Vol. 493: 23–30, 2013
doi: 10.3354/meps10516

MARINE ECOLOGY PROGRESS SERIES
Mar Ecol Prog Ser

Published November 20

Oyster-mediated benthic–pelagic coupling modifies nitrogen pools and processes

Ashley R. Smyth[1,2,*], Nathan R. Geraldi[1], Michael F. Piehler[1]

[1]The University of North Carolina at Chapel Hill, Institute of Marine Sciences, 3431 Arendell St. Morehead City, North Carolina 28557, USA

[2]Department of Biological Sciences, Virginia Institute of Marine Science, College of William and Mary, Gloucester Point, Virginia 23062, USA

ABSTRACT: Removal of nitrogen through enhanced denitrification has been identified as an ecosystem service provided by oysters. In this study, we assessed the effects of an individual oyster (*Crassostrea virginica*) on nitrogen dynamics. Fluxes of N_2, O_2, nitrate/nitrite (NO_x) and ammonium (NH_4^+) were measured from continuous-flow microcosms that contained a live oyster, sediment, or a live oyster + sediment. Net N_2 fluxes were indicative of nitrogen fixation in the sediment treatment and denitrification in the oyster and oyster + sediment treatments. Organic matter deposition and ammonium production associated with oyster biodeposits and excretion likely decreased N limitation, and thus the demand for fixation of new nitrogen, while increasing nitrification and subsequent denitrification. Oyster-mediated denitrification accounted for 48% of the total inorganic nitrogen efflux in the oyster microcosms and 35% in the oyster + sediment microcosms. Despite high rates of ammonium production, inclusion of the eastern oyster did not increase the pool of bioavailable nitrogen but shifted the microcosms from a nitrogen source to a nitrogen sink.

KEY WORDS: *Crassostrea virginica* · Denitrification · Nitrogen · Nitrogen fixation

INTRODUCTION

Oysters and the reefs they form are ecologically and economically valuable habitats. However, 85% of oyster reefs have been lost globally due to overharvesting, pollution and disease (Burreson et al. 2000, Lotze et al. 2006, Beck et al. 2011). Restoration of these ecosystems has been proposed to recover the lost fishery and provide additional ecosystem services (Officer et al. 1982, Cerco & Noel 2007, Beck et al. 2011, Grabowski et al. 2012). Oysters can alter a variety of ecosystem processes through suspension feeding, including the biogeochemical cycling of nutrients and water quality. Oysters filter large amounts of particulate matter from the water column, reducing phytoplankton and seston biomass (Grizzle et al. 2008). While a portion of this material is assimilated into oyster biomass (Carmichael et al. 2012), the undigested (pseudo-feces) and the unassimilated portions (feces) are transferred to the sediments as biodeposits (Newell & Jordan 1983). The transformation and transfer of material modifies conditions in the surrounding sediments and can affect biogeochemical processes, including denitrification (Newell et al. 2002, 2005, Porter et al. 2004, Piehler & Smyth 2011, Smyth et al. 2013).

Denitrification is the microbially mediated reduction of biologically available nitrate to largely inert N_2 gas. Denitrification has been identified as an important removal mechanism for nitrogen in estuaries (Seitzinger 1988, Nixon et al. 1995). Rates of denitrification are influenced by a variety of environmental factors, including oxygen concentration, availability of nitrate and the quality and quantity of organic

*Email: arsmyth@vims.edu

matter (Cornwell et al. 1999, Fulweiler et al. 2013). Oyster reefs are considered 'hot spots' for denitrification, often having higher rates of denitrification compared to areas without reefs (Piehler & Smyth 2011, Kellogg et al. 2013, Smyth et al. 2013). The concentration of organic-rich biodeposits on aerobic sediments coupled with the physical structure of the reef and reef-associated organisms contribute to the high rates of denitrification associated with oyster reef ecosystems. However, the contribution of an individual oyster to enhanced N_2 production remains unclear.

Oysters affect denitrification by changing oxygen concentrations and modifying carbon and/or nitrate availability. These changes may be attributable directly to the individual oyster or indirectly as the oyster modifies resources and conditions in the sediment. Oysters, like other bivalves, can directly affect O_2 concentrations in the water through respiration (Gelda et al. 2001), and indirectly through the increased oxygen demand associated with remineralization of organic-rich biodeposits (Bruesewitz et al. 2008). Oysters may alleviate nitrate limitation for denitrification by increasing rates of nitrification — the conversion of NH_4^+ to NO_3^-. Oysters increase ammonium directly through excretion and indirectly through remineralization of biodeposits (Dame et al. 1984, Lavrentyev et al. 2000, Newell et al. 2005). Furthermore, oysters and oyster reefs provide habitat for nitrifying bacteria. The shell and tissue of bivalves may harbor nitrifying bacteria and contribute to the enhancement of nitrification activity (Welsh & Castadelli 2004). Additionally, selective grazing by bivalves can remove bactivorous protozoa that otherwise consume nitrifying bacteria, a mechanism which has been linked to increased rates of nitrification (Lavrentyev et al. 2000).

