significant correlations ($p = 0.05$) in **bold**

	C	N	C:N	SOM	N_2	SOD	NO_x
C							
N	**0.99**						
C.N	−0.16	−0.28					
SOM	**0.87**	**0.88**	−0.22				
N_2	**0.7**	**0.7**	**−0.18**	**0.69**			
SOD	**0.52**	**0.54**	−0.19	**0.56**	**0.59**		
NO_x	−0.27	−0.27	0.27	−0.12	−0.28	−0.24	
NH_4^+	0.35	0.39	−0.23	**0.72**	0.38	**0.46**	0.24

DISCUSSION

In this experiment, the biological activity of oysters had a greater influence on sediment nitrogen cycling, SOD, SOM and sediment C and N content than the physical structure of our experimental reefs. Benthic–pelagic coupling by oysters has a clear influence on sediment biogeochemistry (Newell 2004, Smyth et al. 2013), and oyster reef ecosystems tend to have enhanced denitrification relative to control sites without reefs (Kellogg et al. 2014). Our experiment was focused on understanding the effects of the oysters' physical structure and biological activity on sediment nitrogen cycling. We found the live oyster treatment increased denitrification over the control by 61%. Of this increase, 39% was due to allochthonous inputs from the reef structure, and 61% was attributable to production and accumulation of oyster derived material. This result suggests that actively filtering oysters contribute more to the enhancement in denitrification found in our experimental reefs than 3-dimensional reef structure. Although the shell treatment did not significantly enhance denitrification, there was a 24% increase in denitrification compared to the control, suggesting that structure alone contributed to a portion of sediment denitrification in the live oyster reef treatment. Distinguishing between the effects of the physical reef structure and oyster feeding provides insight into the direct and indirect effects of oysters on sediment nutrient cycling.

Nitrogen dynamics in our experimental plots were mainly influenced by changes in SOM. In oligotrophic systems, oysters modify the supply of organic matter to the sediment and stimulate carbon- and nutrient-limited processes, such as coupled nitrification–denitrification. The supply of organic matter may help prime oyster reef sediments to increase denitrification in response to nutrient pulses during storm events (Smyth et al. 2015). In eutrophic systems, nitrification can be decoupled from denitrification (Seitzinger et al. 2006), and nitrogen from oyster biodeposits will likely be regenerated as NH_4^+ through enhanced mineralization (Hoellein & Zarnoch 2014). Increased biodeposition from live oysters increases SOD, which may inhibit nitrification, further contributing to NH_4^+ flux. Therefore, eutrophication would likely result in enhanced denitrification of water-column NO_3^- but depressed coupled nitrification–denitrification in the sediment. In eutrophic systems characterized by high levels of chlorophyll in the water column, the water quality benefits of oysters are often linked to filtration and improved water clarity (Officer et al. 1982, Cerco & Noel 2007, Coen et al. 2007). Under these conditions, the presence of live oysters would be more important than reef structure in mitigating the impacts of eutrophication. Studies in oligotrophic and eutrophic systems are needed to gain a better mechanistic understanding of how oyster-mediated denitrification is affected by water quality.

The effects of oysters on the sediment nitrogen cycle will depend on the environmental characteristics of the site. Factors such as residence time, water-column NO_3^- concentration, salinity, temperature and microbial community composition can affect sediment nitrogen cycling properties (Nixon et al. 1996, Seitzinger et al. 2006, Lisa et al. 2015). Moreover, aspects of the oyster reef such as location in the tidal prism (Byers et al. 2015), relief (Gregalis et al. 2008), habitat context and oyster density (Smyth et al. 2015) may also contribute to differences in sediment metabolism. In our experiment, the 3-dimensional structure contributed to enhanced denitrification, but the activity of the oysters had the greatest effect on sediment nitrogen dynamics. Because these effects were also influenced by reef size, oyster density and duration of experiment, expanded research is important to increase our understanding of the interaction between oyster restoration and nutrient dynamics.