The production and accumulation of oyster biodeposits can also increase denitrification. Biodeposits are a source of labile organic matter, which acts as the electron donor during denitrification. When biodeposits settle on aerobic sediments, nitrogen removal can be stimulated through increased coupled nitrification–denitrification (Newell et al. 2005). However, organic matter deposition can change sediment oxygen penetration depth and minimize the zone where nitrification can occur. Thus, organic matter loading can diminish coupled nitrification–denitrification, but enhance direct denitrification when nitrate is available in the overlying water (Caffrey et al. 1993, Cornwell et al. 1999). Organic matter deposition may also shift dominant nitrogen reactions in the sediments from nitrogen fixation to denitrification (Fulweiler et al. 2008). Inputs of organic matter from oysters may, therefore, suppress nitrogen fixation and enhance denitrification. However, in organic-rich systems additional organic matter deposition from the oysters may exacerbate reduced conditions, resulting in sulfide accumulation (Tenore & Dunstan 1973, Azandégbé et al. 2012) and increased anoxic microzones (Kemp et al. 1990) that can inhibit nitrification (Joye & Hollibaugh 1995) and subsequently reduce rates of denitrification.

Previous studies designed to examine the effect of the eastern oyster *Crassostrea virginica* on nitrogen transformations have focused on adjacent sediments (Piehler & Smyth 2011, Smyth et al. 2013), mimicked oyster-mediated biodeposition (Newell et al. 2002), or sampled the whole oyster reef community, including oysters and associated biota (Kellogg et al. 2013). We conducted a microcosm experiment to examine the direct effects of an individual *C. virginica* on nitrogen dynamics. We hypothesized that the inclusion of *C. virginica* in the microcosm would enhance denitrification by providing organic matter, removing oxygen and increasing availability of NH_4^+ for coupled nitrification–denitrification. Individual oysters are the basic building block of oyster reefs, and measuring how an oyster alters nitrogen processes is an important step in understanding the mechanisms through which oyster reefs alter nutrient dynamics.

MATERIALS AND METHODS

Sample collection

The experiment was conducted in microcosms (clear polycarbonate 6.4 cm diameter × 30 cm length) that contained a live oyster (*Crassostrea virginica*), sediment, or a live oyster + sediment and incubated in a continuous-flow system. *C. virginica* were collected from Calico Creek, North Carolina, USA (34° 43.40′ N, 76° 41.55′ W), at low tide and stored in saltwater flow-through tanks for 3 d. Prior to the start of the incubation, the outside shell of each *C. virginica* was scrubbed with a brush to remove algae and biofilms, allowing for the impacts of the oyster to be isolated. Average shell height (longest distance from umbo to opposite shell margin) in our experiment was 9.34 ± 0.45 cm, and the average weight of oyster tissue was 1.0 ± 2.5 g.

Sediment samples (17 cm depth) were collected by hand on 4 August 2009 during low tide from an intertidal flat suitable for oyster reef restoration in Bogue Sound, North Carolina. Sediment samples were col-

lected from within a 1 m^2 area of the intertidal flat. Sediments in this area have a similar grain size (171.5 ± 0.62 µm) and percentage of sediment organic matter (0.6 ± 0.02; A.R. Smyth unpubl. data). In addition, sound water (130 l) was collected for use in the continuous-flow incubation. Surface water temperature, salinity and dissolved oxygen were measured prior to sample collection (YSI 600 Series Sonde and Model 650 data logger, Yellow Springs Instruments).

Benthic flux incubations

Immediately after collection of sediment and water, all microcosm chambers were submerged in a water bath in an environmental chamber (Bally) set to *in situ* temperature (24.7°C). Microcosms were randomly assigned a treatment (oyster, sediment, oyster + sediment), and each treatment was replicated 3 times. Any visible invertebrates were carefully removed from the microcosms. Microcosms were sealed with a gas-tight lid equipped with an inflow and an outflow port and incubated in a continuous-flow system, where a peristaltic pump connects the microcosms to the reservoir water. An additional microcosm that contained only water was incubated as a control for a total of 10 microcosms. The potential for replication was limited due to logistical constraints but was at or above levels seen in similar studies (Anderson et al. 2003, Nizzoli et al. 2007, Fulweiler & Nixon 2009, Eyre et al. 2011). Aerated, unfiltered water was constantly passed through each microcosm at a flow rate of 2.0 ml min^{-1}, which gave a turnover time of approximately 3 h. After an initial 20 h acclimation period in the dark, microcosms were incubated for an additional 24 h in a 10 h dark:14 h light cycle. A light intensity of approximately 50 µE was maintained using dual-spectrum compact fluorescent lights. Oxygen in the reservoir water was monitored throughout the incubation with a YSI sonde and remained at about 6 mg l^{-1}, slightly lower than the dissolved oxygen concentration in Bogue Sound when the water was collected (7.02 mg l^{-1}). All *C. virginica* were alive at the conclusion of the experiment. Upon completion of the experiment sediment cores were again inspected for invertebrates, but none were visible.

Samples for dissolved gas and nutrient analysis were collected from the outflow port of each microcosm in sample vials. A bypass line that flowed directly into a sample vial, collected at the same time, was used to determine the concentration of dissolved constituents entering the microcosm. Samples for dissolved gas analysis were collected twice during the dark period and twice during the light period for each microcosm and averaged to give a microcosm-specific value. Samples for nutrient analysis were collected once in the dark and once in the light. Dissolved gas measurements were collected twice to ensure that each microcosm was at steady state (O_2 concentration in the outflow of each microcosm did not change over time). Nutrient samples were collected after steady state had been established.

N_2, O_2 and Ar were measured using a Balzers Prisma QME 200 quadruple mass spectrometer (MIMS; Pfeiffer Vacuum), and concentrations of N_2 and O_2 were determined using the ratio with Ar (Kana et al. 1994, Ensign et al. 2008). MIMS samples were processed immediately after collection. Samples for nutrient analysis were filtered through Whatman GF/F filters (25 mm diameter, 0.7 µm nominal pore size), and the filtrate was analyzed for nitrate + nitrite (reported as NO_x) and ammonium (NH_4^+) with a Lachat Quick-Chem 8000 (Lachat Instruments) automated ion analyzer (detection limits: 0.04 µM NO_x, 0.18 µM NH_4^+).

Flux calculations were based on the assumption of steady-state conditions and a well-mixed water column in each microcosm (Miller-Way & Twilley 1996). Fluxes were calculated using the following equation:

$$J = \left(\left[i_{\text{outflow}}\right] - \left[i_{\text{inflow}}\right]\right) \times \frac{F}{A}$$

where J is the flux in µmol m^{-2} h^{-1}, $[i_{\text{outflow}}]$ and $[i_{\text{inflow}}]$ are the concentrations (µM) of any dissolved constituent leaving (i_{outflow}) and entering (i_{inflow}) the core, respectively, F is the peristaltic pump flow rate (m^3 h^{-1}) and A is the surface area of the core (m^2) (Kana et al. 1998, Lavrentyev et al. 2000, McCarthy & Gardner 2003, Ensign et al. 2008). A positive flux indicates production in excess of demand, and a negative flux is a demand in excess of production within the microcosm. The N_2/Ar technique results in a net N_2 flux (gross denitrification−gross nitrogen fixation); a positive N_2 flux, therefore, indicates that denitrification dominates the net N_2 flux, while negative N_2 fluxes indicate that nitrogen fixation dominates the N_2 flux. Fluxes were corrected for activity in the water column and conditions in the microcosms by subtracting changes observed in the water blank control from those observed in the microcosm treatments. Daily fluxes for each microcosm were calculated as the light flux multiplied by time in the light (14 h) plus the dark flux multiplied by time in the dark (10 h) (Anderson et al. 2003, Nizzoli et al. 2006). For consistency, daily fluxes were divided by 24 h and

expressed per hour. Daily fluxes from each microcosm were averaged for each treatment to calculate mean values and their standard error (n = 3). Net dissolved inorganic nitrogen fluxes (DIN) were calculated as the sum of NH_4^+ and NO_x fluxes minus the N_2 flux.

Statistical analysis

Differences in daily fluxes (light flux multiplied by time in the light plus the dark flux multiplied by time in the dark) between treatments for N_2, O_2, NO_x, NH_4^+ and net DIN were tested with a 1-way analysis of variance (ANOVA). When the 1-way ANOVA was significant, Tukey-Kramer post hoc tests were used to determine which treatments had different fluxes. All analyses were considered significant at the $p < 0.05$ level. Assumptions of normality and homogeneity were tested using Shapiro-Wilks and Levene's tests, respectively. Data were transformed when necessary. Statistical analyses were performed using R 2.13.1 (R Foundation for Statistical Computing 2011).

RESULTS

Daily O_2 fluxes ranged from 94.38 ± 55.32 µmol O_2 m^{-2} h^{-1} in the sediment treatment to –6023 ± 647.3 µmol O_2 m^{-2} h^{-1} in the oyster treatment (Table 1). O_2 fluxes were significantly greater from the sediment compared to the oyster and oyster + sediment treatments. Oyster respiration likely contributed to O_2 demand.