The physical structure and biology of the oysters enhances the amount and quality of sediment organic matter. Biodeposits, which are positively correlated with oyster density, increase the organic and fine-particle content of the sediment (Haven & Morales-Alamo 1966, Widdows et al. 1998, Newell et al. 2002, Thrush et al. 2006, O'Connor et al. 2008). While wave action and tidal currents may disperse biodeposits, the structure reduces water velocity and increases sedimentation of these particles on and adjacent to the oyster reef structure (Lenihan 1999, Widdows & Brinsley 2002, Pietros & Rice 2003). The structure, therefore, helps to trap material that would otherwise distribute diffusely. Additionally, the reef structure provides habitat for infaunal and epifaunal organisms (Tolley & Volety 2005, Humphries et al. 2011, Byers & Grabowski 2013, Oakley et al. 2014), and oyster shells serve as substrate for microbial biofilms (Svenningsen et al. 2012). Although our experimental reefs likely provided microbial substrate and faunal habitat, sediment cores did not contain live oysters or oyster shell, so differences observed between live and shell treatments are not likely to stem from oyster reef-associated organisms. The presence of live bivalves (the Pacific oyster and the blue mussel) compared to shells has previously been shown to

affect organic content of the sediments but not infauna (Norling et al. 2015).

The increase in SOD and higher NH_4^+ production for live and shell treatments suggest enhanced organic matter deposition affected material processing compared to unstructured habitat. SOD may be used as a proxy for carbon quality, where a higher SOD indicates greater organic matter mineralization and more organic carbon loading to the sediments (Borsuk et al. 2001, Ferguson et al. 2003, Ferguson & Eyre 2013). Based on the measured SOD, the live oyster treatment had the greatest rate of organic matter loading and processing, while structure associated with the shell treatment also had an effect relative to the control. These data suggest that the 3-dimensional structure and properties of the oysters increase sediment metabolism. The standing stock of carbon was similar between the control and shell treatments, likely due to rapid utilization of the labile carbon fraction for both treatments. We also observed a positive relationship between SOD and net N_2, which has previously been observed in coastal sediments (Seitzinger & Giblin 1996, Fennel et al. 2009, Piehler & Smyth 2011). This relationship can vary by habitat and sediment type and is driven by the type of organic matter and the oxygen penetration depth (An & Joye 2001, Eyre et al. 2013).

The low level of water-column NO_3^- found in this study system suggests that denitrification is coupled to nitrification. While we did not measure nitrification directly, it can be calculated as the sum of the NO_x and N_2 fluxes (Gonzalez et al. 2013, Kellogg et al. 2013). Calculated nitrification was 165.05 ± 13.0 µmol N m^{-2} h^{-1} in the control, 199.22 ± 7.04 µmol N m^{-2} h^{-1} in the shell treatment and 276.15 ± 4.62 µmol N m^{-2} h^{-1} in the live oyster treatment. Comparing nitrification rates to denitrification (net N_2 production), nitrification represents the main source of NO_3^- for denitrification in all treatments, accounting for >96% of denitrification. A likely explanation is that the deposition and subsequent mineralization of biodeposits supplies NH_4^+ to nitrifying bacteria, leading to enhanced nitrification–denitrification when biodeposition occurs on aerobic sediments (Newell et al. 2005).

Treatments were net sources for reduced nitrogen (NH_4^+). Fluxes of oxidized nitrogen (NO_x) were directed into the sediment; however, these fluxes were not different from zero, and NO_x concentrations were near the detection limit. SOD and SOM were significantly and positivity correlated with NH_4^+ efflux, suggesting that a portion of the NH_4^+ production was due to mineralization of organic matter (Tobias et al. 2003). The positive relationship between sediment C and N content, SOM and SOD further supports this conclusion. It is possible that the shell structure increased habitat for infaunal organisms (Waldbusser et al. 2004, Humphries et al. 2011) and bioturbation created microzones for nitrification (Rysgaard et al. 1995), resulting in an increased demand for oxygen; however, we did not assess infaunal community or O_2 profiles in the experimental plots or cores.

Sediments from bivalve systems are often associated with high concentrations of NH_4^+ in sediment and fluxes of NH_4^+ to the water column, which may support primary production (Dame et al. 1984, 1989). Despite this, oyster-mediated denitrification can still be a sink for nitrogen. Although rates of denitrification were higher for the live oyster treatment than for the shell or control treatments, denitrification efficiency was not. The highest efficiency was associated with the control treatment because of the low total DIN efflux. Denitrification efficiency for the live oyster treatment was lower than efficiencies from sediments of natural reefs (Piehler & Smyth 2011). However, the efficiency was similar to experiments containing pieces of subtidal oyster reefs in Chesapeake Bay (Kellogg et al. 2013). The DIN efflux may have been enhanced in the live oyster and oyster shell treatments because of direct excretion from the benthic community and increased mineralization of organic matter. In our experiment, denitrification relied on NO_3^- from nitrification; however, deposition of organic matter and subsequent mineralization consume O_2 and lead to reduced conditions that limit nitrification. This results in NH_4^+ release to the water column and decreased denitrification, which lowers the sediment denitrification efficiency.

This study used experimental oyster reefs to answer questions about the role of both the physical structure and biology of oysters in modifying sediment nitrogen fluxes. Our sample size of 4 replicates per treatment was similar to or higher than previous studies examining nitrogen dynamics. The 4 wk duration was necessary for maintaining the shell-only treatment (oyster recruitment was evident after 4 wk). The reef treatment size was similar to some natural, intertidal oyster reefs found in this area (Macreadie et al. 2012), and similar sized reefs have been used to investigate the role of oyster reefs in coastal ecosystems (Grabowski et al. 2005, O'Connor et al. 2008, Kimbro et al. 2014, Geraldi 2015). Although our experimental reefs were not identical to natural reef ecosystems, this design allowed us to discern the contribution of biological and physical characteristics of oyster reefs on denitrification

locally. Reef size and age may affect biogeochemical processes, and further investigation of these factors is needed.

Oyster reef restoration often begins with additions of hard substrate to initiate oyster recruitment and provide habitat for mobile fauna. This initial step in restoration is analogous to the shell-only treatment in our experiment. Based on results from this experiment, the addition of the complex structure to the sediment can have many benefits, including increased nitrogen removal through enhanced denitrification and presumably increased habitat for infauna and mobile fish (Grabowski et al. 2005, Humphries et al. 2011). These benefits of adding hard substrate will likely increase with greater reef complexity and height off the benthos. A recent study comparing the effects of oyster reefs and oyster aquaculture on denitrification found whole reefs had higher rates of nitrogen removal than off-bottom aquaculture (Humphries et al. 2016). These differences were attributed to enhanced biodeposition from the oysters but also to the reef structure. Our study supports this conclusion and highlights the role both reef structure and oyster function have on sediment nitrogen dynamics.

CONCLUSIONS

Oysters influence sediment nitrogen dynamics through alteration of material deposition from reef structure and production of biodeposits. Increased nitrogen removal is a valuable benefit of oyster reefs; however, the difficulty associated with measuring denitrification and the complexity of oyster reefs have made it challenging to incorporate this benefit into restoration management plans. Although this was an experiment, our results provide new information on mechanisms through which oyster filtration and reef structure modify sediment processes.

A basic goal of conservation and restoration of oyster reefs is to maintain viable reefs with abundant oysters. This will also result in enhanced denitrification and facilitate control of eutrophication through oysters. While the 3-dimensional structure of oyster reefs supports many ecosystem functions, the results of our experiment suggest that the water quality benefits associated with oyster reefs are primarily driven by the biological activity of the oyster. By extension, oyster reefs with low densities of live oysters, possibly due to disease or predation, will have limited effects on nitrogen cycling but may increase organic matter deposition and provide habitat (Norling et al. 2015). The impact of oyster function and reef structure on sediment biogeochemistry exemplifies the role of oysters as ecosystem engineers in estuarine ecosystems.

Acknowledgements. We are grateful to L. Dodd, B. Von Korff, C. Martin, V. Pinkerton, W. Rodgers, M. Simpson, and M. Vozzo for help with this experiment. L. Weaver and North Carolina Coastal Federation provided access to the study location. Discussions with A. E. Murphy, I. C. Anderson, J. H. Grabowski, C. H. Peterson, B. K. Song and comments from 3 anonymous reviewers greatly improved this manuscript. This research was supported by funding from North Carolina Sea Grant and the National Science Foundation (NSF OCE-1233327 and NSF OCE-0961929 to M.F.P.).

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Editorial responsibility: Ronald Kiene, Mobile, Alabama, USA

Submitted: August 31, 2015; Accepted: October 4, 2016
Proofs received from author(s): November 9, 2016