There was an efflux of N_2 from the oyster and oyster + sediment treatments and an uptake of N_2 from the sediment treatment (Fig. 1), indicating that the sediment treatment had net nitrogen fixation, while the oyster and oyster + sediment treatments had net denitrification. Daily N_2 fluxes differed significantly between the treatments ($p < 0.05$), with the oyster treatment having significantly higher N_2 fluxes (more positive) than the oyster + sediment and sediment treatments. Daily N_2 fluxes were greater in the oyster + sediment treatment than in the sediment treatment.

There was uptake of NO_x for the oyster treatment and sediment treatment, but an efflux was measured from the oyster + sediment treatment, although it was not significantly different (Fig. 1; $p = 0.18$). Unlike NO_x fluxes, daily NH_4^+ fluxes were distinctive among treatments ($p < 0.05$). There was an efflux of NH_4^+ from the oyster and oyster + sediment treatments; the NH_4^+ flux was negligible for the sediment treatment (Fig. 1). Daily NH_4^+ production was similar in the oyster and oyster + sediment treatments ($p = 0.38$) and significantly higher in both treatments compared to the sediment. Net DIN fluxes were positive and similar for all treatments (Fig. 2), indicating that all treatments were a net source of inorganic nitrogen to the overlying water.

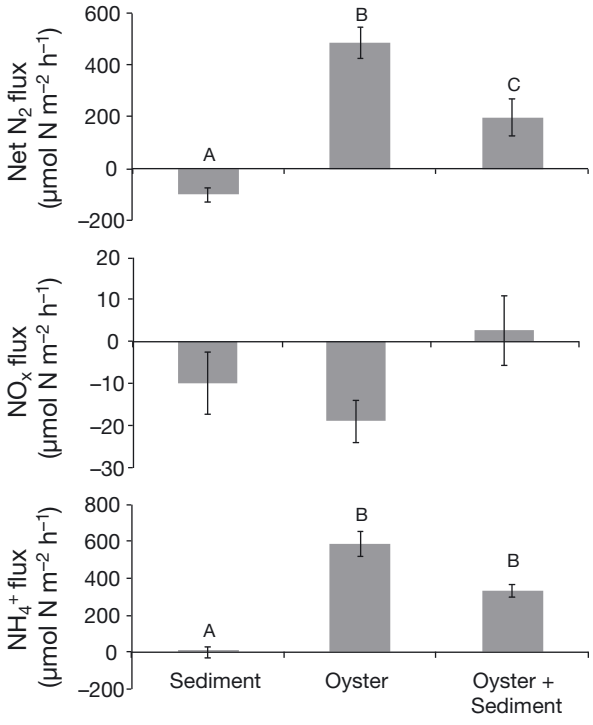

Fig. 1. Daily fluxes of N_2, NO_x and NH_4^+ for each treatment calculated from the sum of light and dark fluxes multiplied by the hours of the light and dark periods. Fluxes were corrected by subtraction of the water blank (procedural control). Error bars represent 1 standard error for 3 replicates. Treatments with the same letter indicate means that are not significantly different from each other. A positive flux indicates that the microcosm was a source to the overlying water

Table 1. Mean ± SE daily oxygen fluxes (µmol O_2 m^{-2} h^{-1}) for each treatment (n = 3). A negative flux indicates that there was a demand for O_2 from the microcosms, and a positive flux indicates that the microcosm was a source of O_2 to the overlying water. Treatments with the same letter indicate means that are not significantly different from each other

Treatment	Daily oxygen flux
Sediment[a]	94.38 ± 55.32
Oyster[b]	–6022.92 ± 647.26
Oyster + Sediment[b]	–4174.45 ± 1256.55

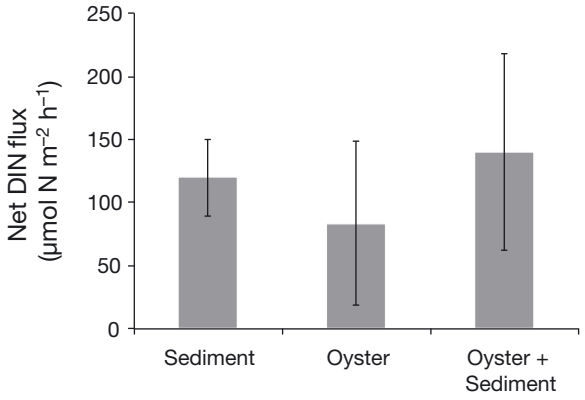

Fig. 2. Daily net dissolved inorganic nitrogen (DIN) fluxes for each treatment. Fluxes were calculated as the sum of NO_x and NH_4^+ fluxes minus N_2 flux. Error bars represent 1 standard error for 3 replicates. A positive flux indicates that the microcosm was a source to the overlying water

Oyster-mediated denitrification (N_2 production) accounted for an average of 41.5% of the total DIN efflux, ranging from 35% from the oyster + sediment microcosm to 48% from the oyster-only microcosm.

DISCUSSION

Our understanding of the processes through which *Crassostrea virginica* alters estuarine nitrogen dynamics is developing. The fundamentals of a mechanistic understanding are built by measuring the impact of individual oysters on nutrient processing. Recent studies have used *in situ* incubation chambers to examine the benthic fluxes associated with the whole oyster reef community (Kellogg et al. 2013) or continuous flow incubations using oyster reef sediments (Piehler & Smyth 2011, Smyth et al. 2013). These incubations make it difficult to isolate changes in fluxes resulting from oysters. Results from our experiment suggest that *C. virginica* alone can contribute to N_2 production. *C. virginica* in microcosm incubations allowed us to capture the direct effects of the oyster on biogeochemical processes.

Rates of N_2 production from the oyster + sediment treatment were similar to those found in previous studies from oyster reef sediments but lower than rates from whole community incubations (Piehler & Smyth 2011, Kellogg et al. 2013). This difference suggests that only a portion of the high rates of denitrification found from whole reef community experiments are attributable to an oyster. However, the use of sediments from a site suitable for restoration rather than oyster reef sediments does not account for the effects of the reef-associated community. We found the highest rates of N_2 production from a microcosm with *Crassostrea virginica* alone, which indicates that denitrification is associated with the animal itself. Denitrifying bacteria have previously been found in the guts of oysters (Pujalte et al. 1999). Differences between the oyster and oyster + sediment treatments are attributable to processes occurring in the sediment, which include competition for available nutrients and diffusion into the pore space.

This study focused on assessing the impact of *Crassostrea virginica* on nutrient dynamics. We hypothesized that the inclusion of *C. virginica* in the microcosm would enhance N_2 production (denitrification) by supplying organic matter, removing oxygen and increasing availability of NH_4^+ for coupled nitrification–denitrification. *C. virginica*-mediated denitrification accounted for 48% of the total DIN efflux in the oyster microcosms and 35% in the oyster + sediment microcosms. We found that denitrification dominated the net N_2 flux when *C. virginica* was present and that when *C. virginica* was absent the N_2 fluxes were dominated by nitrogen fixation. The change from N_2 production to demand suggests that the presence of *C. virginica* shifts the dominant nitrogen cycling pathway from nitrogen fixation to denitrification, likely through increased NH_4^+ availability. However, the net DIN flux was not different among treatments because of the magnitude of nitrogen fixation in the sediment-only treatment. Nitrogen fixation is a source of new nitrogen, while NH_4^+ production from *C. virginica* is a recycling of particulate nitrogen in phytoplankton back to the water column (Dame et al. 1985).

Crassostrea virginica biodeposits contain significant amounts of organic carbon, nitrogen and extractable ammonium that can supply resources to the microbial community (Haven & Morales-Alamo 1966, Grenz et al. 1990, Giles & Pilditch 2006, Higgins et al. 2013). The eastern oyster produces about 1.33 to 16.8 mg C per gram of oyster tissue d^{-1} as biodeposits (Haven & Morales-Alamo 1966, Higgins et al. 2013). Given the turnover time in our incubation and the clearance rates of an oyster, we estimate that 2.66 ± 6.65 to 33.6 ± 84 mg of particulate C was retained within the microcosm as a result of filtration. The bulk of the carbon and nitrogen in the biodeposits could be used for heterotrophic metabolism leading to anoxic micro-zones and conditions favorable for denitrification. Biodeposits have been associated with denitrifying bacteria and enhanced denitrification (Grenz et al. 1990, Azandégbé et al. 2012); however, N_2 production rates from individual *C. virginica* were

higher than rates associated with the biodeposits alone (Higgins et al. 2013) and removed a large percentage of nitrogen compared to experiments where pelletized phytoplankton were used to mimic biodeposits (Newell et al. 2002).

Quantifying the effects of *Crassostrea virginica* on ecosystem function is challenging, given the methodological difficulty in measuring denitrification and the complexity of the reef ecosystems. Our results agree with previous studies which concluded that oyster-mediated denitrification occurs through coupling between nitrification stimulated by biodeposition and ammonium production from the oysters (Boucher & Boucher-Rodoni 1988, Newell et al. 2002, Piehler & Smyth 2011, Smyth et al. 2013). Nitrate/nitrite fluxes were low and directed into the microcosm for the oyster and sediment treatments, while there was low production from the oyster + sediment treatment. In the oyster treatment, the nitrate/nitrite demand was not enough to support the rate of N_2 production measured. This could have resulted from nitrifying bacteria on the shell or within the tissue of the oyster, as has been found with other filter-feeding bivalves (Welsh & Castadelli 2004). If nitrifying bacteria associated with oysters are responsible for increased nitrate supply, this would also be present in the oyster + sediment treatment. Oyster presence has stimulated nitrification in sediments (Boucher & Boucher-Rodoni 1988), and high rates of NO_x production within oyster reefs also suggest that oysters have high rates of nitrification (Kellogg et al. 2013). The low production of NO_x from the oyster + sediment treatment coupled with the positive N_2 flux, and low ambient nitrate/nitrite concentration suggest that sediment nitrification was promoted in the presence of *C. virginica*.

The shift detected between nitrogen fixation in the sediment treatment to denitrification with *Crassostrea virginica* is likely the result of the combination of an increase in ammonium supply and high-quality organic matter. When ammonium is high and there is ample supply of labile organic matter, denitrification tends to dominate the net N_2 flux; when ammonium is low and organic matter is recalcitrant, nitrogen fixation tends to dominate (Fulweiler et al. 2013). The sediments used in this experiment had low sediment organic matter, and the inclusion of *C. virginica*, which was an organic matter addition, increased the recycling of nitrogen through remineralization (as indicated by the high rates of ammonium production). The combination of these effects decreased the need for the fixation of new nitrogen. In a recent study, Atkinson et al. (2013) found an increase in abundance of N-fixing blue-green algae when mussels were not present and an increase in abundance of diatoms when mussels were included, implying that the presence of mussels can elevate nitrogen limitation. Our results indicate that *C. virginica* may have the same effect, and that the increase in ammonium associated with *C. virginica* alleviates N limitation and reduces the need for fixation of new nitrogen. However, if there was ample supply of nitrogen in the water column these effects may not be as apparent.

While continuous-flow microcosms provide insight into the effects of *Crassostrea virginica* on nutrient dynamics, extrapolating these data to oyster reef ecosystems is problematic. For example, the density of bivalves has been shown to affect nutrient fluxes across the sediment–water interface (Nizzoli et al. 2007, Green et al. 2012). The results presented here do not take into account any density-dependent interactions. Additionally, the use of cleaned oysters reduced the effects of any shell-attached organisms. In the natural environment, tide, density, light, water depth, salinity, ambient nutrient concentration, temperature and reef structure will influence the denitrification associated with *C. virginica* and the reefs they form. It is possible that continuous-flow microcosms overestimate the rates of denitrification associated with *C. virginica.* For instance, the increase in O_2 demand from *C. virginica* combined with minimal competition for available nutrients from native benthic organisms and the accumulation of organic material could increase denitrification. However, continuous-flow incubations likely reduce this potential bias as compared to batch incubations, which do not have any water circulation. While the current study used sediments suitable for oyster reef restoration, the addition of oysters to oyster reef sediments would likely provide a realistic representation of the nitrogen fluxes associated with the bivalve ecosystems, because it combines the effects of the oyster reef sediment community with activity directly associated with the oyster and the oyster shell.

This study contributes to the growing body of evidence that *Crassostrea virginica* can enhance denitrification (Newell et al. 2002, Piehler & Smyth 2011, Kellogg et al. 2013, Smyth et al. 2013). We found that in the absence of *C. virginica*, sediments were a net source of reactive nitrogen through nitrogen fixation, whereas the addition of oysters increases organic matter deposition, alleviating carbon limitation and increasing denitrification. While *C. virginica* caused a shift in N_2 processes, the lack of differences in the net DIN flux between treatments

suggests that all treatments affected the pool of nitrogen equally. Thus, restoration is not likely to add additional nitrogen and will provide the valuable ecosystem service of nitrogen removal. This study provides early insight into the effects of *C. virginica* on nitrogen biogeochemistry upon which future research on bivalve impacts on nutrient removal can build.

Acknowledgements. This study was supported by the NOAA Ecological Effects of Sea Level Rise Program, North Carolina Sea Grant, and National Science Foundation awards OCE-0961929 and OCE-1233327. Comments from S. Thompson, A. Murphy and 3 anonymous reviewers greatly improved this manuscript. We thank K. Siporin, M. Simpson, M. Vozzo, L. Brown, L. Dee, W. Rogers, C. Martin, V. Pinkerton and C. Biddle for their assistance in the field and laboratory.

LITERATURE CITED

Anderson I, McGlathery K, Tyler AC (2003) Microbial mediation of 'reactive' nitrogen transformations in a temperate lagoon. Mar Ecol Prog Ser 246:73–84

Atkinson CL, Vaughn CC, Forshay KJ, Cooper JT (2013) Aggregated filter-feeding consumers alter nutrient limitation — consequences for ecosystem and community dynamics. Ecology 94:1359–1369

Azandégbé A, Poly F, Andrieux-Loyer F, Kérouel R, Philippon X, Nicolas JL (2012) Influence of oyster culture on biogeochemistry and bacterial community structure at the sediment–water interface. FEMS Microbiol Ecol 82: 102–117

Beck MW, Brumbaugh RD, Airoldi L, Carranza A and others (2011) Oyster reefs at risk and recommendations for conservation, restoration, and management. Bioscience 61: 107–116

Boucher G, Boucher-Rodoni R (1988) *In situ* measurement of respiratory metabolism and nitrogen fluxes at the interface of oyster beds. Mar Ecol Prog Ser 44:229–238

Bruesewitz DA, Tank JL, Bernot MJ (2008) Delineating the effects of zebra mussels (*Dreissena polymorpha*) on N transformation rates using laboratory mesocosms. J N Am Benthol Soc 27:236–251

Burreson EM, Stokes NA, Friedman CS (2000) Increased virulence in an introduced pathogen: *Haplosporidium nelsoni* (MSX) in the eastern oyster *Crassostrea virginica*. J Aquat Anim Health 12:1–8

Caffrey J, Sloth N, Kasper H, Blackburn T (1993) Effect of organic loading on nitrification and denitrification in a marine sediment microcosm. FEMS Microbiol Ecol 12: 159–167

Carmichael RH, Walton W, Clark H, Ramcharan C (2012) Bivalve-enhanced nitrogen removal from coastal estuaries. Can J Fish Aquat Sci 69:1131–1149

Cerco C, Noel M (2007) Can oyster restoration reverse cultural eutrophication in Chesapeake Bay? Estuar Coast 30:331–343

Cornwell J, Kemp W, Kana T (1999) Denitrification in coastal ecosystems: methods, environmental controls, and ecosystem level controls, a review. Aquat Ecol 33:41–54

Dame R, Zingmark R, Haskin E (1984) Oyster reefs as processors of estuarine materials. J Exp Mar Biol Ecol 83: 239–247

Dame R, Wolaver T, Libes S (1985) The summer uptake and release of nitrogen by an intertidal oyster reef. Neth J Sea Res 19:265–268

Ensign S, Piehler M, Doyle M (2008) Riparian zone denitrification affects nitrogen flux through a tidal freshwater river. Biogeochemistry 91:133–150

Eyre BD, Ferguson AJP, Webb A, Maher D, Oakes JM (2011) Denitrification, N-fixation and nitrogen and phosphorus fluxes in different benthic habitats and their contribution to the nitrogen and phosphorus budgets of a shallow oligotrophic sub-tropical coastal system (southern Moreton Bay, Australia). Biogeochemistry 102:111–133

Fulweiler R, Nixon SW (2009) Responses of benthic–pelagic coupling to climate change in a temperate estuary. Hydrobiologia 629:147–156

Fulweiler R, Nixon S, Buckley B, Granger S (2008) Net sediment N fluxes in a coastal marine system — experimental manipulations and a conceptual model. Ecosystems 11: 1168–1180

Fulweiler RW, Brown SM, Nixon SW, Jenkins BD (2013) Evidence and a conceptual model for the co-occurrence of nitrogen fixation and denitrification in heterotrophic marine sediments. Mar Ecol Prog Ser 482:57–68

Gelda R, Effler S, Owens E (2001) River dissolved oxygen model with zebra mussel oxygen demand (ZOD). J Environ Eng 127:790–801

Giles H, Pilditch CA (2006) Effects of mussel (*Perna canaliculus*) biodeposit decomposition on benthic respiration and nutrient fluxes. Mar Biol 150:261–271

Grabowski J, Brumbaugh R, Conrad R, Keeler A and others (2012) Economic valuation of ecosystem services provided by oyster reefs. Bioscience 62:900–909

Green DS, Boots B, Crowe TP (2012) Effects of non-indigenous oysters on microbial diversity and ecosystem functioning. PLoS One 7:e48410

Grenz C, Hermin M, Baudinet D, Daumas R (1990) *In situ* biochemical and bacterial variation of sediments enriched with mussel biodeposits. Hydrobiologia 207: 153–160

Grizzle R, Greene J, Coen L (2008) Seston removal by natural and constructed intertidal eastern oyster (*Crassostrea virginica*) reefs: a comparison with previous laboratory studies, and the value of *in situ* methods. Estuar Coast 31: 1208–1220

Haven D, Morales-Alamo R (1966) Aspects of biodeposition by oysters and other invertebrate filter feeders. Limnol Oceanogr 11:487–498

Higgins CB, Tobias C, Piehler MF, Smyth AR, Dame RF, Stephenson K, Brown BL (2013) Effect of aquacultured oyster biodeposition on sediment N_2 production in Chesapeake Bay. Mar Ecol Prog Ser 473:7–27

Joye S, Hollibaugh J (1995) Influence of sulfide inhibition of nitrification on nitrogen regeneration in sediments. Science 270:623–625

Kana T, Darkangelo C, Hunt M, Oldham J, Bennett G, Cornwell J (1994) Membrane inlet mass-spectrometer for rapid high-precision determination of N_2, O_2, Ar in environmental water samples. Anal Chem 66:4166–4170

Kana T, Sullivan M, Cornwell J, Groszkowski K (1998) Denitrification in estuarine sediments determined by membrane inlet mass spectrometry. Limnol Oceanogr 43: 334–339

Kellogg ML, Cornwell JC, Owens MS, Paynter KT (2013)

Denitrification and nutrient assimilation on a restored oyster reef. Mar Ecol Prog Ser 480:1–19

Kemp W, Sampou P, Caffrey J, Mayer M, Henriksen K, Boynton W (1990) Ammonium recycling versus denitrificaiton in Chesapeake Bay sediments. Limnol Oceanogr 35:1545–1563

Lavrentyev PJ, Gardner WS, Yang L (2000) Effects of the zebra mussel on nitrogen dynamics and the microbial community at the sediment–water interface. Aquat Microb Ecol 21:187–194

Lotze HK, Lenihan H, Bourque B, Bradbury R and others (2006) Depletion, degradation, and recovery potential of estuaries and coastal seas. Science 312:1806–1809

McCarthy M, Gardner W (2003) An application of membrane inlet mass spectrometry to measure denitrification in a recirculating mariculture system. Aquaculture 218: 341–355

Miller-Way T, Twilley RR (1996) Theory and operation of continuous flow systems for the study of benthic–pelagic coupling. Mar Ecol Prog Ser 140:257–269

Newell RIE, Jordan SJ (1983) Preferential ingestion of organic material by the American oyster *Crassostrea virginica*. Mar Ecol Prog Ser 13:47–53

Newell R, Cornwell J, Owens M (2002) Influence of simulated bivalve biodeposition and microphytobenthos on sediment nitrogen dynamics: a laboratory study. Limnol Oceanogr 47:1367–1379

Newell RIE, Fisher TR, Holyoke RR, Cornwell JC (2005) Influence of eastern oysters on nitrogen and phosphorus regeneration in Chesapeake Bay, USA. In: Dame R, Olenin S (eds) The comparative roles of suspension-feeders in ecosystems. NATO Science Ser IV: Earth and Environmental Sciences, Vol 47. Springer, Dordrecht, p 93–120

Nixon S, Granger S, Nowicki B (1995) An assessment of the annual mass balance of carbon, nitrogen, and phosphorus in Narragansett Bay. Biogeochemistry 31:15–61

Nizzoli D, Welsh DT, Fano EA, Viaroli P (2006) Impact of clam and mussel farming on benthic metabolism and nitrogen cycling, with emphasis on nitrate reduction pathways. Mar Ecol Prog Ser 315:151–165

Nizzoli D, Bartoli M, Cooper M, Welsh DT, Underwood GJC, Viaroli P (2007) Implications for oxygen, nutrient fluxes and denitrification rates during the early stage of sediment colonisation by the polychaete *Nereis* spp. in four estuaries. Estuar Coast Shelf Sci 75:125–134

Officer CB, Smayda TJ, Mann R (1982) Benthic filter feeding: a natural eutrophication control. Mar Ecol Prog Ser 9:203–210

Piehler MF, Smyth AR (2011) Habitat-specific distinctions in estuarine denitrification affect both ecosystem function and services. Ecosphere 2:art12

Porter ET, Cornwell JC, Sanford LP (2004) Effect of oysters *Crassostrea virginica* and bottom shear velocity on benthic–pelagic coupling and estuarine water quality. Mar Ecol Prog Ser 271:61–75

Pujalte MJ, Ortigosa M, Macian M, Garay E (1999) Aerobic and facultative anaerobic heterotrophic bacteria associated to Mediterranean oysters and seawater. Int Microbiol 2:259–266

Seitzinger S (1988) Denitrification in freshwater and coastal marine ecosystems: ecological and geochemical significance. Limnol Oceanogr 33:702–724

Smyth AR, Thompson SP, Siporin KN, Gardner WS, McCarthy MJ, Piehler MF (2013) Assessing nitrogen dynamics throughout the estuarine landscape. Estuar Coast 36: 44–55

Tenore K, Dunstan W (1973) Comparison of rates of feeding and biodeposition of the American oyster, *Crassostrea virginica* Gmelin, fed different species of phytoplankton. J Exp Mar Biol Ecol 12:19–26

Welsh DT, Castadelli G (2004) Bacterial nitrification activity directly associated with isolated benthic marine animals. Mar Biol 144:1029–1037

Editorial responsibility: Erik Kristensen, Odense, Denmark

Submitted: February 8, 2013; Accepted: August 14, 2013
Proofs received from author(s): November 3, 2013